Anthony A. Gaspari
Stephen K. Tyring
Daniel H. Kaplan
Editors

Clinical and Basic Immunodermatology

Second Edition

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Preface

Building on Our Foundation

In 2008, we published the first edition of *Clinical and Basic Immunodermatology*. Over the past 9 years, much has changed in the basic science and clinical arenas, stimulating Steve Tyring and me to update our textbook. We have the good fortune that Dr. Daniel H. Kaplan, a highly accomplished immunodermatologist, joined our editorial team, which has augmented our knowledge in the field and expanded our network of experts to address the ever-broadening horizons of cutaneous immunology.

There continues to be a tremendous number of advances in the fields of cellular, molecular, innate, and adaptive immunity, as well as immunopharmacology, which have been translated to a better understanding and treatment of a number of dermatologic diseases. There are also a number of new therapeutic agents that are targeted therapies, or have an immune mechanism. All of these developments have occurred in the backdrop of the information age. Our goals of this textbook remain the same as with the first edition of *Clinical and Basic Immunodermatology*. We have recruited national and international experts to author chapters on their respective areas of expertise. Hence, our approach for this important endeavor is that of a multiauthored collection of chapters that would be integrated into this book. Our goal is to present the latest information related to fundamentals of the skin immune system, as well as a disease-focused textbook in the same concise, readable, and easily digested format that was initially developed by Dr. Dahl with his original *Clinical Immunology* textbook in 1981. We have recruited new experts to provide information summarized in their chapters. We have added new subject matter such as the expanded role of innate lymphocytes in the immune system and their role in dermatologic disease, a section on antimicrobial peptides, a chapter focused on auto-inflammatory diseases, as well as a chapter on the role of cell death in skin homeostasis and dermatologic diseases.

We thank the authors for their outstanding contributions. We remain grateful to Dr. Dahl for his vision and his original book, which has had profound influence on generations of dermatologists. We have strived to enhance the teaching of cutaneous immunology, particularly as related to skin disease, to the next generations of young dermatologists who will be caring for patients afflicted with immune-based skin diseases. We would be delighted if our textbook triggered the kind of interest in immunology that was stimulated in us, the editors, during our training.

Over the next few years, we look forward to watching the progress unfold in the field of immunodermatology that will lead to the third edition of our textbook *Clinical and Basic Immunodermatology*.

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Innate and Adaptive Components of the Cutaneous Immune Barrier: The Central Role of Dendritic Cells

1

Georg Stingl, Marie-Charlotte Brüggen, and Mariana Vázquez-Strauss

Abstract

Immune responses initiated in the skin can be extremely powerful at both a local and systemic level. One of the milestones in elucidating the mechanisms underlying this phenomenon was the discovery of the T cell response-inducing function of Langerhans cells (LC). In the meantime, we know that the family of dendritic antigen-presenting cells in the skin is much bigger and, in addition to LC, includes dermal dendritic cells (DDC), CD141+DC, CD14+DC, inflammatory DC and plasmacytoid DC. Depending on the cellular and molecular milieu, these cells can function as either sensitizing or tolerizing elements. Signals transmitted from (innate) receptors recognizing damage- or pathogen-associated patterns are involved in directing these different functions in DC. Toll-like pathogen recognition receptors (TLR) have been particularly well investigated in this regard. The distinct distribution of TLR on LC and other DC subsets allows the immune system to elegantly orchestrate the regulatory and pro-inflammatory functions of these cells. Intriguingly, TLR signaling in DC/LC not only allows to initiate adaptive immune responses, but also directly induces innate effector functions. This is demonstrated by our findings on the mechanisms underlying basal cell carcinoma (BCC) regression upon treatment with the pharmacological TLR7 agonist imiquimod. We observed that in imiquimod-treated BCC, plasmacytoid DC directly kill tumor cells via the apoptosis-inducing molecule TRAIL. Melanoma cells can also become TRAIL-susceptible, but the magnitude of this phenomenon varies from patient to patient. Our recent findings show that TRAIL susceptibility of melanoma cell lines can be increased upon exposure to the anti-inflammatory compound diclofenac.

Taken together, we begin to understand the exact position of LC and DC in the highly complex circuits of the immune system in the skin and how these cells could be manipulated for therapeutic purposes.

Keywords

Dendritic cells • Antigen-presenting cell • APC • BCC • Basal cell carcinoma • Contact hypersensitivity • Dermal dendritic cell • DC • DDC • Lipopolysaccharides • Lipotechoic acid • Adaptive immunity • TLR-transmitted

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Abbreviations

APC	Antigen-presenting cell
BCC	Basal cell carcinoma
CHS	Contact hypersensitivity
DC	Dendritic cell
DDC	Dermal dendritic cell
dsRNA	Double-strain RNA
LC	Langerhans cell
LPS	Lipopolysaccharides
LTA	Lipotechoic acid
MHC	Majory histocompatibility complex
PAMP	Pathogen-associated molecular pattern
pDC	Plasmacytoid dendritic cell
PRR	Pattern recognition receptor
S.	Staphylococcus
ssRNA	Single-stranded RNA
TLR	Toll-like receptor
TRAIL	Tumor necrosis factor related apoptosis inducing ligand
UV	Ultraviolet
poly I:C	Polyinosinic:polycytidylic acid

Introduction

One of the first documented examples revealing the potency of immune responses initiated in the skin is the successful smallpox vaccination by Edward Jenner [1]. In this heroic experiment performed on the son of his gardener, Jenner introduced scraping material obtained from an infectious cowpox pustule (of a dairymaid) into the skin. A few weeks later, upon re-inoculation with material from a fresh smallpox lesion, the boy was protected from the disease.

It took quite a while until it was realized that immunization via the skin can result in a stronger, longer lasting immune response than immunization via other routes. This is impressively illustrated by the induction of cancer immunity, which succeeded following the repeated intracutaneous, but not extracutaneous application of cancer (murine sarcoma) homogenates [2]. During the following decades, considerable efforts were made to unravel the mechanisms underlying this phenomenon.

The enigma seemed resolved when it was discovered that epidermal dendritic cells (namely Langerhans cells (LC)) can evoke very robust proliferative responses in naive and sensitized T cells [3, 4], which were greater in magnitude than those induced by mononuclear phagocytes (see also Chap. 9, Dendritic cells). Streilein et al. could show in a murine model of allergic contact dermatitis termed contact hypersensitivity (see Chap. 22) that the epicutaneous application of a hapten only led to sensitization when the skin of the application site contained LC but not when it was devoid of these cells [5]. Based on these findings, the idea evolved that an antigenic encounter in the skin/epidermis invariably results in LC-mediated T cell activation and thereby sensitization. Were this concept true, one would expect an army of hetero- or even autoreactive T cells to constantly populate, attack and injure the skin. Luckily, this is not the case. The possibility that the mere presence of LC is not necessarily predictive of the occurrence of productive T cell responses came from the observation that LC evoked robust T cell stimulation only upon receipt of activating stimuli [6]. These stimuli include the disruption of skin homeostasis and the exposure to danger signals (e.g. immunogenic haptens, microorganisms) that results in the release of proinflammatory cytokines and other mediators. By contrast, resting LC [7], LC from corticosteroid-treated patients [8] or LC from ultraviolet (UV) irradiated skin (via keratinocytic RANKL expression, [9]) lead to an expansion of CD4+/CD25+/GITR+/FoxP3+ regulatory T cells (Treg) capable of down-modulating proliferative and cytotoxic T cell responses. The exclusive role of LC in the initiation of T cell responses via the skin was further questioned by the discovery of a second DC population in normal human skin, namely CD1+ dermal dendritic cells (DDC). DDC are equally potent as LC with regard to their immunostimulatory capacity *in vitro* but exhibit certain phenotypic features that allow distinguishing them from LC (cf. Fig. 1.1a). The relative contribution of LC and DDC in the elicitation of sensitizing and tolerizing skin-derived immune responses is a matter of conjecture and heavy debate. On the one hand, the positive correlation between epidermal LC density and the success rate of epicutaneous sensitization (e.g., contact hypersensitivity (CHS), epicutaneous vaccination) clearly argue in favor of an important role of LC in this process. On the other hand, elimination of epidermal LC in mice by genetic manipulation results in an enhanced CHS response as compared to wild-type mice [10].

From a conceptual viewpoint, it makes perfect sense to assume that danger signals not reaching beyond the epidermis, i.e. the site of LC, will not mobilize all the armed forces the immune system is capable of activating. On the other hand, virulent microorganisms that breach the dermo-epidermal junction and thereby can reach DDC should trigger a massive host defense response that can successfully eliminate the pathogen. From this perspective, one would expect that LC mainly act as regulatory antigen-presenting cells (APC) inducing a state of antigen-specific non-responsiveness. Following this reasoning, DDC should remain inert under homoeostatic conditions and mature into potent sensitizing DC upon receiving appropriate activation signals. The “black and white” picture of the roles of LC vs. DC is probably not tenable under all circumstances. As an example, in response to an overwhelming microbial insult, LC can engage themselves

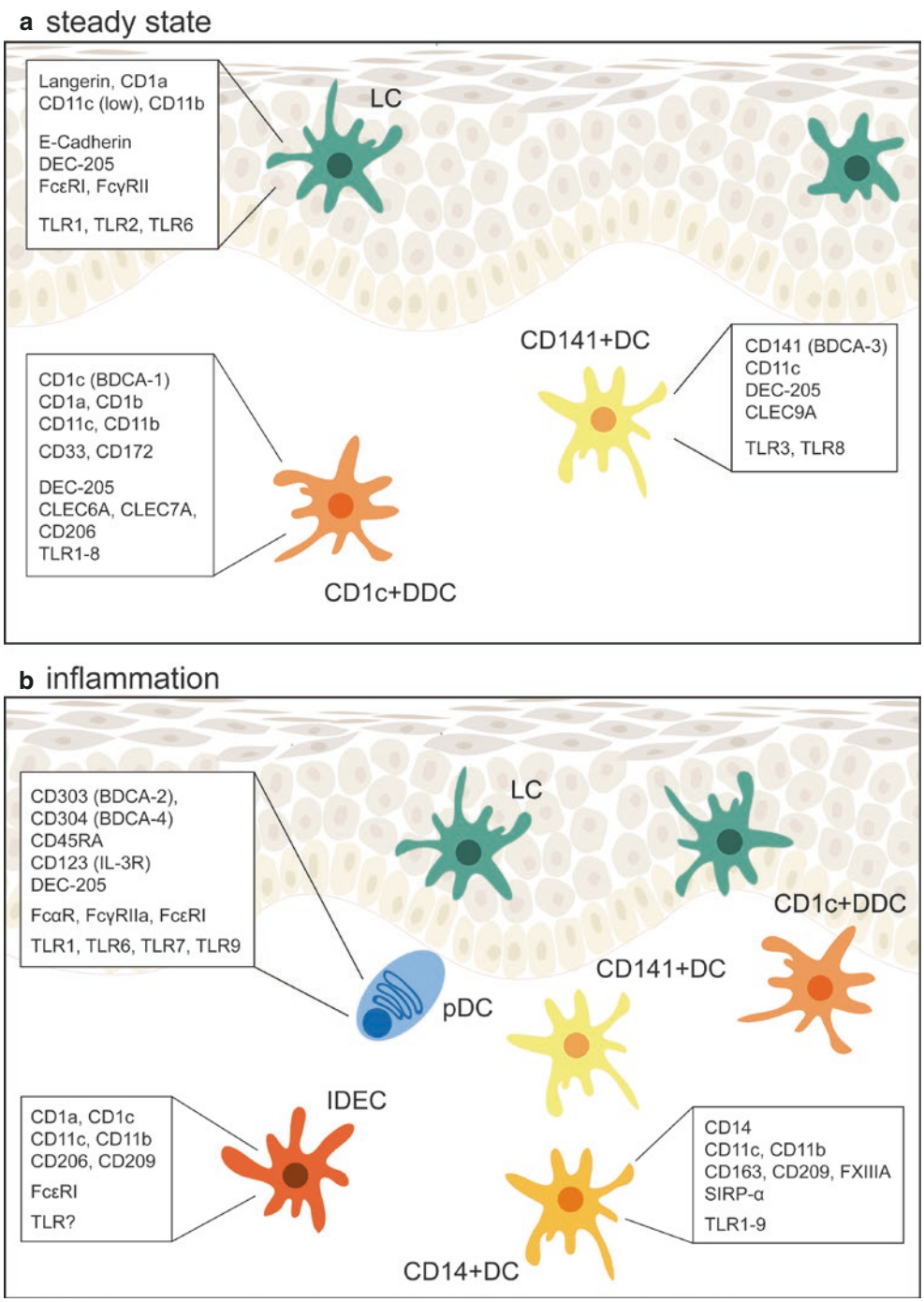

Fig. 1.1 LC and DC populations in human skin. (**a**) LC and DC residing in human skin in the steady state and (**b**) infiltrating the skin during inflammation. The main suface markers and pattern recognition receptors of the respective subpopulations are depicted

in the promotion of a T effector cell response [7]. Although we have gained more insight into the LC and DC network in the skin, we are still far from understanding it in all its complexity. This is illustrated by the recent finding of another (although quantitatively minor) DC subset residing in steady-state skin, i.e. CD141+ DC (Fig. 1.1a) [11]. These DC seem to be mainly engaged in antigen cross-presentation [12].

In an inflammatory setting, skin-resident LC, DDC and CD141+ DC undergo phenotypic and functional changes. In addition, various other types of DC are entering the stage (cf. Fig. 1.1b). These blood-derived DC include the so-called plasmacytoid DC (pDC), various DC with an inflammatory phenotype (inflammatory DC, IDEC) and CD14+ (monocyte-derived) DC. All of them exhibit distinct features with regard to their antigen presentation properties and interaction with other immune cells (for review, cf. [13, 14]). They play major roles in the pathogenesis of various skin conditions such as atopic dermatitis (Chap. 22) and psoriasis (Chap. 21). Their involvement

in these diseases will be discussed in the respective chapters of this book.

Toll-Like Receptors: Bridging Innate and Adaptive Immunity

Pattern Recognition Receptors: Sensing the Danger

Until quite recently, it was essentially unknown by which mechanisms danger signals (such as immunogenic haptens and microorganisms) trigger the activation and maturation of LC and other DC in the skin and ultimately initiate an adaptive immune response. A series of discoveries (for review, cf. [15, 16]) has shed new light on this issue by revealing that DC function and development are essentially modulated by innate immune receptors recognizing damage- or pathogen-associated molecular patterns (DAMP and PAMP; listed in Table 1.1) (see Chap. 2). Among this growing family of pattern recognition receptors (PRR), the so-called toll-like pathogen recognition receptors (TLR) have been particularly well investigated. Ten TLR have been described in humans so far (listed in Table 1.2). TLR can be broadly divided into two groups (extra- vs. intracellular). Extracellular TLR (TLR1, 2, 4, 5, 6) essentially recognize bacterial and fungal products. Briefly, TLR2 combined with TLR1 or TLR6 mostly recognizes motifs of gram-positive bacteria (e.g. lipoproteins, lipotechoic acid (LTA)), while TLR4 senses gram-negative bacteria-associated lipopolysaccharides (LPS). Bacterial flagellin is recognized by TLR5. The intracellular receptors TLR3 and TLR7-9 recognize mostly virus-derived nucleic acids, i.e. double-stranded RNA (dsRNA; TLR3), single-stranded RNA (ssRNA) (TLR7-8) and CpG oligodeoxynucleotides (TLR9).

The potency of TLR-mediated danger signals in triggering immune responses cannot be reduced to their impact on DC and other cells of hematopoietic origin. In fact, keratinocytes [17] express a series of TLR (at the mRNA level: TLR1-6 and 9-10; functionally: TLR3, 4, 5 and 9, [17, 18]). Engagement of their respective ligands can trigger (as illustrated in the following paragraphs) both innate and adaptive responses.

As far as LC and DC are concerned, studies investigating their TLR expression have yielded partially divergent results [18–21], probably due to differences in the experimental setting, e.g. culture conditions. It seems clear that LC and the various DC subsets do not share the same TLR expression patterns (cf. Fig. 1.1a, b) and, in consequence, exhibit differ-

Table 1.1 Pattern recognition receptors (PRR) and their principal ligands

A) Principal PRR families

Group of PRR	Examples of PRR	Principal PAMP/DAMP(s)
Nucleotide-binding oligomerization domain (NOD)-like receptors (NLR)[a]	NOD1 (CARD4) NLRP1B (NALP1) NLRP3 (NALP3)	iE-DAP, GM-tripeptide Anthrax letal toxin MDP, DNA, RNA, toxins
Retinoic acid inducible gene I (Rig1)-like receptors (RLR)	DDX58 (RIG-1) DHX9, DHX36	Short ds-RNA, ss-RNA DNA
C-type lectin receptors (CLR)	CD207 (langerin), CD209 (DC-SIGN), CLEC6A	Fucose, mannose High mannose High mannose
Toll-like receptors (TLR)	(cf. below)	

[a]For details, cf. [16, 55]

B) TLR

Localization	TLR subtype	Principal PAMP(s)	Mostly expressed on
Extracellular	TLR1/TLR2[a]	Lipoproteins	Gram-positive bacteria, mycobacteria
	TLR2	Lipoproteins, peptidoglycan (PGN)	Gram-positive bacteria
	TLR4	Lipoproteins, lipopolysaccharides (LPS)	Gram-negative bacteria
	TLR6/TLR2[a]	e.g. lipoteichoic acid (LTA)	Gram-positive bacteria, mycoplasma
	TLR10	Not known	
	TLR5	Flagellin	Flagellated bacteria
Intracellular	TLR7	Single-stranded RNA (ssRNA)	Viruses
	TLR8	ssRNA	Viruses
	TLR9	CpG oligodeoxynucleotide	Bacteria; DNA viruses
	TLR3	Double-strain RNA (dsRNA)	Viruses

[a]Heterodimerized; for review, cf. [15]

ent reactions to microbial or other immunogenic stimuli. This distinct distribution of TLR on DC allows the immune system to elegantly orchestrate innate and adaptive responses, which is why growing efforts have been put into the development of vaccine formulations making use of these mechanisms.

TLR as Gatekeepers of Tolerance Towards Bacteria?

According to the idea that LC are responsible for maintaining tolerance and DDC for initiating immune reactions, one would expect that LC do not react to the epidermal invasion of harmless, gram-positive bacteria belonging to the commensal skin flora. This theory seems to be supported by the finding that DDC abundantly secrete IL-6 and TNF-α when exposed to bacterial components (such as Pam3CSK, a synthetic TLR1/2 ligand, LPS and PGN [21]), while LC secrete IL-6, -8 and -10 only upon exposure to PGN [18, 21]. PGN-induced IL-10 could, via its inhibitory effect on the antigen presentation function of LC [22], contribute to LC-modulated tolerance towards commensal bacteria. The concept that TLR-mediated signals can contribute to maintaining tolerance is further strengthened by evidence from keratinocyte studies. The latter have shown that in keratinocytes engagement of LTA belonging to the commensal bacterium *Staphylococcus (S.) epidermidis*, but not to *S. aureus* induces an inhibitory effect on TLR3-triggered IL-6 and TNF-α expression [23] and even promotes the expression of antimicrobial peptides [24].

Orchestration of TLR-Transmitted Signals in Viral Infections

As far as viral infections are concerned, it was even before the discovery of TLR that the so-called pDC were identified as a rich source of the type I interferon IFN-alpha (IFN-α) in response to viruses (review in: [25]). IFN-α is a potent tool in the antiviral defense and acts against viruses both indirectly (by enhancing adaptive immune functions) and directly. Later, it was found that the abundant IFN-α production in pDC is triggered by signals from TLR recognizing virus components, i.e. ssRNA (TLR7) and CpG oligonucleotide (TLR9). In contrast, DDC as well as freshly isolated LC do not seem to undergo phenotypic or functional changes in response to direct exposure to these TLR ligands [18]. In the presence of CpG-stimulated keratinocytes however (which abundantly produce IL-1α, TNF-α and GM-CSF), LC up-regulate major histocompatibility complex (MHC) class II and the costimulatory molecule CD86 [26]. The complexity of TLR-transmitted "danger signals" is illustrated by the finding that dsRNA, the virus-associated ligand for TLR3, does not elicit any response in pDC (for review, cf. [25]) but instead enhances different functions in LC and DDC. In LC, the synthetic TLR3 ligand polyinosinic:polycytidylic acid (poly I:C) induces changes that promote an adaptive antiviral response. These include maturation, IL-6 production and upregulation of CD70 (a potent promoter of $CD8^+$ T cell responses) [27]. Meanwhile, exposure of CD141+ DC to poly I:C results in IFN-γ production in CD141+ DC [11] and enhances (in a skin explant model) maturation and migration [20]. Keratinocytes respond to poly I:C by up-regulating surface molecules such as MHCII, CD40 and the Fas receptor [17] and by abundantly secreting TNF-α and IL-6 [23].

TLR-Transmitted Danger Signaling Beyond Skin Infections

A role of TLR danger signals has been demonstrated in various skin conditions beyond infections including acne vulgaris (Chap. 24), roseacea, skin cancers and psoriasis (Chap. 21) (for overview, cf. [28]). In the case of CHS, it had long been known that immunogenic haptens induce the secretion of proinflammatory cytokines in keratinocytes, LC [29] and DC and that skin inflammation is required for the development of sensitization to a hapten. The molecular events behind this remained obscure. An involvement of certain TLR in CHS was indicated by studies revealing that TLR2/TLR4 double-deficient mice are completely resistant to CHS development (see also Chap. 23). The finding that germ-free mice still develop CHS pointed towards a role of endogenous (and not necessarily microbial) ligands in eliciting inflammation during the sensitization phase. In mice, some allergens (such as 2,4,6-trinitro-1-chlorobenzene, oxazolone, and fluorescein isothiocyanate) seem to indirectly activate TLR [28]. Meanwhile, Goebeler et al. were able to demonstrate in elegant experiments that Ni^{2+} ions directly bind to the human TLR4 and, by doing so, initiate a signaling cascade resulting in the generation of proinflammatory signals [30]. The respective role of keratinocytes, LC and DC in TLR-mediated inflammation during the sensitization phase of CHS remains to be elucidated. The lack of TLR expression on LC for instance did not dampen CHS development in a mouse model [31].

In atopic dermatitis (see also Chap. 22), patients exhibit reduced expression of TLR2 on keratinocytes and monocytes/macrophages [32, 33]. TLR2 recognizes *S. aureus*-associated patterns and enhances the expression of certain tight junction molecules [34]. The deficiency of TLR2 in atopic dermatitis patients could thereby not only contribute to their susceptibility to *S. aureus* infections but also reinforce barrier dysfunction, a major feature of the disease.

TLR-Driven Innate Effector Functions of DC

Imiquimod: A Pharmaceutic TLR Ligand

In the early 1990s, it was reported that incubation of peripheral blood leukocytes with certain imidazoquinolines (e.g. imiquimod, resiquimod) results in the production of IFN-α by these cells. Soon, it became clear that imiquimod acts as an artificial ligand of TLR7 [35], single-strand sensing receptor important in triggering IFN-α in pDC [36].

Given the crucial role of IFN-α as a first line defense against viral infections, imiquimod has been developed into a topical cream compound (Aldara®) for the treatment of viral acanthomas such as genital warts [37]. In the years to come, Aldara® cream was also proven to be efficacious in superficial basal cell carcinomas (BCC), lentigo maligna and actinic keratoses (review in: [38]).

pDC as Effector Cells in Imiquimod-Induced Tumor Regression

We as well as other investigators set out to unravel the mode of action of topical imiquimod. In a first series of experiments, we observed that application of Aldara® cream to murine ear skin for only a few days causes massive infiltration of neutrophils, macrophages and, particularly noticeable, pDC. In subsequent experiments we transplanted a (murine) melanoma cell line into the skin of mice. After several weeks, melanomas had appeared and were then treated with either Aldara® cream or vehicle. Aldara® but not the vehicle regularly induced resolution of tumors not exceeding a volume of 130 mm^3. Again, pDC were conspicuously present around and within the regressing melanoma cell islands [39]. All these findings led us to hypothesize that pDC were, in one way or the other, involved in Aldara®-induced tumor regression. In a subsequent study, we treated sporadic superficial BCC from seven patients with topical imiquimod for a total of 6 weeks and examined the clinicopathologic features of the tumor during the course of therapy [40]. After 2 weeks of treatment, BCC lesions showed signs of severe inflammation that quickly resolved after termination of therapy and left behind an area of normal-appearing skin histopathologically free of cancer cell nests (Fig. 1.2a). Immunohistological analysis of lesional skin after 2 weeks of imiquimod treatment revealed changes similar to those seen in our murine model. This was evidenced by a considerable number of apoptotic cancer cells and tumor cell islands surrounded and/or infiltrated by a dense inflammatory infiltrate that contained considerable numbers of inflammatory DC of both the myeloid and the plasmacytoid type (Fig. 1.2b, c). When we evaluated by immunohistochemistry the expression of lytic molecules, we surprisingly found granzyme B and perforin mainly on myeloid DC and TRAIL (tumor necrosis factor related apoptosis inducing ligand) mainly on pDC. Strikingly, the apoptosis-inducing TRAIL-receptor 1 was expressed on BCC (Fig. 1.2c). These in vivo data received experimental support by *in vitro* studies demonstrating the capacity of imiquimod to induce TRAIL on peripheral blood pDC in a strictly IFN-α-dependent manner. TRAIL-expressing, but not unstimulated pDC were perfectly capable of lysing MHCI – bearing tumor cell targets [40, 41] implying that TRAIL-positive pDC in BCC are directly responsible for the killing of the cancer cells. The presence of the pro-apoptotic TRAIL receptor 1 on BCC cells supports this notion [40] as do studies in melanoma-bearing mice treated with imiquimod [42].

Melanoma

In the case of human melanoma, the situation is more complex. We have recently reported that pDC that had been rendered TRAIL-positive by imiquimod stimulation were capable of lysing certain melanoma cell lines, but not others [41]. Further investigations revealed that these differences in TRAIL sensitivity are due to distinct expression patterns of pro-apoptotic TRAIL receptors on different melanoma cell lines and, more importantly, of pro- and anti-apoptotic effector molecules within these cell lines (Fig. 1.3a, b) [43, 44]. When searching for ways to increase the TRAIL susceptibility of resistant cell lines, we found in accordance with previous reports [45] that the anti-inflammatory compound diclofenac was able to do so (Vazquez-Strauss et al., in preparation). In fact, diclofenac led to an enhanced expression of pro-apoptotic TRAIL receptors on melanoma cells as well as to an upregulation of pro-apoptotic and, vice versa, a downregulation of anti-apoptotic molecules within the cancer cells [46]. It will be interesting to explore whether the beneficial effect of diclofenac in the treatment of certain cancers is, at least partly, due to this phenomenon and, if so, whether ways can be found to maximize this tumoricidal effector mechanism.

Conclusions and Outlook

As a result of intensive and increasingly sophisticated research, we begin to understand the cellular and molecular pathways operative in immune responses starting and terminating in the skin. It has become apparent that a highly complex interplay between the innate and adaptive immune system is required to maintain skin homeostasis and initiate host defense. LC and other DC play a central role in this complex network due to their multifaceted roles under physiologic and pathologic conditions.

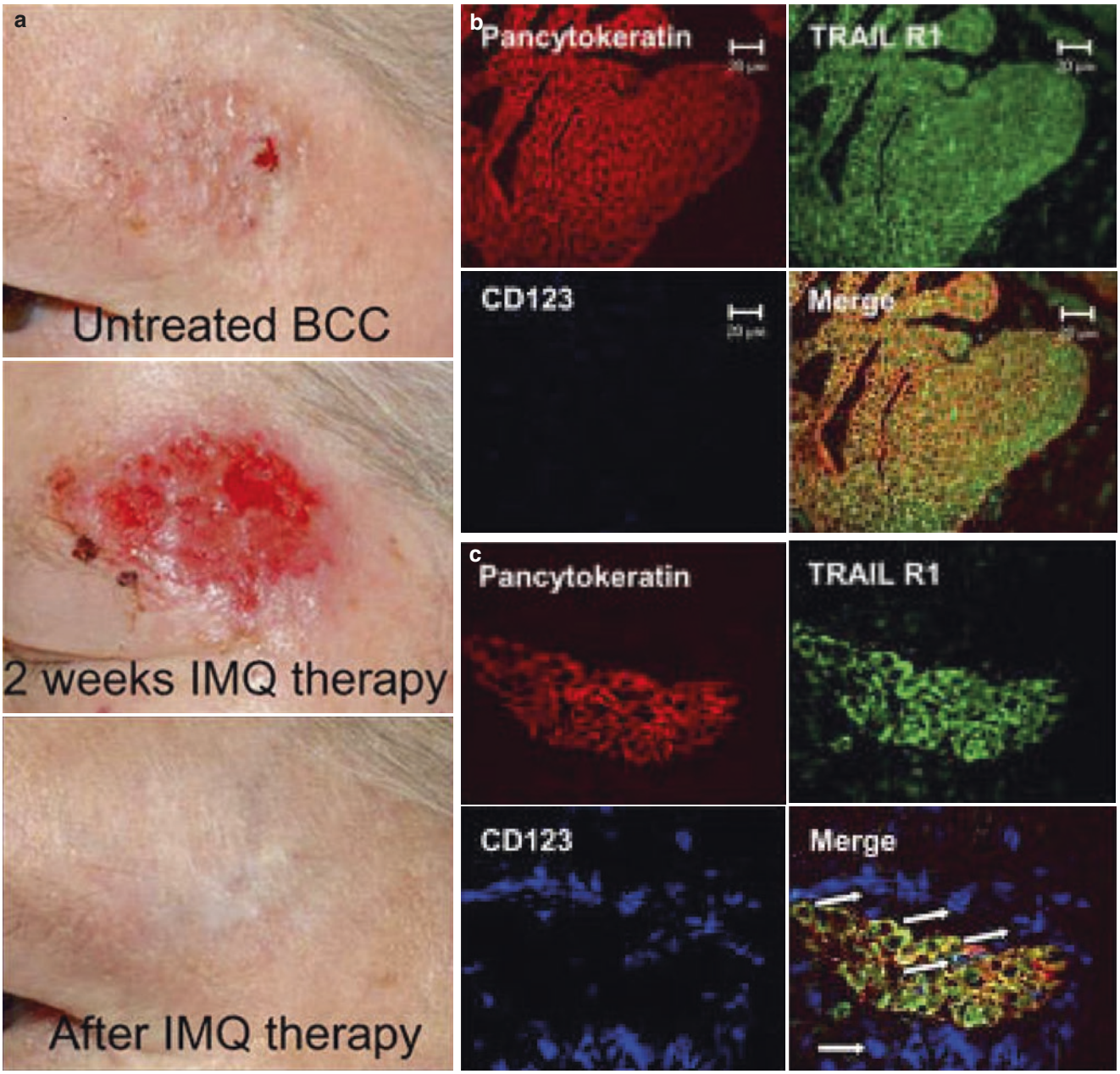

Fig. 1.2 Effects of imiquimod on BCC. (**a**) Imiquimod topically applied to superficial BCC five times a week for a period of 6 weeks led to a local inflammatory response, which resulted in a complete clinical and histopathological tumor clearance in all patients treated. The clinical pictures are representative for all patients (n = 7) treated with imiquimod. Immunofluorescence triple labeling of (**b**) untreated and (**c**) imiquimod-treated BCC with anti-pancytokeratin (TRITC), anti-TRAIL-R1 (A488) and anti-CD123 (Cy5) reveals TRAIL-R1+ BCC cells surrounded by CD123+ cells (*arrows* in **c**) (© 2007 Stary et al. [40])

In the recent past, methods have been developed to exploit the functional diversity of LC and other DC subpopulations for therapeutic purposes. This is exemplified by the development of new and better intradermally delivered vaccines (for review, [47]). In this setting, instead of simply injecting an antigen, the latter is selectively "addressed" to LC and/or certain DC subpopulations. Depending on both the type of ligand to which the antigen is coupled and the nature of the target structure, this allows to direct DC antigen-presenting function in one or the other direction. A good example is the engagement of C-type lectin receptors on DC surfaces such as DEC-205/CD205, langerin/CD207, DC-SIGN/CD209, Dectin, Clec9A, DCIR 1 and 2. These receptors facilitate antigen uptake and sometimes (e.g. TLR3 and TLR7,8 agonists, CD40) induce maturational events in these cells. Clinically, this results in robust humoral and cellular CD4+

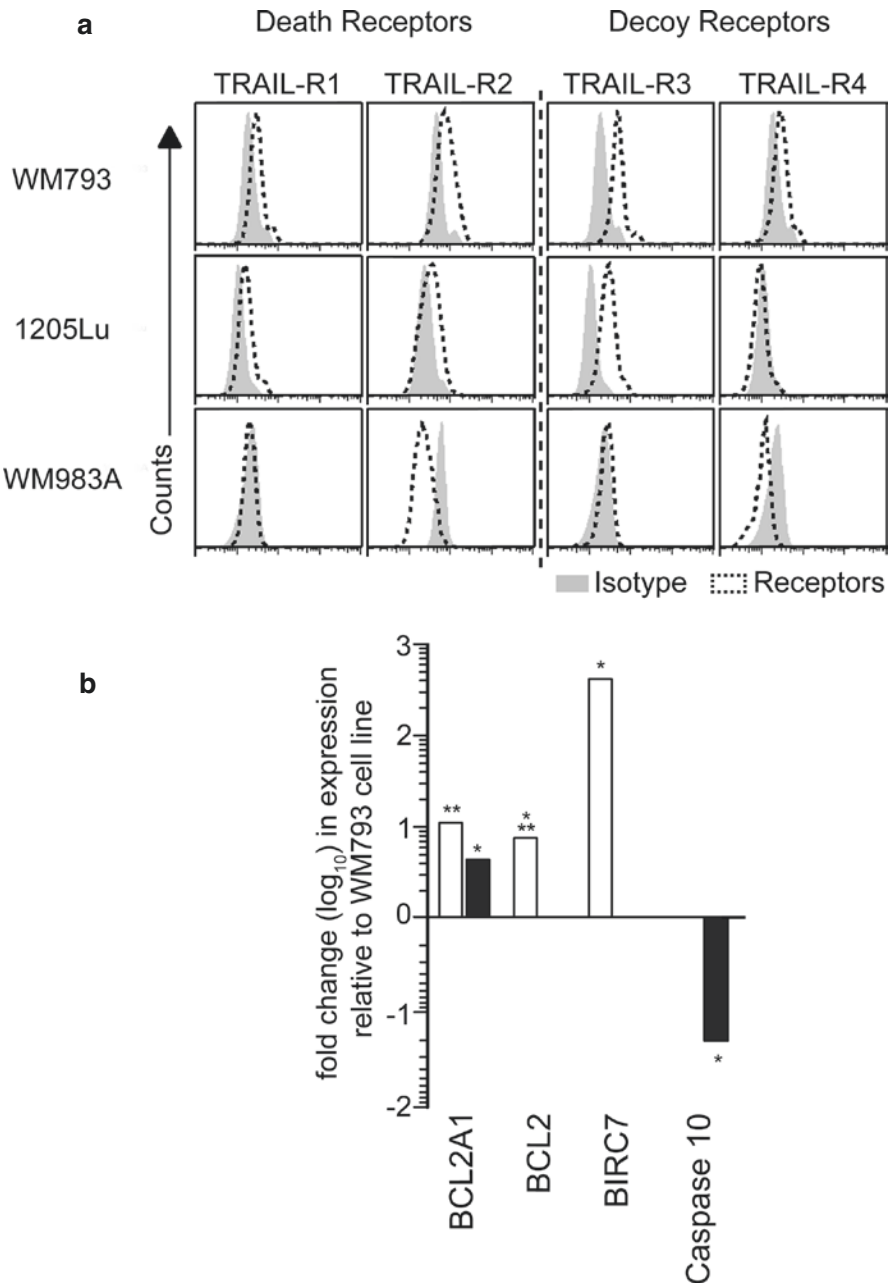

Fig. 1.3 Expression of TRAIL receptors and apoptosis-related genes in TRAIL-resistant and -susceptible melanoma cell lines. (**a**) The expression of TRAIL receptors was assesed by flow cytometry in in two resistant melanoma cell lines (WM983A and 1205Lu) and one susceptible melanoma cell line (WM793). Death receptors (TRAIL-R1, TRAIL-R2) and decoy receptors (TRAIL-R3, TRAIL-R4) for TRAIL were analyzed. Histograms representative for three experiments are shown. (**b**) Using qPCR array technology, the expression of 91 apoptosis-related genes was screened in the same three melanoma cell lines (TRAIL resistant: WM983A and 1205Lu; TRAIL-susceptible: WM793). Three biological replicates were performed. Data represent the mean fold (log_{10}) change in mRNA expression of the depicted molecules in the resistant cell lines as compared to the susceptible WM793 cell line. Some of the most significantly up- and down-regulated genes (>4-fold change in expression) are displayed. *p<0.05; **p<0.01; ***p<0.001

and CD8+ T cell responses. In the absence of adjuvants, however, targeting DEC-205+ DC *in vivo* can induce tolerance [48]. Activation of Clec9A promotes potent antibody responses and facilitates cross presentation [49]. Particularly efficient in this latter regard is the CD40 receptor, probably because of its relatively poor uptake and intraendosomal degradation [50]. By contrast, targeting the lectin-like receptor DC-asialoglycoprotein favors the generation of IL-10-producing CD4+ suppressor cells [51]. Other approaches resulting in either sensitization or tolerization include the use of nanoparticles [52, 53] as well as of cholera toxin [54]. On the other hand we could show that the use of TLR7, 8 agonists can drive innate effector functions in certain DC, i.e. their transformation into killer cells. Thus, the prospect to an efficacious DC-based immunotherapy, tailored to the needs of the individual patients, is realistic, yet challenging.

Questions

1. Which one of the following statements on LC in the skin is correct?
   A. UV-irradiation and the treatment with corticosteroids enhance the ability of LCs to induce cytotoxic T cell responses

B. LC are exclusive stimulators of Th1 cells
C. Under certain conditions, LC can induce the expansion of Tregs and down-regulate proliferative and cytotoxic T cell responses
D. In contrast to keratinocytes, LC cannot produce any inflammatory cytokines
E. LC are found in the dermis but not in the epidermis

2. Which statement regarding TLR is true?
   A. TLR are exclusively expressed on cells of hematopoietic origin
   B. While TLR signaling is a major modulator of innate immune responses, it does not have any effect on adaptive immune responses
   C. TLR recognizing lipids are located on the outer cell membrane while those recognizing proteins are found intracellularly
   D. The different DC subsets in skin express the same TLR repertoire
   E. TLR belong to the PRR family that includes receptors recognizing damage- and pathogen-associated molecular patterns

3. Which statement does not describe parts of the mechanism underlying the imiquimod-induced clinical regression of BCC?
   A. Imiquimod acts as an artificial TLR7-ligand
   B. Imiquimod induces pDC to kill BCC cells in a mostly TRAIL-mediated fashion
   C. Imiquimod induces the killer molecule TRAIL on peripheral blood pDC in an IFN-α-dependent manner
   D. Imiquimod-treated BCC become selectively infiltrated by NK cells
   E. Imiquimod application leads to the apoptosis of BCC cancer cells

Answers

1. C
2. E
3. D

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Toll-Like Receptors

2

Jessica Shiu and Anthony A. Gaspari

Abstract

There are two major arms of the immune system: the innate immune response and the adaptive immune response. Innate immunity is the first line of defense against microbes and serves to limit infection within the early hours after exposure to a pathogen. It is classically associated with the recognition of pathogens by phagocytic cells via specific receptor recognition molecules or through complement fixation. Essential components of the innate immune response include neutrophils, natural killer cells, natural killer T cells, mast cells, complement, and antimicrobial peptides. Innate immune activation via pattern recognition receptors results in a specific expression of co-stimulatory molecules and cytokines. This inflammatory milieu shapes the subsequent adaptive response, which involves B cell activation and T cell-mediated recognition of foreign antigens presented on major compatibility complexes (MHC) I and II on the cell surface of antigen-presenting cells (APCs). Activated B and T lymphocytes then undergo clonal expansion to provide an antigen-specific immune response.

Keywords

Dermatitis • Inflammation • Proteins • Toll • Keratinocyte

There are two major arms of the immune system: the innate immune response and the adaptive immune response. Innate immunity is the first line of defense against microbes and serves to limit infection within the early hours after exposure to a pathogen [1]. It is classically associated with the recognition of pathogens by phagocytic cells via specific receptor recognition molecules or through complement fixation [1–3]. Essential components of the innate immune response include neutrophils, natural killer cells, natural killer T cells, mast cells, complement, and antimicrobial peptides. Innate immune activation via pattern recognition receptors results in a specific expression of co-stimulatory molecules and cytokines. This inflammatory milieu shapes the subsequent adaptive response, which involves B cell activation and T cell-mediated recognition of foreign antigens presented on major compatibility complexes (MHC) I and II on the cell surface of antigen-presenting cells (APCs) [3–5]. Activated B and T lymphocytes then undergo clonal expansion to provide an antigen-specific immune response.

The discrimination between innate and adaptive immunity has long been recognized but the mechanisms that linked the two major arms of immunity were largely unknown until Charles Janeway first proposed the theory of pattern recognition in 1989 [2]. He suggested that highly conserved microbial molecular constituents called pathogen associated molecular patterns (PAMPs) activate germline-encoded receptors on innate cells coined 'pattern recognition receptors' (PRRs). Janeway's pattern recognition theory was later confirmed by the discovery of the toll-like receptor (TLR) family as well as other PRRs such as NOD1 and the family of NOD-like receptors (NLRs) [6–8]. TLRs represent

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Key Points

- Toll-like receptors (TLRs) represent a key receptor family of the innate immune response that recognize pathogen associated molecular patterns as well as damage associated molecular patterns
- TLRs play essential roles in shaping both innate and adaptive immune responses
- TLRs work through two pathways:
  - Adaptor protein myeloid differentiation factor 88 (MyD88) to activate transcription factor NF-κB and MAP kinases (used by all TLRs except TLR3)
  - Adaptor protein TIR domain-containing adaptor protein inducing interferon-beta (TRIF) dependent pathway used by TLR3 and TLR4 that results in type I interferon expression
- TLRs play diverse roles in multiple dermatologic diseases and mutations in TLR signaling pathways have been mapped in human patients, some examples include:
  - TLR2, TLR9 and TOLLIP polymorphisms have been identified in atopic dermatitis patients
  - Activation of TLR4 by nickel, cobalt and palladium in allergic contact dermatitis
  - LL-37, an antimicrobial peptide, complexes with self DNA and activates plasmacytoid dendritic cells to create a DAMP, and drive psoriatic inflammation
- Studies in modulating TLRs for treatment strategies have yielded promising results in a variety of dermatological diseases including treatment of psoriasis, melanoma etc.

a key component of the innate immune system involved in sensing danger. Depending on the particular stimulatory antigen involved, specific downstream components of the signaling pathway are activated, which leads to the generation of an inflammatory response that shapes the subsequent adaptive immune response. Thus, TLRs play an essential role in bridging the gap between innate and adaptive immunity. In support of this notion, studies have implicated TLRs in a variety of human diseases – TLR5 mutations have been linked to an increased susceptibility to Legionnaire's disease [9] while TLR3 deficiency has been associated with herpes simplex encephalitis [10]. In the skin, TLRs have been shown to impact a variety of skin diseases and some widely used dermatologic drugs may possibly exert their therapeutic effects through TLR signaling (Table 2.1) [76]. This chapter will review recent evidence that demonstrates how TLRs affect a variety of skin diseases and infections.

Discovery of TLRs in Humans and Its Expanding Role in Immunity

After Janeway proposed the theory of pattern recognition, based on what was then known about other innate immune receptors, his group was in search for cell-surface receptors expressed on APCs that resulted in NF-κB activation [77]. Lemaitre et al. first identified the antifungal function of Drosophila Toll and demonstrated that it plays a key role in regulating antibacterial gene expression through the NF-κB-like signaling pathway [78]. This seminal discovery paved the path for the discovery of its human counterpart in which Janeway et al. [79] demonstrated that the mammalian Toll homolog induced expression of genes encoding B7 and cytokines that affect the adaptive immune response, providing confirmation for the theory of pattern recognition. Researchers began a fervent search for the ligand of human Toll (now known as TLR4). The first clue came when researchers found that C3H/HeJ mice were unresponsive to bacterial lipopolysaccharide (LPS) and mapped the genetic locus required for LPS responsiveness to *TLR4* [80, 81]. Subsequent studies that attempted to clarify this ligand-receptor interaction proved to be difficult until the other protein in the receptor complex, MD2, was discovered [77, 82]. Since then, studies by many groups have identified multiple other members in the TLR family and elucidated many of their ligands [83]. For their efforts in discovering the toll receptors in Drosophila, Bruce Beutler and Jules Hoffmann won the Nobel Prize in Physiology or Medicine in 2011. TLRs are now the most well characterized PRRs and it is established that different TLR members recognize a variety of PAMPs. Up to 13 TLRs have been identified in mice but only 10 are present in humans as TLR11, 12 and 13 have been lost from the human genome [84]. In contrast, the C-terminal of TLR10 in mice is disrupted by a retrovirus insertion and is nonfunctional. For a detailed look at the history of TLRs, see Table 2.2.

As our understanding of TLRs has expanded in the past couple of decades, increasing evidence has indicated that TLRs are not limited to recognizing PAMPs but can also bind to signals released from damaged tissues, a notion first pioneered by Polly Matzinger who proposed the danger theory as an alternative to the mechanism of immunity initiation [92]. Non-pathogen associated material that leads to tissue injury and other endogenous ligands released during cellular injury such as chromatin bound high mobility group 1 and heat shock proteins also bind and activate TLR signaling [93–97]. Thus, in addition to being the first line of defense against pathogens, TLRs also survey the expression of danger-associated molecular patterns (DAMPs) seen in tissue injury (Fig. 2.1). TLR activation by DAMPs results in sterile inflammation that may play a role in chronic skin

Table 2.1 Toll-like receptors (TLRs) in dermatological disease

TLR	Disease	Comments
1	Tuberculoid	TLR1 favors Th1 phenotype [11]
	Leprosy	*TLR1* I602S mutation protects from *M. leprae* [12]
	Psoriasis	TLR1 expression increased in keratinocytes [13]
	Lyme disease	*TLR1* polymorphism associated with severe disease [14, 15]
	Syphilis	Increased neurosyphilis risk in *TLR1* polymorphisms [16]
2	Acne vulgaris	*P. acnes* stimulates TLR2 and causes hypercornification of sebaceous glands [17]
		Retinoids exert anti-inflammatory effects via TLR2 [18–20]
	Atopic dermatitis	*TLR2* R753Q mutation associated with severe disease [21–23]
		TLR2 signaling necessary for skin barrier repair [24–26]
		TLR2 skews cytokine profile towards a Th2 phenotype [27–30]
	Psoriasis	Increased TLR2 expression in keratinocytes [13]
	Staphylococcus aureus infection	TLR2 deficiency led to increased susceptibility [31, 32]
	Lepromatous leprosy	Associated with $Arg^{677}Trp$ mutation in Korean population [33]
		$Arg^{677}Trp$ mutation: decreased cytokine production [34]
	Syphilis	Lipoproteins stimulate TLR2 [35]
		Increased neurosyphilis risk in *TLR2* polymorphisms [16]
	Lyme disease	Outer surface proteins stimulate TLR2 [36]
		Patients with $Arg^{753}Gln$ mutation secreted less proinflammatory cytokines [37]
	Candidiasis	Phospholipomannans and glycans stimulate TLR2 [38, 39]
	HSV	Glycoproteins stimulate TLR2 [40, 41]
		$TLR2^{-/-}$ animals are more susceptible to HSV encephalitis [42]
3	Psoriasis	Mutation in *AP1S3*, gene required for TLR3 trafficking, associated with pustular psoriasis [43]
	HSV	$TLR3^{-/-}$ astrocytes fail to produce type I IFN [44]
		Humans with TLR3 deficiencies are more susceptible to HSV encephalitis [10]
4	Acne vulgaris	*P. acnes* LPS stimulates TLR4 [45]
	Allergic contact dermatitis	Nickel, cobalt and palladium binds and activates TLR4 signaling [46–48]
	Psoriasis	Increased HSPs that can activate TLR4 signaling [49, 50]
	Syphilis	Lipoproteins stimulate TLR4 [35]
	Candidiasis	Polysaccharides activate TLR4 [38, 39]
		Important for neutrophil recruitment [51]
	UV exposure	TLR4 hyporesponsiveness leads to impaired TNFα production [52]
		TLR4-MyD88 axis deficiencies led to increased cell survival and upregulation of necroptic markers [53]
		TLR4 deficiency led to increased nucleotide excision repair [54]
6	Syphilis	Increased neurosyphilis risk in *TLR6* polymorphisms [16]

(continued)

Table 2.1 (continued)

TLR	Disease	Comments
7	Psoriasis	Imiquimod, TLR7 agonist, drives psoriasis formation [55, 56]
	Systematic lupus erythematous (SLE)	pDCs bind self nucleic acids to stimulate IFN production via TLR7 and 9 [57]
		Small nuclear RNA binds and activates TLR7 and 8 [58]
		Gene duplications of TLR7 increases autoantibody production [59]
		Chronic TLR7 and 9 stimulation leads to glucocorticoid resistance [60]
		Dual TLR7 and TLR9 inhibitor led to decreased autoantibody production in animals and being tested in humans [61, 62]
	Melanoma	Imiquimod and 852A, TLR7 agonist, has been shown to have antitumor effects [63, 64]
	Mycosis fungoides	Imiquimod shown to have clinical responses [65]
	UV exposure	Imiquimod enhances DNA repair and decreased DNA damage [66]
8	SLE	Small nuclear RNA binds and activates TLR7 and 8 [58]
9	Atopic dermatitis	Polymorphisms associated with disease [67]
	Psoriasis	DNA complex with LL-37 stimulates TLR9 to drive IFNα-mediated inflammation [68]
	SLE	pDCs bind self nucleic acids to stimulate IFN production via TLR7 and 9 [57]
		Paradoxical role as TLR9 deficient mice promoted SLE development [69, 70]
		Chronic TLR7 and 9 stimulation leads to glucocorticoid resistance [60]
		Dual TLR7 and TLR9 inhibitor led to decreased autoantibody production in animals and being tested in humans [61, 62]
	Melanoma	PF-3512676, TLR9 agonist, currently being tested in melanoma patients with other modes of therapy [71–73]
	Mycosis fungoides	TLR9 agonist demonstrated to have antitumor activity [74, 75]

Table 2.2 Historical timeline: discovery of Toll-like receptors

	Discovery
1979	Identification of the *dorsal* mutation [85]
1984	Characterization of *toll* mutation and other dorsoventral mutations
1989	Janeway proposes the theory of pattern recognition [2]
1993	Demonstration that NF-κB is required for Drosophila antimicrobial resistance 209
1996	Drosophila *Toll* identified; found to be required for resistance to fungal infections [78]
1997	Human homologue of *Drosophila* Toll, signals activation of adaptive immunity [79]
1998	TLR4 is lipopolysaccharide receptor [80, 86]
1999	MD2 identified as coreceptor for TLR4-LPS interaction [82]
2000	TLR9 recognizes bacterial DNA [87]
2000	TLR2 can pair with TLR6 to recognize bacterial proteins [88]
2000	TLR2 can also associate with TLR1 [88]
2001	TLR3 mediates response to viral double-standed RNA [89]
2001	TLR5 detects flagellate protein in whiplike tails of bacteria [90]
2004	TLR8 (humans), TLR 7 (mice) recognize single-stranded RNA [91]
2011	Bruce Beutler and Jules Hoffmann awarded the Nobel Prize in Medicine for their role in the identification of TLRs

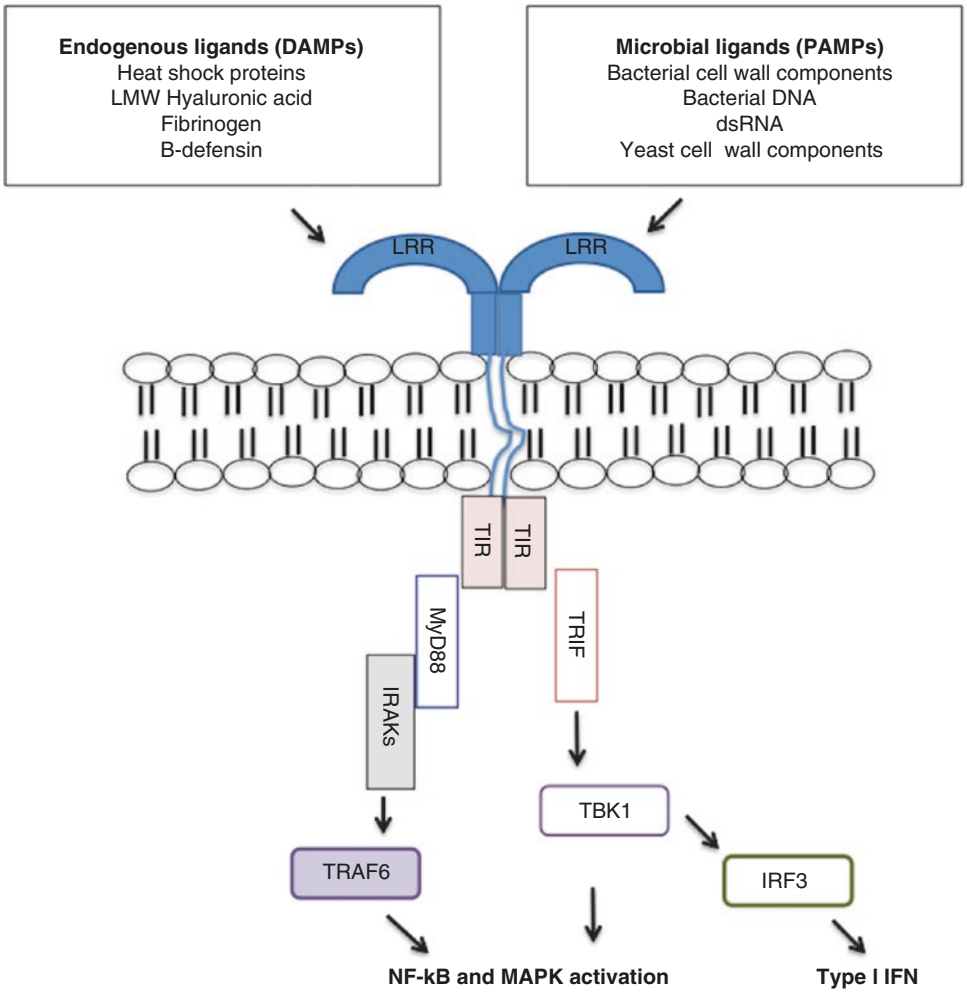

Fig. 2.1 Schematic diagram of TLR activation by various established endogenous and exogenous ligands

diseases such as psoriasis (Fig. 2.2) [99]. For a detailed look at PAMPs and DAMPs that activate specific TLRs, please see Table 2.3.

Toll-Like Receptors in Innate and Adaptive Immunity

As mentioned previously, the pattern recognition theory and identification of TLRs provided the missing link between innate and adaptive immune responses. It is now established that specific ligands activate distinct TLRs and other PRRs, which result in the expression of molecules that shape and fine-tune the adaptive immune response depending on the stimulus involved. On the innate immunity side, activation of TLRs leads to the release of antimicrobial peptides and chemokines that recruit phagocytic cells to the site of infection [120]. TLR activation also induces maturation of dendritic cells to potent APCs via the upregulation of surface expression of MHCII and costimulation markers such as CD80 and CD86 [121].

TLR-mediated effects on the adaptive immune response can be shaped via APCs or T cells directly. It is well known that physical interaction between APCs and T cells requires two signals with signal 1 being the antigen specific signal via MHCII and signal 2 being the expression of costimulation molecules on dendritic cells [122]. TLR stimulation in dendritic cells results in increased expression of MHCII, CD80 and CD86 and is instrumental in promoting both signals required for robust antigen-specific T cell responses [5, 76]. TLR activation on dendritic cells also influences cytokine production, which provides key signals for helper T cell differentiation into different phenotypes with distinct effector functions [123]. For example, TLR-activated dendritic cells produce IFNγ in response to *E.coli* LPS stimulation which is associated with T helper cell 1 (Th1) differentiation while *P. gingivalis* LPS induces expression of IL-5, IL-13 and IL-10, cytokines classically associated with Th2 differentiation [124]. Stimulation of APCs with TLR ligands also leads to interleukin-6 (IL-6) secretion, which can result in the loss of suppressor activity by regulatory T cells, allowing for a

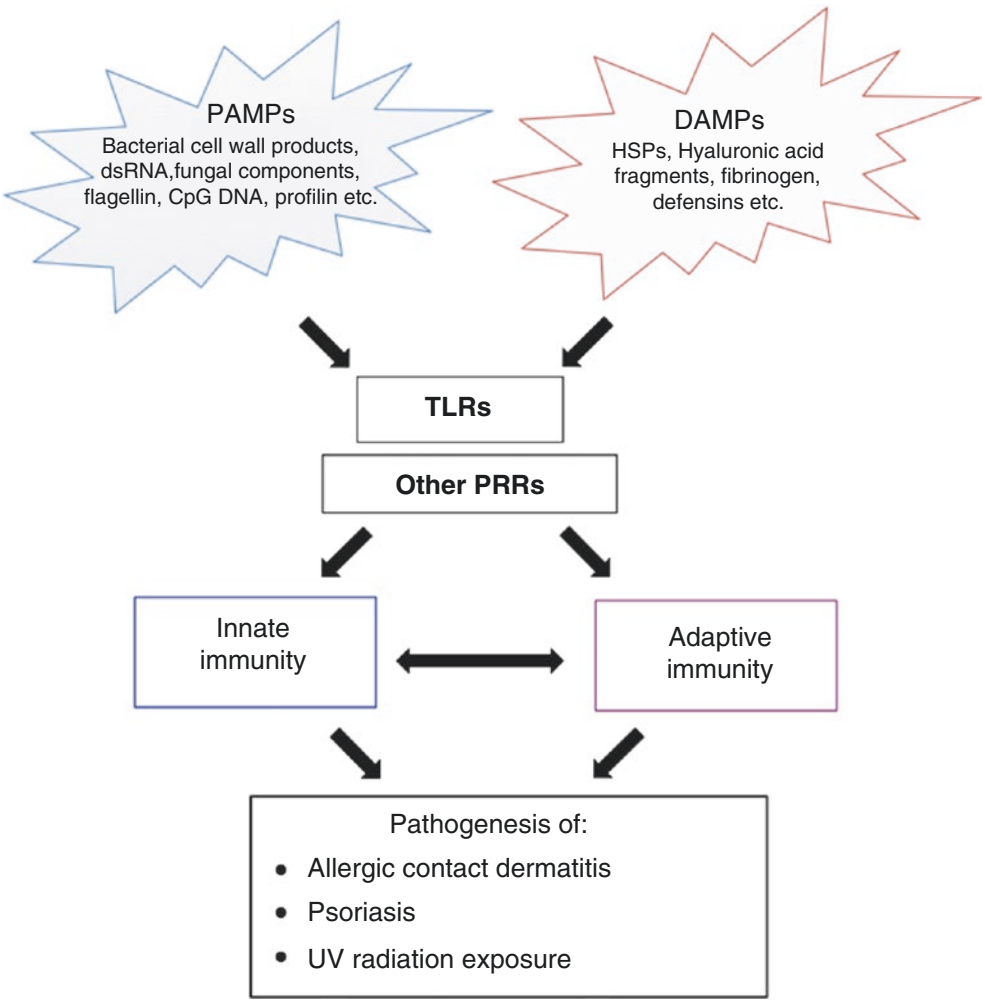

Fig. 2.2 The interplay of PAMPs and DAMPs in the activation of TLRs as well as other PRRs. Activation of these receptors influences both arms of immunity and dysregulation of these pathways can lead to inflammation and the development of a variety of dermatological diseases [98]

more effective immune response [125]. Alternatively, TLRs are also expressed in T lymphocytes and TLR ligands can modulate T cell function directly [126]. Direct TLR2 stimulation of T lymphocytes in the absence of APCs has been shown to induce proliferation of regulatory T cells [127]. Intrinsic B cell TLR activation mediates B-cell proliferation and antibody production to T-dependent antigens and similar results were seen in human B cells [128, 129]. Thus, while TLRs are traditionally associated with the innate immune response, they also play key roles in shaping the adaptive immune response and can directly affect the functions of both T and B lymphocytes.

Expression of Human TLRs in Skin

Based on their cellular localization, TLRs can be broadly classified into two groups [84]. TLRs 1, 2, 4, 5 and 6 are expressed on the cell membrane and recognize predominantly microbial membrane components. TLRs 3, 7, 8 and 9, on the other hand, are expressed in intracellular components such as the endoplasmic reticulum, endosomes and lysosomes and primarily recognize microbial nucleic acids. As the primary physical barrier against the environment, it is not surprising that many cell types residing in the skin express a variety of TLRs to survey for pathogens as well as tissue damage signals.

In the epidermis, keratinocytes constitutively express messenger RNA (mRNA) for TLRs 1–6, 9 and 10 [13, 130]. With the exception of TLR10, many studies have demonstrated that keratinocyte TLRs are functional and respond to their respective ligands [130, 131]. Langerhans cells (LCs) express TLRs 1–10 but are most responsive to TLRs 2, 3, 7 and 8 ligands [132, 133]. In the dermis, stimulation of skin/muscle fibroblasts with ligands to TLRs 2, 3, 4, 5 and 9 led to production of specific chemokines [134, 135]. Expression of human TLRs has also been detected on skin resident and trafficking immune cells such as neutrophils, macrophages, dendritic cells, dermal endothelial cells, mucosal epithelial cells, B cells, and T cells (Table 2.4) [133, 145].

Table 2.3 TLRs: exogenous ligands (PAMPs) vs. endogenous ligands (DAMPs)

TLR	Exogenous ligands	Endogenous ligands	Signaling pathway	
1	Triacyl lipoproteins (w/TLR2)	hBD3	Heterodimerizes with TLR2; MyD88-dependent signaling	[100, 101]
2	Triacyl lipoproteins (w/TLR1) Diacyl lipoproteins lipoteichoic acid, zymosan (w/TLR6)	HMGB1, HSPs, Hyaluronan, Biglycan, Versican, Antiphospholipid antibodies	Heterodimerizes with TLR1 or TLR6; MyD88-dependent signaling	[93, 97, 102–106]
3	dsRNA	Endogenous mRNA from tissue necrosis	TRIP dependent signaling to induce antiviral genes	[100, 107, 108]
4	LPS, viral envelope proteins	HMGB1, HSPs, Hyaluronan, Biglycan, Heparan sulphate, hBD2, fibronectin, s100 proteins Fibronectin extra domain A	MyD88 and TRIF/TRAM dependent signaling	[93, 97, 102–104, 109–113]
5	Flagellin	None identified	MyD88-dependent signaling	[100, 114]
6	Diacyl lipoproteins Zymosan Lipoteichoic acids	HMGB1, HSPs, ECM (with TLR2)	Heterodimerizes with TLR2; MyD88-dependent signaling	[100, 106]
7	ssRNA	Antiphospholipid antibodies ssRNA	MyD88-dependent signaling	[58, 115, 116]
8	ssRNA	Antiphospholipid antibodies ssRNA	MyD88-dependent signaling	[58, 115, 116]
9	CpG-DNA	DNA released from acetaminophen-induced hepatoxicity Mitochondrial DNA Immune complexes	MyD88-dependent signaling	[93, 117, 118]
10	Unknown	Unknown	MyD88-dependent signaling	[119]

HMGB1 high mobility group box 1, HSPs heat shock proteins, double stranded RNA (*dsRNA*), *LPS* lipopolysaccharide, *hBD3* human β-defensin 3, *hBD2* human β-defensin 2, *ECM* extracellular matrix

Table 2.4 TLR expression in different cell types

Cell type	TLR1	2	3	4	5	6	7	8	9	10
Keratinocytes [13, 130]	+	+	+	+	+	+			+	+
Melanocytes [136, 137]		+	+	+	+		+		+	+
LC [132, 133]	+	+	+	+	+	+	+	+	+	+
Skin endothelial cells [138]	+	+	+	++	+	+	+	+	+	+
FB [134, 135]		+	+	+	+				+	
Adipocytes [139, 140]	+	+	+	+		+				
MC [141]	+	+	+	+	+	+	+		+	
mDC[a] [142]	+	+	+	+	+	+		+		+
pDC [142]	+/–					+/–	+		+	+/–
MΦ[b] [143]	+	+	+	+	+	+	+	+	+	+
N [144]	+	+		+	+	+	+	+	+	+
B cell [143]	+	+	+	+	+	+	+	+	++	++
T cell [133, 143]	+	+	+	+	+	+	+	+	+	+

++ strong expression, + expressed, +/– low level expression, *LC* Langerhans cell, *MC* mast cell, *FB* fibroblasts, *mDC* myeloid dendritic cell, *pDC* plasmacytoid dendritic cell, *MΦ* macrophage, *N* neutrophil

[a]Representative of all myeloid DCs, TLR expression varies within myeloid DC subsets

[b]TLR1-10 transcripts are detected but predominantly express 1, 2, 4, 5 and 8

Toll-Like Receptor Signaling

All members of the TLR family are type I transmembrane proteins and contain: (1) extracellular leucine-rich repeats that mediate the recognition of PAMPs, (2) a transmembrane domain and (3) an intracellular tail that contains the Toll/IL-1R (TIR) domain, which bears homology to the IL-1 receptor [84, 146]. Activating ligands lead to homo- or

heterodimerization of one TLR with another TLR and result in the dimerization of TIR domains, which serve as the scaffold for downstream adaptor proteins. Important adaptor proteins in TLR signaling include myeloid differentiation factor 88 (MyD88), TIR domain-containing adaptor protein inducing interferon-beta (TRIF) and TRIF-related adaptor molecule (TRAM). MyD88 and TRIF represent distinct signaling pathways that TLRs utilize that result in activation of specific gene programs in response to different activating stimuli.

MyD88 is an adaptor protein that is used by most TLRs with the exception of TLR3 for the initiation of downstream signaling. It should be noted that TLR4 is unique in that its activation results in both MyD88-dependent and TRIF-dependent pathways. In the MyD88 dependent pathway, MyD88 activation results in the recruitment of interleukin-1 receptor-associated kinases 1 (IRAK1) and IRAK4 [147]. IRAK4 then activates IRAK1, leading to IRAK1 autophosphorylation and the dissociation of both members from MyD88 and downstream interaction with tumor necrosis factor receptor–associated factor 6 (TRAF6), an E3 ubiquitin ligase [146]. This signaling complex results in the activation of NF-κB and mitogen-activated protein kinases (MAPKs) and the production of inflammatory cytokines (Fig. 2.1) [84]. Although all TLRs utilize MyD88 as an adaptor protein, it is important to recognize that each TLR utilizes different combinations of adaptor proteins and kinases to generate an immune response that is appropriate for the initial activating stimuli. For instance, activation of TLR2 by lipoproteins leads to TNFα expression while CpG stimulation of TLR9 results in the expression of IFN-α and TNFα [148].

The TRIF-dependent signaling pathway is mainly utilized by TLR3 and TLR4. TLR3 activation results in TRIF recruitment and subsequent activation of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), a transcription factor required for induction of type I IFNs [148]. TLR4 requires an additional adaptor protein TRAM to stabilize its interaction with TRIF. The discovery of TRIF provided the first molecular explanation for why only TLR3 and TLR4, but not TLR2, can induce IFN-β secretion. Indeed, TRIF-deficient mice were incapable of secreting IFNβ upon stimulation by TLR3 and TLR4 ligands [149]. The TRIF-dependent pathway also results in the activation of NF- κB and MAPKs.

Negative Regulators of TLR Signaling

TLR-mediated signaling plays a key role in the regulation of immunity and excessive TLR signaling has detrimental effects that contribute to autoimmune and inflammatory disease development [150, 151]. Not surprisingly, TLR signaling pathways are tightly controlled and multiple negative regulators of TLR signaling exist at various levels to ensure that immune homeostasis is maintained [100]. IRAK-M, Toll-interacting protein (Tollip) and Suppressor of Cytokine Signaling 1 (SOCS-1) are examples of well-described inhibitors of the TLR signaling pathway. IRAK-M, for instance, is thought to prevent the dissociation of IRAK4 and IRAK1 from MyD88 [152, 153]. Accordingly, IRAK-M$^{-/-}$ macrophages secrete higher levels of inflammatory cytokines and IRAK-M$^{-/-}$ animals are more vulnerable to inflammatory-mediated damage in lupus and lung infection models [154–156]. It is thought that specific genotypes of IRAK-M are associated with sepsis risks (see Table 2.5).

Another negative regulator in the TLR pathway is Toll-interacting protein (TOLLIP), which limits MyD88-dependent NF-κB activation at two different levels [181, 182]. First, overexpression of TOLLIP has been shown to inhibit TLR4– and TLR2–mediated NF-κB activation. TOLLIP also binds directly to IRAK1 to inhibit IRAK1 autophosphorylation and downstream recruitment of signaling proteins required for NF-κB activation [182, 183]. In contrast to IRAK-M$^{-/-}$ mice, TOLLIP deficient animals did not exhibit any overt inabilities to limit the inflammatory response [184]. However, TOLLIP$^{-/-}$ macrophages secreted lower levels of IL-6 and TNFα when stimulated with low doses of LPS, suggesting that TOLLIP is involved in fine-tuning inflammation in response to different levels of stimulation. Polymorphisms of TOLLIP have been associated with atopic dermatitis and inflammatory bowel diseases (see Table 2.5 for other negative TLRs and their association with human diseases). As the role of negative regulators in disease pathogenesis becomes increasingly clear, there is promise that specific targeting of these molecules may lead to the development of new therapeutics.

TLR and Dermatologic Diseases

Acne Vulgaris

Acne vulgaris, a common disorder involving the pilosebaceous unit, is one of the most prevalent conditions in dermatology (see also Chap. 24). It affects more than 45 million people in the United States and is characterized by the presence of inflammatory papules, pustules, nodules and noninflammatory comedones [76, 185]. The pathogenesis of acne is multifactorial but it is generally thought to involve increased sebum production, altered follicular keratinization and an inflammatory response to *Propionibacterium acnes*, a Gram-positive anaerobe that is a part of normal skin flora, a finding that has been confirmed by recent skin microbiome mapping projects [186, 187]. It is thought that the host immune response [188], and not *P. acnes* overgrowth, is the main determinant of disease as PBMCs from acne vulgaris patients produce higher levels of IFNγ, IL-12 and IL-8.

Table 2.5 Negative regulators of Toll-like receptors

Negative regulator	Mechanism of action	Role in human diseases	References
Protein regulators			
IRAK-M	Prevents IRAK1/IRAK4 dissociation Negatively regulates alternative NF-κB activation after TLR2 stimulation	G/G genotype associated with increased sepsis risk A/A genotype is protective against sepsis Possible role in IBD	[153, 157–160]
MyD88s	MyD88 antagonist	Upregulated in septic patients	[161–163]
TOLLIP	Autophosphorylates IRAK1	Polymorphisms mapped in Atopic Dermatitis IBD	[164, 165]
A20	De-ubiquitylates TRAF6	Polymorphisms and mutations associated with rheumatoid arthritis, psoriasis, Sjogren's Syndrome, SLE, lymphomas	[166, 167]
SOCS1	Suppresses IRAK by promoting their degradation	Decreased SOCS1 expression in SLE MS, RA	[168, 169]
SIGIRR	Orphan receptor that suppresses inflammation	No clear demonstrated role in human disease	[170, 171]
ABIN-1	Ubiquitin binding protein that inhibits TLR/C/EBPβ signaling	Protects again psoriasis	[172, 173]
MicroRNAs Targets 3′-untranslated regions to modulate gene expression			
miR-146	Inhibits IRAK1 and TRAF6	RA Psoriatic arthritis	[174–176]
miR-9	Blocks NF-κB	Leukemias Cancer	[177, 178]
miR-21	Blocks NF-κB and PCDC4	Cancer	[175, 179]
miR-155	Stimulates TNFα Blocks TAK1 activation	Cancer	[180]

IRAK-M IL-1R-associated kinase M, *MyD88s* myeloid differentiation factor 88 short, *TOLLIP* Toll-interacting protein, *SOCS1* suppressor of cytokine signaling 1, *ABIN*-1 A20 binding and inhibitor of NF-κB-1, *SIGIRR* single immunoglobulin IL-1 related receptor, *SLE* systemic lupus erythematous, *IBD* inflammatory bowel disease, *RA* rheumatoid arthritis, *MS* multiple sclerosis

However, the notion that the host immune response is the main contributor of disease has been challenged by a recent study that showed that acne vulgaris patients harbor different *P. acnes* strains compared to healthy controls [189].

Early studies demonstrated that soluble factors produced by *P. acnes* stimulated proinflammatory cytokine production but the exact mechanisms were poorly understood [190, 191]. After the discovery of TLRs, Kim et al. demonstrated that *P. acnes*-mediated induction of proinflammatory cytokines was dependent on TLR2 expression and that TLR2 was abundantly expressed on perifollicular macrophages [192]. It was thought that *P. acnes* possessed two potential cell wall components, LPS and peptidoglycan (PG), that can serve as ligands and activate TLR2 and TLR4 to mediate its downstream proinflammatory response [76]. Indeed, distinct strains of *P. acnes* with presumably varied modifications in their cell wall components differentially induced upregulation of hBD2, and IL-8 mRNA levels in keratinocytes in a TLR2- and TLR4-dependent manner [45]. Subsequent studies have also found that expression of TLR2 and TLR4 in keratinocytes increased in the epidermis of inflammatory acne lesions and *P. acnes* exposure led to an increase in TLR2 expression [192, 193]. Other than proinflammatory cytokine production, PAMP stimulation also caused hypercornification of sebaceous glands in a TLR2-dependent manner [17]. While the host immune response is an essential component of acne vulgaris pathogenesis, the molecular mechanisms that differentiate healthy controls and acne vulgaris patients remain poorly characterized. As mentioned earlier, recent studies have showed that different *P. acnes* strains are found in acne vulgaris patients and there is evidence that these strains can modulate cutaneous innate immunity differentially [189, 194]. Specifically, Jasson et al. demonstrated that only some strains have the capacity to recruit TLR2 receptors and trigger a downstream inflammatory response [194]. It will be interesting to see if the differential capacity of TLR2 recruitment by various *P. acnes* strains affects keratinocyte proliferation in pilosebaceous units and have clinical implications in acne vulgaris treatment strategies in the future.

Interestingly, retinoids, one of the treatments commonly used for acne vulgaris, have been shown to exert anti-inflammatory effects by decreasing local expression of TLR2

in vitro [18, 19]. These results were recently confirmed in human patients – systemic administration of isotretinoin in acne patients resulted in downregulation of TLR2 cell surface expression on monocytes and decreased levels of IL-1β, IL-6, IL-12 as well as IL-10 release [20]. Of note, systemic isotretinoin decreased TLR2 cell surface expression to levels comparable to those seen in healthy controls. A similar reduction in proinflammatory cytokines was also evident and this effect was sustained for 6 months after the cessation of therapy.

Atopic Dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory skin condition that affects up to 3 % of adults and 15–25 % of children in the United States (see also Chap. 22) [195, 196]. Multiple defects have been identified in AD patients, including impaired skin barrier function, reduced expression of antimicrobial peptides, concomitant skin infections and Th2 skewing. Moreover, it has been demonstrated that up to 90 % of AD patients are colonized with *Staphyloccus aureus* in both lesional and nonlesional skin, whereas only 5 % of healthy controls exhibit colonization [197]. The molecular details underlying AD pathogenesis are currently under investigation but defects in the TLR signaling pathway have been identified in AD patients. AD patients have decreased TLR2 expression on their circulating monocytes and are impaired in their proinflammatory response to known TLR2 ligands [198, 199]. Werfel and colleagues further reported that a missense mutation in the *TLR2* gene (*R753Q*) is associated with AD patients with a more severe phenotype, higher serum levels of immunoglobulin E (IgE), and greater susceptibility to *S. aureus* colonization [21–23]. TLR9 and TOLLIP polymorphisms have also been shown to be associated with AD patients [67, 164].

TLRs also directly affect skin barrier function by modulating both physical and chemical properties of barrier function [195]. TLR2 signaling has been shown to increase the expression of tight junction proteins and enhance skin barrier repair [24, 25]. Accordingly, $TLR2^{-/-}$ mice demonstrated impaired repair responses to epidermal injury by tape-stripping, suggesting that TLR2 may contribute to a chronic itch-scratch cycle often seen in AD patients. Other than TLR2, TLR3 signaling in response to dsRNA stimulation from epidermal injury also stimulates the expression of genes involved in permeability barrier repair [26]. In addition, TLR signaling is necessary for the keratinocyte production of antimicrobial peptides (AMPs), a key component of cutaneous chemical barrier function. Previous studies demonstrated that human β-defensin-2 (hBD2) and cathelicidin LL-37 (two AMPs important in keratinocyte defense against *S. aureus*) were significantly decreased in acute and chronic lesions of AD when compared to controls and patients with psoriasis [200]. LL-37 and hBD2 production, in turn, is dependent on intact TLR2 signaling after *S. aureus*, *S. epidermis* and skin injury [201–203].

Consistent with their tendency towards a Th2 immune response, AD patients often suffer from other atopic diseases such as allergic rhinitis, asthma and seasonal allergies. Early lesions in AD have a Th2 cytokine profile, which has been shown in murine models to promote preferential binding to *S. aureus* [27]. In support of the key role Th2 cytokines (IL-4, IL-13 and TSLP) play in AD pathogenesis, patients with moderate to severe AD treated with dupilumab, an antibody that targets the Th2 cytokine IL-4, showed remarkable improvement in their symptoms [28]. Increasing evidence suggests that TLRs affect the balance between Th1 and Th2 cytokines in the skin. For example, TLR2 stimulation by purified *S. aureus*-derived diacylated lipopeptitde induces expression of Th2 cytokines like thymic stromal lymphopoietin (TSLP) by keratinocytes [29]. TLR2 ligands also play a role in exaggerating and prolonging Th2-mediated inflammation in AD [26]. TLR2 also has complex roles in modulating other arms of immunity and has been shown to affect mast cell degranulation as well as subsequent IgE antibody production by B cells [30]. Collectively, these data indicate that TLRs, especially TLR2, influence multiple aspects of AD pathogenesis, including barrier function, *S. aureus* colonization as well as skewing of the immune response towards a Th2 phenotype. Further dissection of how TLRs affect the various altered skin functions in AD will likely lead to development of new therapeutic strategies.

Allergic Contact Dermatitis

Allergic contact dermatitis (ACD) is a common skin disorder caused by type IV delayed hypersensitivity reactions to skin-exposed chemical allergens (see also Chap. 23) [204]. In the clinically silent phase of sensitization, dendritic cells migrate to skin-draining lymph nodes and present contact allergens to naïve T lymphocytes, which may take weeks to months of repeated exposures to low molecular weight compounds. Upon re-exposure to the contact allergen, effector T cells are recruited back to the skin to mediate the type IV delayed hypersensitivity reaction (known as the 'elicitation phase') seen in ACD. It is estimated that more than 3000 contact allergens have been described; some of the common contact allergens include nickel, fragrances and hair dyes [98]. Martin et al. [205] first demonstrated a role for TLRs in ACD by showing that mice lacking both TLR2 and TLR4 failed to develop contact hypersensitivity (CHS), the experimental model used to study ACD. Importantly, CHS development was dependent on IL-12 expression that was stimulated by either TLR2 or TLR4 activation of dendritic cells as dendritic

cells from TLR2$^{-/-}$ TLR4$^{-/-}$ double knockout animals were resistant to CHS stimulation in wild type animals. Interestingly, CHS developed normally in germ free animals, suggesting that TLR2 and TLR4 activating signals were most likely derived from endogenous ligands such as DAMPs rather than microbial ligands. Further analyses revealed that contact allergens lead to reactive oxygen species (ROS) production, which stimulates the degradation of high molecular weight hyaluronic acid (HA) to low molecular weight HA products [206]. Low molecular weight HA, in turn, can serve as endogenous ligands for TLR2 and 4 signaling and potentiate an inflammatory cascade [207, 208]. A recent study by Gallo and colleagues [209], however, has challenged this notion that HA alone can cause ACD. The group overexpressed hyaluronidase, an enzyme involved in the generation of low molecular weight HA in mice, and showed that small HA fragments alone did not lead to spontaneous cutaneous inflammation resembling CHS. However, the addition of antigen along with small HA fragments accelerated allergic sensitization in a TLR4-dependent manner. Thus, rather than acting as the inflammatory stimuli for ACD, low molecular weight HA controls the antigen presentation capacity of the skin.

Other than DAMP-mediated activation of TLRs, nickel, cobalt and palladium have all been shown to bind and activate human TLR4 [46–48]. Specifically, binding of human TLR4 to nickel was mediated by histidine residues missing in murine TLR4 and provided molecular evidence for why mice are naturally resistant to nickel-induced CHS [48]. Whether nickel alone is sufficient in driving CHS remains unknown although the natural resistance to nicked-induced CHS seen in mice can be overcome by the addition of LPS [210], suggesting that microbial ligands that activate TLR4 may help to amplify the stimulus to promote sensitization to contact allergens [98]. Together, these studies provide evidence that contact allergens like nickel, DAMPS such as low molecular weight HA and PAMPs are all capable of activating TLRs in ACD. However, the relative contribution of each in either the sensitization phase or elicitation phase remains unknown and whether different TLR-expressing skin cells maybe involved in specific phases present exciting future research opportunities for learning more about ACD pathogenesis.

Psoriasis

Psoriasis is a chronic, recurrent, inflammatory disease characterized by dry, scaly, circumscribed erythematous plaques predominantly located in the scalp, nails, extensor surfaces of the limbs, umbilical region, and sacrum (see also Chap. 21). The pathogenesis of psoriasis, which is characterized by the predominance of Th1/Th17 cytokine profiles, involves hyperproliferation and parakeratosis of keratinocytes, which ultimately leads to thickening of the epidermis [99]. Many advances have been made in understanding the mechanisms involved in psoriasis and developments of new immunosuppressive and biologic treatments. Not surprisingly, TLRs have also been found to play a role in the pathogenesis of psoriasis. A study demonstrated that TLR1 and TLR2 expression was increased in the suprabasal layer of keratinocytes in psoriasis patients compared to skin isolated from normal controls [13]. In contrast, TLR5 expression in basal keratinocytes from psoriatic patients was decreased compared to healthy controls. Other studies have found increased TLR1, 2, 4, 5 and 9 expression in keratinocytes isolated from psoriatic lesions [211]. A recent study also identified mutations in the gene *AP1S3*, a protein involved in TLR3 trafficking, that are associated with pustular psoriasis [43]. Furthermore, application of imiquimod, a known TLR7 agonist, is known to trigger psoriasis in both humans and animal models [55, 56]. It is thought that imiquimod activates TLR7 signaling on DCs to drive psoriatic plaque formation by activating the production of IL-17 and IL-22 by innate lymphoctyes. ABIN-1, a negative regulator of TLR signaling, protects against psoriasis development by preventing exaggerated NF-κB and MAPK signaling in response to TLR7 agonists [172]. Therefore, TLR expression on various cell types in the skin may drive psoriatic pathogenesis and it is plausible that different cell types maybe involved in different phases of disease progression.

In contrast to AD patients who are more susceptible to *S. aureus* infections (see above), it is generally accepted that psoriatic plaques are relatively resistant to *S. aureus* infection [212]. It is thought that increased AMP production such as hBD2 and syndecans seen in psoriatic plaques is partially responsible for this phenotype [213, 214]. Keratinocyte growth factor, TGFα, has been found at high levels in psoriatic lesions and is responsible for increased TLR5 and TLR9 expression as well as TLR-dependent release of AMPs and proinflammatory cytokines [215]. While the increased production of AMPs is beneficial against pathogenic microorganisms, it has been postulated that they may also contribute to inflammation by modulating host immune receptors such as TLRs [185]. For example, LL-37 has been shown to complex with self DNA to create a novel DAMP and activate plasmacytoid dendritic cells (pDCs) via the TLR9 pathway and drive inflammation in psoriatic skin by stimulating IFNα production [68]. A recent study showed that LL-37 and an alternatively processed cathelicidin peptide KS-30 also stimulate keratinocytes to produce more type I IFNs but this was not dependent on its complexed DNA that was important for pDC activation [216].

Other than AMPs, heat shock protein (HSP) expression is also thought to contribute to TLR-mediated inflammation. HSP is induced by exposure to microbial pathogens and

other stressful stimuli [49]. Heat shock protein 27, 60, 70 and 90 have been shown to be overexpressed in psoriasis [49, 50] and can trigger an innate immune response through TLR4 on APCs, resulting in the secretion of TNFα, IL-12, and other Th1 cytokines. They also may act on the adaptive immune response by serving as autoantigens for self-reactive T cells that migrate into psoriatic lesions.

These discoveries are opening doors for novel treatments in psoriasis (see Chaps. 43). It is thought that systemic and topical retinoids used in the treatment of psoriasis may control inflammation through their inhibitory effects via TLR2 [76]. Monomethylfumarate (MMF), a bioactive metabolite of fumaric acid ester, is an immunotherapy for psoriasis that causes decreased production of Th1 cytokines and lymphocytopenia [217]. Monomethylfumarate was shown to decrease DC response to LPS and decreased IL-12p70 and IL-10 production. Etanercept, a TNFα inhibitor that has been successful in psoriasis treatment, has been shown to be associated with decreased LL-37 expression, which may dampen TLR9 activation and further suppress the chronic inflammatory response in psoriasis [218]. Thus, TLR dysregulation appears to play a role in psoriasis pathogenesis although whether a predominant TLR is involved remains unclear. Continued research in these areas will yield interesting findings that will impact treatment options for psoriasis patients.

Bacterial Infections

Bacterial cell wall components were the original ligands shown to stimulate TLR signaling [80, 81]. Accordingly, TLRs have been implicated in the pathogenesis of multiple bacterial diseases.

S. aureus Infections

S. aureus, a gram-positive extracellular bacteria, is the causative agent of a variety of skin infections, including impetigo, folliculitis and cellulitis (see Chap. 16) [219]. It is estimated that 20% of the population is persistently colonized, harboring *S. aureus* on the skin and the nares, while 50% are intermittent carriers [185]. *S. aureus* lipoproteins, peptidoglycan and lipoteichoic acid signal through TLR2/6 and TLR2/2 dimers [220, 221]. Accordingly, TLR2 deficient mice were more susceptible to *S. aureus* infection and harbored higher bacterial loads in blood compared to wild type controls [31, 32]. Animals deficient in MyD88, the key adaptor protein required for all TLR signaling with the exception of TLR3, were also more susceptible to *S. aureus* infection and demonstrated a neutrophil recruitment defect that was not seen in TLR2$^{-/-}$ mice. In corroboration of these animal studies, MyD88-deficient and IRAK4-deficient patients are more susceptible to *S. aureus* infections [222]. Mutations in the IRAK4 kinase that led to premature stop codons have been shown to increase susceptibility to pyogenic infections caused by *S. aureus* as well as *Streptococcus pneumonia* [223]. Cells from patients with this disease did not respond to any known ligands from TLRs 1 to 6 and 9. Consistent with an immune deficient phenotype, these patients suffered recurrent pyogenic infections with minimal febrile or inflammatory responses.

Leprosy

Leprosy, or Hansen's disease, caused by *Mycobacterium leprae*, is a chronic, debilitating disease that encompasses a spectrum of clinical manifestations [76]. At one end, tuberculoid leprosy (TL) presents in patients with a strong cell-mediated immune response, resulting in high resistance to *M. leprae* and few, localized, paucibacillary lesions. At the other end of the spectrum, lepromatous leprosy (LL) patients have a weak immune response, resulting in disseminated, multibacillary disease, including cutaneous and nerve involvement [224]. Other forms of the disease with unstable resistance include borderline tuberculoid, borderline, and borderline lepromatous. The former is Th1 mediated (e.g., IFNγ, IL-12, IL-18, and granulocyte-macrophage colony-stimulating factor), whereas the latter is Th2 driven (e.g., IL-4 and IL-10). There is accumulating evidence to suggest that whether a patient develops one response over the other may be in part due to variations in the TLR signaling pathway.

In 1999, it was discovered that mycobacteria activated macrophages through TLR2, resulting in production of TNFα, a proinflammatory cytokine [225]. An introduction of a dominant negative mutation in TLR2 rendered the receptor unresponsive to *M. tuberculosis*. Furthermore, a mutation in Arg^{677}Trp in TLR2 has been associated with LL in the Korean population [33]. A separate study confirmed that this mutation halts the ability of TLR2 to respond to both *M. leprae* and *M. tuberculosis*, confirming the clinical importance of this polymorphism [224].

Upon stimulation with *M. leprae*, patients with the Arg^{677}Trp TLR2 mutation were found to have decreased production of IL-2, IL-12, IFNγ, and TNFα, and increased IL-10 (an anti-inflammatory cytokine) when compared to those with the wild-type TLR2 [34]. Thus, the mutated TLR2 favored a Th2 phenotype, which is consistent with the observed LL phenotype. Based on these findings, TLR2 appears to play a critical role in the alteration of cytokine profiles and determination of the type of leprosy that develops.

M. leprae products were shown to activate both TLR2 homodimers as well as TLR1-TLR2 heterodimers [11]. Interestingly, TL lesions had higher TLR1 and TLR2

expression compared to LL lesions, suggesting that the expression of TLR2 and TLR1 contributes to the host response. Moreover, this study demonstrated that type 1 cytokines enhance TLR1 and TLR2 activation, whereas the Th2 cytokines inhibited activation. Therefore, not only does innate TLR signaling affect the adaptive immune response, but also the adaptive immune response, through cytokine release, may also influence the innate response. Further evidence that TLRs play a role in *M. leprae* pathogenesis was shown in a recent genetic study. Wong et al. showed that individuals homozygous for the *TLR1* I602S mutation, a functional TLR1 knockout, were protected from *M. leprae* infection, suggesting that *M. leprae* may have utilized TLR1 signaling to enhance its pathogenesis [12]. These findings underline the complexity of the interaction between TLRs and *M. leprae* pathogenesis through evolution and provide additional proof that TLRs are involved in bridging the gap between innate and adaptive immunity.

Syphilis

Syphilis is a contagious, sexually transmitted disease caused by the obligate human pathogen *Treponema pallidum* [76]. There are three stages of syphilis. In primary syphilis, a painless genital ulcer, called a chancre, appears 18–21 days after infection. Secondary syphilis can appear as various cutaneous eruptions—macular, papular, or polymorphous—often with lesions on the palms and soles. Tertiary syphilis occurs 3–5 years after infection. Patients may develop gummas, or necrotic lesions in the skin, mucous membranes, bones, or joints. Other complications of syphilis include neurologic and cardiac involvement.

It is appreciated that the outer cell wall structures of spirochete bacteria like *T. pallidum* are vastly different from the typical outer membranes of Gram-negative bacteria [226]. It is thought that *T. pallidum* has developed multiple strategies to evade the host immune response. For instance, *T. pallidum* lacks LPS and contains a paucity of immunogenic proteins compared to other spirochete bacterium [227]. Thus, during syphilitic infection, *T. pallidum* membrane lipoproteins (LPs) serve as principal proinflammatory mediators [35]. Indeed, it was demonstrated that *T. pallidum* LPs stimulated TLR2- and TLR4-expressing immature murine dendritic cells (DCs) to release proinflammatory cytokines such as IL-12, IL-1β, TNFα, and IL-6. It was long thought that opsonization of spirochete bacteria was essential for *T. pallidum* clearance but mechanistic studies were missing until Silver et al. recently demonstrated that TLR-MyD88 signaling is crucial for phagocytosis and bacterial clearance [227]. MyD88-deficient animals exhibited increased inflammation with a stronger infiltration of neutrophils and lymphocytes but still harbored a high bacterial load due to the inability of MyD88$^{-/-}$ macrophages to opsonize *T. pallidum*. Consistent with these findings, a recent clinical study found that *TLR1*, *TLR2* and *TLR6* polymorphisms are associated with an increased risk of neurosyphilis development, suggesting that the TLR1/TLR2 and TLR2/TLR6 heterodimers are important in protecting against *T. pallidum* [16].

Yersinia pestis

Y. pestis is a gram-negative bacillus that causes plague, a disease that killed millions of people in the "Black Death" pandemic. It is transmitted by the bite of the rat flea *Xenopsylla cheopis*. Clinically, painful buboes form in the axillae or groin, although other skin lesions such as vesicles, plaques, petechiae, and purpura can be seen. *Yersinia* outer membrane protein, V antigen, targets TLR2 and CD14 on the surfaces of APCs [228]. Interestingly, *Y. pestis* has specific variations in its LPS lipid A structure to evade TLR4-mediated host immune recognition [229].

Lyme Disease

Lyme disease is a tick-borne illness caused by the spirochete *Borrelia burgdorferi* and is loosely divided into three stages. The primary stage is characterized by constitutional symptoms and erythema chronicum migrans. The second stage occurs for 5–6 months after the rash resolves. In the tertiary phase, cardiac, neurologic, and rheumatologic complications can occur. Like other spirochetes such as *T. pallidum*, *B. burgdorferi* does not have LPS in its outer membrane structure to stimulate TLR4. *B. burgdorferi* outer surface protein A (OspA) stimulates TLR2 to activate inflammatory signaling [36]. Stimulation with *B. burgdorferi* lysate was found to increase the expression of TLR1 and TLR2 in all peripheral blood monocytes and human brain cells, but not neurons [230]. Consistent with the aforementioned *in vitro* data, TLR2 deficient animals harbored much higher loads of *B. burgdorferi* and TLR2$^{-/-}$ macrophages produced lower levels of proinflammatory cytokines [231]. Peripheral blood monocytes (PBMCs) isolated from patients with TLR2 Arg^{753}Gln mutations also secreted less proinflammatory cytokines [37]. Interestingly, the lower levels of TNFα and IFNγ were protective against late stages of disease such as lyme arthritis development.

Candidal Infections

Candida albicans is a dimorphic fungi that causes cutaneous and mucocutaneous candidiasis and causes severe infections in immunocompromised individuals (see Chap. 19). It has been demonstrated that the immune response against

yeast phospholipomannans and glycans involves TLR2, causing upregulation of TNFα via the NF-κB pathway [38, 39]. Candidal cell polysaccharide mannan most likely activates TLR4 as anti-CD14 and anti-TLR4 antibodies (but not anti-TLR2 antibodies) blocked mannan-induced cytokine production [38, 39]. When stimulated with *C. albicans*, TLR4 defective macrophages expressed lower levels of neutrophil chemokines and impaired neutrophil recruitment [232]. Consistent with the animal model data, killing of *C. albicans* in human keratinocytes was shown to be dependent on TLR2 and TLR4 [51]. More recent work has also implicated a role for TLR7 in IL-12 production in response to fungal RNA [233]. TLR7 and TLR9 deficient animals harbored higher fungal load compared to wild type animals but whether this was dependent on IL-12 was not studied. Together, these studies suggest that TLRs work differently to foster an immune response against *C. albicans* – TLR4 activation leads to recruitment of neutrophils; TLR2 mediates the production of TNFα and TLR7 is important in the IL-12 response against candidal infections.

Herpes Simplex Virus

Viruses are obligate intracellular parasites that rely on host protein machinery to complete their replication cycles (see Chap. 17). Due to their intracellular location, viral nucleic acids are usually recognized in intracellular components such as endolysosomes by various TLRs. Viral proteins released during replication may also stimulate TLRs on cell surfaces. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are double-stranded DNA (dsDNA) viruses that commonly infect skin and mucosa. HSV-1 generally produces vesicular outbreaks at the orolabial or ocular mucosa, whereas HSV-2 typically infects genital mucosa and renders patients more susceptible to other sexually transmitted infections. However, both strains of the virus can infect either physical location.

Herpes simplex virus glycoproteins gH/gL and gB have been shown to stimulate TLR2 and activate NF-κB signaling [40, 234]. TLR2-mediated NF-κB activation, however, may have detrimental effects as TLR2 knockout mice with decreased cytokine responses are resistant to HSV encephalitis [42]. Plasmacytoid dendritic cells recognize HSV through TLR9 to activate interferon production [235, 236]. In contrast to TLR2 deficient animals, TLR9−/− were more susceptible to HSV infection [237, 238]. Furthermore, TLR2/TLR9 double knockout animals exhibited 100 % mortality and had decreased NK cells as well as global cytokine levels. Thus, while TLR9 plays a protective role against HSV infection, the role of TLR2 is complex and further dissection of its role in different cell types is necessary. The importance of TLR signaling is further demonstrated by the fact that a HSV-1 protein, ICP0, that is expressed early during infection accelerates the degradation of MyD88 and inhibits NF-κB activation [239]. Interestingly, Iwasaki et al. [240] showed that HSV is detected in a serial recognition system by DCs – viral glycoproteins are first detected by TLR2 and then viral DNA is recognized by intracellular TLR9. The authors suggested that this serial recognition system helps to mount an optimal antiviral response. Together, this body of work indicates that while TLR2 and TLR9 may have differential effects on the antiviral response, they also work synergistically and the loss of both receptors leads to detrimental effects in the host.

Other than the TLR2 and TLR9 interaction, TLR3, which recognizes dsRNA, has also been shown to play an important role against HSV infection [44]. Vaginal inoculation of $TLR3^{-/-}$ mice led to higher viral loads in the central nervous system compared to healthy controls. Of note, global cytokine production was unaltered in $TLR3^{-/-}$ mice but $TLR3^{-/-}$ astrocytes were unable to produce type I IFN after HSV infection, thereby rendering the host susceptible to extensive CNS infection. Importantly, TLR3 is also protective against HSV in humans as children born with TLR3 deficiencies were more susceptible to HSV encephalitis [10].

Autoimmune Diseases- SLE

The autoimmune connective tissue diseases (AI- CTDs) are a group of clinical disorders that all have circulating autoantibodies (autoAbs) (see Chap. 30). Such disorders include systemic lupus erythematosus (SLE), dermatomyositis, systemic sclerosis, rheumatoid arthritis, mixed connective tissue disease, Sjögren's disease and more [76]. SLE is a disease commonly seen in dermatology, in which patients may exhibit several key diagnostic signs and symptoms, including antinuclear antibody positivity, malar and discoid rashes, photosensitivity, oral ulcers, arthritis, serositis, and renal, neurologic, hematologic, and immunologic disorders. It is generally accepted that IFNα and pDCs contribute to the pathogenesis in SLE – pDCs recognize self-nucleic acids in a TLR7 and TLR9 dependent manner, which leads to the upregulation of IFN production as well as B cell production of anti-DNA and anti-RNP antibodies [57, 61]. These autoantibodies maybe directed against self antigens such as small nuclear ribonuclear protein particles (SnRNP) called U1 and Sm and this interaction leads to the formation of immune complexes with DNA or RNA from dying cells [241]. Recent evidence suggests that TLR7, TLR8 and TLR9 play key roles in mediating an abnormal immune response mediated by pDCs and neutrophils to endogenous ligands, leading to chronic activation that triggers autoimmunity in the skin [57, 242].

Previous work revealed that specific RNA sequences within snRNPs stimulate TLR7 and TLR8 to activate immune cells, such as pDCs and monocytes, to secrete high levels of IFNα and TNFα respectively [58]. Intriguingly, *TLR7* and *TLR8* are both encoded on the X chromosome, which may partially account for why 90 % of SLE cases occur in women [243]. A deletion of a single copy of *TLR7* in mice led to increased survival and reduced autoantibody production and splenocyte proliferation [244]. A direct correlation existed between TLR7 expression and autoAb production, further implicating that TLR7 plays a pathogenic role in SLE. Gene duplication of TLR7 in a specific strain of mice also led to increased autoantibody production [59]. Compared to TLR7, the role of TLR9 in SLE pathogenesis is more complex. TLR9 has been shown to bind single-stranded unmethylated CpG-DNA containing a phosphodiester backbone, a process that is inhibited by chloroquine and quinacrine, suggesting a possible mechanism for the therapeutic effect of these drugs seen in some autoimmune diseases, such as lupus [245]. Moreover, TLR9/MyD88 signaling was crucial for generation of pathogenic autoantibodies in SLE [246]. Based on these studies, it was expected that TLR9 deficient animals would exhibit less severe SLE. Paradoxically, TLR9 deficiency promoted SLE in multiple lupus models, suggesting that the role of TLR9 was more complex [69, 70]. Most recently, it was shown that although TLR9 was indeed required for autoAb formation, TLR9 also plays a role in B cell-mediated tolerance by controlling the life-span of autoreactive B cells [247]. TLR9 also suppressed TLR7-mediated autoAb production and thus has dual roles in SLE pathogenesis [248].

In support of the aforementioned animal data, SLE patients also expressed high levels of TLR7 and 9 [249]. Interestingly, chronic TLR7 and TLR9 stimulation of pDCs led to resistance to glucocorticoid treatment [60]. Inhibition of TLR7/TLR9 with a small immunoregulatory sequence in animal models improved autoantibody production as well as kidney damage and a similar inhibitor has been tested in patients with promise [62]. Other drugs targeting TLR signaling are also under development for SLE and will hopefully lead to drug regimens with more favorable side effect profiles for SLE patients in the future [250].

Melanoma and Mycosis Fungoides

Melanoma is a skin cancer caused by neoplastic transformation of melanocytes and has been increasing in incidence and mortality over the years [251]. It is thought that genetic factors and intermittent high-dose UV irradiation during childhood are both important etiologic factors in melanoma. Although melanoma only accounts for 4 % of all skin cancers, it causes more than 70 % of skin cancer related deaths as metastatic disease often carries a poor prognosis [252]. Since melanocytes express functional TLR2, 3, 4, 5, 7, 9 and 10, it has not surprising that TLR ligands have the ability to modulate melanoma pathogenesis [136, 137]. Indeed, LPS has been shown to stimulate melanocyte IL-8 production in a TLR4 dependent manner [253]. Agonists of TLR 3, 4, 7, 8 and 9 have showed promise as cancer immunotherapy agents and are regarded as having high potential by the National Cancer Institute [254].

Manipulation of TLRs is currently being investigated as a therapeutic option for melanoma as TLR agonists can activate dendritic cells in sentinel lymph nodes (SLNs) of melanoma patients [255]. In animal studies, addition of CpG DNA and poly-I:C (TLR9 and TLR3 ligands respectively) to peritumoral injections have been shown to increase cutaneous tumor rejection and animals remained tumor free after 50 days [256]. TLR7 agonists such as 852A and imiquimod have also been shown to have antitumor effects [63, 64, 66, 252]. Topical application of imiquimod in melanoma patients enhanced influx of CD4+ and CD8+ T cells to the skin as well as SLNs [252]. While commonly used as a topical agent, imiquimod has chemical properties that are not favorable for systemic administration [63], which led to the testing of other TLR7 agonists such as 852A. 852A was well tolerated in metastatic melanoma patients and induced systemic inflammatory responses [64]. In animal models, 852A had significant antitumor activity and stimulated higher levels of type I IFN release [63].

PF-3512676 is an immunomodulating synthetic oligonucleotide that acts as a TLR9 agonist [257]. It is currently under development for the treatment of cancer both as monotherapy and in combination therapy, as well as an adjuvant for vaccines. It acts through TLR9 receptors present on B cells and plasmacytoid dendritic cells to stimulate B-cell proliferation, IFNα and natural killer (NK) cell activity. Used alone as a therapeutic agent, PF-3512676 had a favorable safety profile but only elicited moderate response rates in patients with advanced melanoma [71]. As an adjuvant to other therapeutic modalities, PF-3512676 was shown to be safe in melanoma patients using other modes of therapy such as CTLA-4 blockade [72, 73].

TLR modulators are also being tested in other skin malignancies. Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma (CTCL) and is characterized by malignant clinical proliferation of skin trafficking T-cells [258]. Skin lesions in MF include patches, plaques, tumors, hypopigmented lesions, and erythroderma. Treatment options range from light therapy,

retinoids, nitrogen mustard, topical steroids to systemic interferon [65]. TLR agonists have shown promise as a therapeutic approach – a preliminary pilot study of six patients with patch and plaque stage MF treated with topical imiquimod, a TLR7 agonist, 5 % cream three times a week for 12 weeks reported a histologic and clinical response rate of 50 % [65]. A phase I clinical study administered TLR9 agonist CpG oligodeoxynucleotide (ODN) to MF patients and demonstrated antitumor activity [74]. MF patients who failed standard treatment in a subsequent study using ODN had increased pDC infiltration as well as a decrease in regulatory T cells [75]. Skin lesion regression was noted in one-third of patients but the overall clinical response assessment was limited in this study due to the small patient size. Future studies may yield promising therapies for MF patients who do not respond to standard treatment approaches.

Ultraviolet Radiation

Ultraviolet radiation (UVR) is an established carcinogen that causes genetic lesions in keratinocytes and contributes to skin cancer development (see Chap. 10) [259]. UVR causes the formation of cyclobutane pyrimidine dimers (CPDs) and DNA single-strand breaks [260], which activates DNA repair enzymes that are vital for maintaining genome integrity. Irreversibly damaged keratinocytes that cannot be repaired undergo cell death and are sloughed off to maintain an intact skin barrier. Additionally, it has long been known that UVR causes widespread immune suppression by depleting Langerhans cells (LCs), inhibiting APC antigen presentation and upregulating immunoregulatory cytokines such as IL-10 [259]. UVR stimulates the upregulation of HSPs from keratinocytes that are known to stimulate TLRs (see section "Psoriasis") and lead to the release of IL-10 and TNFα [76]. Moreover, C3H/HeJ mice that are TLR4-hyporesponsive exhibit impaired TNF-α production after UVB exposure and are resistant to UVB suppression of CHS [52]. More recent studies have demonstrated that UVR can damage self noncoding RNA that contain stem-loop structures and activate TLR3 as DAMPs [261]. Additionally, TLR signaling may determine the form of cell death that takes place after UVR damage as deficiencies in TLR4-MyD88 axis led to increased cell survival along with upregulation of markers of necroptosis [53]. Therefore, multiple TLRs are activated after UVR exposure and have multiple downstream effects that may affect the development of malignant lesions.

The power of UV light and the importance of DNA repair machinery is demonstrated in xeroderma pigmentosum (XP), a rare, autosomal recessive disorder characterized by photosensitivity, premature skin aging, and malignant tumor development due to an inability to repair DNA damage induced by UV light [76]. Gaspari et al. [262] discovered that NK cells from XP patients had a defect in IFN production in response to poly-I:C (a TLR3 ligand) stimulation. Subsequent studies have further expanded on the role of TLRs in XP and the DNA repair machinery. TLR4 deficient animals expressed higher degrees of nucleotide excision repair after UV damage due to activation of XP complementation group A (XPA) expression [54]. The ligand involved in TLR4 stimulation was not studied but it will be interesting to determine whether PAMPs or DAMPs are involved in TLR4 activation after UVL damage. In contrast to the inhibitory role of TLR4, TLR7 agonist imiquimod was shown to enhance DNA repair gene expression and decreased DNA damage detected in local lymph nodes when applied topically [66]. Other repair functions in response to UV damage has been shown to be dependent on TLRs as well as TLR3 was shown to be required for effective skin barrier repair after UVR exposure [263]. Collectively, evidence suggests that TLRs play an important role in sensing and modulating the downstream response to UVR damage. Whether these TLR modulating properties by UVR can be harnessed to protect against DNA damage and prevent tumor development in XP patients remain to be investigated.

Conclusion

Since the discovery of TLRs more than 20 years ago, the family of PRRs continues to grow and be implicated in human disease. Evidence continues to accumulate to suggest that TLRs, the most well characterized group of PRRs, play an essential role in bridging innate and adaptive immune responses. Up to 13 mammalian TLRs have been identified and it is believed that TLRs 1–10 are functional in humans and that TLRs not only respond to PAMPs but also endogenous ligands produced after tissue damage coined DAMPs. Both PAMPs and DAMPs can contribute to the activation of TLRs, which has downstream effects on both innate and adaptive immunity (Fig. 2.2). Dysregulation in TLR activation can lead to the development of dermatological diseases such as psoriasis and allergic contact dermatitis. Thus, TLRs play an integral role in countless dermatologic diseases but many questions remain and future studies are necessary to address precise molecular mechanisms that are involved. It is certain that many more discoveries will be made to further characterize and understand this group of receptors, their role in skin diseases, as well as the potential to manipulate signaling through these TLRs to use them for diagnostic and treatment purposes.

Questions

1. Which of the following represent a negative regulator (inhibitor) of TLR function?
   A. IRAK-M
   B. TOLLIP
   C. SOCS-1
   D. All of the above
   E. None of the above

Correct answer: D-All of the above. IRAK-M, TOLLIP and SOCS-1 are all TLR negative regulators

2. Which skin disease have TLR negative regulators been associated?
   A. Non-melanoma skin cancer
   B. Psoriasis
   C. Atopic Dermatitis
   D. Cutaneous T-cell lymphoma

Correct answer: (C)-TOLLIP mutations have been associated with Atopic dermatitis. However, the exact role of these mutations in the pathophysiology of this common skin disease remains unclear

3. How do TLRs mediate pro-inflammatory cytokine production in acne vulgaris?
   A. PAMPs from P. acnes activate TLR2 and TLR4, inciting the production of pro-inflammatory cytokines
   B. PAMPs from S. aureus induce TLR2 activation
   C. TLRs are not involved in the pathophysiology of acne
   D. PAMPs from the pilosebacious unit activate TLR7,8,9

Correct answer: (A)-*P. acnes* microbial products such as LPS and peptidoglycan activate TLR2 and TLR4 to active the production of proinflammatory cytokines in the skin. It is thought that P. acnes strains in healthy controls may regulate TLR expression differently when compared to P. acnes strains in acne vulgaris patients

4. In allergic contact dermatitis (ACD), what is the predominant TLR involved in the pathophysiology of nickel allergy?
   A. TLR4
   B. TLR7
   C. TLR2
   D. TLR9
   E. None of the above

Correct answer: (A) Nickel, cobalt and palladium can bind and activate human TLR4s and activation of CHS. dependent on histidine residues that are specifically found in human TLR4, thus explaining why mice are naturally resistant to nickel-induced CHS

5. Why are mice genetically resistant to ACD to Nickel?
   A. Nickel does not penetrate mouse skin
   B. Their TLR are not activated by nickel
   C. Their Tregulatory cells suppress the response
   D. Mice have a high level of nickel in their diet

Correct answer: (B)-TLR4 in mice lacks the amino acid histidine in the extracellular domain. In humans, TLR4 normally expresses the amino acid histidine. TLR4 activation by nickel is dependent on histidine residues that are specifically found in human TLR4, thus explaining why mice are naturally resistant to nickel-induced CHS

6. How are TLRs involved in DNA repair?
   A. TLR sense DNA damage
   B. TLR activation directly induces a DNA repair response
   C. TLR activation triggers inflammation, which may stimulate DNA repair
   D. TLR7 agonists applied can increase DNA repair in the skin
   E. All of the above
   F. None of the above

Correct answer: (D)-TLR engagement may stimulate DNA repair by multiple mechanisms. This phenomenon is relevant to UV light exposure, and recovery of skin derived antigen presenting cells

7. Which of the following diseases is associated with impaired TLR signaling via TLR3?
   A. Discoid lupus
   B. Alopecia areata
   C. Psoriasis
   D. Xeroderma pigmentosa

Correct answer: (D)-XP patients NK cells are defective in IFN production in response to TLR3 stimulation

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Innate Lymphoid Cells in the Skin

3

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Abstract

The skin forms the body's primary interface with the environment and, as such, is equipped with a network of immune cells to provide the first line of defense against infection and injury. Recently, a new family of lymphocyte-like immune cells has been described that does not express rearranged antigen receptors. These cells have been termed innate lymphoid cells (ILC), and comprise several subsets that are defined by the expression of certain transcription factors and cytokines. Emerging evidence has implicated ILC in the pathogenesis of inflammatory and neoplastic skin diseases. Here, we review the biology of ILC and their role in skin pathophysiology.

Keywords

Innate lymphoid cells • Cytokines • Cell receptors • Cell markers • Skin disease • Immune cell development • NK cells

Introduction

ILC are a heterogeneous family of innate effector cells that are characterised by their lymphoid morphology and absence of rearranged antigen receptors. ILC lack expression of lineage markers for T cells, B cells, myeloid cells and dendritic cells [85]. ILC have the ability to respond rapidly to environmental and pathogenic stimuli or other cytokines by producing 'signature' effector cytokines. Based on their cytokine secretion profiles and the expression of key transcription factors involved in their function, ILC have been grouped into three major subsets. Group 1 ILC, which include conventional natural killer (NK) cells, express the transcription factor T-bet and produce the Th1 cytokine IFN-γ; Group 2 ILC express the transcription factor GATA-3 and secrete the Th2 cytokines, in particular IL-5 and IL-13; Group 3 ILC express the retinoic acid receptor-related orphan nuclear receptor RORγt and secrete the Th17-associated cytokines IL-17 and/or IL-22. All three ILC subsets have been identified in mice and in humans, and are predominantly located at tissue barriers where they have roles in preserving epithelial integrity, restricting commensal bacterial spread, and providing immune defence against pathogens. ILC are also crucial for lymphoid tissue formation. ILC are some of the earliest responders to tissue injury, infection or tumorigenesis, prior to establishment of adaptive immune responses. Cytokines produced by ILC can also regulate stromal cells during homeostasis, or promote tissue remodelling and wound healing after injury or infection. Whilst ILC function in an antigen-independent manner, their cytokine responses may set the stage for subsequent adaptive

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immune responses that might be conditioned by the cytokine milieu.

In addition to the gastrointestinal mucosal tissue and the lungs, ILC are also enriched in murine and human skin compared to the blood. Healthy human skin has been shown to harbour all three ILC subsets described, although their functions in the skin are not yet well understood. However, based on the roles of ILC in other organs, it is likely that skin-resident ILC are also involved in maintaining skin homeostasis, barrier integrity and in sensing of environmental and pathogenic insults. The inappropriate accumulation and activation of ILC contributes to several inflammatory conditions in the gut and lung. Recent studies have demonstrated that increased abundance of ILC2 and ILC3 are associated with skin lesions of patients suffering from atopic dermatitis and psoriasis, respectively. Therefore, increased understanding of ILC regulation and function during homeostasis, inflammation and infection could lead to better therapies and improved diagnoses of inflammatory skin conditions.

ILC Classification and Identification

ILC are absolutely dependent on signalling through the common cytokine gamma-chain (γc) for development, and these $CD45^+$ lineage-negative (Lin^-) ILC, with the notable exception of NK cells, also express the alpha chain of the IL-7 receptor (IL-7Rα, or CD127). Most murine ILC subsets also express CD90 (Thy1), Sca-1 and variable levels of the stem cell growth factor receptor, c-Kit (or CD117). In humans, all ILC subsets, except a subset of group 1 ILC, express CD127.

ILC are currently classified into three groups based on their distinct patterns of cytokine production [84]. Members of the three ILC groups also express distinct families of transcription factors and depend on different sets of transcription factors for their development and maintenance. The phenotypes of murine and human ILC are summarised in Table 3.1. ILC have been identified and characterised using a number of gene and protein expression studies, fate-mapping approaches, and in reporter and knockout mice [84], some details of which are described in the following sections.

Group 1 ILC

ILC1s produce the Th1 signature cytokine IFN-γ and express the transcription factor T-bet. They include conventional NK cells as well as several recently-discovered IFN-γ-producing ILC1 subsets in the tonsils and intestines. ILC1 have also been found in human skin.

Conventional NK cells are the prototypical members of Group 1 ILC as they produce large amounts of IFN-γ. They co-express the T-box transcription factors T-bet and eomesodermin (Eomes), which, together with E4bp4 (Nfil), is required for their development and/or maturation [86]. All NK cells depend on IL-15 signaling for their development, survival and maintenance. Mouse NK cells are identified by their expression of the natural cytotoxicity receptors (NCRs) NKp46, and in certain mouse strains, NK1.1. Mature $CD11b^{hi}CD27^{lo}$ and immature $CD27^{hi}CD11b^{lo}$ NK subsets are found in the spleen and bone marrow, whereas "tissue-resident" NK subsets are found in the liver, salivary glands, skin and uterus. In addition, there is a population of thymic-derived $CD127^+$ NK subset in mice. In humans, NK cells are identified by CD56 and CD16 expression. Both $CD56^{bright}CD16^-$ and $CD56^{dim}CD16^+$ NK cells are found in the blood and tissues. The $CD56^{dim}$ NK cells are highly cytotoxic and release perforin and granzyme upon encountering target cells, whereas $CD56^{bright}$ NK cells specialise in IFN-γ production in response to IL-12 and IL-18 [13]. NK cells also express germ line-encoded inhibitory and activating receptors of the C-type lectin-like family and immunoglobulin superfamily, which include the killer inhibitor receptors (KIR) (e.g., the Ly49 and CD158 series of molecules) and activating receptors (e.g., NKG2D, NKG2c) that control NK cell licensing and proliferation [46].

An additional ILC1 population, distinct from NK cells, was identified in 2013, in the tonsils and gut mucosa [6, 22, 45]. These ILC1 also express T-bet and NK cell-associated

Table 3.1 Surface phenotype of mouse and human ILC

		Mouse		Human	
		Unique	Shared	Unique	Shared
ILC1	NK	IL-12Rβ2	CD127 (not on NK) CD90 (not on NK, ILC1) CD117 (not on NK, ILC1, NCR- ILC3)	IL-12Rβ2	CD127 (not on some NK) IL-1R (all but NK) NKp46, NKp44 (on NK and NCR+ ILC3 only)
	ILC1	IL-12Rβ2		IL-12Rβ2	
ILC2		ST2, IL-17RB, ICOS, CD25		CRTH2, ST2, IL-17RB	
ILC3	LTi	CD4, IL-23R		IL-23R, CD117	
	NCR+ILC3	IL-23R			
	NCR-ILC3			ND	ND

Adapted from Spits et al. [84]

markers and produce IFN-γ but are developmentally and/or functionally distinct from NK cells. In particular, ILC1 derive from an Id2-expressing progenitor that does not give rise to NK cells and are dependent upon GATA-3 for their development, in contrast to NK cells. Phenotypically, non-NK ILC1 cells lack surface expression of the NK cell-specific Ly49 receptors, and possibly death receptors. Most ILC1s, including NK cells, also express the surface receptor IL-12Rβ2, consistent with their responsiveness to IL-12 stimulation.

One of the human non-NK ILC1 populations identified by Fuchs et al. within human tonsils was the NKp44$^+$CD103$^+$ subset [22]. The NKp44$^+$CD103$^+$ cells also secreted high amounts of IFN-γ when stimulated by IL-12 and IL-15, producing similar levels to that produced by blood CD56^{hi} conventional NK cells. Some of these NKp44$^+$CD103$^+$ cells expressed perforin and granzymes and could degranulate, but unlike NK cells, they did not respond to IL-18 stimulation. These cells were also distinct from ILC3 (see below) as they did not respond to IL-23 stimulation, and they expressed higher levels of T-bet and lower levels of RORγt and the aryl hydrocarbon receptor (AhR) when compared to ILC3. Importantly, these cells were conserved in *Rorc*$^{-/-}$ and *Ahr*$^{-/-}$ mice which lacked ILC3. The murine counterparts of the NKp44$^+$CD103$^+$ subset were identified as CD160$^+$NKp46$^+$NK1.1$^+$ cells in the intestine and tonsil. These cells were absent in Nfil3- or T-bet-deficient mice but still present in IL-15Rα-deficient mice, in contrast to conventional NK cells. Non-NK ILC1 localized specifically in the epithelial layer of the tonsils and in the intraepithelial layer of the small intestine, and had a phenotype that resembled tissue-resident memory CD8$^+$ T cells (i.e., high levels of αEβ7 integrin and CD49a).

Independently, Bernink et al. [6] identified a population of cells in human tonsils that expressed T-bet and produced IFN-γ when stimulated with IL-12. After human ILC2 (CRTH2$^+$) and ILC3 (cKit$^+$NKp44$^+$) were excluded from their analyses (discussed below), two additional populations were observed in the tonsils: the cKit$^+$NKp44$^-$ and cKit$^-$NKp44$^-$. The cKit$^-$NKp44$^-$ population produced IFN-γ but was distinct from the ILC1 population identified by Fuchs et al.: these cells did not express NKp44 and localized to the lamina propria instead of the intraepithelial layer of the small intestine. These cells were also distinct from NK cells, as they did not express perforin and granzyme, nor did they express the NK cell markers CD16, CD94 and IL-15Rα. Interestingly, Bernink et al. observed that a portion of these ILC1 could differentiate from cKit$^+$NKp44$^-$RORγt$^+$ ILC3 precursors that had been isolated from both foetal gut and tonsil when they were stimulated with IL-2 and IL-12. This plasticity of ILC1 development from ILC3 to form an "ex-RORγt" subset was consistent with that reported in a RORγt fate-mapping study [95], where ILC3 cells in mice were shown to convert into IFN-γ producers (potentially ILC1). Therefore, there is some degree of plasticity between the ILC1 and ILC3 subsets, and the level of T-bet expression was suggested to promote the ILC3 to ILC1 differentiation (see later). Nonetheless, there also remains a separate ILC1 population that develops independently of RORγt. Notably, no ILC1 were found in the foetal gut, suggesting they may develop after colonisation with commensals.

Another group of researchers identified non-NK ILC1 after investigating the role of T-bet in ILC3 development [45]. They found that a third of NCR$^-$ ILC3 and almost all NCR$^+$ ILC3 in the lamina propria expressed T-bet (see later). They also described a population of CCR6$^-$RORγt$^+$T-bet$^+$ ILC that differentiated from the CCR6$^-$RORγt$^+$ ILC. These cells emerged postnatally under control of the aryl hydrocarbon receptor (AhR) in response to environmental stimuli (presumably commensal microbiota) and IL-23 stimulation. The increased expression of T-bet in this population in turn promoted expression of IFN-γ and NKp46.

Therefore, ILC1s are currently believed to contain IL-7Rα$^+$ conventional NK (cNK) cells, intraepithelial ILC1s [22] and "ex-RORγt" ILC3s [6, 45, 95].

Group 2 ILC

ILC2 produce the Th2 cytokines IL-5, IL-13, as well as IL-4, IL-9 and amphiregulin in response to IL-25 and IL-33, which may be further augmented by TSLP. ILC2 depend on the transcription factors GATA-binding protein 3 (GATA3) and the RORα for their development and function [35, 58, 99]. What is now known as ILC2 were first observed in 2001 as a non-B/non-T population that produces IL-5 and IL-13 in response to intranasal IL-25 administration [21]. In 2006, a population of Lin$^-$cKit$^+$CD90.2$^+$ cells was described in the context of helminth infection in mice, whereby IL-25-dependent expulsion of *Nippostrongylus brasiliensis* occurred independently of B and T cells [20]. However, it was not until 2010 that ILC2 cells were properly phenotyped and functionally characterised, in which they were identified as "natural helper" cells [60], "nuocytes" [61] or "innate helper" cells [69], before the consensus term ILC2 was coined.

Murine ILC2 consistently lack expression of lineage markers CD3, B220, CD11b, Ter119 and Gr-1 [60, 69]. They express CD127 and the IL-17BR (IL-25R) and ST2 (IL-33R) receptors, consistent with their ability to respond to IL-25 and/or IL-33, respectively. Murine ILC2 cells, defined as a Lin$^-$Sca$^-$1$^+$Thy1.1$^+$CD127$^+$T1/ST2$^+$ population, were first found in the mesenteric fat-associated lymphoid clusters, mesenteric lymph nodes, spleen, liver and intestines of mice, and have since been detected in the bone marrow, liver, blood, airways and the skin. There is some variability in

expression of other markers on ILC2, which may be due to the activation status and tissue-specificity of ILC2. For instance, lung ILC2 express lower expression of Sca-1 and CCR9 compared to those from the mesenteric lymph nodes (mLN); and skin ILC2 express CD103. Murine ILC2 also express the high-affinity IL-2 receptor alpha chain (IL-2Rα, CD25) [73], ICOS, and variable levels of major histocompatibility class II molecules and c-Kit (CD117) [33, 53, 54, 61], consistent with the fact that they do not rely upon stem cell factor (SCF) signaling for survival [60, 69, 72]. ILC2, in contrast to the other ILC subsets, do not express CD2, which further serves to discriminate ILC2 cells from conventional NK cells and most T cells [72]. ILC2 also lack the NK and ILC1-associated markers NK1.1 and NKp46, and unlike ILC1 and ILC3, they have not been shown to develop into other ILC.

The human ILC2 equivalents were first identified in the foetal gut and lung tissue [58] as a $Lin^{-}CD127^{+}CD45^{hi}$ population that also expressed transcripts encoding IL-13, IL-17RB, ST2 and CRTH2 but low levels of RORγt. ILC2 have also been identified in human blood, skin, lungs and nasal mucosa. In humans, ILC2 cells are identified by lack of expression of lineage markers CD3, CD19, CD94, CD1a, CD11c, CD123, BDCA2, CD14, FceR1 and CD34 and their expression of CD127, the pan-human-ILC marker CD161 and, uniquely, CRTH2 [58]. Similar to murine ILC2, human ILC2 also express CD25 [59] and variable levels of c-Kit, but do not express NKp44. Human ILC2 cells, like their murine counterparts, are also responsive to IL-25, IL-33 and TSLP, indicative of functional receptors to these cytokines [55, 57, 58, 89].

Group 3 ILC

ILC3 depend on the transcription factor RORγt and produce IL-17 and/or IL-22 in response to IL-1β and IL-23 stimulation [34, 82]. ILC3 are a complex family. The prototypical member is the lymphoid tissue-inducer (LTi) cell that is crucial for lymphoid organ formation during embryogenesis (Peyer's patches, lamina propria) and in the neonate (cryptopatches and isolated lymphoid follicles), and that also has a role in lymphoid organ repair after infection [51, 52]. These cells express CD127, CD117, α4β7, CD4 and CCR6 in mice, although a population of $CD4^{-}$ LTi also exists. Two other non-LTi ILC3 subsets, distinguished as those that express natural cytotoxicity receptors (NCR) and those that do not, were also identified in nonlymphoid tissues, i.e., the gut mucosa and tonsils [10, 14, 50, 75, 77] where they are important for barrier protection. They include $CCR6^{+}CD4^{-}$ cells and the $CCR6^{-/lo}$ population that are rare in the foetal liver but expands rapidly during the first month of life dependent upon the expression of AhR. The $CCR6^{-/lo}$ ILC3s are further subdivided into an $NKp46^{-}$ and $NKp46^{+}$ subsets, i.e., NCR^{-} ILC3 and NCR^{+} ILC3.

The NCR^{-} ILC3 cells do not express IL-17 or CD4 but produce IL-22. The subset now collectively termed NCR^{+} ILC3 were also known as NCR22 cells, $NKp46^{+}$ ILC, ILC22s and NKR-LTi. Although these cells expressed NKp46, they are functionally distinct from NK cells as they do not express cytotoxic molecules such as perforin, granzymes and death receptors. These cells also did not produce IFNγ or TNFα, but instead expressed IL-22. They are also developmentally distinct from NK cells, as fate-mapping experiments using RORγt-reporter mice showed that many of these cells had a history of RORγt expression whilst NK cells never did [50, 76, 77].

ILC Development, Diversification and Plasticity

The lineage-specific development, function and maintenance of ILC depend on a restricted set of transcription factors. Strikingly, these are the same set of transcription factors, i.e., T-bet, GATA-3 and RORγt, that are expressed by distinct $CD4^{+}$ T helper (Th) cells, to coordinate the developmental and functional diversification of Th1, Th2 and Th17 subsets (Fig. 3.1). These transcription factors regulate a similar gene expression program in ILC as they do in T cells, resulting in unique developmental programs, which endow the individual ILC subsets with their specific effector functions. This striking parallel has led to the notion that ILC represent innate counterparts of Th cells, and are important for early immune defence, whilst setting the stage for coordination of the cytokine milieu between innate and adaptive immunity.

ILC are derived from the common lymphoid progenitor (CLP), which is derived from a pluripotent haematopoietic stem cell that has successively shed erythroid and myeloid potential. All ILC also rely on the common γ-chain (γc) used by various cytokine receptors and show a developmental requirement for the transcriptional regulator Id2 (inhibitor of DNA binding 2). Accordingly, genetic ablation of Id2 cripples the development of all known ILC lineages [100]. ILC are also absent in γc-deficient mice [76]. Expression of Id2 in the CLP is thought to inhibit its developmental potential of CLP into T and B cells while favouring ILC generation [35].

Earlier work had identified CLPs that expressed the integrin α4β7 in the foetal liver that were able to differentiate into NK cells and all subsets of $ROR\gamma t^{+}$ ILC [68]. The severe reduction of $\alpha4\beta7^{+}$ CLPs in *Id2*-deficient mice indicates that Id2 lies upstream of Rorγt in the developmental pathway [11]. Hoyler et al. had also previously identified a precursor of ILC2 cells, termed ILC2P. Clues to the identity of the 'elusive' common ILC precursor was recently further revealed. The use of the Id2 reporter mouse by Klose et al. led to

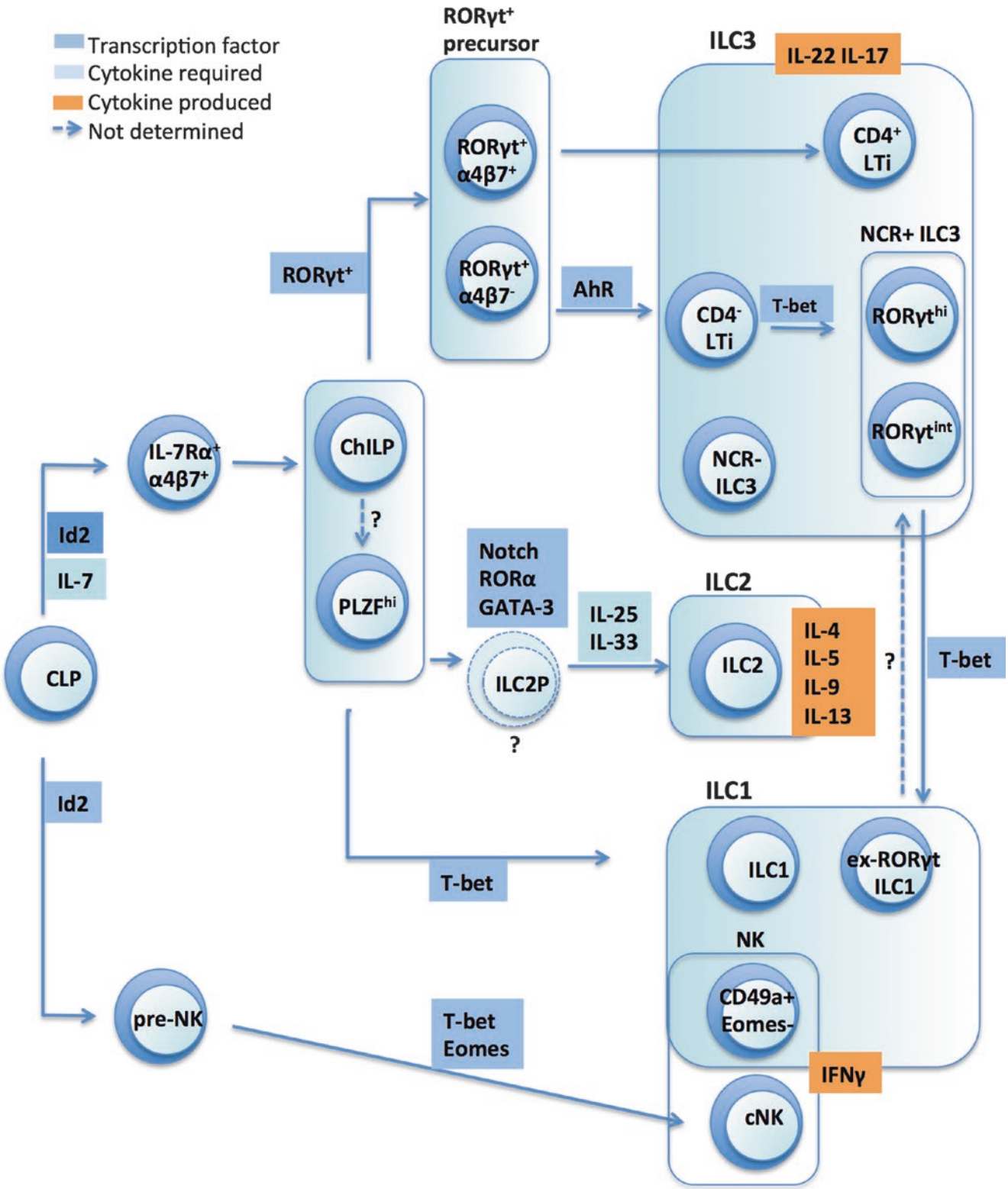

Fig. 3.1 ILC development is orchestrated by transcription factors. Inhibitor of DNA binding 2 (*Id2*), Retinoid-related orphan receptor (*ROR*), eomesdersmin (*Eomes*), Aryl hydrocarbon receptor (*AhR*), conventional NK cell (*cNK*), common helper-like innate lymphoid cell precursor (*ChiLP*), ILC2 precursor (*ILC2P*)

identification of a rare Id2$^+$α4β7$^+$Lin$^-$IL-7Rα$^+$ population in the bone marrow, which they termed ChILP (for common helper ILC precursor) [44]. Whilst the ChILP expresses the integrin α4β7, it does not express Flt3 or CD122, the recently described NK progenitor marker. It also does not express CD25 and may hence be distinct from the CD25$^+$IL-33R$^+$ ILC2P described by Hoyler et al. [35]. In adoptive transfer studies, the ChILP gave rise to ILC2 cells and ILC3 cells (including both LTi and NKp46$^+$ ILC3 subsets), as well as an unusual non-NK ILC1 subset (which was CD49a$^+$NKp46$^+$T-bet$^+$IL-7Rα$^+$ but did not express Eomes). Notably, the ChILP did not give rise to conventional NK cell subsets.

Bendelac and colleagues created PLZF (promyelocytic zinc finger) fate-mapping reporter mice, chiefly to pursue their interest in NKT development [12]. As predicted, they found that NKT cells expressed GFP and were also 'fate-mapped', but, unexpectedly, also discovered a large fraction of GFP$^-$ ILC that were fate-mapped, i.e., had expressed PLZF at some stage during their development. They showed that there was a PLZF^{hi} population within the α4β7$^+$Lin$^-$IL-7Rα$^+$ progenitor cells that gave rise to ILC2 cells, mucosal CD4$^-$ ILC3 cells, and the same peculiar NKp46$^+$CD49a$^+$ ILC1 liver subset described by Diefenbach and colleagues [44]. Therefore, both the ChILP and the PLZF^{hi} progenitors were α4β7$^+$Lin$^-$IL-7Rα$^+$ and generated ILC2, ILC3, and the peculiar hepatic NK subset (now thought to correspond to tissue-resident NK cells), but did not give rise to conventional NK cells, suggesting they may be overlapping populations. Indeed, a large fraction of ChILP express PLZF. On the other hand, PLZF^{hi} precursors also express Id2 and could be differentiated from PLZF$^-$α4β7$^+$Lin$^-$IL-7Rα$^+$ cells in vitro. Since the ChILP generates a broader range of ILC3 cells (including CD4$^+$ LTi cells), it may be possible that the PLZF^{hi} ILC precursor is a subset of the ChILP population but this remains to be determined.

Downstream of this committed ILC progenitor, the induction of GATA3, RORα, T-bet and RORγt transcription factors, together with contributions from the Notch signalling pathway and additional transcription factors such as TCF-1 and TCF-7, governs the polarization of individual ILC subsets (described below and recently reviewed by Tanriver and Diefenbach [87]).

NK cells and ILC1 Unlike ILC, NK cells do not require RORγt or IL-7 for their development but are dependent upon IL-15. There are several developmentally distinct NK subsets, including bone marrow and thymic-derived NK cells, conventional and tissue-resident subsets, that are developmentally driven by the transcription factors Tbx21, and Eomes, and as recently determined, E4bp4 (Nfil3), which promotes transcription of Eomes and Id2 GATA-3 [36]. A pre-pro NK cell (Lin$^-$CD122$^+$NK1.1$^-$CD49b$^-$Id2$^-$GFP$^+$) was also recently identified in the Id2-GFP reporter mouse [9]. Eomes is up-regulated during the transition of CD49b$^-$ immature NK cells to CD49b$^+$ mature NK cells during which they acquire effector function [26]. In contrast, T-bet expression is already found in immature NK cells and T-bet regulates the egress of mature NK cells from the bone marrow [91]. Recently, two groups identified a subset of liver CD49a$^+$ NK cells that developed independently of Eomes and E4bp4 (Nfil3), and that were thus distinct from 'conventional' Eomes$^+$ NK cells [15, 80]. This peculiar liver NK population was also generated from committed ILC precursors identified by Bendelac's and Diefenbach's groups and further studies might reveal if there are additional NK cell-restricted ILC precursors [12, 44].

From their investigations of ILC3 development using RORγt fate-mapping, Klose et al. identified CD127$^+$ ILC1s, primarily found in the intestine, that are distinguished from *bona fide* NK cells [45]. Hence, whilst all IL-15Rα$^+$ NK cells are RORγt fate map negative (RORγt^{fm-}) and did not have a history of RORγt expression, two additional CD127$^+$NK1.1$^+$NKp46$^+$ ILC1 subsets were identified as either RORγt^{fm+} or RORγt^{fm-}. In humans, Fuchs et al. further identified a subset of intraepithelial ILC1s (CD3$^-$CD56$^+$NKp44$^+$CD103$^+$) that expressed T-bet but did not express RORγt. Their development required expression of E4bp4 and T-bet. This subset produced IFN-γ after stimulation with IL-15 and IL-12, displayed some cytotoxic activity, but was still present in IL-15Rα-deficient mice. Bernink et al. identified another ILC1 subset (CD127$^+$Kit$^-$NKp44$^-$) that resided in the lamina propria of the intestine and expressed T-bet and low levels of RORγt mRNA [6]. Interestingly, cells with this phenotype could also be derived from the RORγt$^+$ ILC3 upon IL-12 stimulation. These "ex-RORγt+" cells indicate that there exists some developmental plasticity between ILC3 and ILC1, which has not been described for ILC2.

ILC2 are developmentally dependent upon on the transcription factors Id2 [35, 60], GATA-3 [35] and RORα [28, 29, 97]. In contrast to ILC3s, ILC2s are not well represented in the newborn gut but are generated in the bone marrow. An ILC2 precursor (ILC2P) was identified in earlier studies in the bone marrow, which is defined as Lin$^-$Sca-1^{hi}Id2^{hi}GATA-3^{hi} ('LSIG cell'). This precursor expressed IL-33R and IL-17RB but lacked KLRG1 expression. IL-33 stimulation could convert these cells into strong producers of IL-5 and IL-13. GATA-3 is required for the differentiation of ILC2Ps, as well as the peripheral maintenance of mature ILC2s [35]. Furthermore, two recent studies revealed the additional functions of GATA-3 in dose-dependent control of ILC2 development and intracellular phosphorylation pathways [23, 43]. ILC2 also express high levels of RORα, which is required for their development. Staggerer mice (*Rora*$^{sg/sg}$), which carry a functional null mutation of RORα, have an intrinsic defect in the development of ILC2s and are also impaired in their

ability to clear worm infection [97]. In addition to GATA-3 and RORα, T-cell factor 1 (TCF-1), acting downstream of Notch signalling, is also required for ILC2 development.

ILC3 depend on the transcription factor RORγt. ILC3 include LTi cells that are crucial for lymphoid organ formation [45, 95] and in the foetal liver, the Lin$^-$α4β7$^+$IL-7Rα$^+$ progenitor differentiates into LTi precursors upon Id2 and Notch signaling [11]. The additional RORγt$^+$ ILC3 population resides in the intestinal lamina propria and can be further subdivided into CCR6$^+$CD4$^-$ cells and a CCR6$^{-/lo}$ population that expand in the intestines during the first month of life, requiring AhR expression. The CCR6$^{-/lo}$ ILC3s are further subdivided into NKp46$^-$ and NKp46$^+$ subsets, i.e., NCR$^-$ ILC3 and NCR$^+$ ILC3. In addition to RORγt, expression of T-bet is also important for the generation of some ILC3. Hence, T-bet is highly expressed by NCR$^+$ ILC3 in the mouse lamina propria [45, 79], and together with TCF-1 is also involved in the differentiation of some NCR$^-$ ILC3 to NCR$^+$ ILC3 [45, 70, 79]. It has been suggested that an increasing T-bet gradient controls the sequential upregulation of NKp46 and NK1.1 on NCR$^-$ cells with the concomitant downregulation of RORγt [45, 95]. Rankin et al. recently showed that T-bet was indeed an essential transcription factor essential for the NCR$^+$ ILC3 population, as NCR$^+$ ILC3 were absent in T-bet-deficient mice. The progenitor of LTi, NCR$^-$ ILC3 and NCR$^+$ ILC3 had been a matter of controversy, but in the same study, Rankin et al. showed that NKp46$^+$ ILC3 was derived only from the CD4$^-$ LTi population. Therefore, NCR$^+$ ILC3 and CD4$^-$ LTi were distinct from CD4$^+$ LTi and may be generated from *bona fide* Rorγt$^+$α4β7$^-$ subset which also gives rise to a significant population of NKp46$^+$ cells.

The lineage relationships between ILC populations and the developmental plasticity between ILC subsets that might be driven in response to environmental stimuli, is an exciting area of research. Recent information on the developmental pathways of mouse ILC has further led researchers to suggest that NK cells and CD127$^+$ ILC could be considered innate forms of CD8 and CD4 T cells, respectively [31]. It is fascinating that the same conserved set of cell fate-determining transcription factors were 'selected' to drive the development and diversification of innate lymphoid cells, as well as T cells, their more contemporary counterparts.

ILC in the Healthy Skin

Three groups recently determined the composition of ILC subsets in human adult skin, which was found to harbour all the ILC subsets described to date [18, 89, 94]. Dyring-Andersen et al. analysed cells isolated from dermal explants that were cultured in IL-2 for 11 days [18]. Since the lineage cocktail they used for exclusion of non-ILC did not include the NK cell markers, NK cells were also included in their analyses. The expression of CD56 and RORγt was used to delineate the ILC3, ILC2 and NK cell subsets. Hence, RORγt$^+$CD56$^+$ cells were identified as the NCR$^+$ ILC3 population. This NCR$^+$ ILC3 were also shown to express NKp44, NKp46 and CD117. The RORγt$^+$CD56$^-$ cells that were CD117$^+$ were termed NCR$^-$ ILC3, whereas RORγt$^-$CD56$^-$ cells were identified as NK cells after the exclusion of ILC2 (which lacked NCR expression) within this population. In healthy skin, ILC2 and NCR$^-$ ILC3 were the most prevalent subsets and total ILC was estimated to constitute ~9 % of the CD45$^+$ cells. In contrast, Teunissen et al. and Villanova et al. examined freshly prepared cells from whole skin, or cells prepared from separated dermis and epidermis, in addition to explant cultures. In both studies, skin ILC were first identified as CD45$^+$Lin$^-$CD127$^+$ populations, thereby excluding most NK cells. The CD45$^+$Lin$^-$CD127^{hi} ILC were reported to represent ~1.3 % of CD45$^+$ cells. In both of these studies, skin ILC2 were identified by CRTH2 expression, and the remaining subsets were distinguished by CD117 and NKp44 expression: CD117$^+$NKp44$^+$ cells were identified as NCR$^+$ ILC3 and CD117$^+$NKp44$^-$ cells were identified as NCR$^-$ ILC3, whereas subsets that lacked expression of both CD117 and NKp44, but expressed CD161, were identified as non-NK ILC1. The ILC2, ILC1 and NCR$^-$ ILC3 populations were present in similar proportions in freshly isolated dermal cells. However, NCR$^+$ ILC3 were notably rare in these samples. Instead, NCR$^+$ ILC3 were present in significant proportions in dermal explant cultures, consistent with the representation of ILC3 in explants observed by Dyring-Andersen et al. Teunissen et al. further showed that isolated NCR$^-$ ILC3 could convert into NCR$^+$ ILC3 when stimulated with IL-1β and IL-23 in vitro. This plasticity between ILC3 and ILC1 was similar to that reported for NCR$^-$ ILC3 isolated from human tonsils and foetal intestine, which could differentiate into NCR$^+$ ILC3 after IL-1 and IL-23 stimulation [6]. Villanova et al. also showed that CD3$^-$IL17$^+$ cells could be found in both the dermis and epidermis of healthy skin, whereas CD3$^-$IL-22$^+$ cells could only be identified in the epidermis, but not the dermis [94]. Compared to the blood which contained <1 % of ILC, the skin was enriched for ILC. The blood also comprised ILC that had skin-homing potential, as ILC in healthy control blood expressed CLA at high frequencies (~33 %) compared to T cells (11–18 %). CLA expression was particularly high on the NCR$^-$ ILC3 subset. Thus, ILC could potentially migrate into and populate the skin, although the cues that guide this process are still unknown.

ILC2, ILC3 and NK cells have also been positively identified in the mouse dermis [40, 64, 72, 90]. ILC2 and ILC3 accumulation have been described in a few studies in the context of atopic dermatitis and psoriasis, respectively. As

the skin ILC populations are only recently beginning to be mapped, some key functions of ILC in other tissues will be discussed in the following sections to provide insight into their potential roles within the skin.

Functional Specialisation of ILC and Their Potential Roles in the Skin

Group 1 Innate Lymphoid Cells

In addition to IFN-γ, NK cells produce cytotoxic molecules including granzyme A, perforin and death receptors, which are crucial to their key function of eliminating infected cells or tumours. Their function within the skin is less clear, and whether NK cells are involved in the pathogenesis of inflammatory diseases, such as psoriasis, remains controversial. NK cells have been detected in psoriatic skin [8, 63, 90], and are thought to exacerbate skin inflammation. Some leukocytes isolated from biopsies from psoriatic skin showed a $CD56^{bright}$ $CD16^{-}$ phenotype, which classifies them as either *bona fide* NK cells and/or ILC1 [63]. Nevertheless, a cell population that expressed the NK inhibitory receptors CD158b, CD94 and NKG2A, therefore more likely representing NK cells, were also found in psoriatic lesions [8]. In contrast, a recent study observed a substantially lower percentage of $CD57^{+}CD56^{+}CD16^{+}$ NK cells in lesional psoriatic skin compared to control skin. NK cells were also decreased in the peripheral blood of psoriatic patients [49]. Some evidence in support of a role for NK cells in psoriasis pathogenesis came from a (SCID) mouse xenograft model, whereby NK cells from psoriatic, but not normal donors that were injected into autologous non-lesional human skin grafts could induce histopathology that resembled psoriasis. In summary, the role of NK cells in the skin during homeostasis and inflammation remains unclear.

The other ILC1 subsets are distinct from NK cells in development and function. These cells have been described in mucosal tissue, and the non-NK ILC1 subset has also been identified in human skin. Non-NK ILC1s express T-bet and produced IFN-γ upon IL-12, IL-15 or IL-18 stimulation [6, 22, 45]. These ILC1 populations include the $NKp44^{+}CD103^{+}$ cells first described within human tonsils. Their murine counterparts reside in the intraepithelial layer of the tonsil and small intestine. Based on their tissue localisation and their phenotype, which resembles tissue-resident memory $CD8^{+}$ T cells (T_{RM}; $\alpha E\beta 7^{hi}CD49a^{hi}$) that are observed after viral infection, the ILC1 cells identified in human skin might be viewed as the innate counterparts of T_{RM} which are poised for prompt effector functions. It is possible that epidermis-associated ILC1 might provide early responses to stress or infection. Notably, some of these ILC1 bear hallmarks of TGF-beta imprinting (i.e., they express CD103, CD9 and NEDD), suggesting that skin ILC1 might be modulated by this cytokine, similarly to Langerhans cells.

The second subset of ILC1 was identified by Bernink et al. in human tonsils, and in the intestinal lamina propria, but was not localized to the epithelium [6]. These cells also produced IFN-γ in response to IL-12 (instead of IL-18 and IL-15) but were not cytotoxic. The third description of ILC1 was by Klose et al., who identified a population of $CCR6^{-}ROR\gamma t^{+}T\text{-}bet^{+}$ ILC1 that differentiated from the $CCR6^{-}ROR\gamma t^{+}$ ILC. In the gut at least, these ILC1 may impact protection and pathology. For instance, CCR6-$ROR\gamma t^{+}$ ILC that produced IFN-γ mediated protective immunity against *Salmonella typhimurium* infection by promoting mucus secretion. These early IFN-γ responses might sensitize antigen-presenting cells and shape the local microenvironment to influence subsequent effector T cell responses. Conversely, ILC1 can also contribute to intestinal pathology. Both the intraepithelial $CD56^{+}NKp44^{+}CD103^{+}$ subset and the $CD56^{-}c\text{-}Kit^{-}NKp44^{-}$ subset in the lamina propria were increased in the inflamed ileum mucosa of patients with Crohn's disease. Murine ILC1 have also been shown to accumulate in two mouse models of gut pathology, i.e., DSS-induced gut inflammation in mice reconstituted with human PBMC, and in an innate immune-mediated colitis model, in which $RAG^{-/-}$ mice were administered agonistic anti-CD40. Whether they have similar dual roles in the skin remains to be elucidated.

Group 2 ILC Functions

ILC2 are found in the respiratory and gastrointestinal tissues as well as in skin. Whilst ILC2 were originally described as important in protective type 2 immunity against helminth infection, studies from mouse models of asthma and atopic dermatitis also suggest a role for ILC2 in promoting allergic inflammation.

ILC2 in Airway Allergic Inflammation

Allergic inflammation is characterized by increased expression of Th2 cytokines (IL-4, IL-5, and IL-13), resulting in tissue eosinophilia, mast cell degranulation, mucus production and IgE production. Although allergic responses are traditionally thought to be primarily driven by $CD4^{+}$ Th2 cells, there is increasing evidence that ILC2 are also important, as they too produce significant amounts of IL-5 and IL-13, both constitutively and upon activation. ILC2 have been shown to contribute to type 2 lung inflammation in murine models of allergic airway inflammation induced by different allergens, including fungal [4], house dust mite, glycolipid [41], or ovalbumin [2, 28, 29, 42] in the absence of T cell-derived cytokines. In these models, ILC2 can be activated by epithelial-derived cytokines IL-25, IL-33, and TSLP to produce

IL-5 and IL-13, as well as IL-9, effector cytokines which contribute to airway hyperreactivity, eosinophilia and mucus production. Under certain conditions, lung ILC2 are also able to produce IL-4, for instance, when stimulated with TSLP and leukotriene D4 [17]. Overall, these reports suggest that ILC2 can direct distinct pathogenic lung inflammatory responses depending on their various cytokines produced. On the other hand, ILC2 has been shown to produce the EGFR ligand amphiregulin to promote epithelial regeneration after allergen or viral challenge [17, 59]. Therefore, ILC2 can have dual roles in pathogenecity and repair in epithelial tissues, and the dysregulation or inappropriate activation of ILC2 are associated with allergic responses that are classically thought to be driven by $CD4^+$ Th2 cells.

ILC2 in the Skin

Apart from the gastrointestinal tract, lymphoid tissue, lungs and airway mucosa, ILC2 have been identified in both murine and human skin. In murine skin, ILC2 preferentially localise in the dermis, where they constitute 5-10 % of all dermal $CD45^+$ cells [72]. Murine dermal ILC2 are highly enriched in the skin compared to the blood and to other tissues (with the exception of fat tissue). Consistent with the developmental requirements of all ILC subsets, dermal ILC2 are absent in $Id2^{-/-}$, $IL7^{-/-}$ and $Rag\text{-}1^{-/-}$ mice. Interestingly, dermal ILC2 were enriched in $RAG^{-/-}$ mice, suggesting that they may share and compete for survival factors with T cells that are otherwise present in wildtype murine skin.

The ILC2 in murine dermis differ slightly from those found in other organs: they express CD103, which recognizes E cadherin, and they are c-Kit negative, suggesting that there are local factors that dictate ILC2 phenotype and function in the skin. Dermal ILC2 also express CXCR6, which enabled Roediger et al. to capture ILC2 behaviour in the dermis of $CXCR6\text{-}eGFP^{+/-}$ mice using intravital multiphoton microscopy [72]. The dermal ILC2 cells displayed a patrolling movement which was slower compared to T cells. They were also observed to make prolonged contacts (of up to 30 min) with mast cells. Functionally, during steady-state, ILC2 were found to be the primary contributors to homeostatic IL-13 production in the skin. Roediger et al. further found that IL-9 enhanced mast cell release of IL-6 and TNF-α *ex vivo*, while IL-13 had the opposite effect and suppressed mast cell release of IL-6 and TNF-α [72]. It was postulated that one of the functions of ILC2 in steady-state could be to suppress mast cell activation through constitutive IL-13 production.

ILC2 have also been identified in healthy human skin [40, 74, 89]. Human skin ILC2, like other ILC subsets, does not express the common leukocyte lineage markers. Human ILC2 express CD127 and CD161, and are further distinguished from other ILC by expression of CRTH2, ICOS, CD25, IL33R, TSLPR and IL-25R. Compared with the paucity of circulating ILC2 in the blood (<0.2 % of lymphoid cells), ILC2s are highly enriched in healthy human skin tissue (up to almost 3 % of lymphoid cells). Furthermore, a significant proportion of skin ILC2 expressed the skin-homing chemokine receptors CCR4, CCR10 and CLA, and thus may be distinct from circulating ILC2. Interestingly, Salimi showed that whilst human ILC2 do not express the NK cell-related KIRs, NKp46 and NKp44, they expressed KLRG1 [74]. The authors demonstrated that the interaction of E-cadherin, an adhesion protein important for maintaining epithelial integrity, with KLRG1 on ILC2 inhibited production of cytokines and amphiregulin in vitro. They proposed that expression of KLRG1 may provide a barrier-sensing mechanism, whereby the normal activity of ILC2 is dampened by keratinocytes or LCs that migrate through the dermis. Upon the loss or cleavage of E-cadherin during skin inflammation, such as that often observed during atopic dermatitis [92], ILC2 inhibition may be discontinued, resulting in their inappropriate activation [74].

The enrichment of ILC2 in the skin suggests they may have important roles in homeostasis and barrier defence. It has also been proposed that ILC2 may have a role in skin homeostasis, as they also produce amphiregulin, may participate in skin remodelling by stimulating keratinocyte proliferation and promoting wound healing responses. Studies that expand upon our understanding of their interactions with other skin-resident cell types would help us decipher their roles and how they contribute to immune responses and maintenance of barrier function in the skin.

ILC2 in Atopic Dermatitis

There have only been a few studies characterising ILC2 during skin inflammation, and these were performed in the context of atopic dermatitis (AD), a chronic inflammatory skin condition associated with skin barrier disruption, eosinophilic infiltration, and high serum IgE levels. This disease affects up to 30 % of children of the industrialized world and is precipitated by both genetic and environmental factors [19]. Barrier dysfunction is a key early event in the pathogenesis of AD which is further complicated by inflammation driven by type 2 cytokines. Indeed, null mutations in the filaggrin gene that is involved in barrier integrity are found in patients with severe AD [38].

The three key ILC2 activators, IL-25, IL-33 and TSLP, are produced by a variety of cell types found in the skin, including dermal fibroblasts, epithelial cells and myeloid cells. IL-25 is a member of the IL-17 family and has been associated with Th2-like inflammation and disease. IL-33 is a member of the IL-1 family that binds the ST2 receptors that are preferentially expressed on Th2 cells and a range of innate immune cells. IL-33 is involved in Th2 polarisation, and also acts as an alarmin when released during tissue necrosis, or when cleaved by caspase 1 during programmed

cell death. TSLP is also thought to be involved in type 2 responses. It has been proposed that epithelial damage can lead to the production of these "initiating cytokines" (IL-25, IL-33, TSLP), which are potent activators of ILC2. Indeed, during AD, elevated levels of IL-25, IL-33 and TSLP, in addition to both pro-inflammatory and Th2 cytokines have been reported [78, 83].

ILC2 in humans also express CRTH2, the receptor for prostaglandin D_2. $CRTH2^+$ ILC2 isolated from human skin have been shown to respond to IL-33 and TSLP stimulation by secreting IL-13 [89]. ILC2 lines generated from the skin also produced IL-13 when stimulated by PMA/ionomycin, and TSLP could synergise with IL-25 to increase their production of IL-13. Interestingly, in one study, IL-33 did not further enhance IL-13 production by skin-derived ILC2 lines [40], which was somewhat different to the responses of human ILC2 isolated from nasal polyps and blood, in which IL-33 and TSLP synergistically enhanced type 2 cytokine production [55]. In contrast, Salimi demonstrated a hierarchy of human ILC2 responses upon *ex vivo* stimulation, with IL-33 being most potent at inducing cytokine production and ILC2 migratory behaviour [74]. Activation of CRTH2 by prostaglandin D_2 has been shown to synergise with IL-25 and IL-33 to induce IL-13 release from circulating ILC2 [3]. Recently, Ogg and colleagues demonstrated that endogenous prostaglandin D_2 released by mast cells could stimulate $CRTH2^+$ ILC2 isolated from human skin to migrate and produce a variety of pro-inflammatory cytokines, in addition to IL-4, IL-5 and IL-13 [98]. Together, the results indicate that ILC2 primary cells or cell lines cultured from human skin can be regulated by TSLP, IL-25, IL-33 and prostaglandin D_2. The differences between studies could indicate flexibility in ILC2 responses to different stimulators, or the presence of different subsets that may have intrinsically different cytokine profiles within the skin.

In recent human studies, Kim et al. and Salimi et al. reported that ILC2 numbers were increased in human AD skin lesions compared to healthy control skin [40, 74]. Although ILC2 function was not determined, Salimi et al. noted that ILC2 from AD lesions had upregulated expression of some receptors (ST2, IL17BR, TSLPR, and KLRG1), and it is tempting to speculate that such ILC2 are primed to be more responsive during skin inflammation [74]. Using a suction blister model, patient skin was challenged with house dust mite extract, one of the most common aeroallergens associated with exacerbation of AD symptoms. Increased ILC2 infiltration was documented within 26 h. This rapid increase suggests there was recruitment of ILC2 from the blood, rather than local expansion at the site of allergen exposure. Therefore, ILC2s are resident in human skin but the frequencies of ILC2s are elevated in the setting of AD and could be expanded after allergen challenge.

The role of ILC2 in AD has also been investigated in mouse models. Using MC903-induced dermatitis as a TSLP-dependent model of AD-like inflammation, it was shown that dermatitis was associated with ILC2 infiltration [40, 74]. Furthermore, significantly reduced inflammation was observed when ILC2 were depleted using anti-CD90.2 and anti-CD25 antibodies in $Rag\text{-}1^{-/-}$ mice [59] and RORα-deficient chimera mice [97]. Kim et al. also showed that TSLP was an essential cytokine for inducing skin inflammation and that this response was independent of IL-33 [40] in C57BL/6 mice, which was also noted by Salimi in experiments performed in the C57BL/6 background [74]. Nevertheless, overexpression of IL-33 in the skin in transgenic mice expressing IL-33 driven by a keratin 14 promoter recapitulates many features of atopic dermatitis, such as dermal eosinophilia, increased Th2 cytokines, spontaneous itching, epidermal thickening, increased mast cells, high blood histamine and total IgE levels [37]. In this model of IL-33-driven AD-like inflammation, IL-5-producing ILC2 were significantly increased in the lesional skin, peripheral blood, and regional lymph nodes, and dermatitis with eosinophil infiltration was improved by the administration of an anti-IL-5 antibody. Some of these conflicting findings were partially resolved by Salimi et al., who showed that IL-25 and IL-33 acted redundantly in the skin to mediate ILC2 activation [74]. Roediger et al. showed that dermal ILC2 could also be expanded by IL-2 and that this resulted in higher levels of type 2 cytokines in the skin and was associated with spontaneous dermatitis in mice, indicating that ILC2 can drive pathology in vivo. Collectively, these findings also indicate that multiple upstream triggers can promote ILC2 activation and expansion.

Collectively, it is tempting to propose that TSLP, IL-33 and IL-25, by promoting ILC2 activation, could contribute to AD. Current evidence does not indicate whether ILC2 represent a late event in established disease or if they contribute to the initiating/primary inflammatory responses in AD. Nonetheless, the possible redundancy of cytokine-mediated activation of ILC2 may have important therapeutic implications.

Group 3 ILC Functions

In addition to lymphoid tissue inducer cells, several mucosal ILC3 subsets have also been identified in murine and human tissues. In humans, these include the $CD56^+RORγt^+$ and the $NKp44^+RORγt^+$ cells found in the tonsil, Peyer's patches and small intestine. ILC3s accumulate in the inflamed intestine of patients with Crohn's disease and produce IL-17 [24]; ILC3 that produce IL-22 have been found in healthy intestinal tissues. In murine models, ILC3 and their signature cytokine IL-22 are crucial for gut tissue protection. IL-22 produced by ILC3 prevents the systemic dissemination of commensal

microbes and is thus important for their anatomical containment [81]. IL-22 produced by NCR$^-$ ILC3 that were stimulated by IL-23 was also important for defence against infection by the bacterium *Citrobacter rodentium* in RAG2$^{-/-}$ mice that lack T and B cells [77]. Conversely, ILC3 can be proinflammatory and have been linked to autoimmune disease. In a model of innate intestinal inflammation using infection with *Helicobacter hepaticus*, IL-23 promoted IL-17 production by a subset of ILC3s that was responsible for pathology [7]. IL-17 production by ILC3 is not always detrimental, however, and has been shown to be important for protection against mucosal Candida infections [25]. Therefore, ILC3, which are often found in close proximity to epithelial tissue, can display dual and complex roles in epithelial protection and inflammation.

In addition to a possible role in maintaining skin homeostasis and regulation of commensal bacteria, an important function of ILC in the skin might be to sense and amplify danger signals. ILC3 are activated by IL-23 and IL-1β, cytokines that can be produced by activated macrophages and DC in the skin. TLR agonists can activate macrophages, DC, or epithelial cells, which could lead to indirect activation of ILC3. Furthermore, ILC3 also express receptors that can directly sense endogenous or exogenous molecules. For instance, ILC3 express the aryl hydrocarbon receptor (AhR), which senses a range of metabolites, some of which may be diet or microbiota-derived. Engagement of the NKp44 receptor itself in the absence of cytokines has been found to be sufficient to induce TNF and IL-2 cytokine production, whereas cytokine stimulation (IL-23, IL-1, and IL-7) preferentially induces IL-22 and GM-CSF expression in ILC3. Thus, depending on stimuli, ILC3 may be able to switch between modes of cytokine production, and integrate or synergise signals received by engagement of cytokine receptors and NKp44. IL-22 belongs to the family of IL-10-related cytokines which have roles in maintenance of epithelial barrier function [1, 96]. The engagement of the IL-22 receptor which is exclusively expressed on epithelial cells leads to production of antimcrobial peptides, mucus, and cytokines, including the anti-inflammatory cytokine IL-10, and might therefore promote wound healing. Thus, ILC3, which are important in maintenance of the epithelial barrier function and in innate immune defence against extracellular pathogens in the gut, may also perform similar roles in the skin.

The Increasingly Recognised Role for ILC3 in Psoriasis Pathogenesis

Psoriasis is one of the most common chronic inflammatory disorders of the skin, affecting 2–3 % of people worldwide [62]. Psoriatic lesions are often highly inflamed and scaly due to keratinocyte hyperproliferation and epidermal acanthosis, accompanied by infiltration of different immune cell subsets [62]. Although psoriasis is a mutifactorial disease that is linked to both genetic and environmental factors, T cells were traditionally thought to be causally involved in psoriasis pathogenesis. In recent years, the role of innate immune cells, in particular γδ T cells and the group 3 innate lymphoid cells in psoriasis is increasingly being appreciated.

Th1 and Th17 cells, via their production of the pro-inflammatory cytokines IFN-γ, TNF-α, or IL-17, are thought to drive chronic inflammation in psoriasis. Increased numbers of circulating Th1, Th17 and Th22 cells have been measured in patients with psoriasis compared to controls [5, 39], and increased numbers of IL-22 and IL-17-producing CD8 T cells have been isolated from psoriatic skin compared to control skin [71], suggesting they too have a role in pathogenesis. Since its discovery in 2000, IL-23, a heterodimeric cytokine that shares a common p40 subunit with IL-12, has also been recognised as a key player in psoriasis. IL-23 is mainly produced by activated myeloid cells, as well as epithelial cells, endothelial cells and keratinocytes. Its expression is enhanced in psoriatic skin lesions compared to healthy skin [47, 67]. In the "IL-23/Th17" model of psoriasis [16], IL-23 produced by dermal dendritic cells induces T cell activation and production of IL-17, IL-22 and IFN-γ. These cytokines induce epidermal hyperplasia and act on keratinocytes, which in turn, sustain and amplify the inflammatory process by producing more IL-23. Activated keratinocytes also produce pro-inflammatory mediators, including members of the S100 family of defensins, antimicrobial peptides and chemokines, in particular, the chemokine CCL20, which is able to recruit more CCR6$^+$ Th17 cells [30]. Consistent with the important roles of TNF-α, IL-17 and IL-23/12 in maintaining psoriatic pathology, therapies using antibodies directed against TNF (etarnercept, [27]), against the common p40 subunit of IL-12 and IL-23 (ustekinumab, [48, 65]), and recently, against the IL-17 receptor (brodalumab, [66]), have been clinically successful in treating severe psoriasis [88].

ILC3 respond to IL-23 (and IL-1β) stimulation by producing IL-17A and IL-22. The potential role of ILC in psoriasis was first demonstrated in murine models, in which topical application of imiquimod, a TLR7/9 agonist, induces IL-23-dependent psoriasiform lesions. Whilst Th17 cells played a role in inflammation and pathology, psoariasiform skin inflammation was still observed in treated Rag-2$^{-/-}$ mice, despite the absence of lymphocytes and NKT cells [32, 64]. In these mice, RORγt$^+$ γδ T cells and RORγt$^+$ ILC were shown to be the innate source of IL-17 and IL-22, consistent with an earlier report that T cells were not required for IL-22-dependent formation of skin lesions [93]. Pantelyushin et al. further demonstrated that backcrossing the Rag-2$^{-/-}$ with IL2Rγc$^{-/-}$ mice, which lack ILC, eliminated the response to imiquimod [64]. Repeated intradermal injections of IL-23 also induce psoriasisform inflammation

that was preserved in Rag-2$^{-/-}$ mice. As mice do not naturally develop psoriasis, it was unclear if these findings would be relevant in humans.

Three recent papers provided evidence for a pathogenic role of ILC3 in human psoriasis. These papers showed that ILC3, but not the ILC1 and ILC2 populations, were increased in the lesional and non-lesional skin of patients with psoriasis compared to healthy controls. This skewing toward the NCR$^+$ ILC3 subset was not observed in patients with contact nickel allergy. NCR$^+$ ILC3 were also increased in peripheral blood of psoriatic patients, a significant proportion of which expressed CLA and therefore had skin-homing potential. From these results, it was unclear if a local cytokine milieu was established in the skin to alter the local ILC balance, or if the increased NCR$^+$ ILC3 in the skin arose from local conversion of NCR$^-$ ILC3 (they converted into NCR$^+$ ILC3 upon *ex vivo* stimulation), or if indeed, ILC3 were generated elsewhere to migrate into the skin.

Both skin and blood NCR$^+$ ILC3 produced IL-22 when stimulated with IL-1β and IL-23, whereas NK and ILC2 did not. Villanova et al. suggested that ILC in the blood were major contributors to IL-17 and IL-22 production, accounting for ~20 % of IL-17-producing cells and ~40 % of IL-22-producing cells respectively, but they did not trace this to individual ILC subsets [94]. Teunissen et al. did not directly demonstrate ILC3-derived IL-17A in human psoriasis skin [89]. Nonetheless, these results indicate that human ILC3 may serve as a significant source of pathogenic cytokines in psoriatic skin. The ILC1 population, which is primarily induced by IL-12, was not altered in psoriasis, raising the possibility that the effects of IL-23 may dominate in this disease. Based upon the capacity of IL-23 to drive differentiation and secretion of IL-17A/IL-22 from ILC3s, it would be interesting to determine if IL-12/23 inhibitors, or novel inhibitors that only target IL-23, would also target ILC3s in psoriasis. Importantly, both Teunissen et al. and Vilanova et al. demonstrated a correlation between disease severity (measured by the psoriasis area severity index (PASI)) score with ILC3 numbers. Villanova et al. further showed a 75 % decrease in ILC3s numbers that corresponded to disease improvement after anti-TNF-α therapy [89, 94]. Together, these findings indicate that the accumulation and/or activation of NCR$^+$ ILC3 contribute to psoriasis, and understanding these relationships might guide the use of therapeutics to restore ILC balance in the skin. For instance, topical therapy with vitamin D3 analogues that are currently used to treat milder forms of psoriasis may increase TSLP and decrease IL-23, restoring balance of ILC subsets toward ILC2.

In conclusion, all members of the ILC family are present in healthy human skin and may have several important functions in tissue homeostasis. To date, studies of inflammatory skin disorders have revealed the outcome of ILC dysregulation: ILC2 are accumulated in human and experimental AD; ILC3 are accumulated in psoriasis plaques; and they have the ability to drive disease independent of other lymphocytes.

Learning Objectives Classification of NK cells and other ILC subsets
- Development of ILC
- Transcription factor profile of ILC
- Functions of ILC in normal skin
- Role of ILC in skin inflammation

Review Questions

1. How does the developmental pathway of NK cells and ILC differ from B cells and T cells?
   A. Their developmental pathway is identical to B-cells and T-cells
   B. They lack a rearranged antigen receptor
   C. Their cell surface markers are different
   D. Their transcription factors are unique

Correct Answer: (B) NK cells and ILC lack rearranged antigen receptors, since they are innate immune cells

2. What are the key subsets of ILC?
   A. Group 1 ILC (IFN-gamma producing)
   B. Group 2 ILC (IL-4, IL-5, IL-9, IL-13 producing)
   C. Group 3 ILC (IL-17 and IL-22 producing)
   D. All of the above
   E. None of the above

Correct Answer: (D) All of the above are critical polarized subsets of ILC

3. How are ILC functionally similar to TCR αβ T cells?
   A. Can proliferate and secrete cytokines
   B. Exist as polarized subsets
   C. Interact with conventional T-cells
   D. Express unique transcription factors
   E. All of the above

Correct Answer: **(E)** All of the above. ILC share many features with conventional TCR αβ T cells

4. What is the evidence that ILC participate in the pathogenesis of atopic dermatitis?
   A. Activated ILC2 are increased in atopic dermatitis lesions
   B. ILC1 over-produce IFN gamma from the peripheral blood of atopic dermatitis patients
   C. Deficient ILC2 activation is found in atopic dermatitis
   D. ILC3 are critical in early atopic dermatitis skin lesions

Correct Answer: (A) Activated ILC2 are increased in atopic dermatitis skin lesions compared to uninvolved skin

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Gamma-Delta T Cells in the Skin

4

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Abstract

The skin forms the body's primary interface with the environment and, as such, is equipped with a network of immune cells to provide the first line of defence against infection and injury. While the role of conventional alpha-beta (αβ) T cell receptor (TCR) expressing lymphocytes in skin homeostasis and pathology has been studied in great detail, emerging evidence also points to an important role of gamma-delta (γδ) TCR expressing T cells. The aim of this chapter is to review the biology of γδ T cells in the pathophysiology of the skin.

Keywords

T cells • Gamma-delta T cell receptor • Skin • NKG2D • Cell receptors • Cell markers • Skin disease • Cytokines

Introduction

Gamma delta (γδ) T cells are a population of unconventional T lymphocytes that are enriched at mucosal surfaces and possess both innate and adaptive properties. Unlike conventional αβ T cells, γδ T cells express a TCR comprising paired γ and δ chains of limited diversity. γδ T cells recognise ligands in an MHC unrestricted manner, independent of the CD4 and CD8 coreceptors.

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γδ T cells have unique ontogenic, phenotypic and functional features that make them ideal sentinel cells for stress surveillance in the periphery. They are able to mount rapid effector responses towards biochemically heterogeneous antigens of self and microbial origin without the need for clonal expansion. The majority of γδ T cells exist in a pre-programmed state of semi-activation, allowing them to respond with rapid kinetics. The ability to rapidly detect unprocessed antigens allows γδ T cells to serve as a first line of defence, before the delayed response of αβ T cells can occur. Nevertheless, γδ T cells also show adaptive, memory-like properties and are capable of limited clonal proliferation. γδ T cells thus display pleiotropic potential to develop into effector cells, antigen presenting cells and, at a later phase of the immune response, facilitators of tissue repair and healing [33, 42, 54, 104, 144, 154].

γδ T Cell Development

Although tissue-specific γδ TCR repertoire oligoclonality is often seen in human and mice, in cattle and sheep, in which γδ T cells make up 20 % of peripheral blood T cells and 40 % of T cells, the γδ TCR repertoire is heterogeneous. Most γδ T cells are generated from the thymus, although extrathymic

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pathways of γδ T cell production exist [5, 31]. The ontogenesis of γδ T cells in the thymus precedes that of the αβ T population, and commences on embryonic day 14 in mice. αβ and γδ T cells share a common precursor, and TCR γ, δ and β rearrangement is initiated at the CD4/CD8 double negative (DN) 2 stage. At the DN3 stage, successfully rearranged TCR β on the cell surface pair with the surrogate α chain (pTα) to form the pre-TCR signalling complex, which promotes survival, proliferation and differentiation. Upon expression of this surrogate receptor, the cells commit to the αβ lineage, progress through the 'β-selection' checkpoint and enter the $CD4^+CD8^+$ DP stage, where they undergo selection and are subsequently exported as mature CD4 or CD8 single positive (SP) T cells. For cells committing toward the γδ lineage, the surface expression of TCR γ and TCR δ suffice without the requirement for a surrogate TCR complex. Fate-mapping analysis showed that γδ T cells do not undergo a DP stage in ontogeny, suggesting that they emerge directly from the DN precursor stage, consistent with the DN phenotype of γδ T cells in the periphery [31].

The molecular changes driving fate commitment of γδ T cells are still being elucidated. Two models have been proposed to explain the mechanisms underpinning lineage choice made by DN cells: the stochastic model and the signal strength model [55, 76]. There is evidence that lineage commitment may be initiated in thymic precursors independent of TCR-mediated signals. Differential expression of the IL-7 receptor among DN2 thymocytes that have yet to express TCRs is associated with a propensity to adopt an αβ or γδ cell fate, with cells expressing higher levels of IL-7Rα biased towards the γδ lineage [72]. Others found that differential Notch receptor-ligand activation mediates the divergent differentiation between γδ and αβ fates in human thymic cells [141, 142]. More recently, CD73 was reported to be an early marker of cells that have newly committed towards a γδ cell fate in the thymus [25]. SOX13 is a high mobility group (HMG) transcription factor differentially expressed between αβ and γδ thymocytes. High expression of SOX13 in DN2 cells correlates with a γδ lineage bias. In SOX13-overexpressing transgenic mice, the αβ lineage is ablated, while SOX13 deficiency reduces γδ T cell number in a gene dose-dependent manner. SOX13 antagonises TCF-1 (T Cell Factor-1), another HMG transcription factor that is required for αβ T lineage development [92]. The precise role of SOX13 in γδ lineage commitment remains unclear, as recent analyses of mice with a spontaneous *sox13* mutation have revealed that it may not be required to commit to the γδ lineage but may be important for acquisition of effector function in some γδ T cell subsets [45].

γδ T cells expressing invariant TCR and with distinct tissue tropism are generated in a specific temporal order and are exported to the periphery in waves [1]. This sequential development of γδ T cell subsets is partly due to ordered Vγ-region gene expression but recent evidence suggests that other factors such as foetal versus adult thymic precursors, or age-specific thymic stromal components, may also have a role [50, 54, 61]. In mice, the first wave of γδ T cells consists of $Vγ5^+Vδ1^+$ (Tonegawa's nomenclature used here*) cells destined to seed the epidermis as dendritic epidermal T cells (DETC). This is followed by a second wave of cells rearranging Vγ6Vδ1 that are exported to the mucosal tissue of the uterus, tongue and the lungs [64]. Both the first and second waves of γδ T cells are generated perinatally and consist exclusively of monoclonal canonical TCR sequences. Upon homing to the destination tissue, these cells expand and continuously self-renew by slow cycling *in situ* throughout the lifetime of the host [132].

At around birth, rearrangement of Vγ7 and Vγ1 occurs, resulting in γδ T cells that seed the gut epithelium. This is followed by the postnatal production of $Vγ4^+$T cells that become circulatory γδ T cells in peripheral blood and secondary lymphoid organs. These TCRs have gene segments with highly diverse junctional sequences. Unlike the first two waves of γδ T cells, which are slow-cycling and radioresistant, the circulatory γδ T cells in the spleen and lymph nodes are radio-sensitive, and are amenable to replenishment by adult bone marrow derived precursors [132].

In humans, the first population of γδ T cells emerging from the foetal thymus express Vδ1 paired with multiple different Vγ chains and home to epithelial tissues in the skin and intestine [42, 58]. $Vδ1^+$ cells comprise a large proportion of human intraepithelial γδ T cells but a minor fraction of peripheral blood, where $Vδ2^+$ cells predominate.

In contrast to their scarcity in the adult, γδ T cells make up the majority of peripheral T cells in the neonate and may have an important immuno-protective role in early life, conferring resistance towards intestinal protozoa such as *Eimeria vermiformis* and *Cryptosporidium parvum* [112, 152]. Neonatal γδ T cells also show stronger and more pleiotropic functions compared to their αβ counterparts, suggesting that these cells are competent for immune protection at a time while the αβ T compartment is still immature [41].

γδ TCR Repertoire Generation and Modes of Antigen Recognition

The γδ TCR shares conserved structural features with the αβ TCR. The TCR γ chain, like the TCR α chain, is generated by VJ recombination while the TCR δ chain and TCR β chain through VDJ recombination.

Although a common pool of V genes is available to be used as TCR α and TCR δ gene segments, in actuality the usage does not overlap, limiting the TCR δ pool to 8 genes in mice and 10 in humans. In addition, heterogeneity of TCR γδ is further constrained by restrictions in Vγ and Vδ pairing.

However, the Complementarity Determining Region (CDR) 3 region of the TCR δ gene has the highest potential diversities among all the known TCR/Immunoglobulin chains, owing to the large number of D genes available, the availability of the D genes to be read in all three open reading frames, and nucleotide insertions and deletions at the VJ junctions of TCR γ chain [21]. Nonetheless, the actual γδ TCR repertoire has more limited diversity compared to the αβ T repertoire.

γδ and αβ TCRs have distinctive structural features that impact on their modes of ligand recognition. It is known that the length of CDR3 profoundly affects its shape and hence its ligand recognition capacity. The CDR3 region of both TCR α and TCR β chains are short and constrained in length, being evolutionarily selected for engagement of the peptide:MHC complex. The CDR3 of TCR γ and TCR δ are of uneven lengths, and the longer TCR δ chain has a heterogeneous length distribution, consistent with the capacity of these receptors to recognise a diverse range of ligands [2, 117]. This makes them structurally similar to immunoglobulins, which are also capable of a broad range of ligand recognition.

The spectrum of ligands recognised by γδ TCR includes self- and microbial-derived danger-associated patterns, stress-associated molecules, and evolutionarily conserved, invariant antigens or metabolite intermediates [21]. The recognition of such molecules, often associated with early events of barrier rupture, inflammation or tissue damage, without need for processing or restricted presentation by antigen presenting cells (APCs), is highly relevant to the role of γδ T cells as sentinel cells in stress surveillance at peripheral sites.

Unlike the engagement between αβ TCR and peptide:MHC complex, various γδ TCRs bind peptide:MHC complexes in unconventional manners [21]. For example, the recognition of insulin peptide by the Vγ4$^+$Vδ10$^+$ SP9D11 mouse γδ T cell clone is independent of APCs [160]. On the other hand, the interaction between mouse LBK5 γδ TCR and moth cytochrome peptide:IE(k) complex is non-peptide-specific and of low affinity [52]. Operating by yet another different mode of recognition, the binding of human Vδ1$^+$ TCR to lipid:CD1d complex is constrained by the lipid ligand [139].

The KN6 and G8 γδ TCR clones recognize T10 and T22, MHC-like antigens that lack peptide-binding groove, in association with beta-2-microglobulin (β2m) with high affinity. Stress-inducible MHC-like MICA and MICB are recognized by other γδ TCR independent of β2m. Indeed, γδ T cells have been shown to respond to a variety of other stress-associated molecules. These include intracellular proteins such as apolipoprotein A-1 (apoA-I), mitochondrial ATP synthase, low-molecular-weight phosphorylated molecules from host and microbial metabolic pathways, endothelial protein C receptor (EPCR) and histidyl tRNA synthase [21].

γδ TCR Ligation and Programming of Effector Function

Positive and negative selection by MHC-restricted presentation of agonist ligands in the thymus shape the repertoire of αβ T cells. Unlike αβ TCR, the canonical γδ TCR repertoire in the foetal thymus is generated mainly by genetic recombination of variable gene segments and not by thymic selection. Although earlier studies in γδ TCR transgenic systems found evidences of positive and negative selection in a manner similar to αβ T cells [30, 107], the relevance of agonist:MHC -mediated selection was contradicted by the absence of a γδ T cell deficit in mice which lack the β2m molecule required for assembly and surface expression of MHC I [124]. In addition, wildtype mice and *Tcrd*$^{-/-}$ mice contain the same V region recombination and junctional sequences for the first two waves of invariant γδ TCR cells, indicating a lack of repertoire shaping by selection [63].

In the mouse, 0.1–1 % of adult γδ T cells recognise the non-classical MHC-like antigens T10 and T22 in a β2m-dependent manner. Using a T22 tetramer staining reagent to enumerate antigen-specific γδ T cells in non-transgenic strains that do or do not express these antigens, Jensen et al. [68] found no evidence of a requirement for ligand-mediated selection in the generation and development of these cells. Unexpectedly however, ligand recognition in the thymus profoundly affects cell fate. Ligand-naive cells in the periphery produce IL-17 upon TCR-mediated activation, while ligand-experienced cells produce IFN-γ. This cytokine production pattern is wired in the thymus and persists in the peripheral γδ T cells even after infection and inflammation [68, 114].

The importance of ligand-mediated programming of effector function is evident in the development of DETC. The Vγ5 TCR of these cells likely recognizes an as yet to be discovered antigen on keratinocytes [53, 59]. DETC must contact antigen in the thymus for full maturation and export. Skint1 is a butyrophilin-like molecule expressed by keratinocytes and thymic epithelial cells (TECs). In the presence of skint1, foetal thymic γδ T precursors expressing the Vγ5 TCR are pre-programmed into IFN-γ producers prior to being exported to the epidermis to become DETC [138]. Skint1 ligation induces upregulation of the transcription factor Egr3, which corroborates with NFAT and NFkB activation to suppress SOX13 and RORγt and upregulate T-bet, diverging the cells from a constitutive IL-17 producing phenotype into an IFN-γ producing pathway. The Egr3-Id3 pathway is necessary and sufficient to confer Notch-independent differentiation into IFN-γ producing effectors [80]. The transcriptional reprogramming is concomitant with a change in surface phenotype, as the suppression of SOX13 relieves the inhibition on upregulation

of CD27 and NK1.1. In the absence of skint1, the cells remain SOX13$^+$, RORγt$^+$, CD27$^-$, SCART2$^+$, and develop into IL-17 producing cells. The Taconic farm substrain of FVB mice harbours a genetic mutation in the *skint1* gene, and consequentially a deficit of IFN-γ producing Vγ5$^+$ cells in the DETC compartment. Instead their DETC comprises a heterogeneous TCR γδ repertoire and these cells produce IL-17. Nonetheless at present it is unclear if skint1 directly contacts the TCR of DETC.

A third population of γδ T precursors is pre-programmed in the foetal thymus into semi-activated cells capable of rapid production of both IL-4 and IFN-γ [40, 48]. These thy1(dull) cells express a very restricted TCR comprising Vγ1 and Vδ6.3/6.4, and are thought to undergo agonist selection, upregulating the expression of the transcription factor PLZF (promyelocytic zinc finger), which is also essential for the differentiation and acquisition of effector function in iNKT cells [77]. Agonist-ligand binding is often considered to induce a strong TCR signal, however, paradoxically, the attenuation of TCR signal strength such as in Id3$^{-/-}$ or Itk$^{-/-}$ mice promoted the development of NKT-like γδ cells [3, 111, 146]. In addition to TCR-mediated signalling, the development of NKT-like γδ cells is also dependent on costimulatory SAP-SLAM interaction [3].

Of note, not all γδ T cells are pre-wired in the thymus into IFN-γ versus IL-17 producing effectors. For instance, oral infection with *Listeria monocytogenes* induces the expansion of a population of CD27$^-$CD44$^+$ γδ T cells in the mesenteric lymph nodes (LN) capable of making both IFN-γ and IL-17 [128]. Heterogeneity among subsets of γδ T cells will be discussed below.

TCR-Dependent and Independent Activation of γδ T Cells

According to the classical two-signal model of T cell activation, T cells require signal 1 via the TCR and signal 2 via costimulator molecules for activation and avoidance of anergy and/or apoptosis. In addition, optimal activation of CD8$^+$ T cells may require signal 3 in the form of inflammatory cytokines such as IL-12 or type I interferon [27]. This paradigm of activation of αβ T cells does not capture the more variable means of γδ T cell activation, which may occur in a costimulation-independent or even TCR-independent manner.

Apart from the γδ TCR, γδ T cells also express invariant innate receptors including pattern recognition receptors such as Toll like receptors (TLRs), and NK cell activation and inhibitory receptors. The relative importance of the γδ TCR to these innate receptors varies in a context- or population-specific manner. In subsets of γδ T cells, triggering through the γδ TCR alone, but not NKG2D alone, induces cytotoxicity via Vav1-dependent phospholipase C-γ1 signalling. Nonetheless, NKG2D is capable of augmenting the activation of γδ T cells as a costimulator through the same pathway. On the other hand, in human peripheral Vγ9$^+$Vδ2$^+$ cells, constitutively expressed NKG2D rather than the γδ TCR appears to be the predominant activating molecule eliciting anti-tumour functions [96].

In adult human and mice, the majority of γδ T in the peripheral tissues are present in a semi-activated state, and can be fully activated with signals 2 and/or 3 without further TCR crosslinking. For instance IL-17 producing γδ T cells (Tγδ17) in secondary lymphoid organs constitutively express IL-23R and the transcription factor RORγt. These cells can be activated via cytokines (a combination of IL-1 and IL-23), TLR-2 or dectin-1 to produce IL-17 in the absence of TCR ligation [89, 133].

Although provision of signal 1 in the absence of costimulation does not seem to induce anergy in γδ T cells, costimulatory molecules do augment the activation of γδ T cells, leading to increased proliferation and cytokine production. While sharing some of the costimulatory molecules known to play a role in αβ T cells, including CD28:B7 and CD27:CD70, there are also costimulatory ligand-receptors uniquely used by γδ T cells and their relevant APCs (Table 4.1).

Heterogeneity Among γδ T Cell Subsets

Recent studies strongly suggest that γδ T cells can be divided into an innate or natural population and an adaptive or inducible population [22, 73, 144]. γδ T cell populations that are generated in the foetal thymus typically home to peripheral sites such as the skin and the gut epithelium, where they self-renew *in situ*. These cells are pre-programmed in the thymus into distinct effector lineages, express markers indicative of partial activation, and at least a subset of them can be activated in a TCR-independent manner. Collectively, these features place them within the innate spectrum of γδ T cells. On the other end of the spectrum are γδ T cells showing more adaptive-like features, typically circulating in the blood, spleen and lymph nodes, where about half the population has a CD44$^-$CD62L$^+$ naive phenotype. There is no evidence of effector pre-programming in these cells. Cytokine production potential is plastic with more delayed kinetics relative to that of their more innate counterparts. These cells have different derivative requirements and can be generated from adult thymic and bone marrow precursors. Recall response can be elicited from these 'adaptive' γδ T cells in a number of different species [99, 127, 128].

Table 4.1 The role of costimulators in γδ T cell activation

JAML-CAR
DETC express JAML (junction adhesion molecule like protein) at low level in steady state and upregulate its expression upon activation. The binding partner of JAML, the coxsackie and adenovirus receptor (CAR), is expressed by keratinocytes. CAR-mediated JAML clustering recruits PI3K, in a similar mechanism as described for CD28-mediated costimulation [156]. PI3K mediates costimulatory function of JAML through the same binding motif found on B7 family members CD28 and ICOS. This leads to proliferation and production of IFN-γ, IL-2 and TNF-α by DETC. The inhibition of this interaction results in reduced γδ T activation and delayed wound healing in the skin. Based on the expression pattern of JAML and CAR in the intestine, their interaction could also play a role in activation of γδ IEL in this organ.
CD100-plexin B2
DETC express CD100/semaphorin 4D (Sema4D), a member of the semaphorin family of molecules with a known role in axonal guidance. CD100 on DETC engages plexin B2 on keratinocytes, and *in vitro* ligation of CD100 leads to activation of cofilin and ERK activation and the rounding of DETC. DETC from CD100 deficient mice show delayed rounding, which correlates with a wound healing defect in these mice [157]. In the intestine, all IEL express CD100 and epithelial cells express plexin B2. CD100-deficient mice show exacerbated inflammation and tissue damage in the DSS-colitis model. CD100 deficiency renders γδ IEL unable to produce KGF-1, and KGF-1 administration ameliorates intestinal tissue damage in these mice [93]. Collectively these data indicate the importance of CD100 in mediating tissue homeostasis and repair by DETC.
Aryl hydrocarbon receptor (AhR)
The aryl hydrocarbon receptor (AhR) is a member of the basic helix-loop-helix (HLH)/Per-Arnt-Sim (PAS) superfamily known for their roles in sensing of environmental factors. It is a ligand-activatable transcription factor, and regulates several xenobiotic-metabolizing enzymes. Cell-intrinsic expression of AhR is important for the maintenance of DETC in the skin. AhR-deficient DETC have altered morphology and do not extend dendrites to contact neighbouring cells. Although AhR deficiency does not impair thymic generation or export of these cells to the skin, their numbers in the epidermis decline over time [71]. Recent studies found a similar role for AhR in the maintenance of γδ IEL in the gut [83].
CD27
CD27 is a member of the TNF superfamily and its binding partner CD70 is constitutively expressed on thymic epithelial cells. In the thymus, a $CD25^+CD27^+$ DN population contains the precursors that subsequently diverge into the $CD27^-$ $ROR\gamma^+$ IL-17 producing lineage and the $CD27^+$ IFN-γ producing lineage. This divergence is stable and largely not interconvertible even in the face of infection in the periphery [114]. $CD27^{-/-}$ mice contain less IFN-γ producing γδ T cells, indicating that CD27 is not only a marker but also a regulator of effector cell fate determination of γδ T cells.
WC1 (Workshop Cluster 1)
WC1 are germline-encoded coreceptors of the scavenger receptor cysteine-rich (SRCR) family of transmembrane glycoprotein, consisting of 11 extracellular SRCR domains. WC1 is related to the CD163 family, with closest homology to CD163c-alpha/CD163L1 or SCART) [56]. WC1 is uniquely expressed by γδ T cells in cattle, camelids and pigs. Gene orthologues have been described in mice and human but no functional gene product has been found. The cytoplasmic domain of WC1 contains ITAMs. Specific tyrosine moieties are constitutively phosphorylated and associate with Src family tyrosine kinases. Phosphorylation of the second tyrosine residue is required for the role of WC1 in potentiating γδ TCR-mediated proliferation *in vitro*, an observation supporting the role of WC1 as a costimulatory molecules [150]. Bovine γδ T cells are serologically defined based on available antibodies into $WC1.1^+$, $WC1.2^+$ and $WC1^-$ subsets. WC1 are not merely markers but define functional subsets of bovine γδ T cells with distinct age-dependent turnover kinetics [151]; [118]; [119]. $WC1.1^+$ proliferate more and produce more IFN-γ in allogeneic MLR *in vitro* compared to $WC1.2^+$ γδ T cells [118]; [119]. Intranasal BCG vaccination induces higher expansion or recruitment of the $WC1.1^+$ subset into the lung, as well as eliciting higher production of IFN-γ from these cells [110]; [49]. Virus infection induces differential cytokine and chemokine production in the subsets, with $WC1.1^+$ and $WC1^-$ cells producing MIP-1α and GM-CSF while $WC1.2^+$ cells producing IL-10 and TGF-β [90].
SCART
SCART, another member of the SCRC superfamily, shares significant homology with WC1 but is not its gene orthologue. The expression of SCART1 and SCART2 are largely identical. In the thymus, SCART2 upregulation is first detected at the DN2 stage and does not require a TCR signal. However, its expression is downregulated by strong TCR ligation. In the adult periphery, SCART2 positivity defines a population of thymic-derived IL-17-producing γδ T cells enriched for Vγ4 reactivity residing in the skin dermis and skin-draining LN [74].
NKG2D
NKG2D interacts with various stress-inducible molecules including MICA, MICB, ULBP, H60, Rae1 and MULT. In γδ T cells, the co-ligation of NKG2D and γδ TCR potentiates γδ T cell activation, supporting a costimulotary role for this molecule. However, NKG2D is also capable of TCR-independent activation of γδ T cells in other conditions.
BTLA (B- and T- Lymphocyte Attenuator)
BTLA, an inhibitory costimulatory molecule of the B7 superfamily, has recently been described to play a role in the homeostasis and regulation of γδ T cells [6]. RORγt represses BTLA while IL-7 signalling increases its surface expression. BTLA expression limits the responsiveness of γδ T cells to IL-7 mediated proliferation thereby regulating its homeostatic number at steady state. BTLA also inhibits the production of IL-17 and TNF from $CD27^-$ γδ T cells *in vitro*. In a γδ T cell-dependent model of dermatitis, BTLA deficiency exacerbates, and BTLA ligation ameliorates disease.
Other costimulators
γδ T cells and αβ T cells share many common costimulatory molecules. However, while costimulators have a qualitative role determining the outcome of αβ T cell response between activation and anergy, the effect of costimulation in γδ T cells appears to be quantitative. For example, CD28 ligation does enhance *in vitro* response of γδ T cells to TCR crosslinking and to allogeneic DC. However, the expression of CD28 was found to be variable and its importance in the activation of γδ T cells unresolved [54]. In the presence of γδ TCR crosslinking, TNF SF family member CD40L is known to costimulate the proliferation and cytolytic function of thymic γδ T cells [113]. Persistent upregulation of CD30, another TNF SF member, can be induced upon *in vitro* activation of γδ T cells. CD30 engagement leads to enhanced cytokine and chemokine production [7].

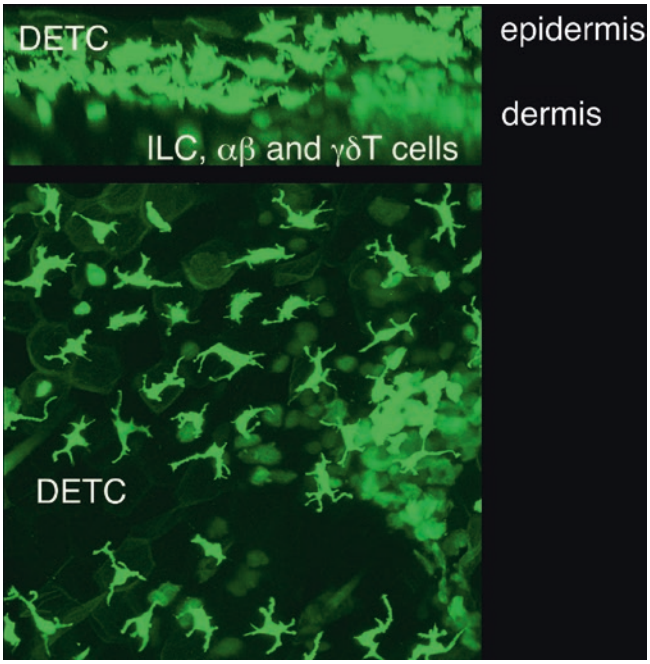

Fig. 4.1 Dendritic epidermal T cells (*DETC*) and dermal lymphoid cells in the skin of Cxcr6$^{gfp/+}$ mice. The non-migratory DETC reside in the epidermis, where their cell processes are anchored at squamous keratinocyte tight junctions, giving the cells their characteristic dendritic morphology. Dermal lymphoid cells, which include αβ and γδ T cells as well as innate lymphoid cells, are more amoeboid in morphology and actively migrate throughout the dermis. The image is a z-projection through a volume of 82 μm of ear skin, including epidermis and dermis

An Example of Microanatomical Specialisation: Dermal γδ T Cells Versus DETC

Several recent studies have highlighted the microanatomical and functional segregation of two populations of γδ T cells in the mouse skin. Using intravital multiphoton imaging of fluorescently tagged mice (CXCR6-GFP knock-in), two groups have identified a novel population of γδ T cells residing in the dermis [47, 132]. In the epidermis, DETC are sessile and constantly extend and retract their dendritic projections, while dermal γδ T cells show a different morphology and migrate in the dermis at a speed of 2–4 μm/min (Fig. 4.1). A subset of these cells also makes contact with MHC-II$^+$ cells, presumably antigen presenting cells, in the dermis. These behaviours suggest an active role for these cells in immune-surveillance of the skin. While DETC are uniformly Vγ5$^+$, dermal γδ T cells are relatively heterogeneous, expressing Vγ5, Vγ4 or other TCRs [47, 132]. In addition, the two populations also show differential expression of γδ TCR levels, CD25, CD43, CCR6 and NK1.1. Both populations are generated perinatally and homeostatically maintained by self-renewal *in situ* in an IL-7 dependent manner. In addition, DETC but not dermal γδ T cells are IL-15 dependent [29, 132]. While DETC are thymically pre-programmed by skint1 ligation to produce IFN-γ [114, 138], dermal γδ T cells constitutively express RORγt and IL-23R, and rapidly produce IL-17 following exposure to IL-1β plus IL-23 [16, 47]. In an intradermal model of BCG infection, dermal γδ T cells are the earliest population showing rapid IL-17 production, which mediates recruitment of neutrophils to the site of antigen deposit. In *Tcrd*$^{-/-}$ mice, the recruitment of neutrophils to the infected ear skin is diminished, and the downstream adaptive response of antigen-specific conventional CD4$^+$ T cells reduced [132]. Nonetheless, these two anatomically segregated of γδ T cells may have functional overlap in certain scenarios, as a recently described subset of DETC is capable of rapidly producing IL-17 in response to wounding and UV irradiation [87, 88].

Interaction Between γδ T Cells and Other Cell Types

Interaction with Other T Cell Subsets

Mature TCR γδ$^+$ thymocytes are reduced in numbers in *Tcrβ*-deficient and *pTα*-deficient, but not *Tcrα*-deficient mice, indicating a role for the CD4$^+$CD8$^+$ DP αβ T precursors on the development of γδ T cells in the thymus. DP cells are capable of conditioning γδ T precursors via lymphotoxin (LT)-mediated RelA and RelB pathways, regulating the expression of transcription factors RORγt and RORα4 to direct their differentiation into Tγδ17 cells [109, 129]. In *Tcrd*$^{-/-}$ mice hyperproliferation of the αβ T cell compartment is often seen, suggesting that γδ T cells may compete with αβ T cells for homeostatic cytokines, such as IL-7. During infection, early cytokine production from γδ T cells may directly or indirectly polarise delayed adaptive responses downstream and impact on the efficacy of pathogen clearance. For instance, infection with *Listeria monocytogenes* or *Nippostrongylus brasiliensis* elicits rapid production of IFN-γ and IL-4, respectively, from γδ T cells of the peritoneal cavity [35].

In models of autoimmune-mediated inflammation, γδ T cells may exacerbate disease. Tγδ17 that are activated by IL-23 during MOG/CFA-induced experimental autoimmune encephalomyelitis (EAE) inhibit the *de novo* induction of regulatory T cells (Treg) from conventional T cells and confer resistance of antigen-specific effector T cells towards Treg-mediated suppression [108]. Early cytokine production from resident mucosal γδ T cells aggravates colitis induced by adoptive transfer of effector CD4$^+$ T cells in lymphopenic hosts, which can be inhibited by adoptive transfer of Tregs [159]. Treg and Tγδ17 are also likely to be engaged in dynamic interactions during the steady state, as the reduction in number and function of CD4$^+$CD25$^+$ Treg in Pdk1-deficient mice allows the expansion of pathogenic Tγδ17 cells that drive chronic intestinal inflammation [103].

Interaction with B Cells

Human Vγ9$^+$Vδ2$^+$ cells are the majority population of γδ T cells in the peripheral blood. A subset of these cells has a CCR7$^+$CD62L$^+$ central memory (TCM) phenotype, expresses the B cell follicle-homing chemokine receptor CXCR5 and the costimulatory molecules CD40L and ICOS, and is capable of producing IL-2, IL-4 and IL-10. *In vitro*, these cells are capable of helper T cell function to drive antibody production and isotype class switching [13]. In patients with genetic lymphopenia, γδ cells remain functional and are capable of providing B cell help in place of CD4$^+$ T cells, leading to the formation of small germinal centres. These patients often show hyperimmunoglobulinemia of IgE, indicative that γδ T cells are capable of driving Ig class switching *in vivo* [34, 95].

γδ T Cells and Antigen Presenting Cells (APC)

The interactions between γδ T cells and antigen presenting cells (APC) in the thymus and the periphery are crucial for their generation, maintenance and function. In the thymus, Vγ5$^+$ precursors and medullary thymic epithelial cells (mTEC) are mutually dependent. LTi (lymphoid tissue inducer) cells as well as Vγ5$^+$ γδ precursor cells provide RANK ligand signal driving the maturation and expression of Aire in mTEC [116]. While Aire expression *per se* has no role in the generation of Vγ5$^+$ DETC, mature mTEC express skint1, which is crucial for the programming of effector functions in these cells.

Intravital imaging revealed that γδ T cells at peripheral sites constantly make contacts with resident antigen presenting cells (APCs) [24]. Examples include contact between DETC and Langerhans cells (LC) in the epidermis, and between dermal γδ T cells and MHC-II$^+$ cells in the dermis. While the depletion of LCs does not affect maintenance of DETCs [134], DETCs are producers of XCL1/lymphotactin, a chemokine known to attract DC [8]. While the activation of naïve αβ T cells strictly requires interaction with mature professional APC ie dendritic cells, immature DC have been shown to be able to activate γδ T cells. Vγ9$^+$ T cells engage in reciprocal interactions with DCs, and are capable of potentiating DC maturation via production of cytokines, as well as relieving inhibitory signals that block DC maturation [62, 104, 149].

γδ T cells may themselves play a role as antigen presenting cells (APCs) thereby bridging the early innate immunity to the delayed adaptive T cell-mediated response [12, 97]. Human Vγ9$^+$Vδ2$^+$ cells have been shown to present antigen to CD4$^+$ T cells, and cross-present antigen to CD8$^+$ T cells. Activated Vγ9$^+$ cells display several salient features that enable them to function as professional APC, including uptake of soluble antigens, phagocytosis, upregulation of CCR7 enabling homing to secondary lymphoid organs, upregulation of MHC-II and the costimulatory molecules CD80 and CD86.

Interaction with Other Myeloid Cells

γδ T cells engage with myeloid populations in a bidirectional manner. Efficient presentation of phosphoantigen to Vγ9$^+$ cells *in vitro* requires the presence of monocytes [94]. Previous studies showed defective maturation of monocytes derived from mice with a deficit in γδ cells [130]. *In vivo*, different subsets of γδ T cells have protective roles in controlling macrophage-mediated immunopathological tissue damage in Listeria infection. Vγ1$^+$ T cells mediate apoptosis of activated macrophages in a Fas:FasL dependent manner [28]. The interaction between Vγ4$^+$ cells and activated macrophages promotes cytokine and chemokine production by macrophages on one hand, while inducing IL-10 production from Vγ4$^+$ cells themselves on the other [137]. Yet both the Vγ1$^+$ and Vγ4$^+$ subsets are protective against Listeria-induced liver injury in independent adoptive transfers.

Interaction with Innate Lymphoid Cells (ILCs)

Administration of chitin elicits type 2 innate inflammation in the lungs involving ILC2-mediated recruitment of eosinophils and alternatively activated macrophages. When innate lymphoid cells are depleted, there is an enhancement of Tγδ17 activation and prolonged neutrophil influx, suggesting inter-regulation between ILC2 and Tγδ17 in determining the identity of infiltrating myeloid cells in response to this allergen [143].

Trafficking of γδ T Cells

Proper homing and localisation is of paramount importance to the generation and function of γδ T cells. The medullary localisation of Vγ5$^+$ precursors is sensitive to pertussis toxin indicating a requirement for G protein coupled protein in this process. Mice deficient for an enzyme required in synthesising E- and P- selection ligands have reduced number of DETC in the skin despite normal generation of DETC in the thymus indicating the importance of these molecules for their homing and/or maintenance in the skin [69]. In the foetal thymus, TCR-mediated upregulation of CCR10 is required for homing of DETC to the skin [70]. CCR9 deficient mice have a deficiency in gut intraepithelial αβ and γδ T lymphocytes [20], indicating that the same chemokine-receptor is used for targeting both populations of T cells to this site.

Despite tissue-specific localisation of γδ T cells of restricted TCR diversity, the individual TCR *per se* is not required to dictate their tissue homing specificity. In Vγ4Vδ5 γδ TCR transgenic mice, DETC expressing the transgenic TCR instead of the canonical Vγ5 are generated and home to the epidermis, albeit at a reduced number [10]. However, the expression of a specific TCR in a specific tissue compartment is clearly of physiological relevance. As evidence, Vγ5Vδ1 deficient mice have increased susceptibility to chemically induced carcinogenesis in the skin [131]. The Taconic strain of FVB mouse which harbours heterogeneous rather than the canonical $V\gamma5^{+}V\delta1^{+}$ DETC is more susceptible to spontaneous and induced dermatitis [82].

The activation of γδ T cells initiates modulation of chemokine receptors and acquisition of differential homing potential. Human $V\gamma9^{+}$ cells constitutively express CCR5, which is downregulated as the cells become activated [43]. Activation is associated with the upregulation of CCR6 and CCR7 [11, 14]. In the lungs, intratracheal instillation of LPS or BCG induces an early TLR dependent infiltration of γδ T cells. Infiltration of γδ T cells is dependent on CCL2:CCR2, as recruitment was diminished in CCL2 (MCP-1) knockout mice and after CCL2 neutralisation, and restored with administration of CCL2 [105]. Hepatic Tγδ17 cells have a protective role in chronic liver injury that is independent of IL-17 production but dependent on CCR6-mediated homing to the liver. In two models of chronic liver injury, $CCR6^{-/-}$ mice develop more severe fibrosis, and adoptive transfer of wildtype γδ T cells ameliorates hepatic inflammation and injury [51]. Notably CCR6-mediated homing of Tγδ17 may be associated with very different outcome depending on the target organ. In a cutaneous inflammation model, CCR6 deficiency precludes the development of inflammation and hyperplasia. CCL20:CCR6 interaction is required for the recruitment of γδ T cells to the epidermis, where these cells exacerbate inflammation via production of IL-17 and IL-22 [86].

Memory Responses in γδ T Cells

There are documented instances of rapid mobilisation and expansion of γδ T cells akin to adaptive memory, however clonal expansion is of a relatively lower magnitude compared to a classical memory response of αβ T cells. In addition, although context specificity is evident, demonstration of cognate antigen specificity is lacking, and probably impractical, given the relatively unknown antigen recognition apparatus and less stringent activation requirement of γδ T cells. Nonetheless, protective effect of γδ T cells in secondary infection is a promising venue to be explored for vaccine development.

Oral infection with *Listeria monocytogenes* elicits the expansion of a population of γδ T cells in mesenteric LN that produce both IL-17 and IFN-γ. These cells are capable of rapid response to secondary oral infection with Listeria but not the unrelated pathogen Salmonella, and to oral but not intravenous infection with *L. monocytogenes*, demonstrating context specificity in response [128]. Infection of *Mycobacterium bovis* in cattle induces rapid activation and IFN-γ production from $CD8^{+}CD45RO^{+}$ γδ T cells that respond more strongly towards *M. bovis* infected rather than BCG-infected macrophages [57]. Primary infection of macaques with BCG induces polyclonal expansion of $V\gamma9^{+}V\delta2^{+}$ cells in the blood. These cells rapidly expand upon secondary BCG infection, and expansion is associated with clearance of bactereamia in the blood. More importantly, in BCG-immune macaques these cells also expand in response to secondary infection with *M. tuberculosis* and showed protection against this usually fatal infection [127]. Within the skin, we have observed an increase in the number of dermal γδ T cells with age, suggesting that these cells may also be capable of exhibiting memory [135].

γδ T Cells in Skin Disease

γδ T Cells in Skin Infections

γδ T cells by themselves are generally insufficient for sterilising immunity. However, $Tcrb^{-/-}$ x $Tcrd^{-/-}$ mice are more susceptible to viral infection indicating that these cells do have a protective role [19, 150]. Different subsets of γδ T cells may be mobilised in temporally and geographically distinct manner for early containment of damage, or later in the response for tissue repair and wound healing [18, 19]. In various models of cutaneous infection and vaccination, γδ T cells are recruited early and produce cytokines, often before the development of an αβ T cell response [100, 125]. The protozoan Leishmania is transmitted to its mammalian host through sandfly bite, and γδ T cell numbers are elevated in the blood of patients presenting cutaneous, mucosal or visceral leishmaniasis [122]. In mice, subcutaneous *Leishmania major* infection leads to a systemic expansion of $V\delta4^{+}$ γδ T cells [121]. Antibody-mediated depletion of γδ T cells culminates in larger cutaneous lesions with higher number of parasites [120]. In $Tcr\alpha^{-/-}$ mice, γδ T cells are instrumental in limiting pathogen spread and pathogen-associated damage, as shown in mouse models of footpad and corneal infection with *Herpes simplex* virus-1 (HSV-1). Mice with double deficiency of αβ and γδ T cells have larger epithelial lesions, higher viral load and dissemination, and succumb to lethal viral encephalitis [125]. In cutaneous *Staphylococcus aureus* infection, mice with γδ T deficiency have significantly larger skin lesions, higher bacterial load, and impaired recruitment of neutrophils to the infected site [23]. The ability of DETC

to rapidly produce IL-17 in an IL-1, IL-23 and TLR2 dependent manner is critical for protection. In systemic *S. aureus* infection, a population of $CD44^{+}CD27^{-}$ $V\gamma4^{+}$ γδ T cells that persists at the infection site and in draining lymph nodes after clearance of primary infection is found to rapidly expand and produce high amount of IL-17 upon secondary infection, displaying features of memory [98].

In addition, γδ T cells may modulate and shape the downstream adaptive response in a multitude of different ways in different models of infection. Depletion of γδ T cells in calves reduces *M. bovis*-specific IgG2 and IFN-γ, and increases IL-4 production [91]. The absence of γδ T cells selectively reduces IgA but not IgG or IgM production elicited by oral immunisation with tetanus toxoid plus cholera toxin [36]. In Chagas disease patients, the presence of IL-10 producing $CD4^{-}CD8^{-}$ γδ T cells in the blood correlates positively with improved clinical parameters of cardiac function [148].

γδ T Cells in Tissue Homeostasis and Wound Healing

In the gut and the skin, intraepithelial γδ T cells do not only have a role in infection and inflammation, but are also indispensable in tissue homeostasis and repair. In the mouse epidermis, DETCs are in constant contact with adjacent keratinocytes as well as Langerhans cells. At steady state, DETC is the primary population of constitutive Insulin-Like Growth Factor-1 (IGF-1) producers supporting the survival, albeit not the proliferation, of keratinocytes [65]. Deficiency of γδ T cells is associated with increased epithelial apoptosis [126].

Wound healing involves coordinated phases of inflammation, proliferation, reepithelialisation, deposition of extracellular matrix and tissue remodelling. During this process, keratinocytes rapidly migrate to and colonise the wound border, produce antimicrobial factors to prevent microbial invasion, and proliferate to re-epithelialise the wound. It is known that γδ T cell deficient mice have a wound healing defect, which can be restored with wildtype DETC [66, 155]. A recent study identified a $ROR\gamma t^{+}$ IL-17A producing subset of DETC to have a role in wound healing [87]. IL-17 blockade delays wound closure in wildtype mice, and conversely administration of exogenous IL-17 or transfer of IL-17-sufficient γδ T cells restore wound healing in $IL\text{-}17A^{-/-}$ mice. IL-17 induces downstream production of a multitude of anti-microbial factors with barrier protective functions [79, 87].

In human skin, both αβ and γδ T cells are capable of producing IGF-1. Elevation of IGF-1 is seen in acute but not chronic wounds [136]. Intraepithelial γδ T cells of the skin and the intestine, but not intraepithelial αβ T cells nor lymphoid γδ T cells, are capable of Keratinocyte Growth Factor-1 (KGF-1) production [9]. Wound healing is delayed in the presence of a dominant negative KGF receptor mutant transgene [153]. Although there is no cutaneous wound healing defect in KGF-1 deficient mice due to functional compensation by KGF-2 in the skin, exogenous KGF-1 rescues the wound healing defect of $Tcrd^{-/-}$ skin organ culture *in vitro* [66]. In normal wound healing, DETC-derived KGF-1 induces hyaluronan production from keratinocytes, which has a role in recruiting macrophages to the site [67]. Consequentially macrophage infiltration is defective in $Tcrd^{-/-}$ mice.

In mice but not humans, wound repair is accompanied by hair follicle regeneration, a process known as wound-induced hair neogenesis (WIHN). This process is dependent on the production of FGF-9 by dermal γδ T cells [39].

γδ T Cells in Psoriasis

The role of the IL23-IL17 axis in psoriasis pathogenesis is well recognised. IL-17A, IL-17 F, IL-22 and IL-21 are found to be elevated in the skin and blood of psoriatic patients, and IL-23 is selectively elevated in psoriatic lesions compared to non-lesional skin. Intradermal injection of IL-21 or IL-23 causes epithelial hyperplasia or acanthosis, one of the hallmarks of human psoriasis. IL-17A also upregulates the expression of keratin 17, a classical psoriatic disease marker, on keratinocytes [15]. IL-23R polymorphism is implicated in the pathogenesis of psoriasis [17]. While earlier studies have focused on Th17 cells, it is now known that innate cells including γδ T cells [16] and NKT cells [26] are major producers of IL-17 in psoriatic skin lesions.

In murine models of spontaneous psoriasitic dermatitis, disease progression correlates with a loss of $V\gamma5^{+}$ DETC and infiltration of $IL\text{-}17^{+}$ dermal γδ T cells [4, 38]. There is compelling evidence indicating a pathogenic role of IL-17 producing dermal γδ T cells in various models of spontaneous and induced psoriatic dermatitis in mice [15, 16, 84, 86, 101, 140]. These models include topical application of imiquimod cream, a TLR7 agonist, or intradermal injection of IL-23 . IL-17A, IL-17 F and IL-22 deficient mice are protected from psoriatic dermatitis indicating the pathogenic role of these cytokines [101, 140]. Moreover, infiltrating dermal γδ T cells and $ROR\gamma t^{+}$ ILC, rather than Th17 cells, have been shown to be the primary IL-17 producers, and are necessary and sufficient for psoriatic pathogenesis. More recently, Gray et al. found SOX13-dependent $V\gamma4^{+}$ Tγδ17 cells to be the specific pathogenic population in their model of psoriatic dermatitis [45]. These cells proliferate in skin draining lymph nodes and home to the inflamed skin. In a $CD45.1^{+}$ C57BL/6 substrain, which carries a *Sox13* mutation, the neonatal development of these $V\gamma4^{+}$ T cells is impaired, and these mice are protected from psoriasis development.

In human psoriasis patients, dermal IL-17-producing γδ T cells are found to be increased in psoriatic lesions [16]. Specifically, a population of CLA^+CCR6^+ $V\gamma9^+V\delta2^+$ cells is reduced in the peripheral blood and increased in psoriatic skin lesions suggesting disease-associated redistribution of these cells. These cells produce IL-17A, and are capable of activating keratinocytes *in vitro* in a TNF-α and IFN-γ dependent manner [78].

There have been much recent efforts geared towards targeting the IL-23/IL-17 axis in the clinic. While targeting the p40 chain of IL-23/IL-12 is potentially risky due to its pleiotropic effect, the anti-IL-17 antibodies AIN457 (secukinumab) and LY2439821 (ixekizumab), and anti-IL-17R antibody AMG 827 (brodalumab) have shown promising efficacy in phase II clinical trials involving cases of chronic psoriatic plaques [15, 60, 81, 102].

Clinical Aspects of γδ T Cell Biology

Focus on Human $V\gamma9^+V\delta2^+$ Cells as an Example

$V\gamma9^+V\delta2^+$ cells constitute the major γδ T population in the peripheral blood of healthy humans. These cells are uniquely able to recognise self- and microbial- derived phosphoantigens in a TCR-dependent, MHC-unrestricted manner. This TCR binds isopentenyl pyrophosphate, an isoprenoid intermediate of the human mevalaonate pathway, and also the microbial isoprenoid (HMBPP), an intermediate of the 2-C-methyl-D-erythritol-4 phosphate (MEP) pathway for isoprenoid biosynthesis [96]. This pathway is shared by a broad spectrum of prokaryotic and eukaryotic pathogens. The ability of these cells to bind an evolutionarily conserved group of invariant molecules via the TCR is consistent with their purported role as early rapid effectors. It has been shown that phosphoantigen recognition requires the presence of APC but is independent of MHC, MR1 or CD1. The nature of presentation of phosphoantigen remains to be resolved. The mitochondrial protein F1 ATPase (F1 adenosine triphosphatase) is a candidate phospho-antigen presenting molecule, but its involvement remains to be proven. Recent studies provided compelling evidence for an antigen-presenting role of BTN3A1, a butyrophilin-like extended B7 family member, in the binding of phospho-antigen to the Vγ9Vδ2 TCR [123, 145].

$V\gamma9^+V\delta2^+$ T cells may have promising clinical potential especially in anti-tumour therapy [44, 96, 147, 158]. These cells are able to recognise a broad range of tumour cells, and have been demonstrated to be cytolytic towards a variety of tumour cells *in vitro*. Interestingly, transformed cells have been shown to upregulate the mevalonate pathway and accumulate IPP intracellularly, pointing to possibilities of *ex vivo* expansion of these γδ T cells and targeting a conserved pathway of tumour cell metabolism. The identification of the minimal binding moiety for γδ TCR, which is a five-carbon alkenyl chain with a pyrophosphate moiety, facilitates the mining and synthesis of phosphoantigen-based agonists for *ex vivo* manipulation and expansion of these cells.

In mouse models γδ T cells have been shown to mediate anti-tumour immunity via rapid early production of cytokines [37, 85]. In a murine sarcoma model, chemotherapy induces an early rapid infiltration of Tγδ17 cells into the tumour bed that is required for the subsequent infiltration of $CD8^+$ CTL and anti-tumour chemotherapeutic efficacy [85].

Tools for Probing γδ T Cells

The inherently complex biology of γδ T cells has proven to be challenging in efforts to elucidate their behaviour and functions. In recent years, the combination of several different tools has revealed previously unappreciated aspects of their biology. The development of tetramer-staining reagents has provided new information on the role of TCR ligation in generation of γδ T cells [68, 75]. Further structural studies are likely to yield useful novel insights on the mechanism by which γδ TCR recognise a diverse range of antigens.

The vast majority of γδ T cells in mice and humans are tissue resident cells, which pose a particular challenge to flow cytometry-based studies that require tissue disintegration and single cell isolation. Enzymatic digestion has to be sufficient for single cell isolation on one hand and preserve important yet enzyme-sensitive surface molecules on the other. The availability of genetically fluorescent tagged mice and powerful intravital imaging platforms have solved some of these issues, offering the opportunity to study the cells *in situ* in real-time [20, 32, 47, 132]. The discovery that the skin contains two segregated γδ populations with distinct biology in the epidermis and the dermis is a testimony of the power of the combination of such technologies. More recently, immunological synapses between keratinocytes and DETC have been characterised using intravital dynamics-immunosignal correlative microscopy [24].

Summary

γδ T cells have unique features that make them highly relevant to immune surveillance in the host, and not a vestigial population from the evolutionary perspective. These cells recognise self-derived stress signals as well as microbial invariant molecular patterns without the need for antigen processing or presentation in an MHC restricted manner. They are localised and self-renew at peripheral sites, thus increase the probability of detecting antigens.

Some γδ T populations express invariant or oligoclonal TCR. Although not a universal feature of γδ T cells, in combination with a restricted localization, this allows the cells to respond rapidly and at an effective magnitude without

having to clonally expand, with a TCR specificity evolutionarily selected to recognise antigens likely to be encountered at peripheral sites. γδ T cells are often present in a semi-activated state, allowing them to rapidly acquire effector function without TCR ligation. The myriad interactions between γδ T cells and other cell types allow them to polarise downstream responses to facilitate an efficacious immunological outcome.

Appendix

*There are different nomenclatures for the different γδ TCR V genes, namely the Tonegawa & Heilig, Garman and Hayday systems. For example Vγ5 in the Tonegawa's system corresponds to Vγ3 in Garman's and GV1S1 in the Hayday's system. The Heilig & Tonegawa system is used in this manuscript, and in the International Immunogenetics Information System (IMGT).

Learning Objectives

- Classification of T cells in the epidermis and dermis
- Development of epidermal and dermal γδ T cells
- Functions of epidermal and dermal γδ T cells
- Role of γδ T cells in skin infection and inflammation

Review Questions

1. What T cell receptors are expressed by epidermal and dermal γδ T cells?
2. When do skin γδ T cells develop?
3. Which cytokines are produced by γδ T cells?
4. What is the role of γδ T cells in cutaneous pathology?

Answers

1. TCR gamma and TCR delta
2. at birth
3. IL17; IFN gamma; IGF1; KGF1; FGF9
4. tissue repair and wound healing, but they produce IL17 which worsens psoriasis

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Mast Cells: Sentinels of Innate Skin Immunity

5

Nicholas Mascarenhas, Zhenping Wang, and Anna Di Nardo

Abstract

Since its discovery over a century ago, the mast cell has been considered an important effector cell of the innate immune system. From mediating various allergic responses to playing a direct role in eliminating pathogens from the skin, the mast cell carries out its effects through a highly coordinated process of degranulation. Following stimulation by pathogens or other host cells, the mast cell is able to release chemical mediators from its intracellular stores, which include pro-inflammatory cytokines, peptidases, and antimicrobial peptides. These mediators serve to alter the inflammatory environment and allow for the recruitment of other immune cells; however, overactivity of the mast cell response has been shown to lead to a variety of disease processes. As the main drivers of both type 1 hypersensitivity and inflammatory disorders, like rosacea, the mast cell has been studied as a target for many disease-altering therapies. Continued research in the field of mast cell biology has the potential to further unveil the coordinated workings of the innate immune system.

Keywords

Mast cell • Degranulation • Innate immunity • SCF • IgE • c-kit • Antimicrobial peptide

History

Over a century ago, when German scientist Paul Ehrlich first identified the mast cell, the field of immunology was just beginning to emerge. Ehrlich initially described these cells as "granular cells of the connective tissue… characterized by a still undetermined chemical substance… bound to granular storages in the protoplasm [1]." He adopted the term "Mastzellen" from the Greek word meaning "breast," suggesting a nourishing function for the granules; however, he found the key identifying feature of these cells to be their high affinity for blue aniline dye, which allows for visualization of the granules and distinguishes the mast cell from other cell types [2]. As studies in the field of immunology advanced, a strong association was noted between the presence of both mast cells and histamine. Riley and West analyzed the mast cell content of various connective tissues from rodents, oxen and sheep, noting a strong positive correlation between the number of mast cells and the amount of histamine present in tissues [3]. Their preliminary work suggested histamine to be the main granular substance and mediator of mast cell effects, and further studies would confirm the mast cell as a key player in allergic hypersensitivity and anaphylactic shock [4]. Much advancement has been made since the pioneers of mast cell biology made their initial discoveries. In this chapter we will outline the role of these sentinel cells of the skin, focusing on mast cell development, common activating signaling pathways, and associated skin diseases.

Overview

As resident immune cells, mast cells are found in strategic locations, particularly in connective tissues of organs with constant environmental exposure, such as the skin, airways

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and intestine. For example, concentrations of mast cells have been found to be as high as 12,000/mm^3 in the skin and 20,000/mm^3 in the gastrointestinal tract [5, 6]. Within their resident tissue, mast cells further localize around blood vessels, nerves, and lymphatic vessels in order to regulate vascular permeability and to recruit effector cells through release of their granular contents. Specifically in the skin, mast cells are localized near blood vessels, hair follicles, and sebaceous and sweat glands [7].

The morphology of the mast cell can vary depending on the cell's physiological location. Free mast cells and those near blood vessels are more round or ovular in shape than those that are attached to dermal fibers, which can be more spindle or stellate. Mast cells isolated from rat peritoneum have been shown to have an average diameter of approximately 13 μm with a relatively large, acentric nucleus ranging from 4 to 7 μm [8, 9]. Mast cells are typically identified by their cytoplasmic granules that take up aniline dyes, like toluidine blue, upon histological staining. These granules, which number approximately 1000 per mature mast cell, have been shown to contain multiple mediators of cellular activity including inflammatory cytokines, proteases, antimicrobial peptides and vasoactive mediators [10].

Historically, granular staining characteristics have been used to categorize different types of mast cells. In rodents, mast cells are divided into two types based on granular heparin content and their binding properties of different dyes. Connective tissue mast cells (CTMC), which are found in rodent skin, intestinal submucosa and serosa, contain granules with heparin, while mucosal mast cells (MMC) have granules with no heparin but abundant levels of sulfated proteoglycans [11–13]. However, this distinction may not be sufficient, as many more granular mediators have been identified, like mast cell chymases and tryptases, suggesting the need for a more adequate classification system based on mediator production and function. Human mast cells all contain histamine and exhibit no differences upon histological staining; however, the two types of human mast cells can be differentiated under electron microscopy and based on their granular protease content. Mast cells with granules containing tryptase but not chymase (MC_T) exhibit a granular structure that resembles scrolls on cross section and are typically found in mucosal layers of the intestine and lungs. Human mast cells with granules containing both tryptase and chymase (MC_{TC}) lack this scroll-like morphology and have granules with a more lattice-like structure [14]. MC_{TC}s are mostly found in the skin and intestinal submucosa, and those found in connective tissue selectively express the mast cell specific carboxypeptidase A [15].

While histamine, heparin and various peptidases represent some of the main granular contents, numerous other cellular mediators are known to be stored in mast cell granules, such as prostaglandins, leukotrienes, and antimicrobial peptides like cathelicidin [16]. Upon activation, mast cells release their granules, eliciting additional activity in themselves and in neighboring cells, but before fully developed mast cells can evoke their effector functions, they must undergo a process of differentiation that begins in the bone marrow.

Mast Cell Growth and Differentiation

Mast cells have been shown to develop from hematopoietic pluripotent stem cells expressing CD34, primarily found in the bone marrow and spleen, although these progenitor cells can be isolated from cord blood and fetal liver as well [17–20]. Mast cells develop along the myeloid lineage and share many common intermediates with developing monocytes and granulocytes. Mast cells eventually enter into circulation from the bone marrow, but what distinguishes them from the other hematopoietic granular cells, basophils, is their final stage of differentiation in the peripheral tissue [21].

One of the key growth factors that triggers mast cell development is stem cell factor (SCF), also known as kit ligand or steel factor [22]. SCF, which is produced by various stromal cells and fibroblasts, binds to the tyrosine kinase receptor c-kit (CD117) expressed on the surface of mast cell progenitors [23]. Once bound to its receptor, SCF induces homodimerization and autophosphorylation of c-kit, leading to subsequent signaling cascades along the RAS/ERK, Src kinase, and JAK/STAT pathways [24]. CD117 is an important cell surface marker for mast cell precursors, and it remains expressed throughout the lifetime of the cell, unlike basophils, which lose expression of CD117 in their differentiated state [25].

The critical role that SCF plays in mast cell differentiation is highlighted in *KitW/W-v* mice, a mouse strain with two mutant alleles for the gene encoding c-Kit. These mice have been shown to be highly mast cell deficient; however, due to SCF's role in the differentiation of other hematopoietic cells, these mice also exhibit impaired melanogenesis, anemia, and sterility [26]. Furthermore, the *KitSl/Sl-d* mouse strain is deficient in SCF expression and also exhibits mast cell deficiency in all tissues, along with the previously stated defects due to impaired hematopoiesis [27]. More recently, *KitW-sh/W-sh* mice strains have been utilized for *in vivo* mast cell deficient studies, as these mice do not exhibit the anemia and sterility of other mast cell deficient strains. The *W-sash (Wsh)* mutation associated with this strain is an inversion mutation of the transcriptional regulatory elements upstream of the c-Kit transcription start site [28]. As a result, these mice show decreased skin pigmentation and a lack of mast cells in the skin and peritoneal cavity; however, they remain fertile and show normal numbers of erythrocytes [29]. Thus, the *KitW-sh/W-sh* strain has proven ideal for *in vivo* studies of mast cell-deficient skin.

In addition to SCF, mast cell progenitors respond to a variety of cytokines derived from type 2 helper T cells. IL-3, IL-4, IL-6, IL-9 and IL-10 have all been shown to induce human and murine mast cell proliferation *in vitro* [30–32]. For example, when murine bone marrow cells are cultured with IL-3, a uniform population of mature mast cells can emerge [33]. Furthermore, culturing murine mast cell progenitors in combination with SCF and IL-4 produces a distinct population of connective tissue-type mast cells [34]. Murine mast cell proliferation has also been shown to be dependent on type 2 helper T cell-derived IL-3 and IL-4, as these cytokines play a key role in inducing an increase in mucosal mast cell numbers in response to helminth infection [35]. While murine mast cell differentiation and proliferation can be stimulated with a wide variety of cytokines, human mast cell development *in vitro* has been shown to be mostly dependent on the addition of SCF and IL-6 to CD34+ cord blood cell cultures [36]. Some researchers also feel the addition of IL-3 during the early stages of cord cell culture is necessary for mast cell development [37]. In general, the culturing of human mast cells is a tedious process, requiring nearly four months for the cells to reach full maturation [38].

After initial stages of development, mast cells must exit the bone marrow, be retrieved from circulation, and localize to their resident peripheral tissues. This migration into tissue is highly dependent on locally produced cytokines that, when bound to mast cells, can induce up or downregulation of various cell adhesion molecules, facilitating the mast cell's movement through the microvascular endothelium and into tissues.[166] Studies conducted with mouse bone marrow-derived mast cells (BMMCs) suggest a migration behavior similar to that of neutrophils. Mast cell expression of integrin $\alpha_4\beta_7$ on the cell surface is critical for localization to the gut via binding to mucosal addressin cell adhesion molecule-1 (MAdCAM-1) or vascular cell adhesion molecule-1 (VCAM-1) expressed on the endothelial cell surface [39, 40]. It has been shown that mice deficient in various types of integrins lack the presence of mature mast cells in corresponding tissues. For example, mice deficient in $\alpha_4\beta_7$ have reduced mast cell numbers in the gut, while mice deficient in $\alpha_m\beta_2$ show a reduced amount of mast cells in the peritoneum and skin [40, 41].

While cell adhesion molecules are necessary for translocation through the endothelium, chemokines are the molecules responsible for initiation of mast cell recruitment and endothelial translocation [42]. However, much of the current data regarding recruitment by chemokines is conflicting. During mast cell maturation, the only chemokine receptor that is consistently expressed on the mast cell surface is CCR3, which binds chemokines CCL11, CCL24, and CCL26 [43]. While this would suggest a key role for CCR3 in the recruitment of immature mast cells to tissue, studies with CCR3-deficient mice still show high numbers of mast cells in the skin after *Trichinella spiralis* infection [44]. Furthermore, *in vitro* mouse BMMCs express CCR3 mRNA, but protein levels remain undetectable. These cells also fail to respond chemotactically to CCL11 stimulation [45]. However, the fact that CCL11 in combination with SCF can promote an increase in mast cell progenitor numbers suggests that the mast cell response to chemokines may be intricately involved in both development and migration processes [46].

Other chemokine receptors of notable importance include CXCR2 and CCR2. CXCR2 is thought to be associated with recruitment of mast cell progenitors to the lung, and evidence shows that CXCR2 can directly increase endothelial expression of VCAM-1, leading to increased mast cell translocation via integrin $\alpha_4\beta_7$ [47]. Additionally, increased levels of CCR2 have been found in allergen-exposed lung tissue [48]. After similar allergen treatment in mice deficient in CCR2 and its ligand CCL2, mast cell numbers in the lung were highly reduced, suggesting a key role for CCR2 in mast cell recruitment to the lung and possible implications for asthma patients [48].

Mast Cell Signaling

Once localized to their resident tissues, mast cells can be activated to carry out their physiological role by either modulating granular contents or releasing granular contents via three types of exocytosis: granular exocytosis in which individual granules release their contents outside the cell by fusing with the plasma membrane, compound exocytosis in which granules fuse to the plasma membrane and to each other forming channels for maximum biological effect, and piecemeal exocytosis in which small vesicles bud off from granules and then fuse with the plasma membrane [49]. The process of mast cell activation is highly regulated by a combination of stimulatory and inhibitory signals downstream from a plethora of cell surface receptors. In this section, we will cover the classical mast cell activation pathways via the high affinity IgE receptor and toll like receptors, as well as pathways of inhibition. However, before covering these signaling pathways, an overview of the granular contents is warranted.

Mast Cell Mediators

The mast cell secretory granules contain a wide variety of preformed mediators that can be released within seconds to minutes after activation [49]. The most common of these preformed mediators is histamine, which constitutes approximately 10% of the weight of granules [50]. Histamine is produced by both MC_Ts and MC_{TC}s, and within the granule, it is bound to negatively charged proteoglycans and is

released from this complex after secretion by cation exchange [50, 51]. Furthermore, the presence of heparin in the granules serves to stabilize the multimeric complexes of histamine, proteoglycan and other proteases [52]. Because the half-life of histamine is relatively short, its effects are local and brief. While histamine can have broad, systemic effects, it is known to mediate the classic wheal and flare reaction in the skin, due to its ability to induce vasodilation and subsequent plasma extravasation. The combination of these two events leads to local erythema and swelling typical of urticarial reaction [53]. Additional effects of histamine include bronchial and intestinal smooth muscle constriction, increased mucus production in nasal cavities, and neutrophil and eosinophil chemotaxis [54, 55]. The effect of histamine on local tissues depends entirely on the expression of histamine receptors by the target cells. H_1–H_4 receptors all mediate the effects of histamine in a different way, leading to the above-mentioned reactions in different tissue types [56].

In addition to histamine, a wide variety of proteases are contained within preformed mast cell granules, mainly tryptases and chymases. Tryptase can serve as a marker of mast cell degranulation, as its half-life is significantly longer than that of histamine [57]. Furthermore, it is synthesized by all types of human mast cells and has been shown to induce the following physiological effects: inhibition of fibrinogenesis, stimulation of fibroblast proliferation, upregulation of IL-8 and ICAM-1 in bronchial epithelial cells, and recruitment of neutrophils in mice [58–61]. Chymase proteases, which include well-studied cathepsin G, are expressed only in MC_{TC}s found exclusively in the connective tissue and skin. Chymase proteases have been shown to induce conversion of angiotesin I to angiotensin II, degradation of adhesion proteins in the basement membrane zone, and inactivation of bradykinin [62–64]. Another molecule found exclusively in MC_{TC} mast cells is carboxypeptidase A. Functioning to convert angiotensin I to angiotensin II and to degrade toxins like snake venom, carboxypeptidase A has been found to be the most specific mast cell marker, as it has not even been identified in basophils [65, 66].

In addition to preformed mediators, mast cells have the capacity to synthesize newly formed mediators upon activation, primarily derived from cell membrane phospholipids that can be converted to arachadonic acid. This arachadonic acid can subsequently be used to form various eicosanoids, such as prostaglandins (PGs), leukotrienes (LTs), and platelet activating factor (PAF) [67]. While PGs and LTs are vasoactive and can mediate smooth muscle contraction, PAF has been shown to be a chemoattractant for neutrophils, monocytes and macrophages, as well as an inducer of bronchoconstriction and vascular permeability [56]. Additional newly synthesized mediators include various interleukins and TNF-α, which act in the recruitment of other immune cells [56].

Activating Receptors

The classical stimulatory pathway for granular mediator release involves the high-affinity IgE receptor, FcεRI. Upon primary exposure to an antigen, IgE antibodies can be synthesized and can subsequently bind to the mast cell FcεRI via its Fc region [68]. Binding of IgE to the FcεRI is high-affinity with a very slow dissociation, ensuring long-term antigen specificity and memory for the mast cell [69]. Activation of the mast cell requires cross-linking of the FcεRIs, which can be induced by antigen binding to the variable region of two IgE molecules already bound to FcεRIs [70]. A variety of molecules can induce cross-linking such as multivalent antigens, antibodies against IgE or antibodies against the FceRI itself. The FceRI has a tetrameric structure: one alpha chain with two extracellular domains for binding IgE, one beta chain with four transmembrane domains, and two gamma chains with cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs) important for downstream signal transduction, which will be discussed later [71].

The IgG receptors FcγRI in humans and FcγRIII in mice have been shown to be expressed on the surface of mast cells and activate degranulation in response to binding of IgG3 [72]. Some research suggests that immune complexes may be important for activating signaling downstream of the FcγRI, as only antigen-antibody complexes have been shown to bind the receptor and not monomeric IgG [73]. Additional activating receptors include c-kit and complement receptors. While c-kit is critical for mast cell growth and development, binding of SCF via c-kit on mature mast cells has been shown to induce degranulation as well as the production of IL-10, IL-1beta and leukotrienes [74, 75]. Mast cells have also been observed to express receptors for complement peptides C3a and C5a. Studies show that when stimulated with C3a and C5a, skin mast cells release histamine in a concentration dependent manner. Effects are rapid, with complete histamine release occurring within 15 s; however, complement stimulation appears to have little effect on the generation of new mediators like prostaglandins or leukotrienes [76]. Furthermore, when human mast cells were stimulated with C5a and C3a in combination with aggregated IgG the effects of degranulation were additive, suggesting that, even though the signaling pathways of these receptors are independent, parallel pathways may work synergistically for maximal immune response [77].

Inhibitory Receptors

Mast cell degranulation must be kept in tight control by a balance of both activating and inhibitory signals. Most inhibitory receptors have been shown to be associated with

immunoreceptor tyrosine-based inhibitory motifs (ITIMS), unlike the ITAMs found associated with activating receptors [78]. One of the most well studied inhibitory receptors on the mast cell is the IgG receptor, FcγRIIB. It has been shown that when FcγRIIB is cross-linked to FcεRI via IgG-antigen complexes, the normal degranulation response via the high-affinity IgE receptor is greatly diminished [79]. Therapies have been investigated using this method. For example, when exposed to cat-allergic donors' mast cells, IgG-cat allergen complexes led to dose dependent inhibition of histamine release, suggesting a method for de-sensitization to various allergens [80]. Furthermore, evidence exists showing FcγRIIB's role in suppressing signaling from other activating receptors like c-kit [81]. Note that FcγRIIB is distinct from the IgG receptor involved in mast cell activation.

Other receptors, like CD300a (IRp60) and CD200, are expressed on the surface of many myeloid cells and have been shown to have inhibitory effects in mast cells. When ligated to the high-affinity IgE receptor, IRp60 blocked IgE-induced mast cell degranulation [82]. The same has been shown for CD200 in CD200-deficient mice, and although this receptor is not associated with an ITIM, this suggests a potential role in setting a threshold for mast cell activation [83]. Additionally, mouse studies have shown constitutive expression of gp49B1 on the mast cell surface. This protein contains two cytoplasmic ITIMs, and antibody-induced co-ligation of gp49B1 with FcεRI was shown to inhibit release of preformed mediators as well as synthesis of lipid mediators [84]. Furthermore, gp49B1 can constitutively suppress mast cell activation through binding of its ligand, integrin αv3, suggesting its independence from the adaptive immune response [85]. While various inhibitory receptors have been identified, their true physiological significance is still being investigated.

Signal Transduction

Signal transduction associated with mast cell activation involves a complex network of signaling cascades that ultimately function to increase cytokine production, synthesize new lipid mediators (eicosanoids), and to release secretory granules. The most well studied signal transduction pathway in mast cells involves cross-linking of the high-affinity IgE receptor and will be discussed here, as signal transduction for this receptor overlaps with that of other activating receptors in the mast cell. As stated previously, activating signaling begins with FcεRI cross-linking via a multivalent antigen [70]. Each FcεRI has two associated ITAMs, one on the beta chain and one on the gamma chain [71]. After cross-linking occurs, both ITAMs are immediately phosphorylated by Lyn, a protein tyrosine kinase that is constitutively associated with the beta chain ITAM [86]. Once phosphorylated, the ITAMs can serve as binding scaffolds for molecules with Src homology 2 (SH2) domains, such as additional Lyn molecules or Syk family kinases. Syk binds via its SH2 domain to the ITAM of FcεRI gamma chain and is subsequently phosphorylated by Lyn, which is constitutively bound to the beta chain [87, 88].

Activation of Syk is critical, as it is able to phosphorylate a number of proteins including linker for activation of T cells (LAT), SH2-domain-containing leukocyte protein of 76 kDa (SLP-76), and Vav, a guanindine nucleotide exchange factor [89]. Additionally, Fyn, another Src kinase that is activated independently of Lyn, can phosphorylate Grb2-associated binding protein 2 (Gab2) [90]. The association of LAT with SLP-76, Gab2, Grb2, and Vav is responsible for downstream signaling via the mitogen-associated protein kinase (MAPK), protein kinase C (PKC), and Ca^{2+} dependent pathways [89].

Upon cross-linking of FcεRIs, LAT is able to associate with the adaptor protein Grb2 and Sos [91]. This LAT/GRB2/Sos complex can subsequently stimulate the conversion of Ras-GDP to Ras-GTP, which can then activate the Ras/ERK signaling pathway. Ultimately, ERK can induce increased levels of phospholipase A_2 (PLA_2), which is responsible for production of arachidonic acid from cell membrane-derived phospholipids [92]. Arachidonic acid can subsequently be converted to the newly synthesized eicosanoid mediators that are released upon mast cell activation. LAT is also able to bind SLP-76, which can then associate with Vav. Vav can subsequently activate Rac proteins, which play a critical role in cytokine gene transcription and cytoskeleton rearrangement [93, 94]. Finally, the LAT/SLP-76/Vav complex can induce activation of phosphotidylinositol-specific phospholipase C (PLCγ1). Activated PLCγ1 can induce transformations in various cell membrane phospholipids to produce the second messengers diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP3) [95, 96]. DAG is able to activate protein kinase C (PKC), and PKC can in turn phosphorylate a variety of proteins including myosin light chain, thought to be involved in granule release, and c-fos and c-jun, which regulate genes involved in cell cycle progression and anti-apoptotic activity [97, 98]. IP3 on the other hand, plays a critical role in releasing intracellular Ca^{2+} stores from the endoplasmic reticulum [99]. The increase in intracellular Ca^{2+} is required for mast cell granule release. While IP3 mediates initial release of Ca^{2+} stores, TRPC channels expressed on the cell membrane are thought to be involved in extracellular Ca^{2+} influx for a sustained increase in cytosolic Ca^{2+} levels [100, 101].

In summary, mast cell activation is mediated by a wide array of stimulatory and inhibitory receptors, principally the high-affinity IgE receptor. Following the cross-linking of the IgE receptors, activation of LAT, SLP-76, and Vav can induce downstream signals that lead to granule release, synthesis of

eicosanoid mediators, and transcription of various cytokines and cell growth factors.

Mast Cell Response to Microbes

While previously discussed mast cell activation and degranulation mainly involves antibody intermediaries and activation of the adaptive immune response, mast cells also play a key role in innate immune processes and direct recognition of microbes that come into constant contact with the human epidermis. This section will assess the role of masts cell in innate immunity and processes critical to maintenance of the skin microbiome.

Mast cell activation in response to microbes typically occurs via toll-like receptor (TLR) signaling, as these receptors are able to engage in direct contact with microbial pathogens by recognizing structurally conserved molecules of the microbe. TLR signaling does not typically induce degranulation of the mast cell, but rather it stimulates the production of antimicrobial peptides and inflammatory cytokines that aid in the recruitment of other immune cells. Cultured human mast cells have been shown to express TLR-1, 2, 4, 5, and 6 on their cell walls, while TLR-3, 7, 8, and 9 are expressed on the surface of endosomes [102]. Each TLR binds to a distinct class of pathogen associated peptides or molecules. A summary of the most well studied TLRs and ligands is included below:

TLR	**Ligand** [102, 103]
TLR1	Lipopeptide (Pam3csk4)
TLR2	Peptidoglycan, Zymosan, LTA, Lipopeptide (Pam3csk4)
TLR3	PolyI:C (dsRNA)
TLR4	LPS, RSV protein F, *Mycobacterium tuberculosis*
TLR6	Peptidoglycan, Zymosan
TLR9	Bacterial DNA (CpG motifs)

For example, lipopeptides from various gram-positive bacteria can bind to both TLR1 and TLR2 inducing heterodimer TLR1/2 formation and subsequent downstream signaling. Similarly, peptidoglycans (PGNs) and zymosan, components of microbial cell walls, cause heterodimerization between TLR 2 and 6. Despite the wide array of stimulatory ligands and TLRs expressed on the mast cell, a common downstream signaling pathway leads to activation of the transcription factor NFkB and the MAPKs, p38 and JNK [102–104].

Mast Cell Response to Bacteria

Due to the mast cells' strategic location in the skin, they play an important role in innate immunity against bacteria through their ability to phagocytose pathogens, present bacterial antigens to T cells, recruit other phagocytic cells, release mediators and cytokines, and produce cathelicidin antimicrobial peptides [105–107].

Some of the earliest studies in mast cell responses to bacteria show that rodent mast cells are capable of phagocytosing bacteria via complement receptors [108]. Additional studies in complement C3-deficient mice demonstrate a decrease in peritoneal mast cell degranulation, production of TNFα, neutrophil infiltration and clearance of bacteria. Treating these mice with purified C3 protein reversed these defects, confirming that complement activation aids the mast cell's full functionality in innate immune defense [109]. Additional preliminary research shows that certain bacteria are able to induce mast cell degranulation. Formalin-killed bacteria such as *Escherichia coli, Enterobacter cloacae, Staphylococcus epidermidis, Proteus vulgaris* and *Klebsiella oxytoca* in addition to bacterial antigens like hemolysin all have the ability to induce histamine release in mast cells [110–112]. However, some bacteria have been shown to have the opposite effect. For example, *Helicobacter pylori*, a major cause of gastritis, peptic ulcers and gastric cancer, is able to directly inhibit histamine release, perhaps contributing to the bacteria's persistence in the gastric mucosa [113]. High doses of non-pathogenic, commensal *E. coli* have also been shown to be a direct inhibitor of degranulation in intestinal mast cells, suggesting a potential mechanism for this commensal bacteria's survival in the intestine, which could potentially be applied to other commensal bacteria in the skin [114].

Despite the varying effects that different strains of bacteria can have on mast cell degranulation, it is considered a commonality that TLR-2 and TLR-4 play important roles in the mast cell's innate immune response to most bacteria. More specifically, these receptors are required for mast cell release of cytokines and chemokines. For example, upon stimulation with LPS derived from *E. coli*, TLR-4 was shown to be necessary for release of the common inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-13 from BMMCs [115]. Furthermore, mast cell-deficient mice that were reconstituted with TLR-4-mutated BMMCs showed defective neutrophil recruitment and production of inflammatory cytokines in the peritoneum, demonstrating the key role the TLR-4 receptor plays in mediating recruitment of other immune cells [115]. While LPS stimulation is mediated primarily through TLR-4, PGN from *S. aureus* stimulates mast cells in a TLR-2 dependent manner to produce TNF-α, IL-4, IL-5, IL-6, and IL-13 but not IL-1β. Additionally, intradermal injection of PGN promoted mast cell-mediated vasodilation and inflammation via TLR-2 [116]. Further evidence exists suggesting that skin-derived mast cells produce the pro-inflammatory cytokines TNF-α and IL-6, but not degranulation, in response to poly (I:C) stimulation via TLR-3, TLR-7 and TLR-9 [117].

Clearly, the mast cell response and production of cytokines depends greatly on the bacterial stimulus and TLR-mediated signaling.

In addition to cytokine production and release, mast cells are capable of releasing antimicrobial peptides, particularly cathelicidin, that can evoke direct bactericidal and anti-viral effects. Cathelicidin antimicrobial peptide was originally thought to be found only in neutrophils, but it has been identified in other cell types, including macrophages and epithelial cells in response to various microbes and 1,25-vitamin D [118]. Initial studies show that LL-37, the human cathelicidin peptide, is able to bind to high and low affinity receptors on the surface of mast cells. This binding cannot only induce degranulation but also mast cell chemotaxis, suggesting that mast cell recruitment to the site of infection is critical for innate immune responses [119]. However, more recent research shows that LL-37 is also synthesized by cultured murine and human mast cells and is necessary for efficient bacterial killing [120]. Furthermore, using mast cell- and cathelicidin-deficient mouse models, it has been demonstrated that mast cells help protect against invasive group A *Streptococcus* infection in the skin by producing cathelicidin. The LL-37 purified from mast cells has been identified as a unique 28-aa peptide by using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) analysis [121].

In conclusion, conserved bacterial elements not only have both stimulatory and inhibitory effects on mast cell degranulation, but they can also elicit recruitment of other immune cells via cytokine release through TLR signaling [122]. The mast cell's ability to directly clear bacteria through cathelicidin release also demonstrates this cells critical role in the innate immune response.

Mast Cell Response to Viruses

While extensive research has been conducted on the mast cell's role in bacterial infection, less has been studied about the mast cell response to viruses. Early work in this field shows that mast cells can be activated to both proliferate and release their granular contents [123, 124]. For example, Sendai virus can generate permeability lesions in the membranes of rat mast cells and induces release of histamine and various proteases by normal exocytotic mechanisms. [125] Although most research on mast cell interaction with viruses has been modeled in the airway connective tissue and epithelium, it is important to note that certain respiratory viruses, like respiratory syncytial virus (RSV) can induce degranulation as well as an increase in TNF-α production. However, this only occurs in co-culture with RSV-infected airway epithelial cells. Incubation of mast cells with medium from the RSV-infected epithelial cells fails to induce degranulation, suggesting a potential mechanism for crosstalk between infected cells and neighboring mast cells [126].

A similar occurrence has been noted between skin mast cells and epidermal keratinocytes infected with herpes simplex virus (HSV). Studies in mast cell-deficient mice showed increased severity of disease after transdermal injection with HSV2, and reconstitution of these mice with BMMCs reversed the high severity and mortality associated with HSV2 infection. Furthermore, TNF-α and IL-6 production in mast cells was stimulated by IL-33 derived from HSV2-infected keratinocytes [127]. Additionally, Wang et al. showed mast cell degranulation in the context of viral infection is dependent on the presence of a viral envelope, as found in vaccinia virus (VV). Lipid fusion of the VV envelope with the mast cell membrane was shown to be sufficient to induce cathelicidin release and TNF-α release, resulting in the recruitment of neutrophils and antimicrobial activity [128]. This further suggests a key role that mast cells may play in triggering inflammation after communication with virally infected cells.

While it is clear that mast cells assist in immune defense to help neighboring tissues, they have also been shown to be susceptible to direct infection by human immunodeficiency virus type 1 (HIV-1) due to their surface expression of CD4 and chemokine receptors CCR3, CCR5, and CXCR4 [129]. HIV-1 glycoprotein, gp120, can act as a viral superantigen inducing cytokine release from infected mast cells. The subsequent trafficking of infected mast cells to various tissues in combination with the mast cell's long life span may contribute to the widespread and persistent viral reservoir in AIDS patients [130, 131]. However, some studies show immunohistochemical evidence of no active HIV replication in tissue mast cells, challenging the hypothesis of the mast cell HIV reservoir [132]. Additional research is warranted to elucidate the mast cell's exact role in combating HIV.

It was previously mentioned that cathelicidin antimicrobial peptide from mast cells has the capacity to directly kill bacteria in the skin. Further research shows that release of cathelicidin by mast cells also has an effect on viral invasion by preventing vaccinia virus (VV) infection. VV is able to bind to the mast cell via its L1 viral membrane protein. Once bound, the virus is endocytosed leading to the activation of the phospholipid mediator S1P and expression of S1PR2. This S1PR2 receptor can function in an autocrine manner inducing degranulation of the mast cell. The granules released in response to VV are shown to contain cathelicidin, which is critical for maintaining low levels of infection [128]. Despite compelling evidence for the mast cell's role in innate immunity against viral infection, the specific mechanisms of viral response in the mast cell are still under investigation.

Mast Cell Response to Fungi

Fungi are one of the four major groups of microorganisms that affect the skin. While knowledge of the mast cell's role in defense against fungal infection remains extremely limited, some reports have highlighted the importance of the mast cell's capacity to kill *Candida albicans* in vitro. Di Nardo et al. showed that when compared to wild type mice, mast cell-deficient mice exhibit persistently larger skin lesions when inoculated with *C. albicans*, suggesting that mast cells play a central role in protecting the skin during fungal infection. Additional *in vitro* studies using isolated rat peritoneal mast cells show potential for mast cell phagocytosis of opsonized *C. albicans*, while exerting fungicidal effects on non-opsonized fungus outside the cell membrane [133]. These fungicidal effects are carried out by mast cell degranulation, but further studies in the field are needed to further unveil interactions between mast cells and fungal microorganisms.

Mast Cell Response to Parasites

Throughout the past decades, multiple studies have been performed showing the activation of mast cells during parasitic infection. While the majority of this research has focused on peritoneal and mucosal mast cells in rodents, it is important to note the key role that the mast cell plays in regulating the inflammatory response to parasites.

For example, daily injections of phospholipid preparations from *Ascaris suum* or from *Echinococcus granulosus* cysts are able to induce blood eosinophilia and mast cell granule lysis along with mast cell hyperplasia [134]. Infection by the nematode parasite *Trichinella spiralis* has been shown to induce increased numbers of IgE containing mast cells in the intestinal mucosa of mice [135]. In addition to the recruitment of mast cells to the site of infection, parasites like *Toxoplasma gondii* have been shown to induce mast cell degranulation, followed by an observed increase in neutrophil count at the site of infection [136]. This suggests that mast cells are critically involved in parasite-mediated inflammation and recruitment of other immune cells. It has also been shown that mast cells play a role in direct elimination of the parasite. During infection with *Schistosoma mansoni*, a pronounced hepatic mastocytosis, or an abnormally high number of mast cells, is observed in rats. The majority of these recruited hepatic mast cells contain a highly soluble granular chymase that is released systemically into the blood during the period of parasite elimination. Thus, due to these chymases, infection is terminated in the liver before egg laying commences [137].

More relevant to the skin is the mast cell's activity during *Leishmania major* infection. Cutaneous leishmaniasis is a quickly spreading, ulcerative skin disorder caused by protozoan parasitic infection [138]. While some evidence shows mast cells to be the cause of increased lesion size and intensity, this is likely due to the immediate release of mediators like beta-hexosaminidase and TNF-α upon mast cell contact with the parasite [139, 140]. Despite the mast cell's apparent contribution to the appearance of the lesion, it has also been shown that mast cell degranulation may inhibit infection. When mast cells in mice were induced to degranulate before *L. major* challenge, these mice showed lower rates of infection along with high levels of IFN-γ and reduced levels of IL-4 [141].

Despite a large amount of evidence that parasites are capable of inducing mast cell activation and degranulation, it has been repeatedly shown that infection by certain parasitic roundworms leads to inhibitory signaling via TLR-4. In contrast to the binding of TLR-4 to LPS, when bound to the ES-62 protein from roundworms, a resultant inhibition of FcεRI activation occurs. Binding of ES-62 to TLR-4 on the surface of mast cells causes sequestration of PKC, which in turn inhibits downstream signaling of the high-affinity IgE receptor [142].

In summary, the presence of mast cells is strongly associated with parasitic infection, mediating inflammation and the recruitment of other immune cells. Although many mechanisms of mast cell defense against parasites remain unknown, it is now evident that mast cells can amplify protective responses against parasites in innate immunity as well as play a conflicting role in inflammation and pathology at sites of infection.

Mast Cell-Associated Skin Diseases

Hypersensitivity Reactions

Mast cells are the primary mediators of immediate anaphylactic hypersensitivity (type 1) reactions. Tissue mast cells that are sensitized with IgE antibodies bound to the high-affinity IgE receptor can also bind to antigen, inducing receptor cross-linking and subsequent mast cell activation and mediator release, as outlined in the section on mast cell activating receptors. Release of these mediators results in increased vascular permeability, edema and smooth muscle contraction. The most common manifestations include urticarial rash, erythema and pruritus, while extreme cases can lead to anaphylaxis. This type 1 hypersensitivity reaction is associated with both food and drug allergies [143].

Mast cells have also been shown to be considerably active in delayed-type hypersensitivity (type 4) reactions (DTHRs), and most relevant to the skin, contact hypersensitivity reactions. DTHRs depend on the presence of type 1 memory T cells and their ability to produce IFN-γ. Hapten binding is

the initial step of these reactions. Low molecular weight contact allergens, called haptens, are able to penetrate the epidermal barrier and bind to various skin proteins [144]. During the initial sensitization phase of the reaction, the bound hapten can be recognized by Langerhans cells, leading to migration to the lymph nodes and clonal expansion of both CD4+ and CD8+ hapten-specific T cells [145, 146].

The following effector phase of the contact hypersensitivity reaction is less studied, but mast cells have been shown to play an important role. During the effector phase, Ig free light chains are produced by B lymphocytes, and they have been shown to be necessary for full development of contact hypersensitivity in mice [147]. Furthermore, these free light chains have been shown to be important in the sensitization of mast cells and for their activation when coming into contact with allergens [148]. While this evidence suggests that mast cells may be involved in the development of DTHRs, various other studies involving reconstitution of mast cell-deficient mice with mast cells cultured *in vitro* show that in various DTHRs, like allergic encephalitis, contact hypersensitivity and cutaneous responses to microbes, mast cells are needed for full manifestation of symptoms. [149–151] Specifically in the DTHR context, mast cells produce TNF-α for induction of dendritic cell migration, IL-3 for proliferation and activation of T cells, and they may play a role in direct antigen presentation [152]. While mast cells may not be the main mediators of contact hypersensitivity reactions, their presence proves necessary for a coordinated immune response, recruitment of other cell types, and full development of symptoms.

Rosacea

Recent studies show new evidence that demonstrates the mast cell's central role in the pathogenesis of rosacea. With approximately 16 million Americans affected, this chronic inflammatory disease can flare up due to increased temperature, spicy foods, or various other environmental triggers. The resulting, often painful, inflammation can take weeks to subside without treatment [153]. It has also been shown that rosacea is associated with elevated levels of an aberrant form of antimicrobial peptide, cathelicidin LL-37, due to overactivity of kallikrein-related peptidases (KLKs), which can generate LL-37 from its precursor peptide [154]. Studies in mast cell-deficient *KitW-sh/W-sh* mouse strains show that upon intradermal injection with LL-37, *KitW-sh/W-sh* mice fail to develop rosacea-related inflammation, whereas wild type mice exhibit the phenotypically characteristic inflammation of rosacea. Furthermore, it is now known that upon stimulation with LL-37, mast cells respond by releasing metalloproteinase 9 (MMP9), the enzyme responsible for activating KLKs, and proinflammatory IL-6, suggesting a critical role for the mast cell in rosacea inflammation. Ultimately, treatment in human rosacea subjects with 4% cromolyn sodium, a known mast cell stabilizer, significantly decreased rosacea-associated inflammation, and in mouse models, it has also been shown to reduce levels of MMP9 [155]. This data shows that the presence of mast cells in the skin is necessary for development of the rosacea phenotype. Furthermore, mast cell stabilizers may prove to be the treatment of choice for rosacea patients.

Mastocytosis

Mastocytosis is characterized by abnormally high numbers of mast cells localizing in one or multiple tissues. This rare disorder is accompanied by chronic or episodic degranulation and release of mast cell mediators into the tissue. Cutaneous mastocytosis involves only the skin, while systemic mastocytosis affects internal organs with or without involvement of the skin [156]. While pathogenesis of the disease is not fully understood, mastocytosis has been shown to be associated with mutations in the gene encoding the c-kit receptor or with increased expression of SCF. The most common c-kit mutation is a D816V mutation of exon 17. This activating mutation induces SCF-independent activation of c-kit, leading to both clonal expansion and apoptotic defects in the mast cells [157, 158]. Increased levels of free SCF have also been identified in the dermis and extracellular spaces of the epidermis in patients with cutaneous mastocytosis; however, this elevation of SCF may be variable in different patients [159]. Patients with cutaneous mastocytosis typically present with a yellow-tan to reddish-brown maculopapular skin lesion known as urticaria pigmentosa, a fixed accumulation of mast cells in the skin, which may also manifest itself in a plaque or nodular form. More rare presentation involves diffuse mastocytosis, which may present in a bullous form and involve the whole skin, or mastocytomas, which usually present in childhood [160, 161]. Mediator release from the mast cells has a presentation that mimics allergic reactions with the typical symptoms of pruritus and flushing [162]. While children with cutaneous mastocytosis typically clear the disease by adolescence, avoidance of triggers that lead to mediator release like temperature change, friction, and physical exertion is important for the disease to remain asymptomatic. Antihistamines and PUVA have proven to be effective therapies; however, treatment with corticosteroids has not been shown to diminish symptoms of the disease [162].

Conclusions

Mast cells prove to be an extremely important part of the innate immune system in the skin. From their ability to initiate recruitment of critical immune cells to their

central role in the allergic response, mast cells truly are the sentinels and directors of many coordinated immune responses. Furthermore, key therapies like imatinib and similar biologics act by blocking the effect of c-kit, and thus mast cell development, in the context of various types of malignancies. The development of medications that can directly target the mast cells further demonstrates this cell's key role in the innate immune response. While much has been discovered about the mast cell's role in the skin, there remains great potential for further study and development of therapeutics centered on mast cell function.

Questions

1. Which cytokine is critical for proper mast cell differentiation?
   A. IL1
   B. SCF
   C. IL6
   D. TNFα

2. Binding of which of the following molecules to the mast cell surface can induce degranulation?
   A. IgE
   B. IgG
   C. Complement peptides
   D. All of the above

3. Release of which of the following mast cell mediators has been shown to aid defense against microbes?
   A. Cathelicidin
   B. Histamine
   C. Leukotrienes
   D. Prostaglandins

Answers

1. B
2. D
3. A

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Antimicrobial Peptides

6

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Abstract

The skin is traditionally viewed as a physical barrier to environmental insults. Its role in immunity goes beyond its role as simply a barrier, as it plays a dynamic role in regulating immune responses and controlling microbial populations, notably through the use of antimicrobial peptides (AMPs). In 1987, Zasloff and colleagues were one of the first to describe the existence of potent AMPs on the skin of vertebrates. Since then, numerous AMPs have been identified and characterized in human skin. AMPs are a heterogeneous group of small proteins with a wide spectrum of antimicrobial activity against bacteria, fungi, and viruses. These peptides are almost all positively charged and amphipathic, allowing for the peptides to be soluble in an aqueous environment while still retaining an ability to bind bacterial membranes and walls. Once bound to the target membrane, the peptides kill through various mechanisms that involve both the physical perforation of pathogens and triggering of the host immune response. This chapter will focus on known AMPs present in human skin, with special focus given to defensins, cathelicidins, granulysins, S100 proteins, and ribonucleases; and their involvements in the etiology of dermatologic diseases.

Keywords

Antimicrobial peptide (AMP) • Defensin • Cathelicidin • LL-37 • Granulysin • S100 • Ribonuclease (RNase) • Psoriasin • Dermcidin • Secretory leukocyte protease inhibitor (SLPI) • Psoriasis • Atopic Dermatitis • Acne • Rosacea • Mycobacteria • Wound healing

Introduction

The skin is primarily thought of as a physical barrier to environmental insults and microbes. Yet its role extends far beyond simply a physical barrier, as the skin is also a dynamic immune organ that responds with robust defense mechanisms to microbial invaders, notably through the use of antimicrobial peptides (AMPs). AMPs in the skin are of considerable interest not only for their properties in controlling microbial populations but also for their effects on influencing inflammatory and immune responses. In 1987, Zasloff led one of the first groups to describe the existence of a potent antibacterial peptide present in the skin of the African frog *Xenopus laevis* [1]. Since then, numerous AMPs have been described in human skin and their roles in host defense have been extensively characterized.

AMPs are a heterogeneous group of small molecular weight proteins with a wide spectrum of antimicrobial activity against bacteria, fungi, and viruses. These peptides are almost all positively charged and amphipathic, having both hydrophobic and hydrophilic surfaces, allowing for solubility in aqueous environments while still retaining the ability

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to bind bacterial membranes through their hydrophobic surfaces. Once bound to the target membrane, the peptides can kill the organism through various mechanisms. Furthermore, there is a growing body of evidence supporting the ability of AMPs to alter the host immune response (Table 6.1).

This chapter will focus on the main AMPs present in human skin and the relevant dermatologic conditions in which deficiency or overexpression of AMPs is thought to play an important etiologic role. (A summary of described AMPs present in the skin can be found in Table 6.2.)

Defensins

Defensins are a family of AMPs with a characteristic β-sheet fold and six disulfide bonds between highly conserved cysteine residues. There are two main defensin subfamilies, alpha- and beta-defensins, that differ mainly in the pairing of cysteine residues. Members of both subfamilies consist of a triple-stranded β-sheet with a prototypic 'defensin' fold (Fig. 6.1). There is a third defensin family, theta-defensins, that is not expressed in humans but is found in several Old World primates. Theta-defensins are thought to inhibit the fusion of HIV-1 to host cells by inhibiting bundle formation needed for fusion [2, 3].

Defensins are widely distributed in cells and tissues involved in host defense and are found in highest concentrations within phagocyte granules. Alpha-defensins, which have disulfide bridges between cysteines 1–6, 2–4, and 3–5 [4], are found predominantly in neutrophils [5], and have aptly been named human neutrophil peptides (HNPs). In humans, alpha-defensins are stored in azurophilic granules of neutrophils as fully processed mature peptides. Two alpha-defensins, human defensins (HD)-5 [6] and 6 [7], are expressed in Paneth cells of the small intestine and the epithelium of the female urogenital tract [8].

Alpha-defensins show a wide spectrum of antimicrobial activity against bacteria and fungi. They have also been shown to inactivate certain viruses, including adenovirus [9] and polyomavirus [10], and have been implicated as one of the molecules that may be important in the antiviral activity seen in $CD8^+$ T cells of HIV-non-progressors [11]. Immunologically, alpha-defensins contribute to the host inflammatory response, for example, by increasing the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-1 in *S. aureus*-activated monocytes [12]. High concentrations of alpha-defensins are toxic to mammalian cells and may be important in tissue injury and necrosis during inflammation. It has been suggested that the upregulation of alpha-defensins 1–3 from T cells may be involved in the

Table 6.1 Antimicrobial and immunomodulatory properties

	Antimicrobial activity			Regulation
	Gram +	Gram −	Fungi	
α-defensin	+	+	+	Constitutive
HBD-1	±	+	−	Constitutive
HBD-2	±	+	+	Inducible via IL-1/NF-kB dependent mechanism Toll-like receptors
HBD-3	+	+	+	Inducible via TGF-α, and IGF-1
HBD-4	+	+	+	Via NF-kB independent pathways
Cathelicidin	+	+	+	Constitutive and inducible Vitamin-D Toll-like receptors
Granulysin	+	+	+	Toll-like receptors Activator protein-1 dependent pathway
Psoriasin	±	+	−	Calcium All-trans retinoic acid Inflammatory stress UV light EGFR ligands IL-1
Dermcidin	+	+	+	Constitutive
RNAse 7	+	+	+	Constitutive and inducible Inducible via UVB, IL-1β, IFN-γ, TNF-α Bacterial challenge (*S. aureus*, *P. aeruginosa*)

Table 6.2 Summary of major antimicrobial peptides present in skin

	Cell source	Other properties
α-defensins	Neutrophils	Increase (TNF)-α and interleukin (IL)-1 in *S. aureus* activated monocytes Inactivation of adenovirus and polyomavirus
β-defensins	Keratinocytes	Chemotaxis of T cells and Dendritic cells
Cathelicidin (LL-37)	Keratinocytes Ductal epithelium Eccrine glands Mast cells Nail bed	Angiogenesis Wound healing Chemotaxis of neutrophils, monocytes, T cells, and mast cells Induction of IL-1β and IL-6 secretion in keratinocytes Inhibition of IAP-2 via COX-2 Anti-biofilm effects
Granulysin	Cytotoxic T lymphocytes NK cells	Chemotaxis of T cells, monocytes, NK cells, and dendritic cells Cytotoxic to tumor cells Anti-inflammatory Graft Rejection Induction of MCP-1, MCP-3, IL-1, IL-6, IL-10, and IFN-α in monocytes Mediator of keratinocyte cell death in SJS and TEN
Psoriasin	Keratinocytes Follicular epithelium Sebocytes	Chemotaxis of CD4+ lymphocytes and neutrophils Calcium-dependent oleic acid transport and metabolism
Dermcidin	Eccrine glands	Limits bacterial colonization
RNase 7	Keratinocytes	Antimicrobial properties against Gram + and Gram – organisms Antifungal properties

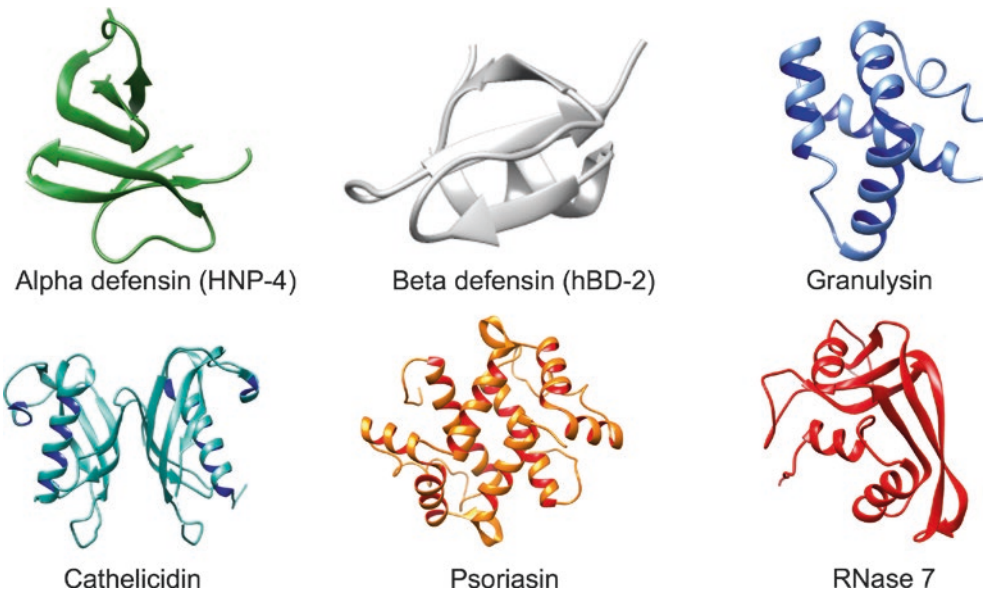

Fig. 6.1 Representative crystal structures of major antimicrobial peptides. Shown here are representative crystal structures of alpha defensin, beta defensin, granulysin, cathelicidin, psoriasin, and RNase 7. Alpha defensin, beta defensin, granulysin, and RNase 7 are shown as monomers. Cathelicidin and psoriasin are shown as dimers

etiopathology of Stevens-Johnsons Syndrome and toxic epidermal necrolysis [13].

Human beta-defensins (hBDs) are also characterized by six cysteine motifs, but are distinct from alpha-defensins through their cysteine cross-bridging. The disulfide bonds of hBDs are instead between cysteines 1–5, 2–4, and 3–6 [14]. hBDs-1, -2, -3, and -4 have been identified in many cell types, including epithelial cells, but their expression, localization, and antimicrobial specificities vary. hBD-1 is expressed predominantly in the urinary tract but is also expressed constitutively in the skin. hBD-2 is largely absent in healthy skin but is induced by bacteria through an IL-1/NF-kB dependent mechanism [15, 16]. The antibacterial spectrum of hBD-2 is broad, encompassing both Gram-negative and Gram-positive organisms. Studies found that hBD-2 is bactericidal against *Pseudomonas aeruginosa*, *Fendegoldia magna*, and *Streptococcus pyogenes*, while only bacteriostatic against *Staphylococcus aureus* [17, 18]. A synergistic effect of IL-1α and epidermal growth factor receptor (EGFR) ligands has been proposed in the induction of hBD-2 [19]. hBD-3 is also a broad spectrum antibiotic for a wide range of bacteria and fungi [20]. As with hBD-2, hBD-3 induction has also been proposed to be dependent on the transactivation of EFGR, yet with its own set of growth factors, including TGF-α and IGF-1 [21]. In addition to its bactericidal effects, hBD-3 has been shown to induce phenotypic maturation of Langerhans cell-like dendritic cells [22]. Lastly, hBD-4 induction is suggested to involve NF-kB independent pathways that have yet to be fully characterized [23].

Both alpha-and beta defensins share a common antimicrobial mechanism. The leading hypothesis proposes that the permeabilization of target membranes is the critical step in defensin-mediated cytotoxicity. In experimental models using *Escherichia coli*, membrane permeabilization resulted in the subsequent inhibition of bacterial metabolism, including RNA, DNA, and protein synthesis (Fig. 6.2) [24]. One study proposed the formation of a stable 25 Å pore, comprised of a hexamer of defensin dimers [25], allowing for small intracellular molecules to leak out of the organism resulting in a decrease in viability. This mechanism, however, only partially explains the antimicrobial activity, as evidence exists for both a transient pore formation and also intracellular sites of action, which may also be important in cell death [26]. In addition, some defensins have been found to bind to membrane glycoproteins with high affinity, giving rise to a possible explanation for antiviral activity [27]. Other proposed mechanisms of action of defensins include modifying cell migration and maturation, inducing cytokines, and triggering histamine and prostaglandin D2 release from mast cells.

Cathelicidins

Cathelicidins are a family of AMPs that contain a conserved cathelin domain, characterized by an N-terminal signal peptide, a prosequence, and a C-terminal cationic peptide [28]. Cathelicidins are expressed by cells in direct contact with the external environment. The propeptide hCAP18 (human cationic AMP, 18 kD) is first synthesized and stored in granules and lamellar bodies of keratinocytes. When hCAP18 is released into the extracellular environment, its antimicrobial C-terminus is cleaved by proteinase 3 in neutrophils and kallikrein in keratinocytes to produce the α-helical LL-37 ("LL" for two leucine residues, 37 for the number of residues present in the peptide) (Fig. 6.1) [29]. The human cathelicidin family is limited to just the one gene with a protein product hCAP18. It was initially identified in keratinocytes at the site of wound healing [30]. It was later found to be constitutively expressed in other locations and conditions, such as in nail beds, eccrine glands [31], and neonatal skin (Fig. 6.3) [32].

LL-37 has a broad antimicrobial spectrum against bacteria, fungi, and viruses. It is bactericidal against both Gram-positive and Gram-negative organisms including *Listeria monocytogenes*, *S. aureus*, *S. epidermidis*, *Salmonella typhimurium*, *E. coli*, and vancomycin-resistant *Enterococci* [33]. Unlike murine and reptilian cathelicidins, LL-37 is the only known

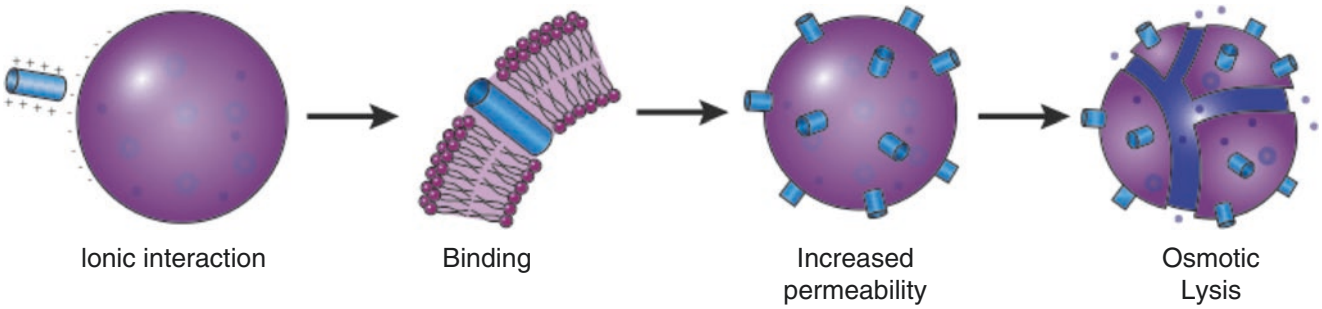

Fig. 6.2 Proposed mechanism of action of antimicrobial peptides. Cationic AMPs (e.g., granulysin) associate with the negative bacterial membranes, resulting in binding and subsequent increased permeability of the phospholipid bilayer. As a result of the increased permeability, irreversible osmotic damage results in cell death

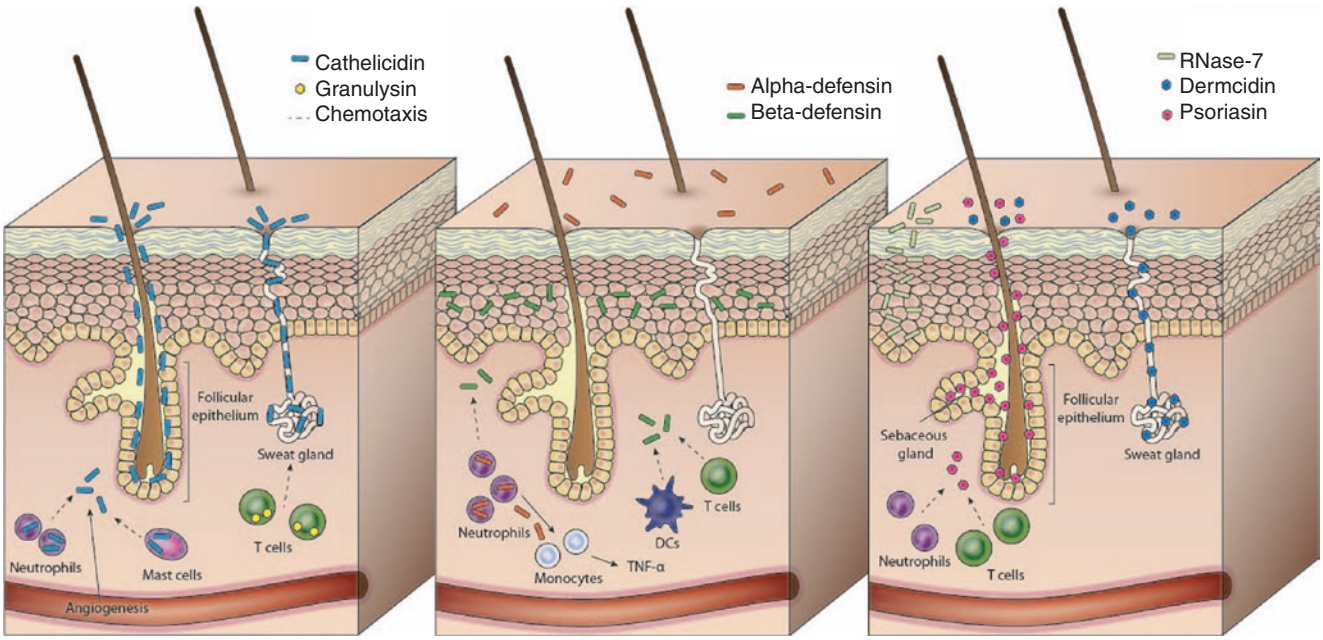

Fig. 6.3 Antimicrobial peptide expression in the skin. The expression of some AMPs by both resident skin cells and infiltrating immune cells is demonstrated in this schematic diagram

cathelicidin with anti-biofilm properties against the opportunistic human pathogens *P. aeruginosa* and *Francisella novicida* [34]. Mice with a mutation in CRAMP (the murine homologue of LL-37) are more susceptible to *Streptococcal* infections when compared to wild-type controls [35]. In humans, LL-37 is further processed into smaller peptides, such as RK-31 and KS-30, which have increasing antimicrobial activity against *Staphylococcal* species [36]. LL-37 is also active against fungal species, most notably *Candida albicans* [37], by disturbing membrane morphology to allow for a swift influx of molecules with masses up to 40 kDa [38]. Furthermore, LL-37 has a broad antiviral spectrum and has been shown to be viricidal against the vaccinia virus [39], influenza virus [40], herpes simplex 1 virus [41], adenovirus 19 [41], varicella zoster virus [42], and HIV-1 [43].

The expression of LL-37 within human skin is both constitutive and inducible. In eccrine glands and ductal cells, LL-37 is diffusely expressed in the cytoplasm of secretory glands and also the ductal epithelium [31]. Serine proteases present within sweat further cleave LL-37 into smaller peptides giving rise to enhanced antimicrobial activity against *Staphylococcal* and *Candidal* species [36]. LL-37 expression is induced in patients with cutaneous lupus erythematosus [44], and is upregulated during wound healing. Studies using a cultured keratinocyte model suggest the importance of IGF-1 in the latter scenario [45].

Vitamin D is frequently mentioned in discussions about LL-37 in the skin as an important regulator. The gene encoding LL-37 contains a vitamin D response element (VDRE) present in its promoter, which explains the dependence of LL-37 expression on vitamin D and its precursors [46]. Liu et al. [47] found that Toll-like receptor (TLR)-2-dependent microbicidal activity was dependent on the vitamin-D induced expression of LL-37 [47]. This identified a novel mechanism for TLR-induced antimicrobial activity and may represent one of the main defenses of infection for both cutaneous and systemic tuberculosis. The relationship between vitamin D and LL-37, however, remains unclear, as calicipotriol, a synthetic form of vitamin D, downregulates LL-37 in keratinocytes prestimulated with UVB, LPS, and TNF-α [48]. This dichotomous relationship poses the hypothesis that vitamin D-induction of LL-37 depends on whether or not the condition in question inflammatory or non-inflammatory.

As with other AMPs, LL-37 also has a role in modulating the host immune response. LL-37 is chemoattractant for neutrophils, monocytes, and T cells by binding formyl-peptide-receptor-like (FPRL)-1. In addition, it recruits and stimulates subsequent production of LL-37 via mast cells, creating a positive feedback loop [49]. In keratinocytes, LL-37 not only has a role in inducing IL-1β and IL-6 secretion [36], but also inhibits the expression of inhibitor of apoptosis-2 (IAP-2) via cyclooxygenase-2 (COX-2), inhibiting apoptosis [50].

Granulysin

Granulysin is an AMP derived from cytotoxic T lymphocytes and NK cells and belongs to a member of the larger saposin-like protein family. Unlike defensins and cathelicidins that are expressed in epithelial cells, granulysin is found exclusively

within the granules of cytotoxic T and NK cells recruited to sights of inflammation. Granulysin co-localizes to the cytotoxic vacuole with perforin and granzymes and acts synergistically to kill intracellular bacteria [51, 52]. Perforin forms pores in the cellular membrane allowing granulysin to access the intracellular compartment in which the pathogen resides, providing a direct mechanism of intracellular pathogen targeting (Fig. 6.3).

Granulysin is only found in humans and is synthesized as a 15 kD protein that is then cleaved to release the final 9 kD peptide [53]. The resultant 9 kD peptide is composed of 5 α-helices joined by short loops (Fig. 6.1) [54]. Granulysin is effective against a wide range of Gram-positive and Gram-negative bacteria, parasites, and fungi. Notably, it has been shown to be antimicrobial against *Mycobacterium tuberculosis* [52], *Cryptococcus neoformans* [55], *Plasmodium falciparum* [56], and *Leishmania* [57]. Granulysin also induces apoptosis of varicella-infected cells [58]. Although the structure of granulysin is significantly different from that of defensins or cathelicidin, it retains the conserved amphipathic nature. The hydrophobic surface is capable of associating closely with the target membrane, and the positively charged surface allows for association with the negatively charged bacterial membrane. In addition, studies on granulysin have shown the importance of the helix-loop-helix domain in the secondary structure. The ability of granulysin to kill *S. typhimurium* and *E. coli* has been localized to helix 2 and 3 of granulysin [57]. The amino acid residues contained within this structural component of granulysin is critical to the antimicrobial activity [59].

Similar to other AMPs, granulysin contains numerous immunomodulatory properties. Several studies show that granulysin is a chemoattractant for monocytes, NK cells, monocyte-derived dendritic cells, and a subset of T cells, including $CD45Ro^+$ memory CD4 and CD8 T cells. Granulysin also induces the expression of multiple inflammatory cytokines in monocytes including MCP-1, MCP-3, IL-1, IL-6, IL-10, and IFN-α [60].

S100 Proteins

S100 proteins belong to a multigene family of proteins with numerous functions including keratinocyte differentiation, epithelial defense, and wound healing. They were first described by Moore et al. in 1965 as nerve-specific molecules from cattle brains "soluble in 100 % ammonium sulfate," thus named S100 [61]. In general, S100 proteins are low molecular weight proteins (9–13 kD) of four conserved α-helical segments, two calcium binding regions, a central hinge, and an amino and carboxy terminal variable domain (Fig. 6.1). Interest in S100 proteins in the skin initially arose in part because many of the genes encoding for this family are located in the epidermal differentiation complex (EDC) [62], and have been implicated in epidermal defense.

In 1990, Celis et al. discovered an intense expression of low molecular weight proteins in the keratinocytes of psoriasis patients [63]. Psoriasin (S100A7) is an S100 AMP widely expressed in the human epidermis. While named after psoriasis, psoriasin is constitutively expressed on the surface of the skin and is also present in other skin diseases characterized by inflammation including lichen sclerosus and atopic dermatitis. Immunohistochemical staining has shown that psoriasin is expressed focally in keratinocytes with high expression in the stratum granulosum and stratum spinosum. As a peptide with antimicrobial properties, it is present in higher levels in areas with high bacterial colonization such as the face, axilla, and palms, and in areas with a high density of sebaceous glands and hair follicles (Fig. 6.3) [16, 64]. In addition to keratinocytes, studies have also shown staining in sebocytes, indicating secretion of psoriasin into sebum [64]. Psoriasin is thought to be preferentially active against *E. coli*, but also has some bactericidal activity against *P. aeruginosa* and *S. aureus* – albeit when psoriasin is at higher concentrations. Psoriasin is also found *in utero* and is thought to protect embryos from infection [65].

The bactericidal properties of psoriasin are attributed to the sequestration of Zn^{2+} [66]. Mutation experiments of calcium-binding and zinc-binding motifs on psoriasin confirm the importance of zinc deprivation in its killing mechanism [67]. Unlike other AMPs, the antimicrobial activity of psoriasin does not depend primarily on forming perforations in bacterial membranes. At pH values less than 6, psoriasin has been shown to permeabilize the bacterial membrane of Gram-negative *E. coli* and Gram-positive *Bacillus megaterium*; however, at neutral pH, the bacteria were killed without disrupting membrane structure in only *E. coli* and not *B. megaterium* [68].

In addition to its antimicrobial activity, psoriasin has numerous other features. Immunologically, it functions as a chemoattractant for $CD4^+$ T cells and neutrophils [69]. Various studies have shown that epidermal production of psoriasin is inducible by many factors such as a through a synergistic induction by EGFR ligands and IL-1 [19], the binding of *E. coli* flagellin to TLR5 [70], and the binding of Th17 cytokines that can be suppressed by vitamin D [71]. Furthermore, UV and all-*trans*-retinoic acid have been shown to be exogenous regulators of psoriasin expression. Psoriasin has also been suggested to have metabolic effects as it is thought to interact with epidermal fatty acid binding protein to modulate calcium-dependent oleic acid transport and metabolism [72].

Other than psoriasin, there are 20 other known S100 proteins in the skin, 11 of which are expressed in keratinocytes including psoriasin (S100A2, S100A3, S100A4, S100A6, S100A8, S100A9, S100A10, S100A12, and S100A15), 1 in Langerhans cells and melanocytes (S100B), and 1 in Meissner's corpuscles (S100P). S100A2 is localized primarily

in the basal layer of the epidermis and in hair follicles and has been shown to be an early marker of oxidative stress [73]. S100A8 and S100A9 form both homo- and heterodimers and are frequently co-expressed. They have low expression in the epidermis and are sometimes found in the granular layer. The heterodimer of S100A8 and S100A9, also known as calprotectin, has some antifungal activity against *C. albicans* [74, 75]. Increased expression of these two S100 proteins is seen in wound healing and psoriasis. S100A10 is found both in the basal and spinous layers of the epidermis [76], and forms a homodimer that binds to a pair of annexin II to form calpactin I heterotetramer [77]. It is thought to be involved in the regulation of cell membrane formation during keratinocyte differentiation. The C-terminal peptide fragment of S100A12 (calcitermin) is capable of killing Gram-negative organisms showing *in vitro* activity against *E. coli*, *L. monocytogenes*, and *C. albicans* under acidic conditions [78]. Although initially described in human airway secretions, S100A12 is also expressed in both basal and suprabasal keratinocytes and is seen in psoriatic skin [79].

Ribonuclease

The RNase A superfamily contains 6–8 conserved cysteine residues forming disulfide bridges, conserved histidines, and a lysine at the center of ribonuclease activity [80]. The human RNase A superfamily currently has 13 known genes, of which 8 genes (RNases 1–8) have been shown to be catalytically active against RNA substrates [81]. Notably, RNase-7 and RNase-5 have emerged as being especially important in acting as AMPs in the skin.

RNase-7 is a 14.5 kD protein that is found in the skin [82]. It is constitutively expressed at a relatively high level in normal skin with the highest levels of expression in the stratum corneum (Fig. 6.3). It may be important in skin disease as greater than two-fold increases of expression is seen in psoriatic skin [16, 83]. It is active against both Gram-positive and Gram-negative organisms, such as *Propionibacterium acnes*, *S. aureus*, *E. coli*, and *P. aeruginosa*; as well as fungi, such as *C. albicans* [82]. RNase-7 has also been shown to be bactericidal against multi-resistant bacteria such as methicillin-resistant *S. aureus* and vancomyin-resistant enterococci [82]. Its relationship with *S. aureus* is notable as the application of RNase-7-neutralizing antibodies to the surface of the skin increased the growth rate of *S. aureus* [84], and as RNase-7 gene expression was found to be significantly reduced in *S. aureus*-positive skin [85].

While the name RNase-7 may suggest antibacterial properties through ribonuclease activity, a study using ribonuclease-inactive recombinant RNase-7 showed no difference in killing activity against *P. aeruginosa*, *E. faecium*, and *E. coli* compared to control [86]. The exact bactericidal mechanism of RNase-7 is still being elucidated. Structurally, RNase-7 contains four disulfide bonds, similar to the defensin family and has been shown to bind and permeabilize bacterial membranes (Fig. 6.1) [87]. While RNase-7 has yet to be demonstrated to be antiviral, it is hypothesized that its ribonuclease activity may have a role against viruses, a hypothesis supported by the observation that RNase-7 expression is upregulated in keratinocytes in response to dengue virus [88]. Immunologically, RNase-7 can be induced by various stimuli including IL-1β, TNF-α, IFNγ [83] UVB [89], *S. aureus* [84], and *P. aeruginosa*. RNase-7 is found in relatively high levels of constitutive expression in normal adult skin and in lower levels in prenatal skin [90].

RNase-5 is found in abundant levels in the stratum corneum and also plays an antimicrobial role in the skin [91]. Initially, RNase-5 was named angiogenin because it was first associated with its capacity to induce angiogenesis [92], but it was later observed to share homology with the RNase A superfamily. RNase-5 has been shown to exhibit bactericidal effects against *S. pneumonia* and limited effects against *E. faecalis*, *L. monocytogenes*, MRSA, and *P. aeruginosa* [91, 93]. This limited killing spectrum compared to RNase-7 is thought to be attributed to the lack of lysine residues required for membrane permeabilization found in RNase-7. RNase-5 also exerts antifungal activity against *C. albicans*, a property attributed to the inherent ribonucleolytic properties of RNase-5.

Other members of the RNase A family have also been demonstrated to possess antimicrobial and immunological properties. RNase-2, also known as eosinophil-derived neurotoxin, attracts dendritic cells and enhances Th-2 mediated responses [94]. RNase-2 also exhibits antimicrobial activity against *S. aureus* [93]. RNase-3, also called eosinophil-cationic protein, has been demonstrated to permeabilize membranes independent of its RNA degrading properties, kill parasitic worms, and induce mast cell degranulation. RNase-3 has also been demonstrated to be antibacterial and antiviral.

Other Antimicrobial Peptides

There is growing evidence to support the presence of multiple AMPs in the human skin in addition to those discussed thus far. Discussion of all the different peptides and proteins with antimicrobial activity present within human skin is beyond the scope of this chapter. However, we would like to highlight a few additional AMPs found within the skin that have recently gained more attention. A summary of some of these AMPs is laid out in Table 6.3.

Dermcidin, a 47 amino acid peptide, is the primary AMP present in sweat, and is constitutively expressed in eccrine ducts (Fig. 6.3) [95]. It is cleaved from a 9.3 kD precursor

Table 6.3 Additional mammalian antimicrobial peptides with relevance to skin

	Cell type	Comments
Adrenomedullin	Blood Skin Mucosal secretions	Regulation of skin growth and wound repair Active against *E. coli* and *S. aureus*
α-melanocyte stimulating hormone (α-MSH)	Keratinocytes	Active against *S. aureus* and *C. albicans*; inhibits HIV-1 replication
Calgranulin A/B	Keratinocytes	Inhibits growth of *C. albicans*
Connective tissue activating peptide 3 (CTAP-3)	Platelets	Microbicidal for bacteria >fungi
Elafin	Keratinocytes	Active against *P. aeruginosa*
Fibrinopeptide A (FP-A)	Platelets	Microbicidal for bacteria >fungi
Fibrinopeptide B (FP-B)		
Lactoferrin	Keratinocytes Neutrophils	Active against Gram-negative bacteria, Decreasing IL-1, IL-2 and TNF-α Enhancing monocyte and NK cell cytotoxicity
Lysozyme	Keratinocytes	Active against Gram-positive and some Gram-negative
Neuropeptide Y	Langerhans cells	Broad spectrum
Neutrophil gelatinase-associated lipocalin (NGAL)	Infiltrating neutrophils	Bacteriostatic; mechanism of action based on iron sequestration
P-cystatin α	Keratinocytes	Inhibits growth of *S. aureus*
Perforin	T cells	Co-localized with granulysin
Platelet basic protein (PBP)	Platelets	Microbicidal for bacteria >fungi
Platelet factor 4 (PF-4)	Platelets	
Polypeptide YY	Langerhans cells	Broad spectrum
RANTES	Platelets	Microbicidal for bacteria >fungi
RNase-2	Eosinophils	Antiviral activity Chemotractant for Dendritic cells Enhances Th-2 mediated responses
RNase-3	Eosinophils	Active against *S. aureus* and *E. coli*
RNase-5	Stratum corneum	Induction of angiogenesis Active against *S. pneumonia* >*E. faecalis*, *L. monocytogenes*, MRSA, and *P. aeruginosa* Antifungal activity
S100A2	Basal layer of epidermis Hair follicles	Early marker of oxidative stress
S100A10	Basal and spinous layers of epidermis	Regulation of cell membrane formation during keratinocyte differentiation
S100A12	Basal and suprabasal keratinocytes	Active against *E. coli*, *L. monocytogenes*, *C. albicans*
Secretory leukocyte proteinase inhibitor (SLPI)	Keratinocytes Glandular epithelium Neutrophils Macrophages	Antibacterial, antifungal, antiviral properties Increased expression in psoriasis and wounds
Substance P	Macrophages Eosinophils Endothelial cells	Active against *S. aureus* Related neuropeptides Bradykinin and Neurotensin with similar, albeit weaker, antimicrobial activity
Thymosin β-4 (Tβ-4)	Platelets	Microbicidal for bacteria >fungi

molecule. *In vitro* experiments have demonstrated enhanced antibacterial activity against a variety of organisms including *E. coli*, *E. faecalis*, *and S. aureus*. In addition, there are reports of activity against *C. albicans* [96]. The function of dermcidin in sweat may be to limit bacterial colonization and protect the host from infection. Levels of dermcidin measured in the sweat of patients with atopic dermatitis compared to control saw a deficit of dermcidin in atopic dermatitis, which may partially explain the greater susceptibility of atopic dermatitis patients to cutaneous infection [97]. The tendency for superinfection in atopic dermatitis as it relates to AMPs is further discussed in the next sections.

Secretory leukocyte protease inhibitor (SLPI) is also an AMP found in keratinocytes. It was first isolated from bronchial secretions, but later found produced by mucosal surfaces, keratinocytes, neutrophils, and macrophages. SLPI is a single-chain polypeptide of 107 amino acids. It is composed of two domains, each with eight cysteine residues that form four disulfide bonds. SLPI is known to have activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*. It also has antifungal properties against *A. fumigatus* and *C. albicans* [98].

Thymic stromal lymphopoietin (TSLP) is an interleukin-7-like cytokine that is expressed in epithelial cells and may be involved in atopic eczema. TSLP was found to have antimicrobial properties particularly against Gram-negative bacteria [99]. Adrenomedullin is a hormone-like peptide of 52 amino acids first isolated from human adrenal pheochromocytoma cells [100]. Adrenomedullin is present in blood, skin, and mucosal secretions. It is also believed to play a role in skin growth, wound repair, and prevention of gastric injury. Structurally, it has a single intramolecular disulfide bond and is separated into hydrophobic and charged regions, similar to defensins. Adrenomedullin has activity against *E. coli* and *S. aureus*.

Psoriasis and Atopic Dermatitis

Human beta-defensin-2 was initially isolated from psoriatic scales, sparking numerous studies to further elucidate the role of AMPs and host defense in human skin disease. Psoriasis is often characterized as having abnormal epidermal proliferation and cellular infiltrates from neutrophils and T cells [101]. It is well-known that psoriasis, a non-infectious inflammatory condition of the skin, is relatively resistant to bacterial superinfection. However, atopic dermatitis, characterized histologically by spongiosis and inflammation, is often secondarily infected by streptococcal and staphylococcal species. One possible reason to explain the discrepancy of superinfection between these two diseases is the disparate expression of AMPs within lesional skin. Ong et al. [102] compared the expression of LL-37 and hBD-2 in psoriatic skin versus atopic dermatitis. Examination of lesional skin revealed that while patients with psoriasis up-regulate LL-37 and hBD-2, atopic dermatitis lesions are characterized by a relatively reduced or absent expression of cathelicidin, defensins, and dermcidin [97]. For unknown reasons, the inflammatory response in atopic dermatitis leads to an impairment in the upregulation of these AMPs. Several theories exist to explain the relative deficiency for LL-37, including an overexpression of IL-4 and IL-13, as well as an overexpression of the anti-inflammatory cytokine IL-10 [103]. In contrast to the relative absence of other AMPs, psoriasin is found upregulated in atopic dermatitis patients. As psoriasin is thought to have preferential killing activity against *E. coli*, this may explain why atopic dermatitis patients typically do not suffer infections of *E. coli*, despite typically experiencing the infection of other bacteria [104]. Furthermore, while RNase-7 is found upregulated in the stratum corneum of psoriasis patients, RNase-7 increased appreciably in atopic dermatitis patients [105].

In addition to bacterial infections, patients with atopic dermatitis are at increased risk for viral infections, particularly HSV and vaccinia virus (VV), implicated in eczema herpeticum and eczema vaccinatum, respectively. Atopic dermatitis patients show a relative lack of hBD-2 and LL-37 [106]. As LL-37 has been shown to have broad antiviral activities against these viruses [39, 41], this may partially account for why these patients experience higher rates of superinfection of these viruses. Moreover, cathelicidin-deficient mice exhibit reduced ability to control VV replication [107]. The local deficiency of LL-37 in atopic dermatitis may in part explain not only the increased risk of bacterial superinfection, but also viral infection in this patient population.

Acne

The pathogenesis of acne is multifactorial and includes follicular hyperkeratinization, increased sebum production, hormones, colonization with *Propionibacterium acnes*, and immunologic influences. Chronnell et al. [108] were among the first to suggest a role of AMPs in acne. They analyzed both the mRNA expression and protein expression of hBD-1 and 2 in the human pilosebaceous unit. High hBD-1 and 2 expression were present in the suprabasalar layers of the epidermis, as well as the distal outer root sheath, sebaceous glands, and the pilosebaceous duct in normal skin. However, hBD-2 expression in lesional and perilesional skin in acne biopsies were higher when compared to normal skin, suggesting a role for beta-defensins in acne vulgaris. A similar, albeit less dramatic, increase in hBD-1 was also observed.

New data has also implicated a role for *P. acnes* in the induction of AMPs. Using primary human keratinocytes, one study found that distinct clinical strains of *P. acnes* could induce the expression of hBD-2, while other strains, including common laboratory strains, did not. Moreover, the expression of hBD-2 was found to be TLR2 and TLR4 dependent [109]. The selective induction of hBD-2 by different strains of *P. acnes* could in part help explain the large clinical spectrum of the disease. Individuals colonized with certain strains of *P. acnes* may produce more hBD-2 as part of host defense and therefore have less severe clinical disease. Other studies have found that *P. acnes* induces hBD-2 production in cultured human sebocyte cell lines [110]. In addition to differential response from different *P. acnes* strains, Nakatsuji et al. demonstrated that sebum free fatty acids play a role in the induction of hBD-2 in human sebocytes [111].

Because there is evidence AMP expression in acne, researchers have aimed to use these AMPs for the treatment of acne. Given the increase in antibiotic resistant *P. acnes*, the need for novel therapeutic agents is growing. McInturff et al. [112] showed the potential use of synthetic granulysin-derived peptides to treat acne. Their work found that synthetic granulysin-derived peptides (amino acids 31–50) possessing a helix-loop-helix domain killed *P. acnes in vitro*. In addition, by substituting a tryptophan for the valine at amino acid 44, the authors were able to increase antimicrobial activity, presumably by increasing hydrophobicity, resulting in a stronger association with the bacterial surface (Fig. 6.4). Finally, using L-type amino acids, they created a peptide that was less susceptible to degradation by proteases. Clinical isolates of *P. acnes* from human microcomedones were also susceptible to the synthetic granulysin-derived peptides. In addition to its microbicidal activity, the peptides also have potential anti-inflammatory effects, as it decreased *P. acnes* induced cytokine expression [112].

Rosacea

Rosacea is an inflammatory skin and ocular disease that affects nearly 3 % of the American population above the age of 30. Rosacea in the skin is often characterized by erythema, papulopustules, and telangiectasia. Evidence indicates that abnormal production of AMPs contributes to the pathogenesis of this disease. In particular, irregularities in the production of cathelicidin in the form of LL-37 are thought to contribute to the disease, through the exacerbation of inflammation and abnormal growth of blood vessels. Rosacea patients generally show abnormally high levels of LL-37 when compared to normal skin. This abnormality has been linked to irregular activity of the stratum corneum tryptic enzyme (SCTE), also known as kallikrein 5 (KLK5), which proteolytically activates hCAP18 to LL-37 [113]. Yamasaki et al. observed that high amounts of LL-37 may also result from abnormal functioning of TLRs. TLR2, in particular, has been shown to be upregulated in rosacea and has been linked to increase protease activity of KLK5 [114].

Mycobacterial Infections

Studies of cutaneous mycobacterial infections, such as leprosy, caused by *Mycobacterium leprae*, have provided great insight into the host innate immune response and the role of AMPs in skin disease. While leprosy remains a relatively rare disease in the United States, it has sparked tremendous research interest as a model for host defense and cutaneous immunity. Leprosy varies widely in its clinical presentation, which can be correlated to the host response launched against *M. leprae*. The tuberculoid form of the disease (TL) is characterized by localized infection, granulomas, and a cytokine milieu favoring cell-mediated immunity. The lepromatous form (LL), however, is a more disseminated

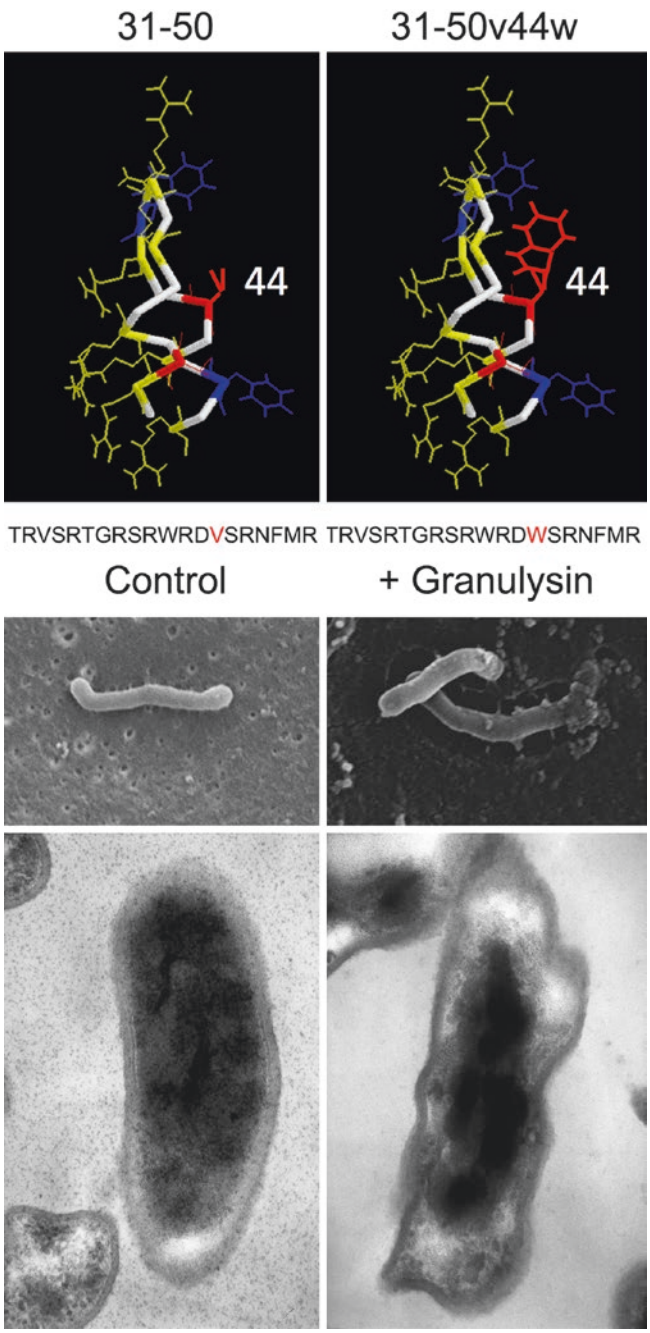

Fig. 6.4 Granulysin derived peptides are antimicrobial. Synthetic granulysin peptide containing amino acids 31–50 was altered by replacing the valine at position 44 with a tryptophan (31–50v44w) (*top panel*). The resultant peptide is more hydrophobic, and has greater efficacy in killing *P. acnes*. Scanning electron microscopy (*bottom two panels*) revealed altered surface topography (10,000X magnification, *bottom panel*) of *P. acnes*, showing decreased fimbriae and a recessed and withered surface in peptide-treated samples

infection, resulting in disfiguring nodules, and the expression of cytokines favoring humoral immunity. LL is characterized by a high organism load within affected tissues and a high level of circulating antibodies that are ineffective at controlling the infection. There also exists several intermediate states of disease, such as borderline forms, in which characteristics of both forms of the disease are present.

Ochoa et al. [115] have proposed a possible mechanism to partially explain the phenotypic mechanism seen in leprosy. Given that T cells are critical to cell-mediated immunity and cytolysis, the authors sought to characterize the level of granulysin expression, a T cell-expressed AMP with a wide antibacterial spectrum. Granulysin-expressing T cells were detected in cutaneous leprosy lesions at a six-fold greater frequency in patients with the localized tuberculoid as compared with the disseminated lepromatous form of the disease. In contrast, perforin, a cytolytic molecule that co-localizes with granulysin in cytotoxic granules, was expressed at similar levels across the spectrum of disease. Within leprosy lesions, granulysin co-localized with CD4 cells and was expressed in CD4+ T cell lines derived from skin lesions. These CD4+ T cell lines lysed targets by the granule exocytosis pathway and reduced the viability of mycobacteria in infected cells. Thus, it appears that granulysin plays a critical role in controlling mycobacterial infection and influences the spectrum of clinical disease.

Evidence has also implicated AMPs in the Toll-mediated cytotoxicity pathway in humans. Although the murine model suggests a nitrous oxide mediated mechanism for Toll-mediated cytotoxicity, evidence using primary human monocytes could not substantiate this hypothesis. However, studies demonstrate a role for LL-37 in the antimicrobial TLR response. Liu et al. [47] demonstrated that stimulation of human monocytes with TLR2 resulted in upregulation of the vitamin D receptor and the vitamin D-1-hydroxylase genes, leading to induction of LL-37 and killing of intracellular *Mycobacterium tuberculosis*. Finally, sera from African-American individuals, known to have increased susceptibility to tuberculosis, had low 25-hydroxyvitamin D and were inefficient in supporting cathelicidin messenger RNA induction. These data support a link between TLRs and vitamin D-mediated innate immunity and suggest that differences in vitamin D production may contribute to susceptibility to mycobacterial infection.

UVB Radiation

Extensive research has focused on the mechanistic links between vitamin D and cathelicidin in the skin. As the production of vitamin D is in the skin is intricately linked with ultraviolet B exposure, a discussion about AMPs would be incomplete without mention of the effects of UVB radiation. UVB has been noted for its modulatory properties on the expression and secretion of various AMPs, including increases of S100A12, S100A8/9, hBD3, RNase-7 [116], and psoriasin, which has been implicated in the recruitment of CD4+ T cells post-UVB radiation [117]. These results are mirrored in skin explants taken from donors exposed to different levels of UV [89]. Glaser et al. measured AMP expression in normal human keratinocytes both *in vivo* and *in vitro* and found after UV radiation, there was a dose-dependent increase of hBD-2, -3, RNase-7, and psoriasin [89]. In addition to improving vitamin D balance, the use of narrowband ultraviolet B as treatment has shown alterations in AMP expression in psoriatic lesions and in atopic dermatitis [118]. After six treatments of narrowband UVB exposure, the skin of psoriasis and atopic dermatitis patients both exhibited an increase in LL-37 expression. Contrastingly, narrowband UV exposure resulted in a decrease in hBD-2 expression.

Wound Healing

AMPs have been described in healing skin of mammals. For example, pig cathelicidin (PR-39) is found in the healing skin of pigs, and has pro-wound healing effects in fibroblasts. In particular, PR-39 increases the expression of certain extracellular matrix proteoglycans, which have been speculated to aid wound healing [119]. In agreement with the porcine data, LL-37 expression is enhanced in wounds [35] and has been shown *in vitro* to stimulate keratinocyte proliferation and angiogenesis. Moreover, inhibiting cathelicidins in pigs leads to increased bacterial colonization and subsequent decreased wound healing [120]. LL-37 has also been implicated in keratinocyte migration through epidermal growth factor receptor (EGFR) transactivation [121]. In addition, LL-37 and alpha-defensins induce cellular proliferation, and subsequent wound closure in airway epithelium [122, 123]. HNP-1 also decreases the expression of the collagen degrading enzymes, e.g. matrix metalloproteinase-1, while simultaneously increasing the expression of procollagen in dermal fibroblasts, suggesting one possible mechanism of action in wound healing.

In addition to LL-37 and alpha-defensins, a study has implicated the role of beta-defensins in cutaneous wound healing. Niyonsaba et al. [124] showed that hBD-2, -3, and -4 was able to stimulate the production of various pro-inflammatory cytokines and chemokines in human primary keratinocytes, including IL-6, IL-10, and monocyte chemoattractant protein-1, *in vitro*. Moreover, they found that hBDs stimulate keratinocyte migration, and that hBDs in fact serve as chemoattractants for keratinocytes. An *in vitro* wound closure assay also showed that keratinocytes incubated with optimal does of hBD-2, 3, and 4 migrated inwardly and covered a larger area of the wound, when compared with untreated or hBD-1 treated samples [124]. Keratinocyte derived growth factors and AMPs may serve as autocrine stimuli for wound healing, promoting keratinocyte migration and collagen synthesis.

In light of serious skin injuries where the structure and function of the epidermis is significantly compromised, the relative decrease in the total amount of keratinocyte derived AMPs may be in part responsible for increased risk of infection. For example, a decrease in AMP can be used to partially explain the increased risk of *Pseudomonas* infections in burn patients. Also, a deficiency in cathelicidins in mice has been reported to result in more severe and longer lasting cutaneous *S. pyogenes* infections [18]. Nonetheless, reports show that while the overall expression of AMPs in serious skin injuries remains relatively lower, individual keratinocytes express an increase in AMPs. A study profiling partial thickness burns and unburned skin tissues observed increases in LL-37, hBD1-4, dermcidin, psoriasin, and RNase-7. The authors acknowledged that this increase in AMP expression did not correlate with clinical observations of wound infections [125].

Concluding Remarks

Over the past 20 years, our knowledge of the function of skin has drastically evolved. What was once seen as simply a stagnant and physical barrier to the outside world, skin has come into light as a dynamic immune organ, capable of mounting specific and effective immune responses. The discovery of AMPs has greatly influenced the way we view the skin. From their initial discovery in invertebrate organisms, AMPs have proven to be a highly conserved and important mechanism of host defense. In addition, our ever-expanding knowledge has shown the importance of overexpression, or lack of expression, of these cationic proteins in various skin conditions. Although they vary in structure and function, the mechanism of action is generally dictated by their cationic and amphipathic nature, which is a hallmark of nearly all AMPs. It may one day be possible to develop synthetic AMPs that can be used as antibiotics and as immune modulators. Given the rapid increase in resistant bacteria, a need to add a potent antimicrobial agent to our armamentarium is imperative. With the increasing research in the field, synthetic AMPs and/or regulation of endogenous AMPs may hold promise in the future of antimicrobial therapy.

Questions

1. Describe the physical property of AMPs.

   Cationic AMPs associate with the negative bacterial membranes, resulting in binding and subsequent increased permeability of the phospholipid bilayer. As a result of the increased permeability, irreversible osmotic damage results in cell death

2. What are some known differences between alpha, beta, and theta defensins?

   Alpha defensins have wide spectrum antimicrobial activity against bacteria, fungi, and some viruses. They contribute to the host immune response by increasing the expression of TNF and IL-1, yet high concentrations are toxic to cells and may be involved in Stevens-Johnson Syndrome and toxic epidermal necrolysis

   Beta defensins differ from alpha defensins in their cysteine cross-bridging. Beta defensins also have a wide spectrum of antimicrobial activity against bacteria and fungi and are thought to have a role in cell proliferation and maturation of dendritic cells

   Theta defensins are not expressed in humans

3. What factors are thought to be important components in the induction of cathelicidin (LL-37)?

   Vitamin D and Toll-like receptor 2. The gene encoding LL-37 contains a vitamin D response element in its promoter. Recent studies show a TLR-2 dependent microbicidal activity on vitamin D-dependent induction of LL-37

4. What are the key differences of the roles of AMPs between psoriasis and atopic dermatitis?

   Psoriasis is often resistant to bacterial superinfection while atopic dermatitis often involves secondary infections. One proposed mechanism behind this phenomenon is a discrepancy of AMPs expressed in these diseases. Psoriatic lesions often upregulate the expression of cathelicidin and beta defensin, while atopic dermatitic lesions are characterized by reduced or absent cathelicidin or defensin expression. The deficiency in cathelicidin in atopic dermatitis has been linked to an impaired immune response

5. What are the roles AMPs play in wound healing?

   AMPs are implicated in keratinocyte migration (LL-37, beta defensins), cell proliferation (LL-37 and alpha-defensins), matrix metalloproteinase induction (alpha defensins), and wound-closure (beta defensins)

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B Cell Biology

7

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Abstract

B cells, central players in the adaptive immune response, produce antibodies and are essential for protective immunity. This chapter provides an overview of the current understanding of B cell biology for the dermatologist and cutaneous biologist. Antibody structure and function are reviewed in detail, outlining the varied antigenic targets of antibodies, enumerating the many effector functions of antibodies and describing how these effector functions differ by antibody isotype. Additionally, an overview of the complement system is provided, highlighting its role in the immune response and mechanisms of its activation. B cell development is summarized, with a focus on generation of the antibody repertoire and establishment of tolerance to self-antigens. Current therapeutic mechanisms of targeted B cell depletion are described and biological caveats of their use are discussed. An overview of the B cell response to infection is presented, highlighting the role of B cell – T cell interactions, formation of the germinal center response and the role of regulatory B cells. Finally, the formation, function and maintenance of long-lived plasma cells and memory B cells, key components of durable immunity, are reviewed.

Keywords

B lymphocyte • B cell • B cell receptor • Immunoglobulin • Antibody • Antigen • Fc receptor • Complement • Tolerance • Follicular dendritic cell • T follicular helper cell • Memory • Plasma cell • Memory B cell

Introduction

B lymphocytes, or B cells, together with T cells and NK cells, constitute the adaptive immune system. The adaptive immune system, which arose in jawless vertebrates more than 525 million years ago [1], complements the more ancient, innate immune system to provide protection during a primary infection. The hallmark of the adaptive immune response is the development of *memory*, or the ability to rapidly mount an effective and specific response to re-infection with a known or related pathogen.

B cells recognize antigen via the B cell receptor, which is membrane-bound antibody. Antibody can recognize and bind a wide variety of antigens, including proteins, polysaccharides, small chemicals, nucleic acids and lipids, and can recognize antigen in its native form. This is in contrast to T cells, which recognize processed protein fragments displayed by other host cells. While B cells are best known for their ability to differentiate into antibody-secreting plasma cells, they also play roles independent of antibody production. B cells are antigen-presenting cells for T cells and secrete cytokines that augment or dampen the immune response. Normal B cell responses are therefore crucial for protective immunity through both direct and indirect interactions with other cells in the immune system.

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B Cells and Antibodies Are Critical for Effective Natural and Vaccine-Mediated Immunity to Infectious Disease

In ancient times it was recognized that prior infection, such as with smallpox, one of the most devastating diseases in human history, could prevent an individual from becoming sick again upon re-exposure. Based on these observations, people in China and the Middle East developed a technique known as variolation to generate prophylactic smallpox immunity. In variolation, pus or powder from smallpox scabs was inhaled or crudely introduced into pierced skin. Despite opposition, variolation was adopted by societies throughout the world, during the seventeenth and eighteenth centuries [2]. While often effective, duration of immunity was variable and variolation carried significant risks, including the development of full-blown smallpox. Furthermore, variolation paradoxically required a constant supply of smallpox patients.

Edward Jenner, an English countryside doctor, built upon the observation that milkmaids infected with cowpox, a virus related to but far less dangerous than smallpox, rarely became sick with smallpox itself. Such individuals were remarkable for their smooth "milkmaid's complexions," which lacked the pitted smallpox scars common amongst others. In 1796, Jenner inoculated James Phipps, the young son of his gardener, with pus from a milkmaid's cowpox scab; a few weeks later, he performed a standard smallpox variolation on the boy. Phipps did not develop mild symptoms of smallpox typical of variolation nor did he become sick after subsequent variolations, indicating that the cowpox had induced a protective immune response. Thus, Jenner proved that effective protection could be induced by immunization with a related but less virulent pathogen. The term vaccination, derived from *vacca*, Latin for cow, was coined and the procedure was adopted worldwide. In 1977, less than 200 years after Jenner inoculated Phipps, the World Health Organization declared that smallpox was eradicated.

It is now known that the induction of highly specific serum antibodies is central not only to the function of the smallpox vaccine [3], but for virtually all currently licensed vaccines [4]. A notable exception is the BCG vaccine for tuberculosis, which appears to rely primarily on the induction of T cell immunity [4]. Robust serum antibody titers correlate with reduced susceptibility to infection [5]. For many vaccine antigens, including vaccinia, measles, mumps and rubella, these titers can last decades, enduring beyond the typical human lifespan [6]. Induction of this specific, protective long-lived immunity requires highly orchestrated interactions between B cells, T cells, follicular dendritic cells and other arms of the immune system.

Improving our understanding of B cells is critical if we are to develop better vaccines and strategies to treat aberrant B cell responses in autoimmunity, allergy and B cell malignancy. This chapter will introduce the basic principles underlying antibody structure and function, B cell development, the B cell response to antigen and the long-lived products of these immune responses – memory B cells and long-lived plasma cells – that mediate protection to subsequent pathogen re-challenges.

Antibody Structure and Effector Functions

Introduction

Antibodies and antisera were first described in 1938 [7] and studies to elucidate both antibody formation and structure garnered researchers Nobel Prizes in the subsequent decades. It was not until the 1960s that B cells, the cells that produce antibodies, were described [8]. B cells display antibodies on their surface, termed B cell receptors (BCRs) and secrete them into the extracellular space. Over 10^9 different specificities can exist in a single individual. One individual B cell, however, encodes only a single specificity of BCR.

Unlike the T cell receptor (TCR), which only recognizes processed, linearized protein antigens presented on MHC Class I or MHC Class II molecules, the BCR and secreted antibodies bind antigens in their native, unprocessed, forms. Like the TCR, the BCR binds protein antigens, but unlike the TCR, it also binds polysaccharides, lipids, nucleic acids and small chemicals. Antibodies bind antigens on microbial surfaces, such as bacterial capsular or viral envelope structures, as well as soluble toxins and native antigens presented by macrophages, dendritic cells (DCs) [9] and follicular dendritic cells (FDCs) [10].

The antibody is typically depicted as a "Y"-shape (Fig. 7.1a). Each antibody molecule contains two identical antigen-binding domains and a single constant domain. The antibody molecule is comprised of two identical heavy chains (IgH) and two identical light chains (IgL), each containing a variable region and constant region. Each arm of the "Y", known as a Fab (antigen binding) fragment, contains heavy and light chain variable regions that together form the molecule's unique antigen-binding domain. The stem of the "Y" is the Fc (crystalline) region, comprised entirely of IgH constant domains. For membrane-anchored antibody, the Fc region associates with Igα and Igβ transmembrane signaling proteins to form the BCR complex, enabling triggering of an intracellular signaling cascade upon antigen binding (Fig. 7.1b). For secreted antibody, the Fc region confers other various effector properties, such as the ability to fix complement and bind Fc receptors.

As antibodies can be raised against a wide range of antigens, they are valuable reagents in clinical and research applications. Antibody molecules can be conjugated to

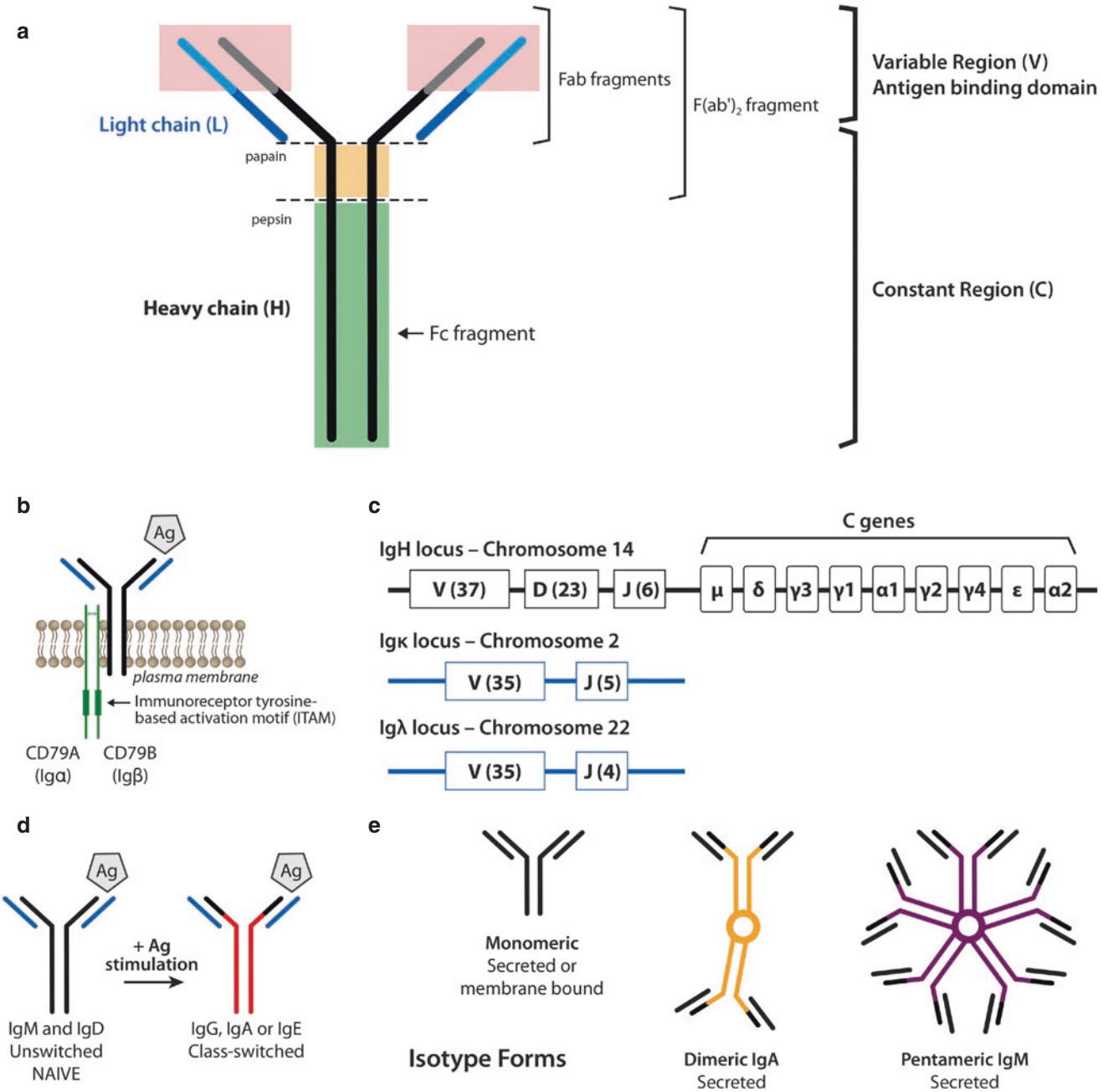

Fig. 7.1 Antibody structure and immunoglobulin locus organization. (**a**) Structure of an individual antibody molecule. Highlighted are the immunoglobulin light chains (IgL, *blue*) and heavy chains (IgH, *black*), the disulfide hinge (*yellow*), two identical antigen binding variable regions (Fab, *pink*) and the crystalline region (Fc, *green*). Cleavage sites for the generation of Fab, F(ab′)2 and Fc fragments, with papain and pepsin, respectively, are indicated. (**b**) The B cell receptor (BCR) signaling complex is comprised of membrane anchored immunoglobulin and a CD79A/B (Ig α/β) heterodimer. Following antigen (Ag) binding to the antigen binding variable region of the BCR, signaling is triggered via the CD79A/B immunoreceptor tyrosine-based activation motifs (ITAMs). (**c**) Key components of the human germline IgH locus and IgL (both k and λ) loci [11–13]. Numbers of estimated functional gene segments are indicated in parentheses following gene segment type. IgH constant (C) genes encode constant regions and dictate isotype. μ = IgM, δ = IgD, γ = IgG (IgG1, IgG2, IgG3, IgG4), α = IgA (IgA1, IgA2) and ε = IgE. (**d**) Antigenic (Ag) stimulation initiates class-switch recombination (CSR). Mature, antigenically-naïve B cells express both IgM and IgD; following Ag stimulation and class switch, they express IgG, IgA or IgE. (**e**) Secreted antibodies can exist in three forms: monomeric, dimeric (IgA only) or pentameric (IgM only) that have implications for avidity and for downstream antibody-mediated effector function

flurochromes, enzymes, toxins and chemical isotopes or used directly (ustekinumab, adalimumab**,** belimumab) to target specific antigens for the treatment of malignancies or autoimmune diseases or for flow cytometric or histologic analysis. To develop reagents that lack background binding from the Fc region, it is often useful to separate antigen-binding domains from effector domains using proteases that cleave the disulfide bonds between Fab and Fc fragments. Digestion with papain yields two identical, but separated Fab fragments (each with a single antigen-binding domain) and digestion with pepsin yields a single $F(ab')_2$ fragment with two antigen-binding domains (Fig. 7.1a). These fragments can also be labeled with fluorochromes and enzymes, etc., as described above. Using genetic approaches, the DNA encoding the variable region can be ligated to alternate Fc regions to create chimeric human-mouse monoclonal antibodies (infliximab, rituximab), which are less immunogenic than complete mouse antibodies, or antibodies with enhanced Fc-mediated functions (obinutuzumab, omalizumab). Additionally, Fc regions can be linked to receptors in order to target ligands of interest (etanercept, abatacept).

Diversity in the Antigen-Binding Variable Region Is Generated via Gene Segment Recombination and Use of Editing Enzymes

The enormous diversity in BCR specificity is generated via several mechanisms. Prior to antigen exposure, diversity in the BCR repertoire results from the recombination of a seemingly limited number of gene segments encoding the heavy (IgH) and light (IgL) variable regions and from variations in the junctional regions between these segments (Fig. 7.1c). This process is analogous to the generation of the TCR repertoire. The IgH locus, located on human chromosome 14, is comprised of three gene segments – Variable (V), Diversity (D) and Joining (J). IgL loci, found on chromosomes 2 (kappa) and 22 (lambda), contain only V and J gene segments. The IgH locus contains an estimated 37 V_H gene segments, 23 D_H gene segments and 6 J_H gene segments [11–13]. There are also approximately 35 Vk gene segments, 5 Jk gene segments, 35 Vλ gene segments and 4 Jλ gene segments [12]. A rearranged IgH will contain a single V, D and J gene segment while a rearranged IgL will contain a single V and J gene segment from either the kappa or lambda locus. Thus, combinatorial diversity yields roughly 10^6 possible IgH and IgL pairings [12].

Recombination of IgH and IgL segments proceeds in an orderly fashion mediated by the V(D)J recombinase enzyme complex, which includes two proteins encoded by genes uniquely expressed in B cells and T cells: recombination activating gene-1 (RAG-1) and recombination activating gene-2 (RAG-2). Recombinases bind signaling sequences flanking the gene segments and then bind to each other to bring the segments together for joining. Mice deficient in the RAG proteins lack mature B cells and T cells and thus have no viable adaptive immune system. Some humans with severe combined immunodeficiency (SCID) have mutations in one or both of the RAG genes, resulting in little to no V(D)J recombination [14]. Additionally junctional diversity/generates more sequences than would be present through mere recombination of the germline genes. Junctional diversity is introduced by exonuclease, which removes nucleotides at the sites of V(D)J recombination, and terminal deoxynucleotidyl transferase (TdT), which catalyzes the addition of random nucleotides.

While the above mechanics generate diversity in the pre-immune antibody repertoire, further diversification in the BCR occurs after antigen exposure and can result in the formation of B cell clones that bind pathogens and immunogens with a much higher affinity than the original clone. This process of somatic hypermutation occurs in the germinal center reaction as a result of T-dependent B cell activation. It is catalyzed by activation-dependent (cytokine) deaminase (AID) and results in the selection of point mutations focused in hotspot hypervariable regions important for binding antigen.

The Heavy Chain Constant Region Dictates Antibody Isotype and Effector Function

The effector function of an antibody is distinct from its antigen binding specificity and is dictated by the class of its heavy chain. There are five antibody isotypes – IgM, IgD, IgA, IgG and IgE – that are each encoded by a distinct constant region (C) gene. The C gene cluster follows the J gene segments on the IgH locus. All mature naïve B cells co-express transmembrane IgM and transmembrane IgD of identical specificities. Other isotypes are expressed only when the B cell is activated by antigen, after an irreversible process known as class-switch recombination (CSR) (Fig. 7.1d). Class-switch recombination does not affect the rearranged V(D)J nor does it use the RAG enzymes. Therefore, a single variable region can elicit a number of different effector functions depending on the C gene with which it is associated.

Functional properties of the various isotypes are summarized in Table 7.1. Secreted IgM is unique amongst the isotypes in that it can pentamerize, which is advantageous in binding repetitive antigen sites, such as those found on encapsulated bacteria and gut flora, and in increasing antibody avidity (binding strength) (Fig. 7.1e). IgD, initially expressed by all naïve B cells, is poorly defined in effector function. IgG is the most abundant isotype in the serum and

Table 7.1 Isotype-specific functions of antibodies

Isotype	Complement fixation	Major functions and unique properties
IgM	+++	Antigen receptor of naïve B cells
		Pentameric secreted IgM
		Fcα/μR binding
IgD	No	Antigen receptor of naïve B cells
IgG1, IgG3	++/+++	FcγR binding – all
IgG2	+	FcγR binding – all except FcγRI, FcγRIIB and FcγRIIIB
IgG4	No	FcγR binding – all except FcγRIIIB
IgA1, IgA2	No (Lectin but not classical pathway)	Mucosal immunity
		FcαR, Fcα/μR binding
IgE	No	Helminth response
		Allergic response
		FcεR1, FcεRII binding

also has the longest half-life. In humans, there are four subclasses of IgG – IgG_1 (the most abundant IgG subclass), IgG_2, IgG_3, and IgG_4. Maternal IgG can cross the placenta, providing protection to the fetus. IgA is produced primarily at mucosal surfaces such as the respiratory and gastrointestinal tracts and, when secreted, can dimerize and cross the epithelium (Fig. 7.1e). Finally, IgE plays an important role in immunity to parasites, in type I hypersensitivity and in common allergic responses. It is unique amongst antibody isotypes in that it can be bound to its high affinity Fc receptor prior to antigen binding.

Class-switch is heavily influenced by the cytokine milieu surrounding the B cell. Cytokines may be produced by $CD4^+$ T cells, macrophages or dendritic cells and can promote, inhibit or augment class-switch to specific isotypes. For example, in mice, IL-4 promotes class-switch to IgG_1 and IgE but blocks class-switch to IgG_{2a} and IgG_3. Conversely, IFN-γ induces class-switch to IgG_3 and IgG_{2a}, but blocks class-switch to IgG_1 and IgE. Along with IL-5, IL-6 and TGF-β, IL-4 and IFN-γ play important roles in influencing B cell effector function.

Effector Functions of Antibodies

Neutralization

Some antibodies function by binding to and directly neutralizing their antigens, a role that does not require the help of other immune cells or immune cell products. Neutralizing antibodies are effective against bacterial toxins, viruses and adhesion factors expressed on bacteria that facilitate host entry. Thus, B cells (through antibodies) are capable of blocking infection before it occurs. Neutralizing antibodies are central to the protective effects of the tetanus toxoid, pneumococcal, hepatitis A and B and HPV vaccines. Development of effective vaccination strategies to induce neutralizing antibodies against human immunodeficiency virus (HIV) and influenza virus have been more challenging, in large part because these viruses have intrinsic mechanisms to mutate or mask vulnerable surface epitopes.

Complement Activation

Most antibodies do not neutralize their targets, but instead initiate a protective response by recruiting other components of the immune system, such as the innate complement system. The complement system is a collection of circulating and cell membrane proteins that play critical roles in host defense against microbes and in tissue injury. The complement system can be activated by pathogens directly or by antibodies, thus linking the adaptive and innate immune systems. Ability to activate the complement pathway varies widely amongst antibody isotypes; IgM and most IgG isotypes are robust at activating the complement cascade while IgE and IgA are not (Table 7.1).

The complement pathway is a series of controlled proteolytic cleavage reactions that ultimately lead to microbe or host cell destruction, neutrophil recruitment and inflammation. There are three complement pathways – classical, mannan-binding lectin and alternative – which all converge on a common intermediate protease, C3 convertase. This convertase is in turn cleaved with its products eliciting various effector functions.

Activation of the classical pathway is initiated by binding of the first complement component, C1q, to the Fc portion of an antibody:antigen complex, known as an immune complex. This binding triggers a series of proteolytic reactions, leading to C3 cleavage and activation. Activated C3b fragments can opsonize (coat) microbes and these fragments in turn bind to specific complement receptors on phagocytes, ultimately leading to microbe phagocytosis. When binding to microbes such as Neisseria, C3b fragments initiate formation of the membrane-attack complex, which creates pores in the pathogen membrane, leading to its lysis. Lastly, C3 activation and subsequent reactions stimulates release of inflammatory

mediators, enhances vascular permeability and triggers neutrophil recruitment and activation.

There are six complement receptors (CR) that vary in cell expression and in effector function. Red blood cells (RBCs) express the most CRs, binding immune complexes for later removal in the spleen. CRs can be found on macrophages, endothelial cells, mast cells, dendritic cells, FDCs and even B cells themselves.

Complement activation can stimulate and enhance the humoral response via binding of B cell-expressed CR1/CD35 and CR2/CD21. CR2 functions as a BCR auxiliary receptor, as recognition of C3 coated pathogens by the BCR and CR2 simultaneously triggers a stronger net signal than BCR alone. Additionally, complement receptors on FDCs bind antigens coated with complement proteins, presenting them to B cells in the germinal center reaction, thus facilitating B cell activation and selection during the immune response.

The complement system is tightly regulated and dysregulation of or deficiencies within the complement cascade can lead to poor pathogen clearance and immune complex-driven disease. Complement opsonization is a primary clearance mechanism for polysaccharide encapsulated bacteria, so individuals with complement deficiencies can have serious Hemophilus, Streptococcal and Neisseria infections [15]. Binding of antigen:antibody immune complexes to complement receptors on red blood cells is critical to their clearance in the spleen and liver. Thus, in systemic lupus erythematosus (SLE), deficiencies in CR1/CD35 on red blood cells and C1q, C2 and C4 contribute to immune complex deposition and subsequent sequelae in the skin and kidney. Conversely, complement activation promotes tissue damage in many autoimmune situations, such as in bullous pemphigoid [16, 17]. Complement measurement is therefore a useful clinical disease metric in both autoimmune and infectious contexts.

Fc Receptor-Mediated Cell Activation

In addition to antigen neutralization and complement activation, antibodies activate other arms of the immune system via binding to specific Fc receptors (FcR) on the surfaces of immune cells. All known human FcRs and their expression patterns and functions are outlined in Table 7.2. Specific FcR-mediated effector functions are described below.

FcR-Mediated Opsonization and Phagocytosis In addition to recognizing antigens opsonized with complement proteins, phagocytes bind immune complexes directly via binding of phagocyte FcR to Fc portions of immune complexes. Macrophages, dendritic cells and neutrophils bind and phagocytose pathogens opsonized with antibody, then degrade the pathogens within phagolysosomes. Antibody-mediated phagocytosis is the major form of defense against encapsulated bacteria such as pneumococcus.

FcR-Mediated Antibody-Dependent Cellular Cytotoxicity FcγIIIA is expressed on natural killer (NK) cells and binds to multiple IgG subclasses. Binding initiates a process known as classical antibody-dependent cell-mediated cytotoxicity (ADCC), in which the opsonized cell is brought into close proximity to the NK cell, which then discharges granules to lyse the cell. ADCC is one mechanism by which therapeutic antibodies deplete target cells in autoimmune disease and cancer.

FcR-Mediated Eosinophil and Mast Cell Degranulation IgE:FcεRI ligation can trigger degranulation by eosinophils or basophils. Parasites such as helminthes evade phagocytosis because of their large size. When opsonized with IgE, they can bind to FcεR1 on eosinophils which then destroy them by degranulation. IgE:FcεRI-induced eosinophil and mast cell degranulation also play important roles in tissue damage in asthma, urticaria and other allergic disease.

FcR-Mediated Antibody Recycling and Transport The neonatal FcR, FcRn, is expressed in the placenta, neonatal intestinal epithelium and adult vascular endothelial cells [18]. It binds maternal IgG in blood or milk and transports it across the placenta or intestinal lumen, providing short-term passive immunity to the fetus and neonate. It also plays a homeostatic role by recycling serum IgG and protecting it from catabolism. In a therapeutic context, it has been hypothesized that high dose intravenous immunoglobulin (pooled IgG from donors) competes with pathogenic auto-antibody for binding to FcRn, resulting in more rapid degradation of pathogenic IgG antibodies [19].

FcR-Mediated Downmodulation of the Immune Response FcR activity is not exclusively activating. FcγRIIB binds all IgG subclasses with low affinity and this binding can mediate endocytosis of immune complexes as well as triggering of negative signaling cascades that dampen the immune response. FcγRIIB is expressed on B cells and tranduces downregulating signals via immunoreceptor tyrosine-based inhibition motifs (ITIMs). FcγRIIB influences peripheral tolerance by binding low-affinity, autoreactive IgG^+ B cells and blocking clonal expansion [20] and, in the context of a productive immune response can negatively regulate B cell activation [20]. FcγRIIB, is also involved in plasma cell survival [21].

Factors That Modulate Antibody: FcR Interactions Antibody glycosylation can affect binding to FcRs and thus can also affect antibody effector function. Additionally, FcγR

Table 7.2 Fc receptors and their functions

Fc Receptor	Ig Ligand (Avidity)	Cell expression pattern	Downstream effector function
FcγRI (CD64)	IgG_1,IgG_3, IgG_4 (high to very high)	Macrophages	Phagocytosis
		Monocytes	Endocytosis
		Neutrophils	Antigen presentation
		Eosinophils	Respiratory burst
		Mast cells	
		Dendritic cells	
FcγRIIA (CD32)	IgG_1, IgG_3 (medium to high) IgG_2, IgG_4 (very low)	Macrophages	Phagocytosis, degranulation
		Monocytes	
		Neutrophils	
		Eosinophils	
		Basophils	
		Mast cells	
		Platelets	
		Langerhans cells	
FcγRIIB (CD32)	IgG_3 (low) IgG_1, IgG_4 (very low)	T cells	Phagocytosis
		B cells	Feedback inhibition of B cells and macrophages
		Macrophages	
		Monocytes	
		Neutrophils	
		Basophils	
		Mast cells	
FcγRIIC (CD32)	All IgGs? (unclear)	NK cells (not in all humans)	Antibody-dependent cell-mediated cytotoxicity (ADCC)
FcγRIIIA (CD16)	IgG_1, IgG_3 (medium) IgG_2, IgG_4 (very low)	T cells	ADCC by NK cells
		NK cells	
		Macrophages	
		Monocytes	
		Neutrophils	
		Mast cells	
FcγRIIIB (CD16)	IgG_1, IgG_3 (low to medium)	Neutrophils	Phagocytosis
		Basophils	
FcRn (*not a classical Fc receptor)	All IgGs	Syncytiotrophoblasts	Transports maternal IgG across placenta
		Endothelia	Maintenance of serum IgG levels
		Mucosal epithelia	
		Hepatocytes	
FcεR1 (CD23)	IgE (high)	Mast cells	Degranulation
		Basophils	
FcεRII (CD23)	IgE (low)	B cells	Regulation of antibody response
		Eosinophils	Degranulation
		Langerhans cells	
Fcα/μR (CD351)	IgM, IgA_1, IgA_2 (medium)	B cells Mesangial cells	Endocytosis
		Intestinal cells	
		Macrophages	
FcαR1 (CD89)	IgA (low to medium)	Monocytes	Phagocytosis
		Macrophages	Degranulation
		Neutrophils	
		Eosinophils	

polymorphisms can affect binding of the receptor to IgG and this in turn can lead to increased susceptibility to autoimmune diseases and infections. Several FcγR alleles have been associated with increased risk for developing systemic lupus erythematosus, rheumatoid arthritis and Wegener's granulomatosis, amongst other autoimmune diseases [22]. In a murine model of bullous pemphigoid, mice deficient in activating FcγRs, but not in the inhibitory FcγRIIB, have a reduced disease phenotype [23]. Thus, dysregulated or deficient expression of FcγRs can have a variety of effects as these receptors both promote and negatively regulate the immune response.

Summary

Antibodies contain two identical antigen binding domains (Fab domains) and a single effector domain (Fc region). Antigen binding diversity in the mature naïve B cell repertoire results from somatic recombination of assorted Variable, Diversity and Junctional (V, D, and J) gene segments and editing at gene segment junctions during the recombination process. Upon activation, some B cells undergo class-switch recombination, resulting in antibodies with the identical antigen specificity but different heavy chain isotypes (IgG, IgA, IgE), each with specialized effector functions. One role of antibodies is to directly neutralize pathogens. Via complement activation or engagement of Fc receptors on a variety of immune cells, antibodies can also trigger effector processes that result in pathogen destruction. Such effector functions include phagocytosis, degranulation and antibody-dependent cell-mediated cytotoxicity and require the recruitment of arms of the innate immune system including NK cells, neutrophils, eosinophils, mast cells and macrophages. Lastly, antibodies play immunomodulatory functions. Antibody binding to Fc receptors on B cells provides feedback inhibition to activated B cells. Binding to Fc receptors on epithelia and endothelia regulates protective antibody levels in the blood and tissues.

B Cell Development and Tolerance

Introduction

In 1965, Max Cooper formally identified the cells that give rise to antibodies and the humoral response [8]. Dr. Cooper was working with chickens, where the cells are derived from the Bursa of Fabricius, and thus the term "B" cell was coined. In mammals, B cell lymphopoiesis occurs primarily in the bone marrow. B cells are continually produced throughout life, though generation slows with advanced age [24].

B cells, T cells and NK cells originate from a common lymphoid progenitor cell, which is in turn derived from a pluripotent hematopoietic stem cell (Fig. 7.2). B cell development can broadly be divided by geography – initial stages (pro-, pre- and immature) occur "centrally" in the bone marrow. The last two developmental stages (transitional and mature) occur in the periphery, which includes secondary lymphoid organs such as the spleen and lymph nodes. B cell developmental stages are defined by the expression of markers that are turned on and off and by rearrangement of the IgH and IgL loci. Developing B cells, like T cells, pass through both positive and negative selection checkpoints. The BCR must bind ligand to be positively selected; however, strong binding to self-antigen will generally trigger negative selection. The vast majority of developing B cells will die or be rendered unresponsive due to unsuitable and/or autoreactive BCRs.

In this section, we will review the basic steps in B cell lymphopoiesis and highlight two monoclonal antibodies that selectively deplete B cells at specific developmental stages. We will also describe B cell-intrinsic and B cell-extrinsic mechanisms – in both the bone marrow and in the periphery – that prevent the proliferation of autoreactive B cells in healthy individuals.

Developing B Cells Must Productively Rearrange Both the Immunoglobulin Heavy and Light Chain Loci to Exit the Bone Marrow

The first cell in the B cell development assembly line is known as a pro-B cell, which differentiates from the common lymphoid progenitor cell. The pro-B cell undergoes rearrangement at the IgH locus (first D to J and then V to DJ). If rearrangement on the first allele results in an in-frame V(D)J, the IgH locus on the other allele is turned off in a process known as allelic exclusion, ensuring that the B cell expresses an IgH of a single specificity. Should the first allele have a nonproductive V(D)J rearrangement (a sequence that cannot result in a properly folded, functional IgH molecule), the IgH locus on the second allele undergoes rearrangement. Nonproductive V(D)J rearrangements on both alleles results in death of the pro-B cell.

Productive IgH rearrangement allows the pro-B cell to progress to the pre-B cell stage. At this stage the IgH protein pairs with a surrogate IgL to form a pre-BCR. Meanwhile, the pre-B cell undergoes rearrangement at the IgL locus (first the kappa (k) light chain genes, then, if they fail, the lambda (λ) light chain genes). Again, the principle of allelic exclusion applies: an in-frame VJ IgL rearrangement on one allele results in the remaining IgL locus on the other allele being turned off. Without productive rearrangement on any allele, the pre-B cell dies. If

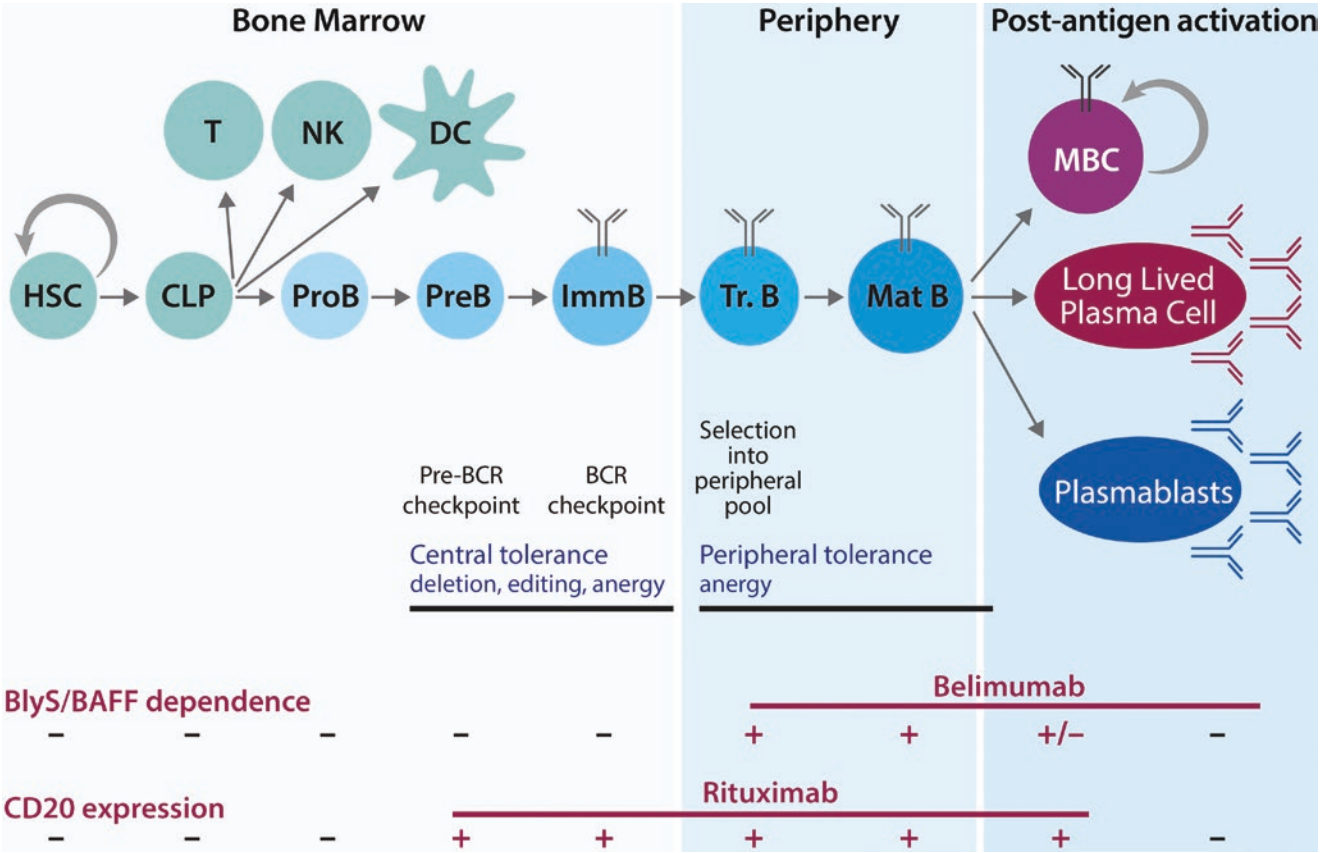

Fig. 7.2 B cells develop in an ordered maturation process that includes central and peripheral tolerance checkpoints and is characterized by distinct marker expression and survival factor dependence. Development stages include the hematopoietic stem cell (HSC), common lymphoid precursor (CLP), pro-B, pre-B, immature (Imm) B cell, transitional (Tr) B cell and the mature (Mat) B cell. Timing of B cell receptor (BCR) checkpoints and tolerance mechanisms are depicted. Following antigen stimulation, mature B cells can differentiate into memory B cells (MBC) or antibody-secreting plasmablasts (short-lived) or long-lived plasma cells. MBCs and long-lived plasma cells constitute humoral memory. Therapeutically, specific developmental populations of B cells can be deleted via the monoclonal antibody, belibumab, which blocks the soluble survival factor, BLyS/BAFF or rituximab, a chimeric antibody which targets $CD20^{+}$ B cells

productive IgH and IgL rearrangements are achieved, the pre-B cell is termed an immature B cell.

Immature B Cells Are Evaluated for Strong Self-Antigen Binding

Immature B cells express a true surface BCR of the IgM isotype and express the co-receptors required for signaling. Via this BCR complex, immature B cells sample antigens within their environment. If there is little to no antigen recognition – indicating low autoreactivity – they shut off the recombinase activating gene (RAG) enzymes, preventing further rearrangement, and exit the bone marrow. If the BCR binds antigen more tightly – indicating potential autoreactivity – they die or undergo receptor editing, described below.

B cells exit the marrow as immature cells and undergo final stages of maturation in the periphery, termed the transitional B cell stage [25, 26]. Early transitional cells that bind tightly to antigen die via apoptosis in another critical checkpoint for potential autoreactivity, and later transitional cells appear to undergo positive selection events into the mature B cell pool [27]. Transitional B cells require stimulation with B cell activating factor (BAFF) to survive, and without it die before transitioning to maturity [28–30]. The half-life of transitional B cells is only a few days and in a healthy adult most cells die without entering the mature pool.

A mature B cell expresses its BCR in both the IgM and IgD isotypes and expresses markers that distinguish it from its precursors. Mature B cells continually recirculate via the blood and lymphatics through the spleen, lymph nodes, tonsils, bone marrow, mucosa and other tissues. These "naïve" (non-antigen activated) mature B cells sample the environment for cognate antigen to bind. Mature B cells do not self-renew and turn over every 2–4 months [31, 32]. They rely on stimulation with BAFF and ligand-independent tonic non-BCR signals for survival. When a naïve mature B cell binds

cognate antigen via its BCR, the cell is activated and an immune response is initiated, as detailed later.

Tolerance Mechanisms Limit the Expansion of Autoreactive Lymphocytes

Given the incredible antigen-binding diversity in the naïve B cell repertoire, it may seem surprising that autoimmune diseases are not more common. In healthy humans there are two major check points where autoreactive B cells are managed: central (during early development in the marrow) and peripheral (during later stages of maturation outside of the marrow) (Fig. 7.2). Central tolerance mechanisms help manage immature B cells. It is estimated that approximately 75 % of the human immature B cell repertoire is self-reactive [33]. If an immature B cell binds antigen strongly, it can undergo receptor editing whereby RAG genes are re-expressed and mediate further recombination of light chain genes in an attempt to generate antibody of an alternate specificity [34]. Immature B cells that continue to bind antigen strongly die by apoptosis (negative selection).

As not all self-antigens localize to the bone marrow, autoreactive lymphocytes must be managed in the periphery as well. Peripheral tolerance is a collection of mechanisms that prevent transitional or mature self-reactive B cells from becoming activated. Peripheral tolerance mechanisms include deletion by apoptosis, functional inactivation by anergy or regulatory cells and physical sequestration of self-antigen. When peripheral tolerance is broken, pathogenic autoreactive responses can be initiated.

Deletion Autoreactive B cells in the periphery can die by apoptosis when they encounter antigen in the absence of co-stimulatory signals. This can occur because cognate helper T cells were themselves deleted thorough negative selection or rendered anergic or because the self-antigens lack innate activating signals characteristic of pathogens.

Anergy and Hyporesponsiveness After encounter with self-antigen, some B cells become functionally unresponsive, or anergic, to further simulation. Such cells express low levels of surface BCR and have an inhibited capacity to be activated. For example, anergic B cells upregulate SHIP1 [35], a phosphatase that negatively regulates BCR signaling [36]. Additionally, self-reactive B cells may be more dependent on BAFF stimulation for survival; in competition with non-reactive cells, many self-reactive B cells are disadvantaged [37–39], although this scenario does not necessarily apply to all self-reactive B cells [40]. Generally, anergic B cells have a reduced lifespan relative to other, non-autoreactive mature B cells [41].

Regulatory Cells Specialized regulatory cells also play a role in the maintenance of peripheral tolerance. These cells are discussed later in the section on the immune response.

Mechanisms of Targeted B Cell Depletion

Unique surface marker 'fingerprints' for B cells at different developmental stages can be used to develop therapeutic strategies to selectively deplete B cell subpopulations. One such marker is CD20 (Fig. 7.2), a B cell-specific transmembrane protein with no defined function or ligand to date [42]. CD20, commonly used as a B cell identification marker, is expressed on pre-, immature-, mature and memory B cells, but is down-regulated on plasmablasts and long-lived plasma cells. Rituximab is an anti-human CD20 chimeric monoclonal antibody that depletes CD20-expressing B cells via a combination of antibody-dependent cellular cytotoxicity, complement-mediated cellular toxicity and directly inducing apoptosis [43]. Rituximab treatment depletes most B cell precursors, mature and memory B cells, but spares lymphoid and pro-B precursors and long-lived plasma cells in the marrow. Thus, following rituximab-mediated B cell depletion, the B cell pool will slowly reconstitute itself. Furthermore, vaccine-induced and naturally acquired standing antibody titers to pathogens are spared. Rituximab is approved by the U.S. Food and Drug Administration for the treatment of B cell lymphoma, rheumatoid arthritis and ANCA-associated vasculitis and is used off-label to treat systemic lupus erythematosus and immunobullous disorders [44].

Another approach to B cell depletion is to target soluble factors required for their survival. One successful target is B cell activating factor (BAFF; also known as BLyS). BAFF is a TNF superfamily ligand that binds BAFF-receptor (BAFF-R) on transitional and mature B cells and is required for their survival [28, 45, 46]. Memory B cell survival as a whole is independent of BAFF [47], although subsets differ in their dependence [48]. While plasma cells utilize BAFF signals to survive, they are not absolutely dependent on BAFF. APRIL, a ligand related to BAFF, can compensate for BAFF loss. BAFF is an appealing therapeutic target in B cell mediated autoimmunity because some autoreactive B cells may be more BAFF-dependent than non-autoreactive populations [49, 50]. Belimumab is a human monoclonal antibody that targets BAFF, thus inhibiting receptor binding. Belimumab treatment depletes immature and mature B cells but spares plasma cells and the majority of memory B cells. As with CD20-targeted depletion, the B cell pool can reconstitute as precursor populations in the bone marrow are spared. In 2011, the U.S. Food and Drug Administration approved belimumab for the treatment of systemic lupus erythematosus (SLE), the first drug approved for the treatment of SLE since hydroxychloroquine in 1955 and aspirin in 1948 [51, 52]. Related drugs are in development, including

ones that block BAFF via alternate mechanisms or that block BAFF and APRIL simultaneously.

Summary

B cell development is an ordered maturation process that starts from a pluripotent hematopoietic stem cell in the bone marrow and concludes with a mature B cell in the periphery. The goal of early stages of development is the formation of a B cell precursor bearing a fully functional B cell receptor of a single antigen specificity. Next, developing B cells are evaluated for autoreactivity (central tolerance). Immature B cells with strongly reactive B cell receptors can be deleted directly or can undergo receptor editing, in an attempt to generate receptor of a different specificity. Next, newly generated transitional B cells exit the bone marrow and undergo final stages of maturation and selection in the periphery. This population includes cells with weak autoreactivity, which can be managed though deletion or other mechanisms that suppress their activity (peripheral tolerance). Finally, a minority of transitional B cells are positively selected into the mature B cell pool. This mature B cell pool is comprised of B cells of diverse antigenic specificities. Mature B cells continually recirculate through the spleen, lymph nodes, marrow and peripheral tissues via the blood and lymphatics, surveilling for infection.

Breaks in central and/or peripheral B cell tolerance underlie autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis and immunobullous skin disease. Malignant proliferation of B cell populations results in lymphoma. Targeted monoclonal antibodies that deplete B cells can be effective therapies for B cell malignancies and B cell-mediated autoimmune disease. Such drugs include rituximab and belimumab, which target CD20 or BAFF-R expressing B cell subpopulations.

B Cells in the Immune Response

Introduction

Upon binding to its cognate antigen, a B cell becomes activated. B cell receptor activation is intrinsically regulated – positively and negatively – through accessory surface proteins. Different types of antigens and the involvement of $CD4^+$ T cells shape the B cell response. A primary goal of the B cell response is to produce antibody, which binds the pathogen or pathogen-produced toxins, mediating a variety of effector functions as described above (Antibody Structure and Function). $CD4^+$ T cell help enables another, more temporally distant goal – that of achieving long-term immunity (memory) to the pathogen.

This section will describe B cell signaling and B cell participation in the immune response. The focus is the T cell-dependent immune response, although the T-independent B cell response is reviewed as well. Specific interactions between T cells and B cells that profoundly influence B cell fate are highlighted.

Activation of the B Cell Receptor Signaling Complex Triggers a Protein Kinase Signaling Cascade

B cell activation begins with ligation of antigen to the BCR complex which triggers an intricate and highly regulated signaling cascade [53]. The BCR does not on its own trigger downstream signaling following antigen binding, but rather, it requires invariant transmembrane proteins Igα (CD79a) and Igβ (CD79b) – which each have immunoreceptor tyrosine-based activation motifs (ITAMs) – to initiate a protein kinase signaling cascade. These proteins associate with a portion of the immunoglobulin heavy chain constant region and are phosphorylated by associated kinases. The phosphorylation cascade culminates in the activation of phospholipase C-γ (PLC-γ) and guanine-nucleotide exchange factors (GEFs). PLC-γ cleaves a membrane phospholipid, resulting in an increase in intracellular Ca^{++} levels. This in turn leads to the activation of two transcription factors: NF-kB and NFAT. Guanine-nucleotide exchange factors activate a MAP kinase cascade that leads to the activation of another transcription factor, AP-1. Together, these three transcription factors turn on genes important for proliferation, differentiation, antigen presentation and cytokine production.

BCR signaling is tightly regulated. At the initiation stage, crosslinking of two or more BCRs with antigen is required for triggering the downstream signaling cascade (Fig. 7.3). Subsequently signaling can be both positively and negatively modulated. The B cell coreceptor complex – CD19, CD21/CR2 and CD81 – is activated by antigens opsonized with complement and augments BCR signaling by recruiting one of the guanine-nucleotide exchange factors. BCR signaling can be negatively regulated by several B cell-intrinsic proteins, such as CD22 and FcγRIIB, that contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails. ITIMs recruit the phosphatases SHIP1 and SHP1, which dephosphorylate and thus deactivate kinases involved in the BCR signaling cascade. SHIP1 and SHP1 are upregulated in germinal center B cells and may be involved in their selection by increasing the minimum threshold for BCR signaling, thus favoring higher-affinity B cells [36]. Anergic B cells – B cells that are unresponsive to antigen stimulation – have chronic activation of SHIP1 [35].

In addition to recognizing antigen via the BCR, B cells engage antigen or modified antigen via surface receptors,

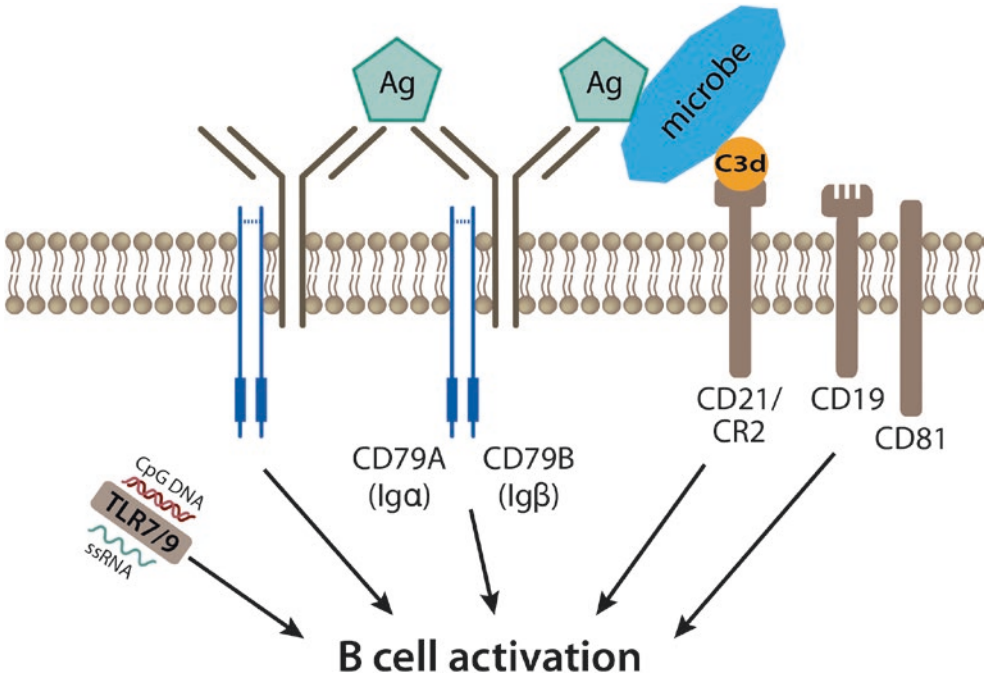

Fig. 7.3 B cell activation requires B cell receptor (BCR) cross-linking and is augmented by additional signals. B cell activation is initiated by antigen (Ag) binding induced BCR cross-linking. Additionally, the BCR co-receptor complex – CD21/CR2, CD19 and CD81 – can further enhance signaling. CD21/CR2 binds complement-coated microbes and activates CD19. Toll-like receptors (TLRs) recognize motifs unique to different classes of pathogens and further activate B cells, thus linking the innate and adaptive immune systems

particularly Toll-like receptors (TLRs), CD21/CR2 and FcγRIIB. The latter two proteins manipulate the BCR signaling cascade by binding complement-bound antigen or IgG-bound antigen respectively. TLR activation can exquisitely tune B cell antigen presentation, differentiation and class-switch during the B cell immune response [54].

B Cells Responses to Lipid and Polysaccharide Antigens Do Not Require T Cells

Humoral responses are the principal defense against polysaccharide and lipid antigens, and T cells are not required to mount these "T-independent" (TI) responses. In TI responses, antigen binding induces B cell activation, proliferation, expansion and differentiation into antibody-secreting plasma cells. Resultant antibodies are typically IgM and low affinity, identical to those produced by their naïve precursors. There are two forms of T independent responses, classified by antigen type. Type I responses involve pathogens that engage B cell Toll-like receptors (TLRs) as well as the BCR. Type II responses involve polysaccharide antigens that exclusively activate the BCR and induce strong cross-linking because of their repeating antigenic epitopes. T-independent type II antigens can elicit both memory B cells and a long-lasting antibody response [55–57], although antibody titers are not boosted upon repeat immunization [58]. Polysaccharide-based vaccines include Pneumovax and Menomune, which provide protection against pneumococcus bacteria and bacterial meningitis, respectively.

B Cell Responses to Protein Antigens Require Cognate B-T Interactions

An effective humoral responses against protein antigens requires both B and T cells is and is termed a "T-dependent" (TD) response. B cell responses primarily occur in secondary lymphoid organs such as the spleen or lymph node. B cell activation occurs when a naïve B cell binds cognate antigen, triggering intracellular signaling and receptor-mediated endocytosis of antigen. Antigen is processed and the resultant peptide fragments presented on MHC class II molecules for display to $CD4^+$ T cells.

B and T cells are located in separate areas of the spleen and lymph node, so during early activation they must be brought into close proximity. Activated B cells alter expression of chemokine receptors, downregulating receptors for chemokines expressed in the B cell follicle and upregulating receptors for chemokines expressed in T cell zones. Thus, activated B cells migrate toward T cells and, similarly, dendritic cell-activated T cells migrate towards B cells. When B and T cells specific for the same antigen meet and have a cognate MHC Class II:TCR interaction, the T-dependent response is initiated. Additional direct interactions between B cells and T cells further stabilize the conjugate.

The B cell response to T-dependent antigen is highly orchestrated and occurs in overlapping stages (Fig. 7.4). The early "extrafollicular" response, so called as it occurs outside of the B cell follicle at the T cell zone – follicle border, is characterized by B cell proliferation and differentiation into short-lived plasmablasts that secrete copious amounts of low affinity

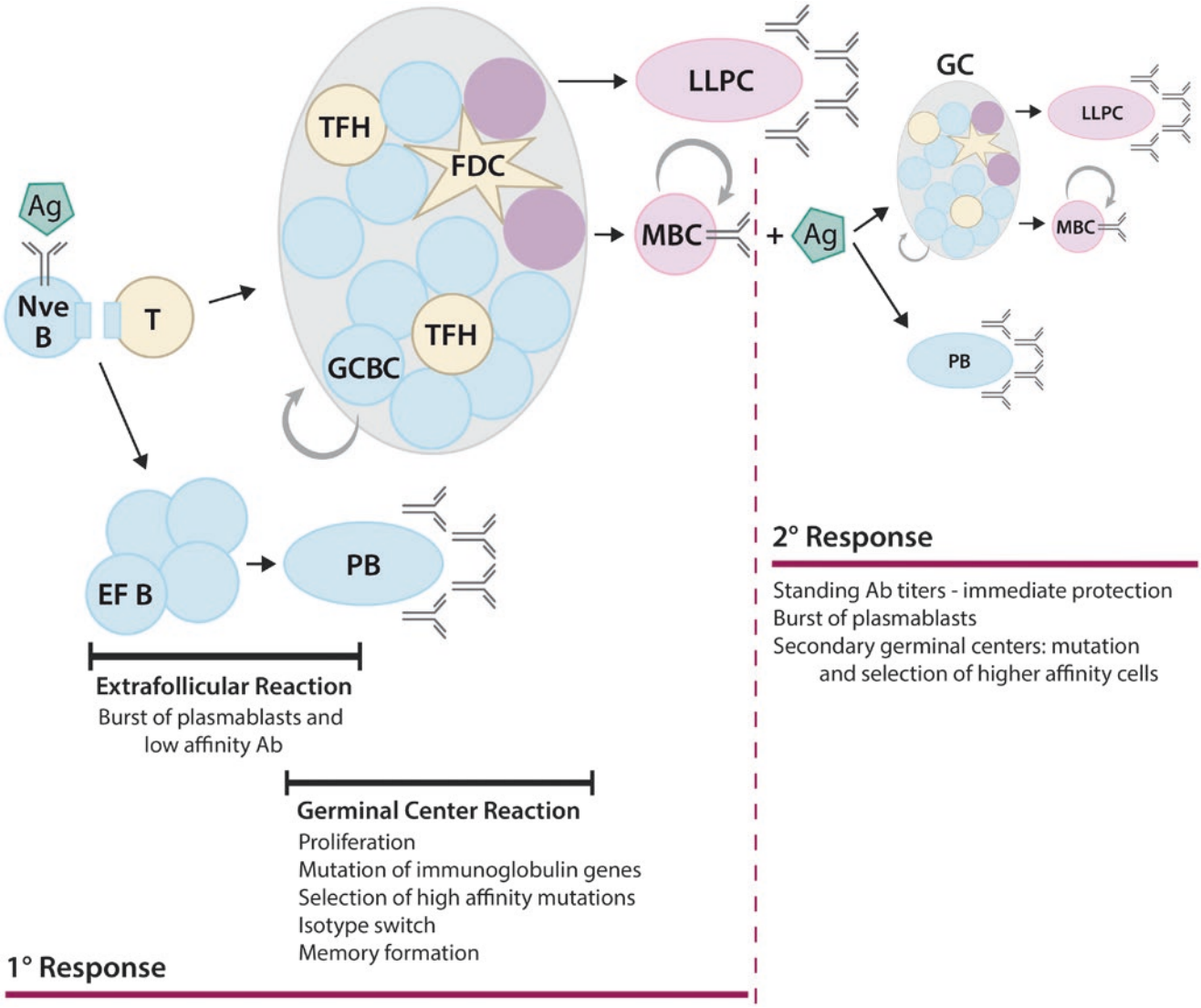

Fig. 7.4 B cells in the immune response. Following binding to its cognate antigen (Ag), the naïve B cell (Nve B) presents antigen-derived peptide to an activated CD4+ T cell and is itself activated. Rapid proliferation results in the formation of an extrafollicular focus of B cells (EF B), which is characterized by rapid differentiation into short-lived plasmablasts (*PB*) that produce a large burst protective low-affinity IgM antibody (Ab). With time, some B and T cells can seed a germinal center (*GC*). Germinal center B cells (*GCBC*) proliferate rapidly and interact with follicular dendritic cells (*FDC*) and T follicular helper cells (*TFH*). Somatic hypermutation, competition between B cell clones and affinity maturation yield B cells with higher affinity for antigen. Class-switch recombination produces clones with switched immunoglobulin isotypes. Ultimately, the germinal center produces long-lived plasma cells (*LLPC*), terminally differentiated cells that exclusively produce antibody, and memory B cells (*MBC*). Upon antigen re-stimulation in a secondary (2°) response, MBC rapidly differentiate into plasmablasts or form new germinal centers and undergo further affinity maturation

antibody. This early extrafollicular response is important for gaining rapid control of a new infection, and in the case of persistent self-antigen, can contribute to autoimmunity [59, 60]. Some memory B cells are formed during this time, but are typically unmutated and of low-affinity compared with those that are generated later in the response [61, 62].

After several days, some interacting B and T cells begin to express the master transcriptional regulator Bcl-6 and migrate into the follicle where they continue to proliferate and form nascent germinal centers (GC). The germinal center is a site of intense B cell proliferation, BCR mutation and ultimately B cell differentiation into memory B cells and plasma cells. Germinal center B cells (GCBC) can undergo somatic hypermutation, potentially altering the structure and affinity of the antigen-binding region of the BCR. They can also undergo class switch recombination, resulting in permanent switch away from IgM and IgD heavy chains to IgG, IgA or IgE. Both somatic hypermutation and class switch recombination are dependent on B cell-intrinsic expression of the DNA-editing deaminase, activation-induced cytidine deaminase (AID). GCBC divide on average every 6 h, with one precursor producing as many as 5000 progeny in a week [63]. There is

intense competition between B cells for critical interactions with cognate T cells, follicular dendritic cells and survival signals, and this competition results in the death of the vast majority of germinal center B cells. Those that survive and continue to propagate tend to be of higher affinity, a process referred to as affinity maturation. Ultimately, a germinal center B cell can undergo one of three fates: death, division (with retention in the germinal center), or positive selection and differentiation into the memory B cell or long-lived plasma cell pools (with exit out of the germinal center).

B cell participation in the germinal center comes at a cost, as germinal center B cells do not immediately contribute to pathogen clearance. Germinal center kinetics differ based on tissue and antigen accessibility. For example, murine influenza virus studies have shown that germinal center B cell frequency peaks later in the lungs than in the lymph nodes or spleen [64], suggesting that rapid production of antibody by plasmablasts (and thus more rapid influenza virus clearance) is prioritized over germinal center participation amongst lung B cells. While the germinal center reaction may be less immediately relevant to pathogen clearance when compared to extrafollicular plasmablast production, its products (memory B cells and long-lived plasma cells) play a vital role in the response to re-infection. Thus, the germinal center is an investment in the immunological future of the host.

Follicular Dendritic Cells Shape the Geography of the Germinal Center

Germinal center B cells (GCBC), T follicular helper cells (T_{FH}) and follicular dendritic cells (FDC) are the critical cellular components of the germinal center. Importantly, FDCs are not professional antigen-presenting dendritic cells. Rather, they capture and retain immune complexes on their surfaces via complement receptors and Fc receptors. FDCs present unprocessed, native antigen to GCBC and help mediate their survival [65]. They also produce chemokines that regulate lymphocyte migration and germinal center architecture. FDC density is varied within the germinal center, providing a structural framework for GCBC migration and division.

T Follicular Helper Cells Interact with Germinal Center B Cells to Promote B Cell Selection, Proliferation, Class-Switch and Differentiation

T cell help to B cells is fundamental to the adaptive immune response and the generation of immunological memory. In the context of a germinal center reaction, this help is provided by T follicular helper cells (T_{FH}), specialized CD4 T cells located in the follicle and defined in part by their high expression of CXCR5, PD-1 and IL-21 [66, 67]. T_{FH} provide critical signals driving B cell proliferation, survival, immunoglobulin isotype class-switch and differentiation into long-lived plasma cells and memory B cells. Both GCBC and T_{FH} express the transcriptional repressor and master regulator Bcl6, which is crucial to their identities and distinguishes them from other B cell and T cell subsets. T_{FH} require two direct signals for optimal activation. The first is provided by the antigen-derived peptide presented by MHC Class II on the surface of the B cell. The second signal, a costimulatory signal, can be provided by a variety of receptor interactions and can further hone the 'help' provided by the T_{FH} cell.

Positive selection of germinal center B cells was long thought to exclusively depend on BCR signaling strength, with the assumption that GCBC with higher affinity BCRs would have stronger downstream signaling. However, it has recently been demonstrated that BCR signaling in GCBC is actually attenuated [36], so BCR signaling cannot be the sole determining factor in selection. Instead, antigen presentation signals appear to dictate GCBC selection. High affinity BCRs effectively capture more antigen and ergo present more antigen to T cells [68]. The resulting enhanced T cell-B cell interaction leads to more B cell division and more somatic hypermutation. Thus, T cell help via peptide-loaded MHC Class II molecules plays a crucial role in deciding GCBC fate. The mechanisms determining when a GCBC will differentiate into a plasma cell or memory B cell and exit the germinal center, rather than divide and remain in the germinal center, however, are not understood and are under active investigation.

B:T Interactions in the Immune Response

B cells express a number of proteins that bind partner proteins expressed on the surface of T follicular helper cells. Figure 7.5 depicts some of the primary T cell-B cell interactions that take place. T cells influence numerous aspects of germinal center B cell fate including maintenance, differentiation, proliferation and class-switch. This complex web of interactions also has consequences for the T cell. Expression of some of these proteins (on both B cells and/or T cells) is dictated by the activation state of the cell as well as pre-existing expression of other proteins.

Direct Interactions Between B Cells and T_{FH} Promote Germinal Center Formation and Germinal Center B Cell Survival

Specific receptor-ligand interactions between B cells and T_{FH} are necessary for germinal center formation. CD40 is constitutively expressed on B cells and binds CD40L, expressed on activated T cells. This interaction is absolutely required for

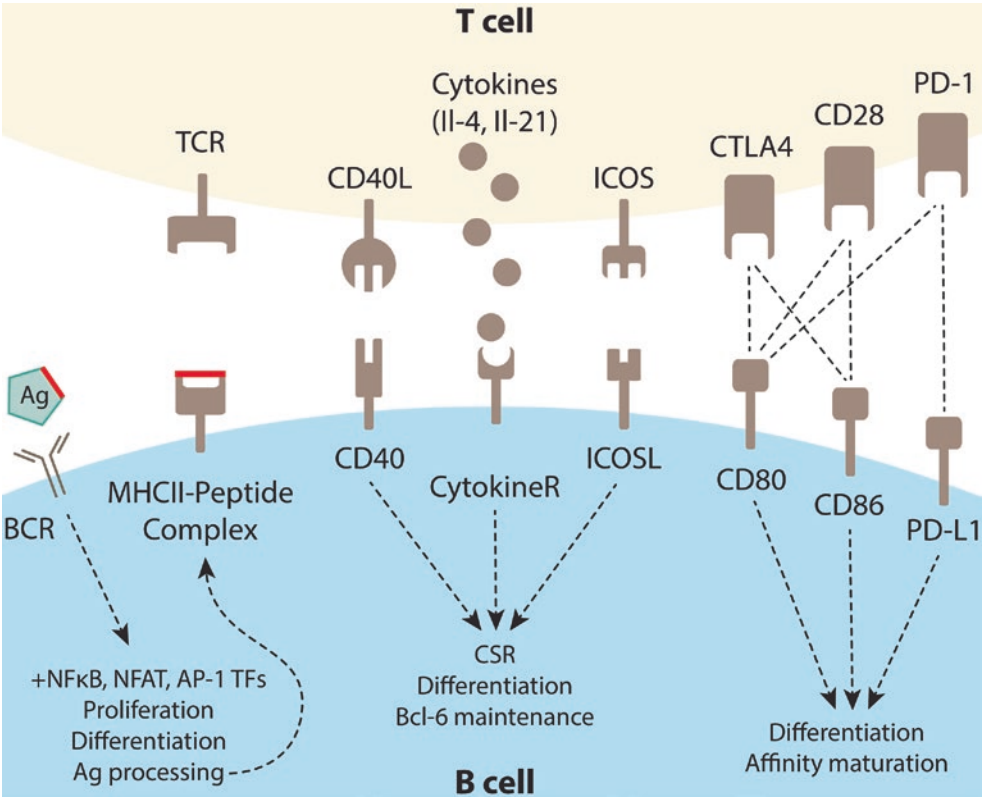

Fig. 7.5 Key interactions between activated B cells and T follicular helper cells (T_{FH}). Following antigen (Ag) binding to the B cell receptor (*BCR*), B cells process antigen and present peptides on MHC Class II to T cells. T cell receptor (*TCR*)-MHC Class II binding is further modulated by CD40/CD40L, ICOSL/ICOS interactions and by T cell-produced cytokines. B7 family interactions modulate the response further [72]. While effects on B cells are highlighted, these B cell-T_{FH} interactions have functional consequences for the T cell as well

germinal center formation. In the absence of CD40L, T_{FH} are reduced [69–71]. Similarly, ICOS and ICOSL – expression of which are induced following T cell and B cell activation respectively – also interact to promote germinal center formation, notably through T cell production of IL-4 [70].

Other direct T_{FH}-B cell interactions may not be absolutely required for germinal center formation, but do influence GCBC survival and selection. One example of this type of signaling involves the B7 family of proteins, which includes CD80, CD86, PD-L1, PD-L2 as well as the receptors CD28 and PD-1 [72]. GCBC upregulate first CD86 and then CD80, both of which can bind CD28, an activating receptor expressed by T cells, and CTLA-4, an inhibitory receptor expressed by T cells. CD80 can further bind PD-1, an inhibitory receptor that is highly expressed on T_{FH} and crucial for T cell secretion of IL-4 and IL-21. Finally, PD-L1 and PD-L2 – additional ligands for PD-1 – are also upregulated on murine GCBC. Murine studies have revealed that the B7 family has an important role in T cell-dependent immune responses with the varied types of interactions having similar, but not always completely redundant, effects. CD80 expression on B cells not only promotes normal T_{FH} development, but also GCBC survival and selection of high-affinity plasma cells [73]. Similarly, interactions between PD-1 on T_{FH} and PD-L1 or PD-L2 on GCBC influence GCBC survival and long-lived plasma cell formation, with fewer but higher-affinity long-lived plasma cells surviving in the absence of PD-1 and PD ligand signaling [74]. Thus, in addition to the binding of the peptide-loaded MHC Class II to the TCR, other direct interactions between GCBC and T_{FH} can influence the GCBC selection process.

T_{FH} Produce Cytokines That Indirectly Influence Germinal Center B Cell Maintenance, Class-Switch and Differentiation

In addition to direct protein-protein receptor binding, T_{FH} produce several cytokines that influence GCBC fate. Most prominently, they secrete IL-21, which binds IL-21R on B cells. In conjunction with IL-6, IL-21 is also reciprocally important for T_{FH} formation. IL-21 promotes the expression of Bcl6 in GCBC. In its absence, there is no quantitative effect on the T_{FH} population but there is reduced GCBC proliferation and a decrease in long-lived plasma cell production [75]. In conjunction with the B7 family interactions described above, this further demonstrates the requirement for functional T_{FH} in long-lived plasma cell output and affinity maturation. Memory B cell output is generally not significantly affected by the absence of B7 family members, but interestingly, there is an increased frequency of memory B cells in the absence of IL-21, suggesting that sustained T cell interactions are not required for GCBC to differentiate into memory B cells. In addition to IL-21, T_{FH} can secrete IL-4, which promotes class-switch to IgG_4 and IgE as well as GCBC proliferation.

A diverse range of direct and indirect stimuli guides the formation, maintenance and ultimate output of the germinal center. While some proteins may be absolutely necessary for germinal center formation, it is likely that differential signaling and variable participation of most ligands, receptors and cytokines finely tune individual germinal centers. This tuning can go awry: T_{FH} can also promote expansion and differentiation of autoreactive B cells. Current research is directed towards better understanding the range of interactions between T_{FH} and GCBC, including the sequence of ligand, receptor and cytokine upregulation as the germinal center reaction matures. Such knowledge will help develop targeted therapies to constrict autoreactive or malignant GCBC proliferation and differentiation and conversely to expand and fine tune the germinal center response to vaccination.

Regulatory T Cells and B Cells Dampen the Immune Response

The immune system exists as a delicate balance – while a healthy individual should mount a vigorous protective response to an invading pathogen, an overly vigorous response could be dangerous. Sometimes, dampening of the immune response is absolutely necessary. Successful reproduction in placental mammals depends upon repression of the maternal immune response to prevent rejection of the fetus. In addition to T_{FH}, other $CD4^+$ T cell subtypes can be involved in regulating the B cell response. Regulatory T cells (Tregs) are generally characterized by expression of the transcription factor Foxp3, the IL-2 receptor CD25 and production of IL-10. They are capable of directly suppressing autoreactive B cells in many situations, including in systemic lupus erythematosus [76]. Effective suppression of autoimmunity requires functional Tregs to be present in adequate numbers in the correct location.

In addition to their roles as antibody-producing cells and antigen-presenting cells, B cells secrete regulatory cytokines. B cells can be subdivided based on cytokine production in a manner similar to $CD4^+$ T cell subtypes. Regulatory B cells (Bregs), like Tregs, are characterized by production of IL-10 or TGF-β [77–79]. In mice, Breg is a catchall term that applies to B cells from different lineages and anatomic niches. In humans, they have been characterized using combinations of surface markers, including markers associated with memory B cells [80]. Like Tregs, they may be involved in suppressing the maternal anti-fetal T cell response in pregnant women [81]. In murine models of autoimmune disease, Bregs have been shown to protect against disease progression. In systemic lupus erythematosus and pemphigus, B cell depletion therapy has been suggested to work in part by increasing the relative amount of tolerogenic B cells.

Summary

B cell activation is initiated when antigen binds the B cell receptor, triggering an intracellular signaling cascade. Responses to polysaccharide and lipid antigens do not require T cell help and are termed T-independent reactions. Responses to proteins do require T cell help and are termed T-dependent reactions. For T-dependent reactions, after B cell receptor ligation, the antigen is endocytosed, processed and presented on MHC class II to $CD4^+$ T cells. When a B and T cell specific for the same antigen make contact, the B cell proliferates. First an extra-follicular focus is formed wherein there is extensive differentiation to short-lived plasmablasts that secrete large amounts of low affinity antibody to provide immediate protection against the infection. Next, a germinal center reaction is formed. Here, B cells interact with T follicular helper cells and follicular dendritic cells and undergo massive clonal expansion. Some germinal center B cells undergo class-switch to IgG, IgA or IgE isotype and some undergo somatic mutation of the B cell receptor, thus generating new clones of differing affinity for antigen. Germinal center B cells compete with one another for access to necessary survival signals and clones with higher affinity are selected, a process termed affinity maturation. The goal of the germinal center reaction is the production of long-lived plasma cells and memory B cells. In the next section, we will discuss describe these cells and their significance.

Long Lived Plasma Cells and Memory B Cells

Introduction

Immunologic memory, the basis of durable immunity to vaccination and natural infection, is the hallmark of the adaptive immune system. Memory is comprised of durable antibody titers and the bone-marrow resident long-lived plasma cells that produce them and antigen-specific memory B and T cells. Long-lived plasma cells and memory B cells differentiate from the germinal center reaction through critical but poorly understood mechanisms, after undergoing multiple rounds of proliferation, somatic hypermutation and selection. Long-lived plasma cells are terminally differentiated cells that continually produce and secrete antibody. In contrast, memory B cells are not terminally differentiated and upon antigen re-stimulation can either differentiate into plasmablasts or can generate or re-enter a new germinal center. In this section, we will review basic properties of both long-lived plasma cells and memory B cells and consider new ways in which optimization of the germinal center reaction could be used to improve vaccine design.

Long-Lived Plasma Cells Exclusively Secrete Antibodies and Lose the Ability to Proliferate or Present Antigen to T Cells

Terminal differentiation of germinal center B cells to long-lived plasma cells (LLPC) requires initiation of a unique transcriptional program and the concomitant blockade of the germinal center B cell transcriptional program [82, 83]. The plasma cell differentiation program relies on increased expression of three transcription factors – Xbp1, Prdm1 (encoding BLIMP1) and Irf4. Together, these expand the size of the burgeoning plasma cell, stop cell cycle progression, downregulate MHC Class II expression and enhance protein manufacturing and secretion. The requirement for these transcription factors in LLPC development and function has been validated in elegant studies utilizing mouse models with gene-specific deletions.

The exact signals required to induce the development of LLPC are unclear, but signals delivered through the BCR and cytokines in the milieu are clearly important. Long-lived plasma cells emerge from the germinal center late in the immune response, in contrast to memory B cells, which tend to arise earlier [84]. The primary and best-characterized niche for LLPC is the bone marrow. Thus, LLPC must egress from the site of formation in germinal centers in the spleen, lymph node or other secondary lymphoid organs and migrate to the marrow. An early step in their development is the downregulation of CXCR5, a chemokine receptor that retains B cells in the follicle. Egress also requires signals delivered through β-integrins [85] and CD138/syndecan. Migration to the marrow is directed in part by the upregulation of CXCR4, the receptor for the chemokine CXCL12, which is produced by bone marrow stromal cells.

The BM-resident plasma cell compartment is long-lived, with plasma cells estimated to survive in mice for their lifespan (at least 1 year) [86]. The lifespan of LLPC in humans is unclear, but antibody titers can be durable over a lifetime. As plasma cells are terminally differentiated, non-dividing cells, longevity of an individual plasma cell is likely quite long. Bone marrow stromal cells provide several key survival factors, including IL-6, IL-21 and BAFF and APRIL. In mice, survival or retention signals are also provided by eosinophils and basophils [87, 88].

IL-21 is a master regulator of B cell differentiation that, among other effects, promotes plasma cell development [89]. IL-21 is produced by T follicular helper cells and regulates B cell expression of Bcl6, Prdm1/Blimp-1 and Aicda/AID. It has myriad effects, resulting from combinatorial signaling provided by other direct and indirect cues, which orient B cell fate. Humans with homozygous loss-of-function mutations in the IL-21R gene have, amongst other problems, reduced serum IgG levels and impaired responses to T-dependent antigen-based vaccines. Additionally, IL-21 and IL-21R gene polymorphisms that result in aberrant production of IL-21 have been associated with several autoimmune diseases [90].

Long-Lived Plasma Cells Utilize Several Mechanisms to Cope with High Protein Output Stress

Ex vivo and without additional stimulation, human bone marrow-derived plasma cells produce 10^7–10^8 Ig molecules/cell/hour [91]. Thus, long-lived plasma cells are highly stressed cells and require several mechanisms to adapt [92]. The unfolded protein response and the ubiquitin proteasome system are part of this transformation. Increased stress from misfolded protein accumulation renders long-lived plasma cells more sensitive to death induced by proteasome inhibitors such as bortezomib [93]. This finding has been applied to treatment of multiple myeloma and proteasome inhibitors may also have a role in the treatment of autoimmune disease.

In addition to modifications in the protein folding and degradation process, murine plasmablasts and plasma cells rely on autophagy. Autophagy is a self-recycling process that rids cells of damaged proteins and organelles while maintaining metabolism under nutrient-poor conditions. Mice whose B cells conditionally lacked a component of autophagy participated normally in germinal centers, but had lower antibody titers and fewer bone marrow-resident LLPC than wild-type mice [94]. While the role of autophagy in supporting human LLPC survival has not yet been studied, it is likely important.

Because plasma cells differ from their B cell precursors in expression of cell surface molecules and in their dependence on survival factors, they often respond differently than other B cell populations to medications used to treat autoimmune diseases and malignancies. Importantly, rituximab, which targets CD20-expressing cells, is effective at depleting naïve and memory B cells but leaves CD20-negative plasma cells and plasmablasts intact. Belimumab, a monoclonal antibody that binds and blocks BAFF activity, depletes transitional and mature B cell populations, but is relatively sparing of certain memory B cell compartments and plasma cells. Therapeutic strategies that directly target signals and proteins important for long-lived plasma cell survival and maintenance may therefore be more effective in the treatment of some autoimmune diseases and multiple myeloma. These signals can include both external survival factors such as BAFF or APRIL as well as internal plasma cell components needed for the unfolded protein response or autophagy.

Memory B Cells Can, Upon Re-activation, Differentiate into Plasmablasts or Reenter the Germinal Center

Like long-lived plasma cells, memory B cells (MBC) are effectively elicited after vaccination and infection and are clonally long-lived [95]. They too are produced in the germinal center and are critical for long-term immunity. Unlike long-lived plasma cells, MBC are not terminally differentiated, and thus are poised to respond dynamically to reinfection with either known or new-but-related antigen. Upon challenge, MBC can rapidly differentiate into antibody-secreting plasmablasts, contributing immediately to short-term protection, or form new germinal centers and undergo further affinity maturation. Successful elicitation of a robust MBC population is likely required for effective vaccination strategies for chronic and/or rapidly evolving pathogens such as HIV and influenza virus, and pathogenic autoreactive MBC may underlie autoimmune disease. Therefore, the study of MBC biology is a dynamic area of investigation.

Memory B Cells Can Be Divided into Phenotypic and Functional Subsets

Phenotypically, MBC resemble naïve B cells and until recently, understanding of MBC biology was severely limited due to a lack of reliable markers to distinguish true, antigen-specific memory cells from their naïve precursors. Historically, the presence of class-switch isotype (IgG) was used as a marker for MBC, however, this definition excludes the significant proportion of MBC that are IgM-bearing [96] while encompassing recently activated but not true memory cells. Recently, we have identified several markers of MBC in mice, including CD80, PD-L2 and CD73, which together define distinct MBC subsets [97, 98]. Some of these markers, such as the B7 family members CD80 and CD86, are also upregulated on human MBC [99, 100] and B cell-intrinsic CD73 plays a role in class-switch recombination [101]. Importantly, CD80 and PD-L2 define subsets of MBC, independent of isotype, that differ functionally from one another [102]. Together with work illustrating differential functionality of MBC subsets defined by isotype [103] and tissue distribution [64], these data demonstrate that the MBC population is heterogeneous both phenotypically and functionally.

In humans, CD27, a member of the TNF receptor superfamily, is upregulated on most MBC [104, 105]. As in mice, MBC are not exclusively class-switched or somatically hypermutated and marker expression can vary across subsets [106, 107]. In addition to CD27, other MBC markers have been identified, including CD21/CR2 [108], FcRH4/FcRL4 [109, 110] and CD148 [111]. Thus, human MBC are also heterogeneous [106]. Intriguingly, additional memory B cell subsets have been described in individuals with HIV infection [108] and systemic lupus erythematosus [112]. These include activated memory B cells ($CD27^{hi}CD21^{low}$), which are a source of plasmablasts and more vulnerable to apoptosis, and tissue-like memory B cells ($CD27^{low}CD21^{low}$), which harbor exhausted B cells enriched in HIV-specificity [108]. Further characterization of these subsets could suggest new vaccine and therapeutic strategies for HIV and other chronic diseases.

MBC are generated from antigen responding B cells in the germinal center reaction. MBC are formed biphasically in the early germinal center, while long-lived plasma cells are formed in the late germinal center [84], suggesting that the germinal center milieu changes with maturation and that MBC generation may be less dependent on prolonged T cell help than LLPC generation. Ultimately, relatively few MBC and LLPC are generated in spite of the high number of germinal center B cells at the peak of the reaction. The mechanism(s) that underlie germinal center B cell differentiation into MBC or LLPC remain poorly understood.

Memory B Cells Respond Quickly and Robustly to Antigenic Stimulation

Compared with naïve B cells in the primary response, MBC mount a secondary response more quickly and robustly, and this response tends to be of higher affinity and more enriched in class-switched antibodies (Fig. 7.4). The mechanisms enabling the rapid and robust memory response are not entirely elucidated, but MBC may have a lower threshold for activation than naïve B cells. They are more responsive to cytokines such as IL-21 and IL-10, which can promote plasmablast differentiation [113]. Moreover, memory, but not naïve, B cells constitutively express proteins such as CD80 and CD86 that enable direct interaction with $CD4^+$ T and upregulate survival factors in response to Toll-like receptor (TLR) stimulation more readily [100]. Upon activation, MBC can either rapidly differentiate into short-lived antibody-secreting plasmablasts, thus providing a burst of antibody for immediate protection, or rapidly form new germinal centers, wherein they undergo further affinity maturation and produce new MBC and long-lived plasma cells that produce antibody with improved affinities for the pathogen. The former fate is critical for protection to known pathogens, while the latter is likely key for protection against new but related pathogens, such as rapidly mutating viruses. Recent studies in mice indicate that these functions are provided by distinct subsets of MBC [102] suggesting the same may be true for human MBC.

Memory B Cells Are Clonally Long-Lived and Self-Renew

A defining characteristic of the MBC compartment is its longevity [95]. MBC longevity is in stark contrast with the

relatively short lifespan of their direct naïve precursors. In part, this longevity can be explained by the fact that MBC survival is relatively independent of BLyS/BAFF signals compared with naïve B cells [114], but the magnitude of independence differs between subsets [48], indicating that other factors are important as well. Interestingly, T cell help does not appear to be required for MBC survival [115]. Furthermore MBC and long-lived plasma cell compartments appear to be maintained independently [6, 116].

Longevity also depends on MBC self-renewal. Homeostatic self-renewal has been observed in careful long-term kinetic studies [117, 118]. In this respect, MBC are more similar to their early hematopoietic stem cells precursors, which self-renew, than their direct mature precursors, which cannot, suggesting a process of neoteny wherein MBC, or a subset of MBC, acquire stem cell-like properties. Genetic evidence for this concept comes from microarray comparisons between murine MBC, memory T cells and hematopoietic stem cells that indicate that memory lymphocytes share an expression signature with hematopoietic stem cells [119]. Furthermore, human MBC and memory T cells (both $CD4^+$ and $CD8^+$) have been shown to share a common genetic signature, which includes some genes important for quiescence [120]. Elucidating the mechanism(s) underlying MBC durability could lead to improved vaccine design strategies and is a focus of active research.

Summary

Long-lived plasma cells and memory B cells are independent B cell populations that emerge from the germinal center and provide protection against re-infection. Plasma cells are antibody-producing factories that maintain durable standing antibody titers for immediate protection to re-exposure to pathogen. Plasma cells have a highly specialized transcriptional program with mechanisms in place to handle the high stress of enormous protein output. Memory B cells resemble naïve B cells in many respects, retaining the ability to enter the germinal center as well as to differentiate into plasmablasts upon re-stimulation. Unlike their naïve precursors, memory B cells are frequently class-switched and often bear mutated, high affinity B cell receptors. Critically, they have a lower threshold for activation and are thus better positioned to adapt to pathogen re-challenge. Unlike their precursors, memory B cells are long-lived and self-renew.

Conclusions

B cells are key components of the adaptive immune system. They produce antibodies, act as antigen-presenting cells for T cells and secrete cytokines that modulate other arms of the immune system. A hallmark of the B cell response is the development of memory, comprised of protective standing antibody titers, long-lived antibody-secreting plasma cells and memory B cells. B lymphocyte responses are thus critical for effective and durable immunity to pathogens but can drive and promote pathology in autoimmune, allergic and malignant disease.

In summary, this chapter broadly reviewed the current field of B lymphocyte biology, providing a conceptual framework for the dermatologist and cutaneous biologist. Significant redundan emphasis was devoted to reviewing antibody structure, isotype variation and effector function, in part because antibodies are critical tools for dermatologists and biologists given their exquisite specificity against protein, small molecule, lipid and carbohydrate molecules. The field of B cell development was reviewed briefly, introducing the fundamental mechanisms by which current and emerging B cell depletion therapies work. The dynamics of the B cell response, central to the pathogenesis of cutaneous autoimmune disease such as immunobullous disease, vasculitis and systemic lupus erythematous, was broadly outlined. The final subsection was devoted to a discussion of B cell memory, laying a foundation for understanding the mechanisms by which vaccination, plasmapheresis and intravenous immunoglobulin therapy function.

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Questions

1. Which of the following is true of class-switch recombination?
   A. It affects the rearranged V(D)J
   B. It uses the RAG enzymes
   C. It is an irreversible process
   D. A single variable region cannot elicit a number of different effector functions

2. Which is the only secreted immunoglobulin isotype that can pentamerize?
   A. IgA
   B. IgD
   C. IgE
   D. IgG
   E. IgM

3. Which immunoglobulin isotype has the longest half life?
   A. IgA
   B. IgD
   C. IgE
   D. IgG
   E. IgM

4. All three complement pathways converge on which common intermediate protease?
   A. C3 convertase
   B. alpha/beta hydrolase
   C. alkaline phosphatase
   D. serine protease
   E. GTPase

5. Which of the following is not a characteristic of regulatory T cells (Tregs)?
   A. expression of Foxp3
   B. expression of CD25
   C. production of IL10
   D. expression of the IL-2 receptor
   E. expression of CD80

Answers

1. C
2. E
3. D
4. A
5. E

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T Cell Immune Responses in Skin

8

Sherrie J. Divito and Thomas S. Kupper

Abstract

The adaptive immune system is historically divided into cell-mediated immunity and humoral immunity, which were considered to provide defense against intracellular and extracellular pathogens, respectively. As the complexity of each element of the adaptive immune system is revealed, it is now clear that extracellular pathogens can also be targeted by cell-mediated immunity, and intracellular viruses by humoral immunity. The two main effector cell types mediating adaptive immunity are T and B lymphocytes (more commonly called T and B cells). Both cell types are characterized by a nearly infinite variety of unique antigen receptors, so that at the genomic level no two naive T cells or naive B cells are alike. There are two hallmarks of the adaptive immune system, specificity and memory, and lymphocytes are the only immune cells capable of both. Naïve lymphocytes are antigen inexperienced, meaning they have not previously recognized and responded to antigen. Their response upon first exposure is termed the primary response. The primary response is characterized by rapid but transient clonal expansion, and this ultimately generates a limited number of long-lived antigen specific lymphocytes, termed memory cells, which upon re-exposure to their cognate antigen produce a more rapid and effective immune response, termed the secondary response. Engineered generation of memory T and B cells to specific pathogens is the basis for vaccination. Specificity to structurally distinct antigen is produced by a combination of genetic events and selection process that allows lymphocytes to recognize and react predominantly to foreign, and much less frequently to self, antigen. The loss of inhibition of recognition of self results in autoimmune disease, and there are a number of mechanisms in place to prevent this from occurring.

This chapter will review development and selection, antigen recognition, effector and regulatory functions, and memory responses of T cells, with special focus on recently described skin resident memory T cells, and with clinical correlations to dermatologic disease and therapeutics.

Keywords

T Cell • Immune system • Cell-mediated • Humoral immunity • B cell • Skin diseasae • Thymic Selection • TCR • T cell receptor • CD4 • MHC • Th1 • Th2 • Cell stimulation

T Cell Development and Thymic Selection

The T cell receptor (TCR) on the surface of all T cells allows for the detection of a diverse array of closely related chemical structures termed antigens (Ag). Peptide antigens are expressed by MHC molecules on the surface of antigen

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presenting cells [94]. For an effective immune response, a T cell must be able to recognize a foreign antigen presented by self-MHC [70, 126, 161]. Antigen receptors (TCR and B cell receptors) are clonally distributed, meaning, that each naive lymphocyte has a particular sequence in the antigen recognition part of its receptor known as CDR3 sequence that is unique to that T cell. For memory T cells, a clone consists of a parent cell and all its progeny which express the same TCR. The vast majority of T cells express a TCR made up of one alpha chain and one beta chain. A smaller number of T cells express a TCR made up of one gamma chain and one delta chain [38]. All four chains contain a variable region (V) which recognizes the antigen, and a constant region (C) which is conserved amongst all chains. Within the V region, there are three hypervariable regions (also called complementarity determining regions, CDR) which is the actual part of the TCR that binds the MHC:Ag complex [37, 48]. Associated with the TCR are intracytoplasmic signaling proteins that together make up the TCR complex. When two or more adjacent TCRs bind antigen, the complexes cross-link which allows downstream signaling proteins to come into close proximity and initiate an enzyme cascade that ultimately results in T cell activation [15, 37]. The lymphocyte repertoire refers to the total collection of lymphocyte TCR specificities. The number of potential viable TCR recombinations is estimated to be over 10^{20} [157]. Estimates of the size of the actual T cell repertoire pool are much lower, for example Artsila et al estimated that there are approximately 2.5×10^7 diverse T cell clones in human blood (with 10^6 unique TCRβ chains paired with 25 different α chains) [1], and Robins et al calculated there to be $3–4 \times 10^6$ unique TCRβ CDR3 sequences in peripheral blood [116].

CD4 and CD8 are coreceptor molecules that enhance the binding of the TCR to MHC molecules and resultant signaling [156]. A mature T cell expresses either CD4 or CD8. The TCR on the surface of CD8 T cells recognizes Ag presented in the context of MHC class I, and the TCR on the surface of CD4 T cells recognizes Ag presented in the context of MHC class II. The genes that code for each chain of the TCR (i.e., α, β, γ, δ) undergo gene rearrangement which generates tremendous diversity of the T cell repertoire.

T cells are so-called because they develop in the thymus (reviewed in [74, 124, 153]). Pre-thymocytes are T cell precursors that develop in the fetal liver and bone marrow where they undergo initial TCR rearrangement. They express neither CD4 nor CD8 on their cell surface. The pre-thymocytes migrate to the thymus where they undergo a series of further TCR gene rearrangements and maturation. Within the thymus, as T cells develop, they express neither CD4 nor CD8 (double negative), and rearrange and express the TCR β or γ chain. They then express both CD4 and CD8 (double positive) and rearrange and express the TCR α or δ chain. These double positive T cells expressing complete TCRs on their surface then undergo thymic selection. Even in αβ TCR T cells, the TCR γ gene locus undergoes rearrangement [125] although the γ chain protein is not expressed.

While in the thymus, immature T cells contact thymic epithelial cells that express self MHC: self Ag complex. If the immature T cells recognize the self MHC they are selected to continue on with maturation, termed 'positive selection'. If the TCR does not recognize the MHC, the T cells fail to receive a life-saving signal and undergo apoptosis, a process termed 'death by neglect' [74], (a T cell that cannot recognize self-MHC is of no use to the immune system). If the bond between TCR and MHC is too strong though, this could result in autoimmunity, so the immature T cell is destroyed, termed 'negative selection'. Medullary thymic epithelial cells express the Autoimmune Regulator (*AIRE*) gene (reviewed in [79]). Aire protein acts as a master regulator controlling expression of tissue-specific self-Ags by thymic epithelial cells and leading to negative selection of self-reactive thymocytes (reviewed in [79]). Deficiency of Aire results in APECED – Autoimmune-Polyendrocrinopathy-Candidiasis-Ectodermal Dystrophy syndrome [105]. APECED is characterized by hypoparathyroidism, adrenal insufficiency and mucocutaneous candidiasis. In addition to mucocutaneous candidiasis, dermatologic manifestations include alopecia, vitiligo, keratoconjunctivitis, dental enamel hypoplasia and nail pits [73].

As T cells proceed through thymic selection, they lose expression of either CD4 or CD8. Only ~5 % of immature T cells will survive thymic selection to be released as mature T cells into the peripheral circulation [153]. Of these mature single positive T cells, ~95 % express TCR αβ, the remaining ~5 % express TCR γδ [38]. There are more CD4 T cells in the periphery than CD8 T cells at roughly a 2:1 ratio. Thymic selection is imperfect, and there are T cells in the periphery capable of responding to self antigen in the context of MHC. T regulatory cells (discussed below) are important for the control of these potentially autoreactive T cells.

Activation of Naïve T Cells

Mature T cells released into the periphery are antigen inexperienced, or naïve T cells. Naïve T cells express CD45RA on their cell surface, a useful marker in determining naïve vs memory T cells in humans (human memory T cells express CD45RO) [41]. Naïve T cells circulate between blood and lymph nodes, entering through specialized high endothelial venules and exiting through efferent lymphatics. As they migrate to the T cell rich areas of lymph nodes, they constantly sample MHC:Ag complexes on the surface of antigen presenting cells (APC), looking for their cognate MHC:Ag [98]. These APC are typically dendritic cells (DC) that have processed and are presenting peptides from proteins found in the tissue drained by the lymph node.

Initiation of a skin adaptive immune response occurs when skin DC capture antigen introduced into the skin, and travel to draining lymph nodes. As DC migrate to draining LN, they mature and up-regulate surface expression of MHC:Ag complex, co-stimulatory molecules CD80, CD86 and CD40, and adhesion molecules that facilitate binding to T cells [7]. Antigen can also be captured by DC resident in LN. Within draining lymph nodes, the mature (or activated) DC interact with naïve T cells searching for cognate MHC:Ag complex. Activation and clonal proliferation, termed priming, of a naïve T cell requires sufficient signaling via TCR bound to MHC:Ag complex, termed signal 1, and signaling via CD28 on the surface of the T cell via binding to CD80 or CD86 on the surface of the APC, termed signal 2 (Fig. 8.1). The combination of robust signal 1 and signal 2 results in an enzymatic cascade that induces production of Interleukin (IL) – 2 and IL-2 receptor by the T cell [27, 66]. IL-2 functions in an autocrine and paracrine manner to promote T cell proliferation (Fig. 8.1). Further, DC secrete cytokines (signal 3) that influence T cell differentiation. The interaction between DC and T cell is not unilateral however. CD4 T cells express on their cell surface CD40L which induces signaling via CD40 on the DC surface that in turn promotes upregulation of CD80 and CD86 and secretion of cytokines by the DC (Fig. 8.1) [7, 50], further amplifying T cell stimulation and proliferation. The robust proliferation of clonal T cells is termed expansion. An activated T cell divides 20 times to expand 10,000 fold in 1 week in response to antigen (reviewed in [35]). Of clinical note, the calcineurin inhibitors cyclosporine, tacrolimus, and pimecrolimus inhibit IL-2 production [66] and therefore T cell proliferation, and are used to quell inflammation in dermatologic conditions [52, 90].

As T cells proliferate, they decrease surface expression of CD28 and up-regulate expression of CTLA-4, an inhibitory molecule, that also binds CD80 and CD86 on the surface of antigen presenting cells [149]. CTLA-4 binds CD80/86 more avidly than CD28 [82]. This serves to limit the T cell proliferative response, and prevent immune-mediated tissue

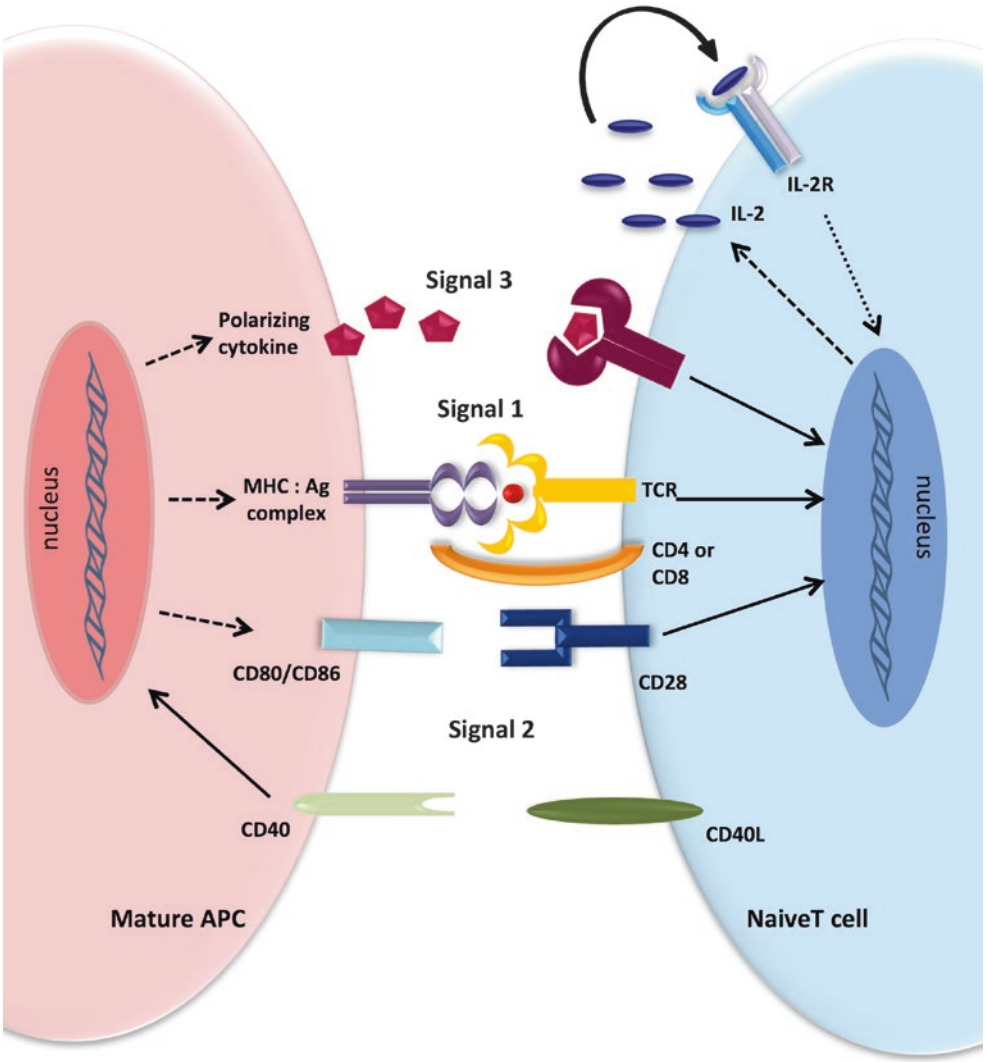

Fig. 8.1 T cell priming by APC. TCR binds cognate Ag presented in the context of MHC on the surface of mature APC to trigger TCR signaling (signal 1). Mature APC also express high levels of the co-stimulatory molecules CD80 and CD86 which bind CD28 on the T cell surface (signal 2). Signaling via TCR and CD28 results in production of IL-2 which acts both in a paracrine and autocrine fashion to stimulate T cell proliferation. Mature APC also express CD40 on their surface that binds CD40L on the T cell surface. CD40 binding of CD40L results in further APC activation. Finally, APC release polarizing cytokines (signal 3) which guide differentiation of T cells

Table 8.1 Notable co-stimulatory and co-inhibitory receptor/ligand pairs on T cells and APC

	T cell	APC
Co-stimulatory	CD28	CD80, CD86
	CD40L	CD40
	ICOS	ICOSL
	OX40	OX40L
	4-1BB	4-1BBL
	CD137	CD137L
Co-inhibitory	CTLA-4	CD80, CD86
	PD1	PDL1, PDL2
	TIM-3	Galectin 9

damage. In metastatic melanoma, the CTLA-4, blocking antibody ipilimumab is employed to block CTLA-4 induced T cell inhibition and therefore promote T cell responses against the tumor [59].

There are several additional co-stimulatory and co-inhibitory molecules expressed on the surface of T cells to promote or inhibit, respectively, T cell stimulation (Table 8.1). Similar to the use of ipilimumab in metastatic melanoma, antibody blockade of the inhibitory molecules PD-1 or its ligands are currently used in melanoma and other solid tumors [39, 55, 89], and one therapeutic antibody targeting PD-1, pembrolizumab, is approved by the FDA for advanced melanoma. Likewise, adhesion molecules expressed on T cells, APC, and endothelium enhance T cell binding and are also potential clinical targets to alter immune responses [13, 62].

If a T cell receives insufficient signal 1 and/or signal 2, the T cell can become 'tolerized' to that antigen, meaning it either undergoes deletion (apoptosis), becomes anergic (refractory to activation), or becomes a regulatory T cell [122, 131]. The result of this T cell inactivation is known as 'peripheral tolerance' [131]. In steady state conditions, APC are constantly sampling self Ag in the human body and presenting this self Ag to cognate naïve T cells in lymph nodes [131]. Since these APC are not activated, i.e. they are immature (express low levels of MHC:Ag complex and low levels of CD80/CD86) [131], T cells that are specific for self Ag undergo this 'tolerization' process rather than becoming activated to self.

T Cell Differentiation and Function

CD4 T Cells

Upon antigen stimulation, T cells not only proliferate, but also differentiate into effector T cells with specialized functions. CD4 effector T cells are termed 'helper' T cells because they function to (i) promote phagocytosis of microbes by macrophages, (ii) promote antibody production by B cells, and (iii) enhance CD8 T cell cytotoxicity (reviewed in [160]). This is achieved via two mechanisms. First, as introduced above, CD4 T cells express on their cell surface CD40L which binds to and stimulates CD40 on the surface of APC [50] (Fig. 8.1). This signaling enhances APC activation and function. Second, CD4 helper T cells produce various cytokines and chemokines that further influence the immune response. The specific cytokines produced depend on their differentiation, or polarization. T helper cell differentiation is guided by the strength of TCR signaling, co-stimulatory molecules and cytokines provided by APC, and inflammatory signals in the surrounding microenvironment, including autocrine signals produced by the CD4 T cells themselves (reviewed in [160]). Teleologically, CD4 helper T cell differentiation evolved to provide ideal effector function to clear the provoking infection.

Historically, CD4 T cells were thought to polarize toward one of two phenotypes, T helper type 1, Th1, cells, and T helper type 2, Th2, cells [103]. However, the past decade has witnessed discoveries of additional phenotypes and functions suggesting additional T cell subsets, identified as Th9, Th17, Th22 and follicular helper T cells, Tfh, on the effector side, and nTregs, iTregs and Tr1 cells on the regulatory side. Each subset will be described below, however it is important for the reader to know that many of these cell types appear to be quite plastic, and may be capable of converting to another phenotype/function given the appropriate stimulation. Figure 8.2 reviews T helper cell differentiation.

Th1

Th1 cells promote macrophage and DC maturation that in turn increases stimulation of CD8 cytotoxic T cells and induces macrophages to destroy phagocytosed microbes. These two responses enhance clearance of intracellular pathogens. Th1 cells produce the pro-inflammatory cytokines IL-2, IFN-γ, and TNF (reviewed in [160]), which further bolster macrophage activation and CD8 cytotoxic T cell responses. Th1 cells also produce chemokines that attract macrophages to sites of infection (reviewed in [65]). The mechanism of Th1 polarization is well-elucidated (reviewed

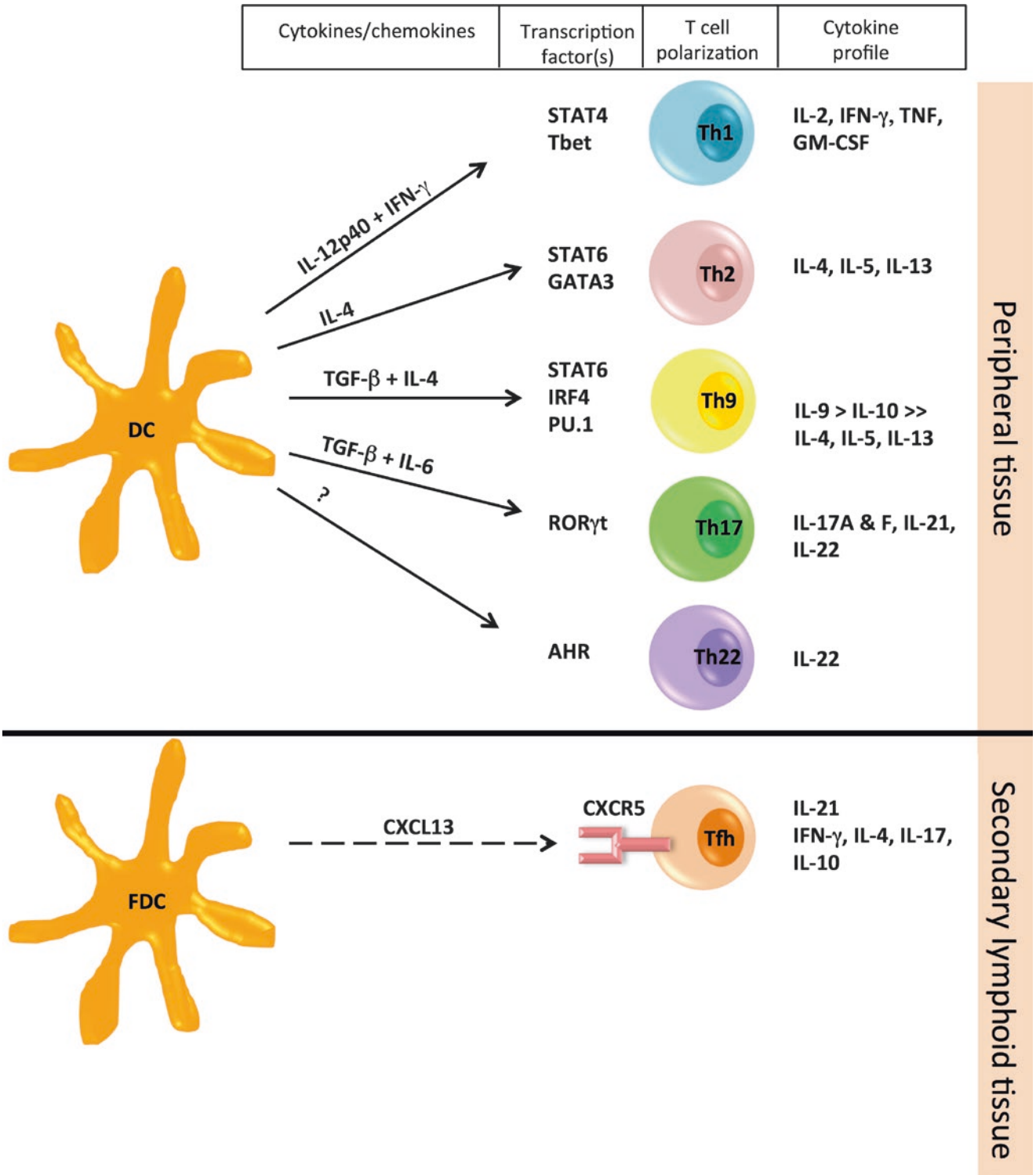

Fig. 8.2 T helper (Th) cell polarization. DC in peripheral tissues produce various cytokines that guide T helper cell polarization via specific transcription factor activation. Polarized T cells in turn release cytokines specific to their differentiation. Follicular (F)DC produce the chemokine CXCL13 which attracts T follicular helper (Tfh) T cells. TFh can produce various cytokines in a similar fashion to polarized Th cells. *AHR* aryl hydrocarbon receptor, *FDC* follicular DC

in [65]). IFN-γ binds to its receptor on the surface of activated T cells and induces the transcription factor T-bet via STAT 1 signaling. Tbet in turn transactivates IFN-γ and IL-12Rβ2. The cytokine IL-12p70 is then capable of binding

to the IL-12 Receptor (a heterodimer consisting of IL-12Rβ1 and IL-12Rβ2) on the T cell surface and induces signaling via STAT4 which further promotes Th1 activity. IFN-γ is the hallmark cytokine for Th1 cells.

Th2

Th2 cells activate naïve Ag-specific B cells to produce antibody which targets extracellular pathogens, particularly parasites and helminths, and they also stimulate eosinophil and IgE responses (reviewed in [65]). Th2 cells secrete the pro-inflammatory cytokines IL-4, IL-5, and IL-13 (reviewed in [160]). Th2 polarization (reviewed in [65]) is induced via TCR and IL-4 receptor signaling, which induces STAT6. Phosphorylated STAT6 in turn induces the transcription factor GATA-3 which transactivates IL-4, IL-5 and IL-13, while down-regulating STAT4 and IL-12Rβ2.

Polarization of T helper cell responses toward Th1 or Th2 is important for skin disease. For example, the bacterium *Mycobacterium leprae* grows inside macrophages, so Th1 responses that trigger macrophages to kill intracellular pathogens limits extent of clinical disease to tuberculid leprosy [102]. Comparatively, Th2 differentiation is largely ineffective against intracellular pathogens and so there is a higher burden of *M. leprae* in patients with a Th2 response resulting clinically in lepromatous leprosy [102]. This is likewise true for the intracellular pathogen Leishmania; Th1 responses are more effective at controlling infection than Th2 responses [151]. The dichotomy of Th1 versus Th2 extends beyond pathogen control to autoimmune disease. Polarization toward Th1 is observed in psoriasis (although Th17 cells appeared to be more pathogenic) [88], while polarization toward Th2 is seen in acute atopic dermatitis [56]. This helps explain why the anti-TNF agents etanercept, adalimumab and infliximab are effective in psoriasis and psoriatic arthritis [14], while anti-IL-5 antibody, mepolizumab, and anti-IL-4Receptor antibody, dupilumab, are therapeutic agents for atopic dermatitis [56].

Th17

Th17 cells secrete the pro-inflammatory cytokines IL-17A and IL-17F, which belong to the same cytokine family and have overlapping functions. Receptors for IL-17A and F are expressed on both hematopoietic and non-hematopoietic cells and receptor binding induces production of IL-6, IL-1, TNF, and IL-22, as well as chemokines that promote inflammation and neutrophil recruitment [11]. Th17 cells function in host defense to help clear bacterial and fungal infections when Th1 and Th2 responses are insufficient [11]. Th17 cells are generated by TGF-β and IL-6, which together induce the transcription factor RORγt, which drives production of IL-17, IL-21 and IL-23Receptor (R) [10, 64, 96, 143]. IL-21 functions in an autocrine manner to amplify differentiation [77, 108] and IL-23 produced by APC and non-hematopoietic cells such as skin keratinocytes binds the IL-23R on the surface of Th17 cells, and signals via STAT3, to further promote expansion and survival [2, 80, 100].

Notably, IL-23 receptor is a heterodimer consisting of IL-23R bound to IL-12Rβ1 [111]. Increasing IL-23R surface expression effectively impairs Th1 differentiation by limiting the ability of IL-12Rβ1 to bind to IL-12Rβ2. Further, both IL-12 and IL-23 share a common p40 subunit. IL-12 (officially called IL-12p70) is a heterodimer consisting of IL-12p40 and IL-12p35, while IL-23 heterodimer consists of IL-12p40 and IL-23p19 [110]. Ustekinumab is a monoclonal antibody developed to block the p40 subunit of IL-12 and is effective clinically in psoriasis patients [54, 83]. Although initially thought to function by decreasing Th1 responses via blocking IL-12, the reality that IL-23 is also blocked by an anti-IL-12p40 antibody implicates Th17 cells in psoriasis pathogenesis. Indeed, IL-23 induces Th17 cells to produce the cytokine IL-22 [84, 159] which has been demonstrated to induce human keratinocyte proliferation and acanthosis, consistent with psoriasis pathogenesis [117]. IL-22 also helps elicit innate immune responses via β-defensins [84] which are upregulated in psoriasis [19]. There are now several antibodies specifically against IL-23 and IL-17 and its receptor for psoriasis [47], and this appears to be the central cytokine pathway for this disease.

Th22

There are CD4 helper T cells that produce IL-22, but not IL-17, IL-4 or IFN-γ. These CD4 helper T cells have been termed Th22 cells, but it is unclear whether they are a distinct helper T cell subset. The master transcription factor for Th22 cells has been identified as aryl hydrocarbon receptor which has been shown to inhibit IL-17 production [115, 142]. Th22 cells reside in normal skin but are increased in lesional skin in psoriasis and atopic dermatitis, and these cells express skin homing markers such as CCR4 and CCR10 [40]. The IL-22 receptor, consisting of IL-22R1 and IL-10R2, is only expressed on non-hematopoietic cells, including epithelial cells such as keratinocytes [46]. In addition to increasing keratinocyte proliferation and epidermal hyperplasia as seen in psoriasis [16, 107], IL-22 has been shown in vitro to decrease filaggrin, loricrin and involucrin [16, 53, 107], suggesting that it contributes to impaired epidermal barrier function as is seen in atopic dermatitis. IL-22 is reduced in psoriasis after treatment [46], and patients with chronic atopic dermatitis treated with narrow-band UVB have reduced IL-22 in their skin [139]. A role for Th22 has also been suggested in allergic contact dermatitis, scleroderma and CTCL [46].

Th9

Th9 cells are a recently described CD4 helper T cell representing a unique T cell subset. Th9 cells are generated via the combination of TGF-β and IL-4 [36, 144]. IL-4 induces

STAT6 and the transcription factor IRF4 and TGF-β activates the transcription factor PU.1 while suppressing the transcription factors Tbet and GATA-3, ultimately inducing production of the pro-inflammatory cytokine IL-9 [158]. Th9 cells also secrete to a lesser extent the anti-inflammatory cytokine IL-10, and only a small amount of IL-4, IL-5 and IL-13 [65]. These latter cytokines parallel those seen in Th2 cells. Th9 cells are pleiotropic, i.e. depending on the environmental milieu, they are also capable of producing Th1 and Th17 cytokines. In fact, in human T cells stimulated via CD3/CD2/CD28, blocking IL-9 limits production of IFN-γ, IL-13 and IL-17 [121]. The IL-9 receptor is expressed on the surface of many immune cell types including T and B cells, mast cells, macrophages and DC, and also on non-hematopoietic cells [44, 65, 87]. Th9 cells seem to play a role in helminth infections [42], asthma and allergy [130].

In regards to skin, Th9 cells were shown to be skin-tropic in humans, with a high frequency of specificity for *Candida albicans*, suggesting a role in protection against extracellular pathogens [121]. Interestingly though, Th9 cells were also increased in number in lesional skin in psoriatic patients suggesting a contribution to inflammatory skin disease [121]. In keeping with this, Th9 T cells were demonstrated in skin biopsies of allergic contact dermatitis [85] and the percentage of Th9 cells in peripheral circulation was increased in patients with atopic dermatitis [91]. Our lab has shown that the pro-inflammatory functions of Th9 cells can be harnessed in tumor immunity, via adoptive transfer of Th9 cells into B16F10 melanoma tumor bearing mice, in which Th9 cells impaired tumor growth [114].

Tfh

Th1, Th2, Th9, Th17 and Th22 cells all function in the periphery to guide and promote immune responses. Conversely, there is a CD4 helper T cell subset that remains in the lymph node follicle, appropriately termed follicular helper T cells, or Tfh. Tfh cells are recognized by their location in germinal centers and by surface expression of CXCR5, ICOS and PD-1 (reviewed in [134, 136]). CXCR5 is a surface receptor that binds CXCL13, a chemokine produced by follicular dendritic cells in the germinal center, thereby attracting $CXCR5^+$ Tfh to that locale (reviewed in [136]). Tfh cells are required for the germinal center reaction in lymph nodes to occur, by which B cells are activated to undergo class switching and produce abundant antibody (reviewed in [134]). They generate the cytokine IL-21, which is a potent growth and differentiation factor for B cells, but also IFN-γ, IL-4, IL-17 and IL-10, suggesting that their cytokine production helps guide antibody class switching (reviewed in [134, 136]). The role of Tfh in skin disease has not been directly studied, however Tfh have been implicated in autoimmune diseases where antibody production is pathogenic. For example, there are increased numbers of $CD4^+CXCR5^+ICOS^{hi}PD1^{hi}$ T cells in circulation of some patients with SLE and Sjogren's syndrome, and this increased number correlates with disease severity [43, 127, 138]. Further, the frequency of $CD4^+CXCR5^+$ T cells was reduced in SLE patients following treatment with steroids [43]. The study of Tfh in human disease is in its infancy, and it is likely that further involvement in cutaneous disease will be unearthed going forward.

CD4 Regulatory T Cells

Immunologic tolerance refers to a state of unresponsiveness to substances or tissues that would otherwise have the ability to stimulate an immune response. We have already seen above one mechanism by which tolerance is induced centrally, by thymic selection, and one by which peripheral tolerance is maintained, by sub-threshold stimulation of naïve T cells. The immune system is more complex still, producing multiple cell types with regulatory function. Here we will review regulatory CD4 T cells, or Tregs.

Like other CD4 T cells, Tregs have been divided into different classifications. Tregs produced in the thymus are referred to as naturally occurring Tregs, nTregs, while Tregs induced in the periphery are termed inducible Tregs, iTregs [112]. iTregs are further divided into two populations, Tr1 cells which are induced by IL-10, and Th3 Treg, which are induced by TGF-β [112], (recall from above that effector Th17 cells are generated by the combination of TGF-β and IL-6, while Th9 cells are generated by the combination of TGF-β and IL-4). Both nTregs and Th3 cells express the transcription factor Forkhead box P3, Foxp3, and are largely phenotypically indistinguishable, while Tr1 Treg do not express Foxp3 [112]. The importance of Foxp3 function is demonstrated by the X-linked genetic disorder IPEX, in which a mutation in the *Foxp3* gene results in multi-organ inflammatory disease, including type 1 diabetes, psoriasis-like dermatitis and enlarged secondary lymphoid organs [8]. The thymic transcription factor, Aire, discussed above, also plays a role in nTreg development (reviewed in [79]), (recall that a mutation in its gene, *AIRE*, results in the APECED).

Tregs maintain tolerance to self-antigens (the body's own tissue) and to harmless or commensal organisms. They function by releasing regulatory cytokines, most notably IL-10 and/or TGF-β, by expressing on their cell surface co-inhibitory molecules such as PD-1, and by cytotoxicity [112]. They also consume IL-2, thereby limiting its availability to effector T cells [112]. They suppress T effector cells that have received low to medium strength TCR signaling, but not high avidity TCR signaling [6] and the ratio of Tregs to Teffector cells seems to be important for suppression of T effector cell responses [20].

Tregs have been heralded as a potential cellular therapeutic against a number of autoimmune diseases and transplant

rejection. Significant time and effort have been spent researching *in vitro* and *ex vivo* expansion, generation and manipulation of Tregs for this purpose. However, Tregs have been shown repeatedly to be particularly plastic, meaning that under different environmental conditions, Tregs gain pro-inflammatory functions, raising concerns about their clinical safety. With that said, clinical studies treating chronic Graft versus Host Disease with low dose IL-2, which promotes Treg responses *in vivo*, has shown initial promising results [76].

CD8 T Cells

Cytotoxicity

CD8 effector T cells recognize intracellular pathogen-derived Ag presented in the context of MHC class I on the surface of an infected cell. In some cases, CD8 T cells may require CD4 effector T cell help to become activated [26]. The main effector function of CD8 T cells is direct target cell killing, or cytotoxicity. They are thus referred to as cytotoxic T lymphocytes (CTLs). When CTLs recognize their cognate Ag presented by MHC on a target cell, the CD8 T cell can employ one of two main mechanisms to induce target cell killing (reviewed in [57]). Firstly, CD8 T cells release granules containing perforins and granzymes. Perforins bind the target cell surface, integrate into the cell membrane, and as the name suggests, induce tiny pores in the surface through which granzymes can enter the target cell cytoplasm. Granzymes are proteases that induce both caspase-dependent and –independent target cell apoptosis. Secondly, CTLs also express on the cell surface Fas ligand (FasL), which binds to Fas on target cells. Binding of Fas on target cells also induces target cell apoptosis.

In regards to skin disease, CTLs have long been suspected in the pathogenesis of toxic epidermal necrolysis (TEN), although their role is controversial [140]. CD8 T cells have been observed infiltrating lesional TEN skin and in TEN blister fluid [34, 81, 106], and granulysin, a cytotoxic mediator of CD8 T cells has been demonstrated in TEN blister fluid [28]. The ability of CD8 T cells to express FasL and keratinocyte Fas, has long been one basis for the [controversial] use of IVIg in TEN, as it is hypothesized that IVIg blocks Fas:FasL binding and downstream keratinocyte apoptosis [145]. Also of interest, is the role of CD8 T cells in alopecia areata (AA), an autoimmune mediated non-scarring form of alopecia. A well-conceived study by Xing et al. demonstrated in both human samples and a mouse model the mechanism by which pathogenic CD8 T cells mediate AA [152]. Defective cytotoxic killing activity is observed in Griscelli syndrome, a rare autosomal recessive genodermatosis with pigmentary defect and immunodeficiency [51].

Cytokine Production

In addition to direct cytotoxicity, CD8 effector T cells directed against intracellular pathogens secrete the pro-inflammatory cytokines IFN-γ and TNF-α, consistent with Th1 cell cytokine pattern, and are therefore referred to as Tc1 (reviewed in [101]). More recently, alternative CD8 T cell subsets have been identified, similar to CD4 T cells. Tc2 cells produce IL-5 and IL-13, Tc9 cells produce IL9, IL-10 and low levels of granzyme B, and Tc17 cells produce IL-17 and IL-21 [101]. Further, suppressor CD8 T cells exist and have been termed CD8 Tregs [101]. The study of these CD8 T cell subsets in skin disease is a burgeoning field.

γδ T Cells

In addition to conventional CD8αβ T cells, as described above there is a second type of CD8 T cell, consisting of one γ and one δ TCR chain, or γδ CD8 T cells. Complicating matters, there are γδ T cells that do not express the CD8 coreceptor (reviewed in [69]). Unlike conventional CD8αβ T cells, γδ T cells do not bind MHC I, but rather recognize and respond to a number of stress-inducible proteins expressed on malignant or stressed cells via activating receptors on the γδ T cell surface (reviewed in [69]). As such, they are classically considered a component of the innate immune system.

γδ T cells exist at low levels in the peripheral blood but are enriched at mucosal interfaces, and in mice, constitute a significant fraction of T cells in the epidermis (where they are called dendritic epidermal T cells-DETCs). DETCs depend on IL-15 for survival. A separate γδ T cell population exists in the mouse dermis [133]. While DETCs express mostly Vγ5 TCR, the dermal γδ T cells are largely Vγ5 negative [133] all activated by IL-23 and produced IL-17. γδ T cells also produce cytokines such as IFN-γ, IL-2 and IL-13 and chemokines such as CCL3, CCL4, CCL5 and XCL1 (reviewed in [58]) and appear in mice to play a role in homeostasis, infection, tumor surveillance, and wound healing (reviewed in [75]). Comparatively, γδ T cells compose only a small fraction of T cells in human epidermis [141], but do appear to play a role in wound healing [141].

T Cell Migration to Skin

Migration of differentiating effector T cells to sites of inflammation is determined by derivation of cognate antigen. T cells express CLA and CCR4 if they encountered antigen first in skin draining LN [21, 23]. CLA binds to E-selectin which is expressed on the surface of post-capillary venules in skin, and up-regulated during inflammation [9, 45, 78, 113]. Skin migrating T cells also express CD44 and CD43 which help facilitate binding to E-selectin [3, 99]. CCR4 binds to two chemokines, CCL17 and 22 [155], which are produced at the site of infection and help attract T cells

[25]. In addition to CCR4, the chemokine receptors CCR6, CCR8 and CCR10 have also been implicated in T cell homing to skin [61, 63, 92]. Modification of selectin ligands on T cells requires α(1,3)fucosyltransferses IV and VII [60, 95, 129] and mice lacking these enzymes are used to study the role of T cells in skin disease [71, 95, 101]. Preferential differentiation toward skin-homing T cells is further promoted by skin itself; skin fibroblasts produce prostaglandin E2 which inhibits DC production of retinal dehydrogenase, thereby impairing gut-homing differentiation [132].

Memory T Cells

Development of a memory response that is capable of more rapid Ag specific immunity upon Ag re-exposure is a major hallmark of the adaptive immune response. How memory T cells are generated in vivo is an avid area of investigation in immunology. It is known that upon primary antigen exposure, naïve T cells expand into an effector cell population that functions to clear an infection. As the infection wanes, the majority of effector cells (90–95 %) die off in a process termed contraction, leaving behind a small population of memory T cells [104] (Fig. 8.3). It is suggested that memory cell generation may be related to the strength of TCR stimulation [128, 135], with higher levels of inflammation and antigen favoring generation of effector cells rather than memory cells [5, 68, 109]. Development of memory cells may also be linked to naïve T cell precursor frequency [4, 97]. Regardless, it is known that distinct subsets of memory T cells exist. Memory T cells are traditionally divided into two categories based on effector function, proliferative capacity and migration potential [119], and are termed central memory T cells, Tcm, and effector memory T cells, Tem. Tcm express the lymphoid organ homing receptors L-selectin (CD62L) and CCR7, but not peripheral tissue homing molecules, and so are confined to secondary lymphoid organs [104, 118]. At baseline, these cells exist in low numbers and can produce IL-2 but not IFN-γ, but upon re-exposure to Ag, vigorously proliferate, migrate to sites of infection, and develop effector functions [104, 118]. Comparatively, Tem express tissue homing molecules but not lymphoid homing molecules, and so remain migrating between peripheral tissues and blood. They produce IFN-γ but have less proliferative capacity. Upon Ag re-exposure, these cells rapidly migrate to peripheral tissue where they provide an early Ag-specific defense against a pathogen [93, 150], while Tcm cells in secondary lymphoid organs are mobilized. A useful marker for memory cells is CD45RO [41]. Therefore CD45RO$^+$ L-selectin$^+$ CCR7$^+$ denotes Tcm while CD45RO L$^-$selectin$^-$ CCR7$^-$ denotes Tem.

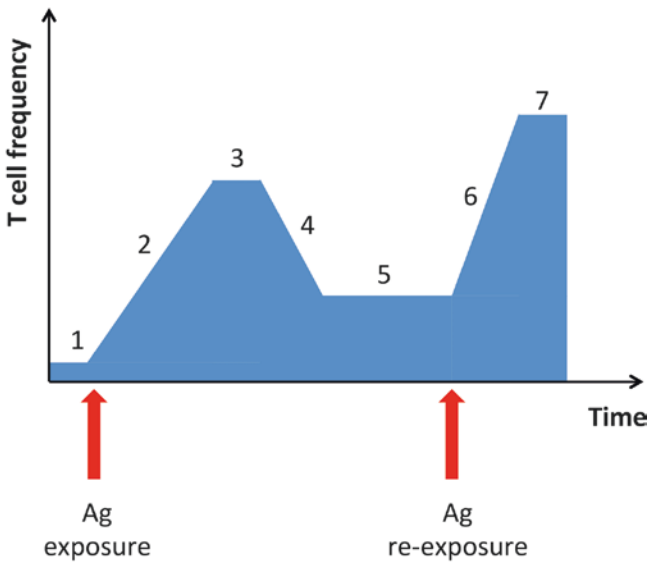

Fig. 8.3 T cell frequency as a function of Ag exposure (infection). (*1*) Naïve precursor T cell pool (*2*) Expansion of Ag-specific T cells (*3*) Ag-specific T effector cells control infection (*4*) Contraction of T effector cell population (*5*) Ag-specific memory T cells remain at a higher frequency than their naïve precursors (*6*) Upon Ag re-exposure, memory T cells require less stimulation, so more rapidly expand and acquire function to control re-infection (*7*) Repeat infection is controlled

Skin-Resident Memory T Cells

In 2006, a method of isolating T cells from human skin via tissue explant cultures allowed for the first time enumeration of T cells in normal human skin [30]. Surprisingly, calculations determined that there are approximately 1 million T cells/cm^2 of skin [29], and based on the average adult total body surface area roughly 20 billion T cells in an adult human's skin [29]. Notably, this is more than is present in an adult's entire blood supply. Further, >95 % of T cells in normal skin are CD45RO memory T cells and the majority express CLA and CCR4, while <5 % are naïve T cells [17, 24, 61, 120]. The majority of these cells also lack expression of the lymphoid homing molecules CCR7 and L-selectin, supporting their categorization as effector memory T cells. The discovery that the vast majority of these CLA$^+$ memory T cells are present in skin under resting conditions (non-inflamed skin), rather than in circulation [29], suggested that this effector memory T cell population is actually resident in skin, and so have been aptly named skin-resident memory T cells. There is also a smaller population of T cells in skin expressing both CLA and CCR4, and CCR7 and L-selectin [29], suggesting an intermediate phenotype population also exists. CD8 resident memory T cells exist primarily in the epidermis, while CD4 resident memory T cells are found primarily in the dermis [93, 154]. A population of T cells that express CLA, CCR4 and CCR7 but not L-selectin

have been recently identified. These migratory memory cells, or Tmm, can recirculate out of skin [162].

The purpose of resident memory T cells is likely to provide a rapid response to antigenic re-challenge (i.e. re-infection). For example, following skin HSV infection in mice, CD8 T cells accumulate in high numbers in skin at the site of infection, are long-lived, and are protective against virus infection [49]. Re-infection of skin with HSV results in a transient population of non-specific T cells recruited from circulation, but also in local proliferation of antigen-specific skin resident CD8 T cells that accumulate in skin [148]. Results were similar in BCG vaccinated humans challenged with the Mantoux skin test, where memory CD4 T cells proliferated locally in skin and were long-lived [147]. Using a vaccinia virus infection model in mice, our lab observed high numbers of protective resident memory T cells at the site of infection, but also noted these cells throughout uninfected skin [67]. Further, skin resident memory T cells were sufficient to clear vaccinia virus in the absence of central memory T cells [67, 86], and utilizing a parabiotic mouse model, Trm were demonstrated to be superior to Tcm in clearing virus [67]. This realization, that a large number of skin resident memory T cells can be generated by skin infection not only at the site of infection, but throughout the skin, and that these cells are poised to act upon re-challenge has heralded great promise in the study of vaccination. Currently, most vaccinations are given intramuscularly or subcutaneously and generate primarily antibody responses which are highly effective against extracellular pathogens and toxins, but less so against viral pathogens. Comparatively, vaccinia vaccination against smallpox, which is performed through skin scarification, prompts a robust and long-lived skin resident memory T cell response [86].

The elucidation of the existence of a skin resident memory T cell population has shed light on the pathophysiology of a number of immune-mediated skin disorders. For example, in psoriasis, the fact that effector memory T cells are present in non-inflamed human skin explains why blockade of T cell entry into skin in a patient with preexisting psoriasis is ineffective at treating disease [12]. This also explains the finding that grafting of 'uninvolved' skin from psoriasis patients to immunocompromised mice results in graft psoriasis despite the absence of human T cells in circulation [18]. In fixed drug eruption (FDE), a skin lesion occurs following ingestion of a causative drug, resolves once the drug is discontinued, then recurs at the same site up to decades later when the same drug is again ingested. FDE is CD8 T cell mediated and following resolution of the FDE, CD8 T cells remain in the epidermis of clinically resolved FDE lesions [137].

Perhaps the best example of the role of skin resident T cells in human skin disease is that of cutaneous T cell lymphoma. In stage IA mycosis fungoides, malignant T cells are confined to stable patches and plaques on the skin [72]. In Sezary syndrome, malignant T cells migrate throughout the entire skin surface, blood and lymph nodes [72]. Interestingly, our group has shown that the malignant T cells in mycosis fungoides are skin resident Trm, while the malignant T cells in Sezary syndrome are consistent with Tcm [22]. Based on the clinical observation that patients with mycosis fungoides treated with alemtuzumab, a monoclonal antibody against CD52, do not have increased susceptibility to cutaneous infections, we have shown that treatment with alemtuzumab is effective in Sezary syndrome, but not mycosis fungoides, as alemtuzumab depletes circulating but not skin resident T cells [33].

Skin Resident Regulatory T Cells (Tcm, Tem)

Approximately 5–10% of skin resident T cells in normal human skin are Foxp3$^+$ Tregs [31] and under inflammatory conditions, these cells can proliferate [32]. Increased numbers of Tregs have been observed in skin injected with PPD and of resolved fixed drug eruptions [137, 146]. Interestingly, it has been shown that Tregs are necessary to control inflammation in non-challenged skin [71, 101] and must actually be present in skin to exert their suppressive effects [71, 101]. Langerhans cells, a type of DC found in epidermis promote skin resident memory Treg proliferation via MHC II:Ag – TCR interaction under both resting and inflammatory conditions [123]. The role of skin resident Treg remains an active area of research and it is likely that these cells will turn out to play a significant role in dermatologic disease and therapeutics.

Conclusions

T cells are clearly a complicated yet essential component not only of the systemic immune system at large but of the skin immune system as well. Their involvement in skin span from protecting against cutaneous pathogens in an otherwise healthy host, to their pathologic targeting of self-Ag leading to cutaneous autoimmune disease, to their own deregulation leading to cutaneous T cell malignancy. As hinted at above, the unique features of these cells are only partially explored. As also suggested in this chapter, understanding of their phenotype and function has led to significant breakthroughs in the treatment of human disease. Taken together, these realizations raise the hope that further investigation of these fascinating cells will continue to impact clinical care of patients with skin disease.

Questions

1. Which of the following binding partners is incorrect?
   A. MHC I : CD8
   B. CD28 : CD80

C. CTLA-4 : CD80
D. MHC I : CD4
E. CLA : E-selectin

2. The genodermatosis APECED results from a defect in which gene:
   A. FoxP3
   B. Calcineurin
   C. CTLA-4
   D. Aire
   E. SPINK5

3. Calcineurin inhibitors (Cyclosporine and Tacrolimus) function by?
   A. Inhibiting production of the effector cytokine IL-17
   B. Promoting IL-2 production thereby decreasing T cell proliferation
   C. Inhibiting IL-2 production thereby decreasing T cell proliferation
   D. Increasing IL-10 production thereby decreasing T cell function
   E. Increasing IL-12 production thereby increasing T cell function

4. Which surface molecules expressed on resident memory T cells allow their migration to the skin?
   A. CD45RO and CCR4
   B. CD4 and CLA
   C. CD28 and CD45RO
   D. CLA and CCR4
   E. Granzyme B and CD8

Answers

1. D
2. D
3. C
4. D

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Cutaneous Dendritic Cells in Health and Disease

9

Sakeen W. Kashem and Daniel H. Kaplan

Abstract

Dendritic cells (DCs) are a heterogeneous cell type found in lymphatic and peripheral tissues. They function as professional antigen-presenting cells (APCs), specialized to acquire antigen from their environment that they process and present to T cells. In addition, DC participate in the generation of local inflammation and suppression of inappropriate immune responses. Thus, they play a critical role in the initiation, propagation and suppression of immune responses that promote health and autoimmune disease. The skin of humans and mice contain several distinct subsets of DC (epidermal Langerhans cells are the most well-known) that are speculated to have varied and versatile functions. In this chapter, we discuss dendritic cells as important players of the innate and adaptive immune system. We will cover their roles in acquiring antigen, inducing T cell responses, and characterizing the functional differences between distinct skin DC subsets. Finally, we will relate DC in relation to human health including their contribution to diseases such as allergic contact dermatitis and psoriasis and their ability to serve as therapeutic targets in vaccinations.

Keywords

Dendtrict cells • DC • Lymphatic tissue • Peripheral tissue • APC • Antigen-presenting cells • Autoimmune disease • Skin disease • Psoriasis • T cell • Allergic Contact Dermatitis • ACD • Langerhans Cells

Historical Perspectives

In 1868, German physician Paul Langerhans observed a network of cells with a dendritic morphology throughout the epidermis [1]. These eponymously named cells were thought to be part of nervous system in the skin. At roughly the same time, Metchnikoff introduced the concept of phagocytosis by macrophages which was later recognized with a Nobel Prize in 1908 [2]. Although Langerhans cells (LC) are related to macrophages, it took more than 100 years for the function of LC to be understood. In 2011, Ralph Steinman received the Nobel Prize for his recognition in 1973 of a novel cell type in murine spleen with an appearance distinct from macrophages that he termed dendritic cells (DC) [3]. He found that DCs were not as apt at endocytosis as macrophages but expressed high levels of major histocompatibility (MHC) molecules and were potent inducers of the mixed leukocyte reaction in mice [4]. Balfour characterized cells resembling LC in the skin draining afferent lymph that were critical for promoting lymphocyte activation [5]. Katz and Frelinger demonstrated mouse LC to be bone marrow derived and, therefore, part of the hematopoietic system [6, 7]. Finally in 1985, Austrian dermatologist Schuler in the Steinman laboratory defined LC as members of the DC family thereby demonstrating the presence of dendritic cells in the skin [8]. In recent years, there has been an explosion of research examining the phenotype and function of dendritic cells.

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Dendritic Cell Paradigm

Dendritic cells that reside in both peripheral and lymphoid tissues (e.g. lymph nodes and spleen) exist in what is termed an immature or unactivated state under normal conditions. They are able to efficiently acquire extracellular material from their environment through a variety of mechanisms. In the case of skin DC, these antigens include self proteins expressed in the skin, products of skin commensal organisms as well as invading skin pathogens. DC in the spleen and lymph node acquire antigens from the circulation or from draining lymphatics, respectively. Immature DC acquire soluble antigens via endocytosis or fluid phase macropinocytosis allowing them to accumulate antigens that are extensively diluted in their environment [9, 10]. DC ingest particulate antigen by phagocytosis. DC also express a variety of antigen-uptake receptors. These include Fc receptors (recognition of Fc domains of immunoglobulin), complement receptors, and many different C-type lectin receptors that recognize microbial carbohydrates [11].

DC activation occurs in response to a large variety of pathogen associated molecular patterns (PAMPs) (e.g. TLR receptors) that are discussed in detail in Chap. 2. Once activated, DC undergo a series of cellular changes that result in mature dendritic cells. As part of the maturation process, DC increase expression of the chemokine receptor CCR7 that is the receptor for the ligands CCL19 and CCL21 [12]. These chemokines are expressed by lymphatic endothelium and direct the migration of antigen-bearing DC from the periphery via afferent lymphatics into the T cell area of regional lymph nodes. Within the lymph node, DC express CCL19 that attracts T cells though the interaction of CCR7 that is also expressed by naïve T cells [13].

Immature DC retain the bulk of the antigen they acquire in an unprocessed form within intracellular endosomes. Activated DC shunt this antigen into intracellular pathways that results in processing and surface presentation of antigen in the context of MHC-II for recognition by CD4+ T cells [14]. Specific DC subsets also have the capacity to present antigen acquired from their environment in the context of MHC-I for recognition by CD8+ T cells, a process termed cross-presentation. In addition, some DC present lipid antigens on nonpolymorphic CD1 molecules to both classic αβ T cells as well as non-classical T cells such as invariant NKT cells [15–17]. Ligation of the T cell receptor (TCR) by antigen-MHC complexes on the surface of DC (or other APC) results in the first signal of T cell activation.

As part of the DC maturation process, activated DC increase surface expression of adhesion molecules that are required for efficient formation of the immunological synapse with the T cell [8, 10, 18, 19]. TCR ligation alone, however, in the absence of co-stimulation results in antigen-specific unresponsiveness, termed anergy, rendering the T cell unable to respond to subsequent antigen encounters (Fig. 9.1a) [20]. DC have a higher capacity to provide co-stimulation compared to other APCs [19]. Surface expression of CD80 (B7.1) or CD86 (B7.2) that bind CD28 on T cells is greatly increased by DC activation. Other co-stimulatory molecules of the B7 family (ICOS ligand, PD-L1, PD-L2, etc.) and TNFR family (CD40L, CD70, OX40L, 4-1BBL, GITRL) are also expressed at high levels by DC and serve significant and diverse roles in inducing and regulating immunity [21, 22].

In addition to TCR ligation and co-stimulation, DC-derived cytokines determine the specific phenotype of T cells that respond (Fig. 9.1b). For instance, DC-derived IL-12 is required for the development of cytotoxic function in CD8+ T cells. In the absence of IL-12, peripheral tolerance is evident in CD8+ T cells and they have reduced ability to kill target cells and to produce IFNγ [23]. Similarly, IL-12 and its homolog IL-27, also have been shown to be important for CD4+ T cell commitment to the Th1 lineage and for the secretion of IFNγ that facilitates immune responses against intracellular pathogens such as viruses, certain bacteria and neoplasia [24–26]. DCs also produce IL-1β, IL-6 and IL-23 that induce Th17 cells. Th17 cells secrete IL-17A that recruits and activates neutrophils as well as IL-22 that signals on keratinocytes to stimulate proliferation and production of antimicrobial peptides [27–32]. Th17 cells are crucial for protection against extracellular skin bacteria and fungi such as *S. aureus* or *C. albicans* (see Chap. 16) [33]. Conversely, over-activity of Th17 is associated with autoimmune conditions such as psoriasis (Chap. 21) [34]. DC are also critical for generation of Th2 responses, which protect against parasites and are also a key cell type involved in atopic dermatitis (Chap. 22) [35–37]. It is important to note that the specific cytokines that DC elaborate and thus the ultimate composition of the T cells response depends both on the specific DC subset and on the nature of the pathogen encountered. The function of individual DC subsets in response to specific pathogens is discussed below.

Steady-State DC Paradigm

The DC paradigm described above was defined based on work with DC that had been activated by inflammatory stimuli. Under steady-state conditions, DC that reside in LN and spleen present self antigens acquired locally as well as filtered from the lymph and blood in the absence of inflammatory stimuli. Antigen that is experimentally targeted to DC in the absence of adjuvant results in transient activation and proliferation of CD4+ and CD8+ T cells, followed by deletion of these cells and the establishment of antigen specific tolerance [38, 39]. Similarly, a genetic approach to limit antigen expression to immature DC resulted in transient activation and proliferation of CD4+ and CD8+ T cells, followed

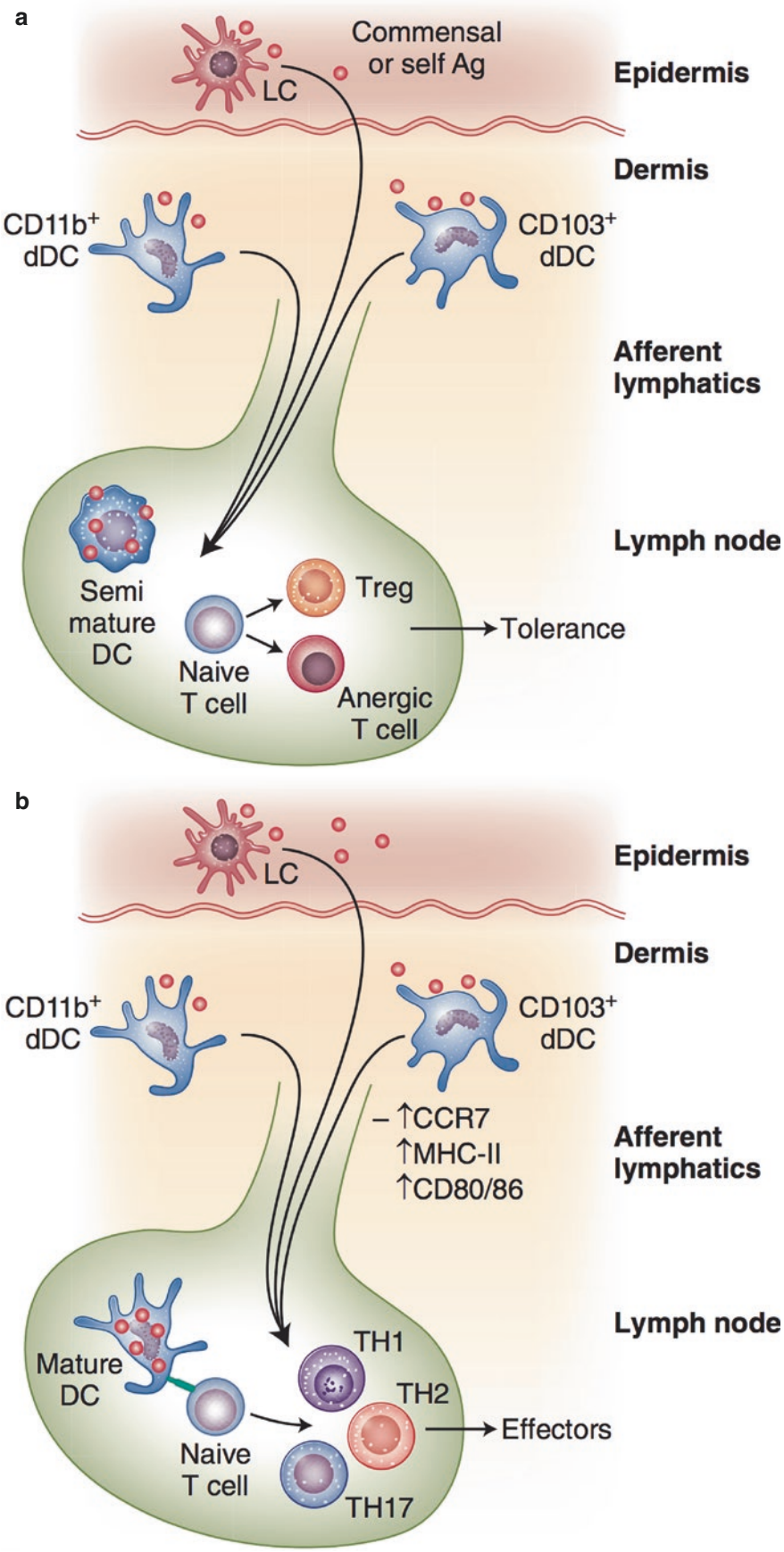

Fig. 9.1 Dendritic cell paradigm during steady state and activation. Cutaneous dendritic cells (DC) include Langerhans cells (LC) in the epidermis and CD11b + and CD103+ dermal dendritic cells (dDC) in the dermis. In the steady state (**a**), cutaneous DC patrol and survey the skin for commensal and self-antigens. Semi-mature DC migrate to the lymph node to present antigen to CD4+ and CD8+ T cells to induce tolerance via the differentiation of regulatory (Treg) and anergic T cells. During inflammation (**b**), DC sample danger and pathogen associated molecular patterns, take up the causative agents, become activated and upregulate CCR7, MHC-II and CD80/86. DC migrate to the lymph nodes, where they present peptides, provide co-stimulation and secrete cytokines to drive the differentiation of effector CD4+ and CD8+ T cells

by deletion of these cells and the establishment of antigen specific tolerance. This was mediated by increased expression of the inhibitory molecules PD-1 and CTLA-4 on T cells and required Treg cells [40–42]. Peripheral DC also migrate in the absence of exogenously added stimuli. These DC, termed "semi-mature", present antigens to T cells that are derived from self proteins and possibly from products of commensal organisms as well. DC that are activated through disruption of E-cadherin mediated DC-DC interaction or by genetic manipulation of β-catenin take on this "semi-mature" phenotype and induce tolerance [43, 44]

In addition to inducing T cell deletion or unresponsiveness, DC have the capacity to induce the formation of Treg cells through the elaboration of specific factors such as transforming growth factor-β (TGFβ) and retinoic acid [45–47]. Interestingly, the number of Tregs in unmanipulated mice correlated with the number of DC [48]. In addition, mice in which peripheral DC are prevented from migrating have reduced number of Treg and develop autoimmunity, implicating peripheral DC in maintaining tolerance [49]. The role of individual DC subsets in Treg induction and maintaining tolerance will be discussed below.

Dendritic Cell Subsets

A major barrier for the study of dendritic cells is their relative rarity and the existence of several distinct subsets of these cells. Initially, DC were distinguished from other leukocytes based on a shared set of characteristics including morphology, distribution, and function. DC have elongated processes termed dendrites that project outward and sample the environment. An irregular shape allows DC to have a large surface area and, while they account of a small population of all cell present, they are able to interact with many neighboring cells. Unlike cells such as T and B cells, there is no single antigenic marker that uniquely identifies DCs. All DCs, regardless of subset, constitutively express the hematopoietic markers CD45, MHC-II, as well as CD11c. They also lack T cell, natural killer (NK) cell, B cell, granulocyte, and erythrocyte lineage markers. CD11c is the classic marker associated with DC but is not unique and is also expressed by several macrophage populations—particularly lung and intestinal macrophages—but also on cDC precursors and other leukocytes [50–52]. The addition of other surface markers in combination with CD11c and MHC-II allows for reliable classification of DC subsets (Table 9.1). To make sense of the complexity of DC subsets, it is easiest to examine them based on their ontogeny and tissue of origin (i.e. peripheral tissues such as skin vs. LN/spleen resident).

Classical DC

Classical DCs (cDC) are a broad category of LN/spleen resident DC that have a high turnover and are constantly replaced by blood-derived precursors. cDCs develop from a hematopoietic lineage distinct from other leukocytes requiring the transcription factor zbtb46 and the cytokine Flt3L [53–56]. Importantly, cDC can be divided into two groups that were

Table 9.1 Mouse APC subsets

		IRF8 cDC	CD103+ dDC	IRF4cDC	CD11b+dDC	LC	Mo-DC	Macrophages
Surface markers	CD8	+	–	–	–	–	–	–
	CD103	–	+	–	–	–	–	–
	XCR1	+	+	–	–	–	–	–
	Clec9A	+	+	–	–	–	–	–
	CD11b	–	–	+	+	+	+	±
	CD207	±	+	–	–	–	–	–
	CD301b	–	–	–	+	±	+	+
	CD172	–	–	+	+	–		
	CD64	–	–	–	–	–	+/low	+
	MERTK	–	–	–	–	–	–/low	+
	CCR2	–	–	–	–	–	+	–/low
	f4/80	–	–	–	–	+	+	+
Transcription factors	Batf3	+	+	–	–	–	–	–
	ID2	+	+	–	–	+	–	–
	IRF4	–	–	+	+	–		
	IRF8	+	+	–	–	+	–	–
Soluble factors/receptors	Flt3	+	+	+	+	–	–	–
	Csf-1R	–	–	+	+	+	+	+
	Csf-2R	+	+	+	+	–		
	TGF-b	–	–	–	–	+	–	–

initially demarcated as "lymphoid" and "myeloid" DC lineages but are now best described as IRF8 dependent and IRF4 dependent cDC. **IRF8 cDC** require the transcriptional factor Basic leucine zipper transcription factor ATF-like (BATF3), interferon regulatory factor 8 (IRF8), and inhibitor of DNA protein 2 (ID2) (Table 9.2) [57–59]. They express high levels of Flt3, proliferate after administration of Flt3L and are nearly absent in Flt3L−/− mice [55, 60]. They can be best identified based on the expression of CD8α but not CD8β and are often referred to as CD8+ cDC. They also express no or low levels of integrin CD11b as well as other macrophage defining markers [61] and represent 20–40 % of the secondary lymphoid organ resident DC [62, 63]. **IRF4 cDC** similarly proliferate in response to Flt3L and are reduced in Flt3- and Flt3L-deficient mice, but to lower levels in comparison to IRF8 cDC [55, 60]. They depend on transcriptional factor IRF4 and zbtb46, but not on BATF3, IRF8 or ID2 for their development [57, 58, 64]. They can be identified based on their expression of integrin CD11b+and the absence of CD8α.

Dermal DC

The best defined DC subsets in the dermis can be classified as **CD103+ dermal DC** (dDC) or **CD11b+dDC**. CD103+ dDC are homologues of the IRF8/CD8+ cDC. They express the integrin CD103 that binds e-cadherin expressed by epithelial cells but lack expression of CD8a [58]. CD103+ cDC lack the macrophage markers CD11b, CD115, CD172a, F4/80, and CX3CR1 [65]. They also depend on BATF3, IRF8 and ID2 [57–59]. In addition to Flt3L, CD103+ cDC also require Csf-2 for their development [66, 67]. This DC subset is fairly infrequent in the dermis but they migrate and are replenished from bone marrow precursors at a high rate [68].

CD11b+dDC comprise the majority of DC in the dermis but are less well studied than CD103+ dDC [68]. They are homologous to IRF4 cDC and can be identified based on expression of CD11b and the absence of CD103. They require cytokine Csf-1, IRF4 and Flt-3 for development [51, 66, 69]. Importantly, these dDC are often confused with macrophages that are also abundant in the dermis and express CD11c, MHC-II and CD11b. CD11b+dDC can be distinguished based on the absence of FcγRI (CD64) and MerTK expression [70].

Langerhans Cells

Langerhans cells constitute the sole APC population in the epidermis under steady-state conditions. Murine LCs are uniformly CD11b+F4/80+ and lack CX3CR1 expression [51]. They express the C-type lectin Langerin (CD207), which is involved in the formation of Birbeck's granules, a pathognomonic marker for LC [71]. LC account for 3–5 % of epidermal cells and stand apart from the other DC subsets through their unique ontogeny and homeostatic properties [72]. During ontogeny, LC precursors seed the epidermis first from hematopoetic precursors in the yolk-sac and then from the fetal liver [73, 74]. In contrast to most classical DC, LC develop independently of Flt3 and Flt3L and require keratinocyte-derived IL-34 signaling on Csf-1R for their development. CSF1R signaling is also required for macrophage development suggesting that LC may be more closely related to macrophages than other DC subsets [75–78]. In addition, LC require Runx3, PU.1, ID2 and BMP7 for their differentiation and autocrine TGF-β for their epidermal maintenance and homeostasis [79–84]. In the adult, LC form a self renewing population that can be replenished from

Table 9.2 Human APC subsets

	pDC	CD1c+	CD141+	CD14+	LC
Location	Blood/lymph/dermis			Dermis	Epidermis
HLA-DR	+	+	+	+	+
CD11c	Low	+	+	+	+
CD1a	–	–	–	–	+
CD14	–	–	–	+	–
BDCA1 (CD1c)	–	+	–	+	+
BDCA2 (CD303)	+	–	–	–	–
BDCA3 (CD304)	+	±	+	–	–
BDCA4 (CD141)	–	–	–	–	–
XCR1	–	–	+	–	–
Clec9a	–	–	+	–	–
Langerin (CD207)	–	–	–	–	+
EpCam	–	–	–	–	+
E-Cadherin	–	–	–	–	+
Murine equivalent	pDC	CD11b+DC	IRF8/CD103+ DC	mo DC/mac	LC

blood derived monocytes after strong inflammatory stimuli such as UV light [85–90]. LC precursors emigrate into the epidermis via the hair follicle [91]. In the absence of strong inflammatory stimuli, LC will remain of host origin after bone marrow transplantation

Human Skin DC Subsets

The use of flow cytometric and gene profiling methods have allowed for refined characterization of DC in the human skin (Table 9.2). This is an active area of research but the current data suggests that human DC subsets are homologous to mice DC subsets but express a different set of identifying markers. DC in humans are defined as lacking lineage markers CD3, CD19, CD14, CD20, CD56 and glycophorin A [92]. Conventional human DC also express MHC-II and CD11c, but do not express markers such as CD303 (BDCA-2) and CD304 (BDCA-4) that are exhibited on plasmacytoid DC. Circulating pre-DC differentiate into conventional DC in peripheral tissue in mice while human skin CD1c+(BDCA-1) and CD141+ (BDCA-3) are found and arise in circulating blood. CD1c+DC represent the major APC population in the human dermis as well as circulation and are similar to the mouse CD11b+conventional DC [93, 94]. CD141+ DC are the only DC to express XCR1 and make up a small population, representing the same lineages as the mouse IRF8 and cD103+ DC [94]. The human dermis also contains CD14+ monocyte derived DC and macrophages. Langerhans cells are the only APC population in the human epidermis, expressing CD1a, and E-Cadherin. In contrast to the mice DC subsets in which LC and IRF8 DC both express Langerin, human LC are the only skin DC to express Langerin [95].

Cutaneous DC Function

Cross Presentation of Exogenous Antigen to CD8 T Cells

An important function of DC is the acquisition of foreign antigen that is then processed and cross-presented in the context of MHC-I in order to activate CD8+ cytotoxic killers (CTL). CD8+ CTLs provide immunity to viral and intracellular bacteria as well as many varieties of neoplasia. Identifying which DC subsets cross-present antigen is of particular importance for designing effective vaccines. Numerous *in vivo* experiments in mice have identified the IRF8 cDC and CD103+ dDC and the primary DC subset responsible for cross-presenation [96–99]. Mice with a selective deficiency of these subsets (i.e. Batf3$^{-/-}$ mice) are unable to mount CD8+ T cell response against subcutaneous infection with West Nile virus, epicutaneous infection with *C. albicans,* and are unable to reject fibrosarcomas [57, 100, 101]. In addition, CD103+ dDC cross present keratinocyte derived antigens to CD8+ T cells that could be important in promoting cross-tolerance [68]. IRF8 cDC and CD103+ dDCs express MHC-I related genes [102, 103] and are a source of IL-12 and IL-15, cytokines that drive differentiation of cytotoxic CD8+ T cells [104]. Moreover, these DC uniquely express the chemokine receptor XCR1. XCL1, the ligand for XCR1, is rapidly produced by CD8+ T cells upon antigen presentation and promotes CTL differentiation [105].

Other DC subsets may also have the capacity to cross-present antigen. CD11b+dDC can present antigen to CD8+ T cells. Whether this occurs in only specific circumstances and whether this is presentation of antigen expressed by the DC itself or represents true cross-presentation is unclear [106–109]. LC grown *in vitro* or isolated from human or mouse skin explants efficiently cross-present antigen to CD8 T cells *in vitro*. In contrast, LC were unable to generate CD8+ T cell expansion in response to skin infection with HSV-1 or *C. albicans in vivo* [101, 110]. It is unclear whether these conflicting results represent disparate functions of human vs. mouse LC, derive from differences in experimental technique, or reflect differences in DC isolated from skin vs lymph node [111]. Interestingly, all DC subsets isolated from human skin explants can prime CD8+ T cells to some extent *in vitro* but CD141+ DC, the human homolog of IRF8+ and CD103+ dDC, are significantly more efficient than other subsets [94, 100, 112, 113].

Allergic Contact Dermatitis

A key event in allergic contact dermatitis (ACD) is the priming of hapten-specific naïve CD4+ and CD8+ T cells in the regional lymph node (see Chap. 23) [114]. Although haptens can drain to the lymph node via lymphatic flow this does not lead to a productive T cell response [115, 116]. Instead, antigen presentation in the lymph node by migratory DCs is absolutely essential for the generation of responses to peripheral antigen [115–118]. Most studies examining the contribution of individual DC subsets have been performed in mice with selective ablations of individual DC subsets using contact hypersensitivity (CHS) assays to small haptens such as DNFB. In one series of studies, mice in which LC are selectively ablated develop enhanced contact hypersensitivity responses to various contact allergens suggesting that LC suppress the development of CHS. The suppressive function of LC occurs during the initial priming step and the absence of LC during the effector phase does not affect CHS [119–121]. LC suppression of CHS responses depends on cognate interaction with CD4+ T cells and LC derived IL-10 [121]. LC have been also demonstrated to suppress CHS by tolerizing

CD8+ T cells and activating regulatory T cells [122]. CD103+ dDC appear to participate in the development of CHS since some mice with a conditional depletion of CD103+ dDC have reduced CHS [123, 124]. This suggests that CD103+ dermal DC, rather than Langerhans cells, promote the development of contact hypersensitivity. Other data, however, particularly using low doses of hapten find that LC are required for the development of CHS [125–127]. Moreover, mice constitutively lacking C103+ dDC and IRF8 cDC develop CHS responses normally [114]. In addition, transplant of hapten primed CD11b+dermal DC can transfer CHS *in vivo* [128]. Thus, the relative importance of individual DC subsets for the induction of CHS remains unresolved. Untangling the functions of skin-resident DC during CHS is hindered by the intrinsic experimental variability of the assay and the difficulty in analyzing hapten specific T cells responses.

Skin Infection

In the setting of skin infection, distinct skin resident DC subsets have diverging functions in mounting T cell differentiation. In the setting of *C. albicans* skin infection, CD103+ dDC are required for the development of CD8+ T cell and Th1 responses through a mechanism that likely involves CD103+ dDC-derived IL-12 [101]. In contrast, Langerhans cells are specialized to drive Th17 differentiation in the setting of an epicutaneous *C. albicans* infection and produce significant amounts of Th17 differentiating cytokines such as TGF-β, IL-1β and IL-6. The function of skin DC may vary with the pathogen used since mice lacking CD103+ dDC and IRF8+ DC are able to mount protective responses to West Nile Virus and cutaneous *Leishmania major* infection [57]. In the setting of *N. brasiliensis* helminth infection, CD11b+and IRF4+ DC are important in inducing Th2 immunity [35, 36]. Similarly, dermal immunization with papain and epicutaneous immunization with FITC promote Th2 responses that required CD11b+dDC [36, 128]. Th2 induction by CD11b+dDC likely involves DC-derived thymic stromal lymphopoetin (TSLP) [129, 130]. LC also express the receptor for TSLP and been shown to initiate epicutaneous sensitization with protein antigens and induce Th2-type immune responses via TSLP signaling [129, 131].

Tolerance

Immunological tolerance describes a state of T cell unresponsiveness. A failure of tolerance to self antigens results in autoimmune disease. As discussed above, "semi-matured" DC present self antigen and participate in tolerance to self antigen. Dendritic cells that are "semi-matured" by repeated injections of TNFα suppress autoimmunity [132]. Few but not all pan-DC depletion models demonstrate the generation of spontaneous autoimmunity [133–135]. It is important to note that while the role of individual DC subsets have been shown to be important in peripheral tolerance, no single constitutive DC subset deficiency models have been demonstrated to lead to autoimmunity [55, 57, 58, 91]. Individual DC subsets do suppress inflammatory responses. LC suppress irritant responses in hapten challenge settings and in mouse model of acrodermatitis entropathica [119, 136]. In addition, LC have been shown to suppress immune responses to *L. major* via the activation of regulatory T cells and promote tolerance to minor-mismatched skin grafts [137, 138]. Receptor activator of NF-kB ligand (RANKL) expression on keratinocytes mediates LC directed Treg activation and regulates UV induced immunosuppression [139]. In addition, CD11b+dDCs are thought to have a superior ability in inducing peripheral Treg differentiation due to their unique expression of aldehyde dehydrogenase (ALDH), an enzyme that metabolizes exogenous vitamin A into retinoic acid that helps regulatory T cell differentiation [140–143]. Steady state targeting of antigens to LC and CD103+ dDC can induce Treg cells [38, 39, 144] demonstrating that most DC are capable of inducing Tregs *in vivo*. Thus, it appears likely, that the ability to suppress effector responses is not limited to an individual "suppressive" DC subset.

Antibody Responses

DC have been shown to be important for B cell mediated antibody responses. Langerhans cells project their dendrites through the epidermis and capture antigens, leading to production of significant IgG1 production [145]. Steady state antigen targeting to DC to various antigen uptake receptors have shown the induction of robust humoral immunity. Specifically, targeting antigen to IRF8+ and CD103+ DC induce the differentiation of antigen specific T follicular helper cells and germinal center B cells [146, 147]. DC induction of antibody response is likely an important contribution to skin autoimmune disease such as pemphigus vulgaris as well as for vaccine therapeutics.

Monocyte, Macrophage and Recruited DC

Macrophages

In addition to subsets of dendritic cells, other subsets of MHC-II antigen presenting cells are present or can be recruited into the skin. Dermal macrophages do not migrate to the draining lymph nodes in mice and form a self renewing population similar to Langerhans cells [148]. Dermal macrophages have a lower capacity to present antigen compared to dermal DC

and function in scavenging and killing microorganisms [148]. They also likely participate in presentation of antigen to T cells that are recruited into the skin [93]. In stark contrast to dermal DC, macrophages as well as monocyte derived DC in the human and mouse dermis express high levels of IL-10 transcript, suggesting a possible anti-inflammatory role [70]. Some dermal macrophages express CD4 and surround post-capillary venules and produce chemokines that promote extravasation of neutrophils into the infected dermis [149].

Monocyte Derived DC

In healthy mouse skin, dDC and monocyte-derived DC have a fast turnover whereas dermal macrophages have a slower turnover and a longer life. Most tissues macrophages are thought to be derived from blood LY6Chi monocytes while some macrophages are established prenatally, and derive from self replicating yolk sac progenitors [73, 150, 151]. In addition, there is a small population of blood derived CD16-expressing monocytes that display an advanced stage of differentiation with effector functions related to antigen processing and presentation [152, 153]. They arise in inflammatory conditions and represent the main producers of inflammatory cytokines such as TNFα and have a high capacity to stimulate antigen-independent T-cell responses. Monocyte derived DC can be further subdivided into 6-sulfo LacNAc (slan) -positive and -negative monocytes that both produce pro-inflammatory cytokines. The expression of slan was initially identified on an inflammatory human DC subset found in the blood and skin [154]. In steady-state, murine classical monocytes continuously extravasate and transport tissue antigens to the LN without differentiating into DC or macrophages [155]. Despite high levels of antigen capture and expression of MHC-II, monocyte derived DC are relatively poor antigen presenters [156, 157]. Alternative mechanisms such as cytokine production or antigen transfer with productive DC and APC may be their role in adaptive immunity.

Inflammatory DC

Inflammatory DCs refer to populations of DCs that are transiently formed in response to various inflammatory stimuli and disappear when the stimuli is resolved. Inflammatory DC development and function remain poorly understood, with the lack of surface markers to identify them. The phenotype of inflammatory DCs is influenced by kinetics and the nature of the stimuli. Inflammatory DCs that accumulate in the LN in response to lipopolysaccharide (LPS) administration express DC-specific transcription factor zbtb46 and fail to accumulate in Flt3L−/− mice, validating them as DC [53, 56]. Another subset of inflammatory DCs identified in mice infected with *L. monocytogenes* was termed TNF-α/iNOS-producing DCs (TipDC) because of their ability to produce high levels of TNF-α and iNOS. TipDC are also found abundantly in psoriatic skin and are believed to be a key effector population [158]. However, in contrast to LPS-induced DC, TipDCs lack zbtb46, suggesting that they are distinct from true DC [53, 56]. The full understanding of inflammatory DC function is lacking, as are subset specific depletion models.

Plasmacytoid DC

Plasmacytoid DCs (pDCs) represent a small subset of DCs that share a similar origin to DC but a distinct life cycle. They circulate in blood and lymphoid tissues and can be found in the skin and other peripheral tissues under inflammatory conditions. They express lower levels of MHC-II and costimulatory molecules in the steady state and display a narrow range of PRRs that include Toll-like receptors 7 and 9. Upon recognition of pathogenic nucleic acids, they produce massive amounts of type I IFNs that are important for resistance to viral infections but also participate in the development of autoimmune conditions such as psoriasis and systemic lupus erythematosus [159–163].

DC and Disease

Syndromes with DC Defects

There are three known genetic DC deficiencies in humans. DCML deficiency syndrome is caused by a mutations of GATA-binding factor 2 (GATA2) and demonstrates a complete absence of blood DCs, pDCs, tissue cDCs, circulating monocytes, B cells and NK lymphoid cells [164]. IRF8 null mutations are found in humans and lead to the absence of monocytes, pDC, cDCs, and dermal DCs and defective IL-12 production and intact epidermal LC, resembling the mice knockout of IRF8 [165]. Mutation of adenylate kinase 2, a phosphotransferase required for nucleotide homeostasis causes a form of severe combined immunodeficiency known as reticular dysgenesis. It is associated with impaired formation of all nucleated blood cells, including neutrophils, lymphocytes, monocytes, and cDCs as well as LCs [166]. Patients with these defects all have increased risk of infections, but it is difficult to pinpoint the specific function of individual DC since the defects affect a broad range of cell types.

Psoriasis

Psoriasis is also another condition in which DC are implicated for pathogenesis. Repeated application of the TLR7 ligand imiquimod to murine skin induces an inflammatory response that recapitulates some similarities to that of human psoriasis, including hyperproliferation and differentiation of keratinocytes and thickening of the epidermis [167, 168].

Infiltrates of neutrophils in the epidermis and DC, macrophages and T cells in the dermis is found after imiquimod induced psoriasis an other genetic mouse models of psoriasis. In the imiquimod model of psoriasis, IL-23 production by DC elicits dermal gamma delta T cells to secrete IL-17 and IL-22 which recruit neutrophils and drive the proliferation of keratinocytes, respectively [169–173]. TLR signaling in DC seems to be necessary and sufficient for the formation of IL-23 mediated psoriasis [174]. While pDC are found in the skin of psoriatic patients and mouse models of psoriasis, conditional ablation of pDC does not lead to attenuation of disease [174]. More recently, it has been suggested that sensory nociception leads to the activation of DC to produce IL-23 after the application of imiquimod and cutaneous denervation leads to attenuation of disease in transgenic mice models of psoriasis [175, 176]. However, which DC or monocyte or macrophage subset is responsible for IL-23 secretion remains controversial [172, 174].

Vaccination

Efficient vaccination requires acquisition of antigen by DC. The density and easy access to DC in the skin makes them an attractive target for vaccination strategies.

Interestingly, the smallpox vaccine, which was the first vaccine developed, is administered intradermally and remains one of the most effective vaccines developed [55, 74, 119, 177]. The density of skin DC is thought to explain the increased efficiency of skin immunization compared with the intramuscular route [93]. Most vaccines, however, bypass the APC rich epidermis and the dermis and target the connective and adipose tissue rich hypodermis. More recently, newly developed delivery systems are becoming more common in order to target the more superficial layers of the skin. For example, microneedle vaccination has been used to introduce encapsulated influenza virus vaccine into the dermis leading to robust cellular and humoral immune responses [178]. Laser-generated micropores have been used for intradermal immunizations to induce potent immune responses [179]. Combinations of novel delivery systems with epitopes coupled to antibodies that specifically target cell surface endocytic receptors (e.g. DEC-205 or Langerin) expressed by APC populations are becoming more popular for researchers to study DC functions as well as for vaccination trials against cancers and pathogens [180, 181]. For example, targeting vaccine antigens directed against receptor Clec9A to CD103+ or CD8+ cDC enables robust cross-presentation and CD8+ T cell response [135, 146, 147]. In addition, antibody responses are generated via the production of T follicular helper cells [147]. Given the potential for DC specific differences in function, it is plausible that specific DC may be targeted for a tailored cellular immune response, such as tolerance in the setting of autoimmunity [38, 39, 144].

Review Questions

1. Identify the two major subsets of dendritic cells found throughout the secondary lymphoid tissues (select all correct answers).
   a. IRF8 DC
   b. Langerhans Cells
   c. CD103+ dDC
   d. IRF4 DC
   e. CD11b + dDC

Correct answer: a and d. Classical DCs (broad category of LN/splenic resident DCs) can be broadly categorized into two distinct subsets by the expression of transcriptional factors IRF4 and IRF8. IRF8 cDCs express CD8, XCR1 and Clec9a while IRF4 cDCs express CD11b and CD172. These 2 DC types are found in lymph node, spleen and have closely related counterparts in most peripheral tissues. Skin DCs can be categorized into three broad categories: Langerhans cells, CD11b+ dDCs, and CD103+ dDCs

2. How are dendritic cells activated (select all correct answers)?
   a. PAMPS
   b. Cytokines
   c. Stochastically
   d. Antigen
   e. B-catenin

Correct answer: a and b. Skin DCs get activated through their pattern recognition receptors (e.g. TLR) as well as by inflammatory cytokines. This results in expression of CCR7 that facilitiates migration into LN and increased expression of MHC-II and co-stimulatory molecules (e.g. B7) that allow for efficient activation of cognate CD4+ and CD8+ T cells

3. What is the function of skin dendritic cells under steady-state conditions (select all correct answers)?
   a. T cell activation
   b. Clean-up necrotic debris
   c. B cell activation
   d. Inhibition of Mast cell activation
   e. T cell tolerance

Correct answer: e. Steady state DCs mediate tolerance to self antigens through a mechanism involving b-catenin as well as other likely mechanisms

4. What role do dendritic cells play during allergic contact dermatitis, Psoriasis and during infection (select all correct answers)?
   a. Activate T cells specific for foreign antigens
   b. Secrete IL-23
   c. Secrete IL-15
   d. Induce Th17 responses
   e. Induce Th2 responses

Correct answer: a,b,c,d,e. The precise role of individual DC subsets during allergic contact dermatitis remains

unclear. Some studies demonstrate that LCs suppress CHS and DTH while others show that LCs induce CHS and DTH responses. Similarly, the data is also controversial for CD103+ and CD11b+ dDCs. For Psoriasis, mice in which DCs are conditionally depleted have less response in imiquimod induced psoriasis inflammation model. TLR signaling on CD11b+ dDCs have recently been demonstrated to be sufficient in inducing IL-23 in response to imiquimod to drive psoriasis like inflammation in mice. Fo infection, In response to *C. albicans, L. major* and West Nile virus infections, CD103+ dDCs mediate Th1 responses. LCs mediate Th17 responses in response to epicutaneous *C. albicans* infection. Finally, CD11b+ dDCs induce Th2 responses to *N. brasiliensis* infection

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Photoimmunology

10

Jake E. Turrentine and Ponciano D. Cruz Jr.

Abstract

Photoimmunology is the study of the effects of non-ionizing electromagnetic radiation (principally ultraviolet light) on the immune system. Ultraviolet (UV) light represents the spectrum of electromagnetic radiation between the wavelengths of 100 and 400 nm. Through its actions on the immune system, UV radiation promotes the development of skin cancers, modulates the development of allergic contact dermatitis, and triggers several specific photosensitivity disorders collectively known as the immunologically-mediated photodermatoses. These disorders include polymorphic light eruption, actinic prurigo, solar urticaria, hydro vacciniforme, and chronic actinic dermatitis. In addition to discussing the molecular underpinnings of UV-induced carcinogenesis and the effects of UV radiation on contact hypersensitivy responses, this chapter reviews the clinical features, epidemiology, pathophysiology and treatment of the immunologically-mediated photodermatoses. Additionally, this chapter highlights the key immunologic mechanisms by which UV radiation is used therapeutically to treat dermatologic diseases, especially T-cell mediated skin disorders.

Keywords

Photoimmunology • Ultraviolet light • Ultraviolet radiation • Photoimmunosuppression • Actinic prurigo • Polymorphic light eruption • Solar urticaria • Hydroa vacciniforme • Chronic actinic dermatitis • Phototherapy

Photoimmunology is the study of effects of ultraviolet (UV) light/radiation (photons) on the immune system. UV light occupies a narrow portion (100–400 nm) of the sun's electromagnetic radiation, which spans ionizing wavelengths (<100 nm) on one extreme and radio- and microwaves (>10,000 nm) on the other. Despite its reported diminution, our stratospheric ozone layer continues to filter solar radiation by blocking all ionizing and UVC radiation and the vast majority of UVB radiation from reaching the earth's surface. What natural exposure to sunlight delivers is some UVB, much UVA and visible light, and considerable infrared radiation (>800 nm) (Fig. 10.1).

The UV spectrum is subdivided into UVC (100–290 nm), UVB (290–320 nm), and UVA (320–400 nm) radiation. The ability of these wavelengths to penetrate the skin depends in great part on their absorption by different chromophores or photoreceptors (Fig. 10.2). UVC and UVB are readily absorbed by DNA within living cells, and thus almost all of these shorter wavelengths are absorbed as they pass through the epidermis, with little to none reaching the dermis. By contrast, UVA and visible light penetrate deeper into the dermis where these wavelengths are absorbed principally by

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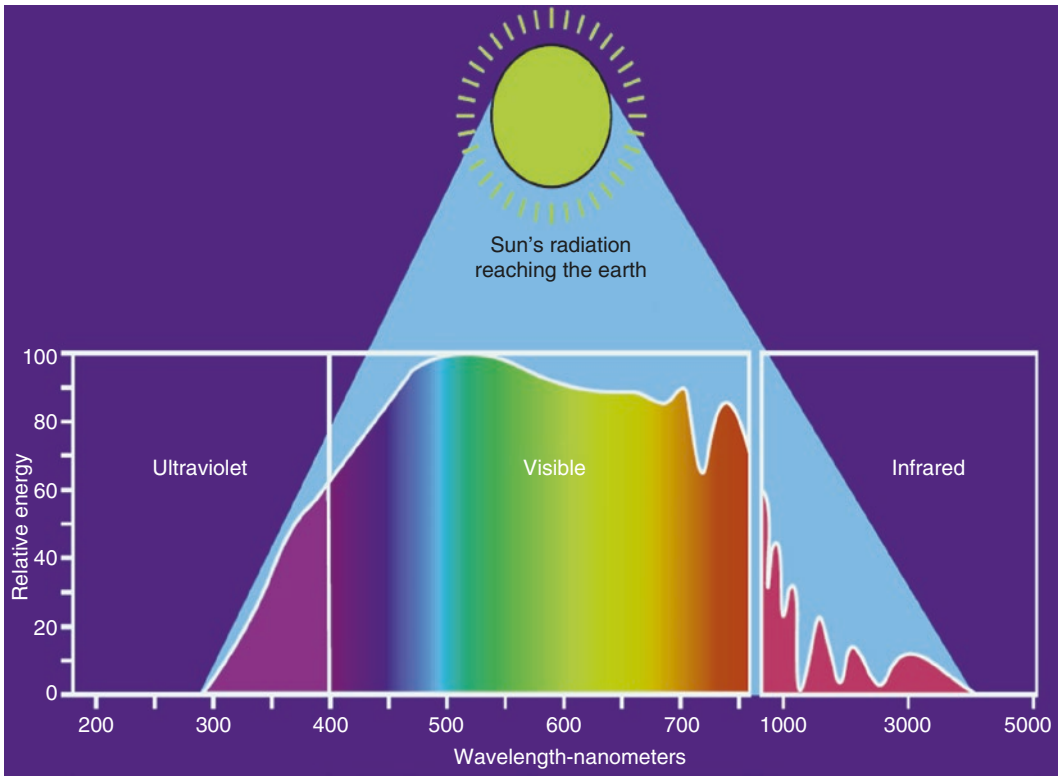

Fig. 10.1 Schematic diagram of the sun's radiation reaching the earth's surface

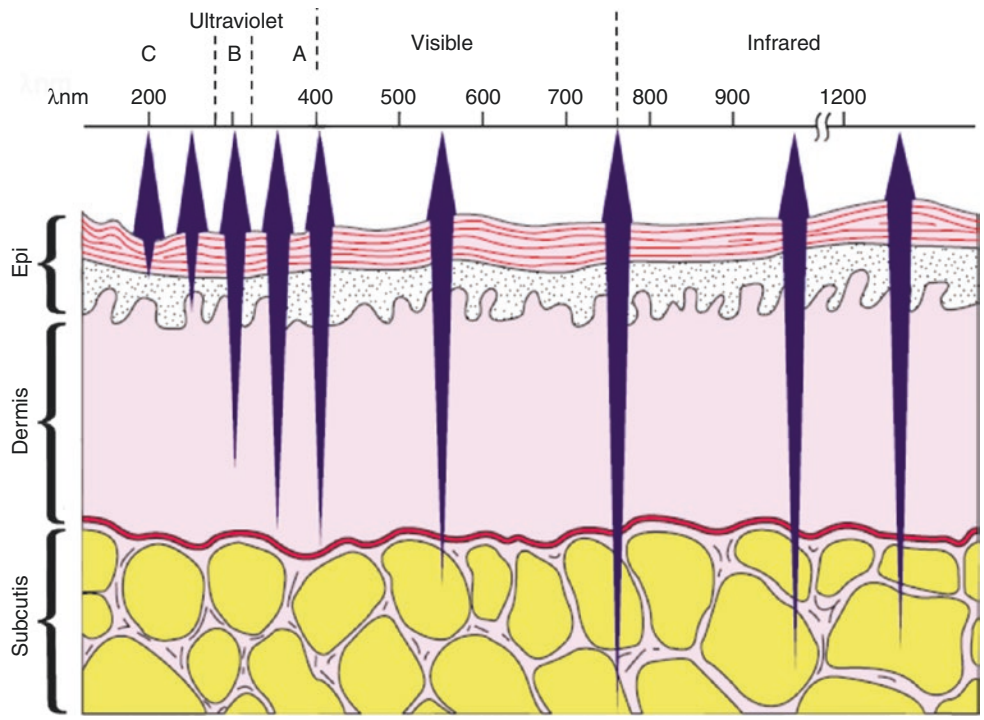

Fig. 10.2 Wavelength and depth of penetration. Within the 200- to 800-nm range, there is a direct relationship between wavelength and its depth of penetration into the skin

aromatic amino acids on cell membrane proteins, and by collagen, elastin, and ground substance [1]. As will be discussed later, the development of high-energy light sources that emit 340–400 nm (UVA1) exclusively and their use as a therapeutic modality that is safer than PUVA (psoralens plus UVA) has led to its distinction from UVA2 (320–340 nm), which more closely resembles UVB. UVA1 has features intermediate between UVB and visible light. UVC and UVB are absorbed primarily by DNA, and are mutagenic. Indeed, UVC (germicidal) lamps are used to kill microbes. Of the solar radiation reaching the earth's surface, UVB is the most carcinogenic. UVB is also the most erythemogenic and is responsible for producing sunburn and immediate tanning. By contrast, UVA is considerably less erythemogenic, but more effective in producing a longer lasting tan.

Photocarcinogenesis

Human epidemiologic and laboratory animal studies overwhelmingly support the concept of UV exposure as the major environmental risk factor for non-melanoma skin cancer (NMSC) [2–5]. In this respect and as cited previously, UVB is most carcinogenic [6], although large doses of UVA also have been shown experimentally to cause non-melanoma skin cancer [7]. A causative role for UV radiation in melanoma is also supported by scientific evidence, albeit at a level less substantial than for NMSC [8–10]. Both UVA and UVB may be involved in the pathogenesis of melanoma [11], but the two types of UV radiation likely act by different mechanisms. Experimentally, UVA-induced melanoma requires the presence of melanin pigment and the DNA damage is caused indirectly by reactive oxygen species, whereas UVB-induced melanoma develops in a melanin-independent manner and is due directly to UV-mediated DNA-damage [12].

UVB causes a variety of DNA mutations, most commonly cyclobutane pyrimidine dimers that have been linked to the genesis of sunburn, tanning, and skin cancer. The biologic consequences of UVB-induced mutations depend on the ability of the host to repair DNA defects and on the specific gene mutations left unrepaired. Mutations that activate oncogenes (e.g., ras) or deactivate tumor suppressor genes (e.g., p53 for squamous cell cancer; PATCHED for basal cell cancer) set the stage for carcinogenesis [13]. It is assumed that people with competent DNA repair mechanisms are able to correct UV-induced mutations. These protective mechanisms may deteriorate with age, allowing some mutations to escape repair, thereby leading to actinic keratoses, squamous cell cancers, and basal cell cancers. Xeroderma pigmentosa, a congenital disorder of absent or deficient DNA repair enzymes, provides a dramatic illustration of the foregoing concepts since afflicted individuals suffer from multiple skin cancers as early as childhood.

UV-Induced Immunosuppression

In addition to its carcinogenic effects, UV radiation promotes cancer growth by suppressing a second host defense mechanism – the ability of the immune system to kill UV-induced skin cancers. That UVB radiation leads to suppressor T-cell activation has been demonstrated not only for UVB-induced carcinogenesis but also for UVB-induced suppression of delayed-type (DTH) and contact hypersensitivity (CH) [14, 15]. The immunosuppression generated is not a generalized one, but specific for the antigen to which the host is being sensitized at the time of UV exposure [14]. Several overlapping pathways have been shown to lead to UV-induced immunosuppression (Fig. 10.3). There is overwhelming scientific evidence supporting the ability of UVB to induce immunosuppression, yet recent evidence has also suggested that UVA wavelengths from 364 to 385 nm may also be immunosuppressive [16], though the immunomodulatory effects of UVA have a complex dose–response relationship [17].

UVB light can induce keratinocytes to secrete soluble factors, of which tumor necrosis factor-α (TNF-α) [18, 19] and interleukin-10 (IL-10) [20, 21] are most critical to UVB immunosuppression; TNF-α regulates Langerhans cells' emigration out of the epidermis into draining lymph nodes, and IL-10 shifts T-cell responses from T-helper-1 (Th1) to Th2 phenotype [20]. UV light can also trigger lipid peroxidation on cell membranes leading to secretion of platelet aggregation factor (PAF), which in turn stimulates prostaglandin E2 (PGE2) production, which in turn prompts IL-4 secretion, and also IL-10 production [21, 22]. UV-induced oxidation reactions can also produce PAF analogs such as 1-alkyl-2-(butanoyl and butenoyl)-sn-glycero-3-phosphocholine that can stimulate the PAF receptor [23]. Trans-urocanic acid, a by-product of histidine metabolism, accumulates in the stratum corneum; UV non-enzymatically transforms trans-urocanic acid into the cis isomer, which contributes to immunosuppression via effects on antigen-presenting cells (APCs) [24, 25]. Immunosuppressive effects of cis-urocanic acid were shown to be mediated through binding to the serotonin (5-HT) receptor [26]. Finally, UVB radiation can trigger release of calcitonin gene related peptide (CGRP) from cutaneous nerve endings, leading to mast cell release of TNFα, which dampens CH responses [27].

UV can directly alter the function of epidermal Langerhans cells (LC) and other APCs. Depending on the UV dose and the manner in which it is administered, UV may induce these APCs to undergo apoptosis, shift their function from stimulators of Th1 (cellular) to Th2 (humoral) responses, or cause them to activate "suppressor" T cells [28–30]. Previously, as LC were regarded as the most important APCs in the skin, it was thought that such antigen tolerance was due to epidermal LC depletion by UV light. [31] Now dermal dendritic cells

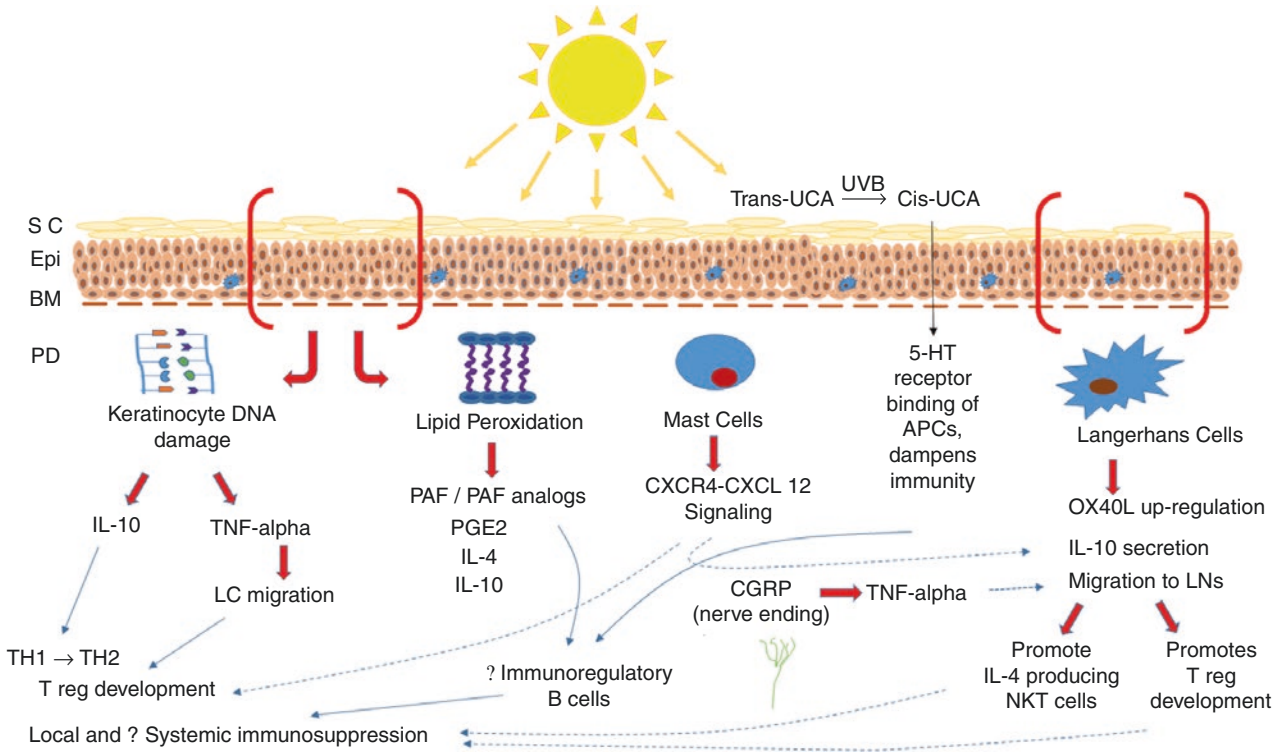

Fig. 10.3 Overlapping pathways accounting for the effects of ultraviolet radiation on the immune system. *5-HT* serotonin, *APC* antigen presenting cell, *BM* basement membrane, *CGRP* calcitonin gene related peptide, *CXCL* CXC chemokine ligand, *CXCR* CXC chemokine receptor, *Epi* epidermis, *IL* interleukin, *LC* Langerhans cell, *LNs* lymph nodes, *NKT* natural killer T, *OX40L* ligand for CD134, also known as CD252, *PAF* platelet activating factor, *PD* papillary dermis, *PGE* prostaglandin E, *SC* stratum corneum, *TH* T-helper, *UV* ultraviolet, *TNF* tumor necrosis factor, *T reg* T regulatory cell, *UCA* urocanic acid

(FITC+, CD11c+, Langerin−) are thought to be critical for antigen presentation and immune stimulation (even in the setting of UV light) [32, 33]. By contrast, LC play a significant role in UV-mediated immunosuppression. UVB radiation leads to LC maturation, up-regulation of surface marker OX40L, and production of IL-10, which upon migration to lymph nodes, supports the development of regulatory T cells [34]. Regulatory T cells express CD4, CD25, and CTLA-4 markers, secrete copious amounts of IL-10, bind dectin-2, and dampen immune responses [35, 36]. LC migrating to the lymph nodes may also mediate immunosuppression by activating a population of natural killer T (NKT) cells to secrete IL-4 [37]. Interestingly, topically applied low-dose green tea extract (3 %) has recently been shown to reduce UV-mediated depletion of LC [38].

Additionally, mast cells and complement activation via the C3 pathway have been shown to participate in UVB-induced immunosuppression [39–42], and evidence continues to mount in support of an integral role for mast cells in UV-induced immunosuppression [43]. Dermal mast cells have been shown to critically mediate UV-induced immunosuppression through the CXCR4-CXCL12 signaling pathway. In fact, blockade of mast cell trafficking with the CXCR4 antagonist AMD3100 prevented UV-induced immunosuppression and reduced the number of cutaneous squamous cell carcinomas in mice, presumably by reducing mast cell migration into local lymph nodes and the tumors themselves [44, 45]. Interestingly, studies have shown an increased mast cell density in buttock skin of patients with basal cell carcinoma and melanoma compared to controls [46, 47].

UV light also leads to the development of a suppressive population of B cells that express high levels of major histocompatibility complex II (MHC II) and B220 but low levels of co-stimulatory molecules. When dendritic cells and B cells isolated from UV-radiated mice were conjugated to antigen ex vivo and injected into naïve hosts, this subset of B cells suppressed dendritic cell activation of immunity (which occurred in the absence of these B cells) [48]. Both platelet activating factor (PAF) and serotonin were shown to promote development of these so-called "immunoregulatory B cells" (FITC+, IL-10-secreting, CD19+, B220+) capable of inducing tolerance to antigens following UV exposure [49].

In addition to attenuation of CH and skin cancer immunosurveillance, UV light has been shown to serve as an immunomodulator for systemic autoimmune diseases, including multiple sclerosis, type I diabetes, and rheumatoid arthritis [50]. Much of the evidence is epidemiologic: the latitude

gradient in these diseases suggests an inverse relationship between disease incidence and UV exposure. However, in a murine model of multiple sclerosis (experimental autoimmune encephalitis or EAE), UVB radiation was shown to induce tolerogenic dendritic cells that circulated systemically and promoted development of regulatory T cells in several organs, including the CNS [51].

UVB Radiation and Vitamin D

UVB causes the non-enzymatic conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D3) in skin. Although vitamin D is well-known for its effects on mineral metabolism and bone health, it has also become apparent that cholecalciferol modulates immune responses in skin and, perhaps, systemically. Vitamin D can modulate UVB-mediated damage to keratinocytes, although the effect is lost at higher doses of UVB irradiation, which may explain increased skin cancer risk associated with chronic, high dose UV exposure [52]. Several laboratory studies have suggested a protective role of vitamin D against basal cell and squamous cell carcinoma as well as melanoma, although further human studies need to be performed. Toll-like receptors (TLRs) activated in response to intracellular bacteria can upregulate vitamin D receptors on macrophages, leading to the induction of canthelicidin and intracellular killing of Mycobacterium tuberculosis [53]. In addition, sera from African Americans, a population known to be more susceptible to tuberculosis, were found to have lower levels of 25-hydroxyvitamin D compared to controls. Vitamin D may also stimulates phagocytosis by monocytes and macrophages [54].

While these examples support an immunostimulatory role for vitamin D, there is also data suggesting that vitamin D may be immunosuppressive. Cholecalciferol may inhibit the production of Th1 type cytokines, suppress activation of Th1 cells, and enhance the function of regulatory T cells [50]. These actions may play be protective or preventative of autoimmunity. Life-long vitamin D supplementation in NOD mice prevented development of diabetes mellitus whereas induced vitamin D deficiency from birth led to early development of type I diabetes mellitus [55]. A promising role for UVB radiation in multiple sclerosis is related to elevated levels of vitamin D in patients treated with UV light. [51] The exact role of vitamin D in skin and systemic immunity remains to be fully elucidated. UVB as the primary external stimulant of vitamin D production has the potential to mediate vitamin D- dependent immunity.

Immunologically-Mediated (Idiopathic) Photodermatoses

The immunologically mediated photodermatoses (IMPs), also known as idiopathic photosensitive skin disorders, are a heterogeneous group of conditions for which exact etiopathologic mechanisms remain elusive though each manifests immunologic underpinnings (Table 10.1). Our focus will be on polymorphic light eruption, actinic prurigo, solar urticaria, hydroa vacciniforme, and chronic actinic dermatitis.

Polymorphic (Polymorphous) Light Eruption

Polymorphic light eruption (PMLE) is the most common photosensitivity disorder, with estimates of prevalence as high as 20% of the general population [56, 57]. PMLE has a

Table 10.1 Photosensitivity disorders

Immunologically-mediated (idiopathic) photodermatoses	Polymorphic light eruption (including juvenile spring eruption)
	Actinic prurigo
	Solar urticaria
	Hydroa vacciniforme
	Chronic actinic dermatitis
Defective DNA repair	Xeroderma pigmentosa
	Trichothiodystrophy
	Cockayne syndrome
	Rothmund-Thompson syndrome
Autoimmune	Lupus erythematosus
	Dermatomyositis
Drug-induced	Phototoxic reaction
	Photoallergic dermatitis
	Pseudoporphryia
Porphyrias	Porphyria cutanea tarda
	Erythropoietic porphyria

predilection for women, an age of onset in the teenage and young adult years, and a tendency to affect people living in temperate climates. Recurrent outbreaks typically occur in spring, with clinical onset occurring several hours following UV exposure. The eruption develops over sun-exposed skin, is usually pruritic, and may take several morphologic patterns (i.e., polymorphic): small to large papules, vesicles, plaques, and even erythema multiforme-like eruptions [56]. Despite the wide variety of morphologic patterns, individual patients tend to present with the same morphologic feature in a recurrent manner [58]. Outbreaks typically last 1–2 weeks, and the eruption frequently improves in the summer (termed hardening). Photoprovocation studies have implicated different wavelengths spanning UVB, UVA, and even visible light ranges [59, 60]. Juvenile spring eruption (JSE) is considered a localized variant of PMLE that tends to occur on the ears of young boys. Outbreaks most commonly occur during the spring after exposure to bright sunlight in cold weather, with body sites lacking hair (e.g., protuberant ears) as favored locations [61].

Pathogenesis is believed to be a delayed cellular hypersensitivity reaction to an endogenous cutaneous antigen possibly generated by the action of UV radiation [62, 63]. Although the identity of the chromophore/antigen remains unknown, studies have shown patients to be relatively incapable of manifesting UVB-induced suppression of immune responses like induction (but not elicitation) of contact hypersensitivity [64], a defect ascribed to inability of UVB to induce emigration of Langerhans cells from the epidermis to draining lymph nodes [65, 66]. Lesions of PMLE have demonstrated an altered immunoregulatory network, with reduced epidermal or dermal expression of TGF-beta-1, IL-10 and RANKL, and a relatively low number of Tregs [67]. This reduced ability of UVB to suppress immune responses leads to an enhanced immune response to cutaneous neoantigens generated from UV exposure. Repeated UV exposure in these patients eventually suppresses immunity, explaining the natural improvement (hardening) during summer months as well as the therapeutic role of prophylactic NB-UVB or PUVA therapy [58].

Most cases of polymorphic light eruption are mild and can be managed by photoprotection plus topical corticosteroids for symptomatic relief. Occasionally in young, otherwise healthy patients, a short course of systemic corticosteroids (0.6 mg–1 mg/kg per day for 1 week) can help control symptoms. For patients with severe, recurrent disease, NB-UVB or PUVA therapy in late winter can prevent seasonal flares during spring [58].

Actinic Prurigo

Actinic prurigo is an immune-mediated photodermatosis most commonly seen in the indigenous Indian and mestizo populations of Central and South America, especially individuals living at high altitudes, although Caucasian and Asian populations are also affected [58]. The disorder is thought to be caused by transformation of an epidermal antigen upon exposure to UV light, leading to an abnormal immune response [68]. Although both UVA and UVB have been implicated in actinic prurigo, the eruption is most often linked to UVA radiation [58, 68]. Typically, actinic prurigo presents in children younger than 10 years (often 4–5 years) with intensely itchy papules, plaques and nodules as well as excoriations and scarring on sun exposed areas. The face, neck, extensor forearms, dorsal hands, and upper chest are usually affected, but involvement of the back and buttocks may occur. Conjunctivitis and cheilitis (lower > upper lip) are characteristically present. Pruritus tends to last throughout the entire year, with exacerbations in the spring and summer.

While actinic prurigo may share with polymorphic light eruption a similar immunologic mechanism, lesion morphology, and delayed-onset relative to sun exposure, there are several clinical differences between the two disorders. As outlined above, actinic prurigo is more likely to affect both exposed and covered sites, can occur in the winter, tends to persist beyond 4 weeks, often exhibits lip or conjunctival involvement, and frequently presents with excoriation and scarring [69]. Furthermore, actinic prurigo has been linked to human leukocyte antigen (HLA) subtypes. Originally HLA subtypes A24 and Cw4 were linked to actinic prurigo in patients of Cree ancestry [70], but subsequent studies in both European and Central American populations demonstrated strongest association with the DR4 allele, with the specific allele HLA-DR4/DRB1*0407 being most common, found in 60–70 % of affected individuals [58, 68, 69, 71].

Unlike polymorphic light eruption, management of actinic prurigo tends to be more challenging. Photoprotection is the most important measure, including use of protective clothing, sunscreens, lip balms, and sunglasses. Multiple therapies may reduce pruritus, including topical corticosteroids, emollients, and oral antihistamines. Occasionally NB-UVB and PUVA are also used, although the level of evidence for their efficacy is lower than for polymorphic light eruption [58]. In actinic prurigo, the most consistently effective form of therapy is thalidomide, although its use is limited by adverse effects including teratogenicity, peripheral neuropathy, and venous thromboembolism [58, 68, 72].

Solar Urticaria

Solar urticaria is a rare form of physical urticarial provoked by exposure to UV light. Most studies support an action spectrum between 300 and 500 nm, although the exact action spectrum tends to vary between studies and individuals. Rare cases due to infrared light have also been reported [73, 74]. Solar urticaria affects about 0.8 % of patients with urticarial

eruptions, and up to 7 % of patients with photodermatoses. The disorder has a variable age of onset, although most patients present in young adulthood. Women tend to be affected more commonly than men. There does not seem to be a racial or ethnic predilection [73].

Unlike polymorphic light eruption and actinic prurigo, solar urticaria typically develops within minutes of exposure to sunshine and manifests as itching, burning, erythema, and wheals [75]. The eruption tends to affect "classic" photodistributed areas such as the V-shaped area of the upper chest and the arms, but urticarial lesions may spare areas regularly exposed to sunlight like the face and hands. Systemic symptoms including headache, nausea, wheezing, dizziness and rarely anaphylactic shock [73].

Solar urticaria is an immediate type of photoallergic reaction caused by immunoglobulin E (IgE) autoantibodies. There are two subtypes of solar urticaria based on our most current understanding of these autoantibodies. In Type 1 solar urticaria, affected individuals have an abnormal chromophore within the skin to which IgE antibodies develop, leading to urticarial lesions. In these cases, passive transfer of serum to a normal individual will variably produce lesions of solar urticaria upon light exposure if the abnormal chromophore is also contained in the serum. By contrast, in Type 2 solar urticaria, affected individuals have IgE antibodies to a normal chromophore in the skin. In these cases, passive transfer of serum to a normal individual will always lead to urticaria upon subsequent light exposure [73]. While this distinction is useful for understanding underlying mechanisms of solar urticaria, passive transfer experiments are not routinely performed for ethical reasons, so the distinction between these subtypes is generally not made in clinical practice.

Treatment of solar urticaria is aimed at strict photoprotection. As in other types of urticaria, symptom relief can be achieved with topical corticosteroids or oral antihistamines. Although phototherapy should be approached with caution due to potential flaring of the disease, both NB-UVB (only to areas of skin that are regularly sun exposed, to produce "hardening") and PUVA have been used effectively in select cases. There is limited evidence supporting the use of beta-carotene, antimalarial medications, plasmapheresis, systemic immunosuppressives, and intravenous immunoglobulin [58, 62].

Hydroa Vacciniforme

Hydroa vacciniforme is a rare photosensitivity disorder found almost exclusively in children, with a mean age of onset around 8 years. Boys are affected more often than girls [58, 76]. Most patients are sensitive to UVA radiation, which causes recurrent crops of 2- to 3-mm erythematous macules that evolve into blisters within a few hours to days. The lesions then become umbilicated with crusting within a few days, often mimicking impetigo or impetiginized herpes simplex virus infection. These lesions heal with pitted varioliform scarring. The face and hands are the most commonly affected sites.

Epstein-Barr Virus (EBV) infection has been linked to hydroa vacciniforme. One case report of EBV-associated hydroa vacciniforme suggested that plasmacytoid monocytes (CD68+ and CD123+) may play a crucial role in the pathogenesis based on immunohistochemistry from the patient's biopsy demonstrating a predominance of this cell type [77]. More recently, a case series evaluating biopsies and peripheral blood of patients with hydroa vacciniforme found an increase in EBV-infected γ/δ T cells in affected patients which was not present in controls with hypersensitivity reactions to mosquito bites [78]. However, the exact pathophysiology to explain the relationship between the viral infection and UVA light is yet to be elucidated.

No treatments for hydroa vacciniforme are consistently successful, so photoprotection remains a critical aspect of management. Occasionally anti-malarials, beta-carotene, PUVA, systemic immunosuppressives, and thalidomide have been used successfully [62]. In severe cases, systemic corticosteroids can be used [58].

Chronic Actinic Dermatitis

Chronic actinic dermatitis is an umbrella term for several forms of chronic photosensitivity disorders including photosensitive eczema, persistent light reaction, chronic photosensitive dermatitis, and actinic reticuloid. It often begins insidiously as nonspecific eczematous lesions in sun-exposed areas that become more persistent, in the absence of known topical or systemic photosensitizers [62]. Eczematous lesions are by far the most common, though papular lesions and pseudolymphomatous "reticuloid" lesions may also occur [79]. It is more common in men and tends to occur in later adult years, although it may occur earlier in patients with a strong history of atopic dermatitis [56, 80]. UV-B, UV-A, and visible light, either alone or in combination with each other, have been implicated in chronic actinic dermatitis [81].

Although the exact etiology is uncertain, the cause appears to be a form of delayed type hypersensitivity to a normal skin constituent that becomes altered by UV light and becomes antigenic in the absence of "normal" UVB-induced immunosuppression [80–82]. In many cases, there is a strong association with allergic contact dermatitis, though it remains unclear whether ACD is directly involved in the pathogenesis of chronic actinic dermatitis or whether allergy develops secondary to abnormal barrier function in the setting of a susceptible cutaneous microenvironment. Sesquiterpene lactone has a well-known association with

chronic actinic dermatitis and remains an important allergen even in recent studies. Other studies have shown a significant increase in patch test positivity to non-fragrance consumer allergens in affected individuals, particularly para-phenylenediamine [83]. Up to 75 % of patients react on patch testing or photopatch testing to allergens suspected to be involved in chronic actinic dermatitis; it is unclear whether the remaining patients have underlying allergy to an untested hapten or develop chronic actinic dermatitis in the absence of allergic contact dermatitis [80, 83].

With appropriate treatment, including avoidance of sunlight, regular use of sunscreen and skin-protective clothing, and avoidance of known allergen(s), chronic actinic dermatitis tends to improve or resolve over the course of many years, with approximately 55 % of patients experiencing improvement and 35 % experiencing resolution over 15 years in one study [84]. Aside from these basic measures, many other therapeutic options may be tried, including emollients, topical corticosteroids, topical tacrolimus, systemic corticosteroids, azathioprine, cyclosporine, methotrexate, hydroxyurea, retinoids, antimalarials, and phototherapy. Azathioprine tends to be quite effective for severe forms of this disorder, and systemic corticosteroids may be beneficial in acute exacerbations. Phototherapy tends to be poorly tolerated, but has been useful in some patients [79].

Phototesting and Photopatch Testing

Both phototesting and photopatch testing may be diagnostically useful in the evaluation of immunologically-mediated photodermatoses. Sensitivity to UV is usually assessed by measuring the minimal erythema dose (MED), which is the amount of UV radiation that will produce minimal erythema. MED can be tested with either UVB or UVA wavelengths but tends to be more reliable for evaluating photosensitivity to UVB. In PMLE, most patients have normal MEDs to UVB and UVA, although some patients have decreased MEDs to UVB and/or UVA. However, photoprovocation testing (repeated exposures to sub-erythemogenic doses of UV radiation for 3–4 days) often reproduces the eruption [58]. Although not necessary for diagnosis, phototesting may be useful in the evaluation of other immunologically-mediated photodermatoses (Table 10.2). Photopatch testing is a procedure in which a typical patch test is performed with addition of UV exposure (usually UVA) upon removal of patches. In addition to evaluation of photo-allergic contact dermatitis, photopatch testing can be useful in the diagnosis of chronic actinic dermatitis [81].

Phototherapy

Along with Mohs micrographic surgery for the treatment of skin cancer and patch testing for the diagnosis of allergic contact dermatitis, phototherapy is among the most quintessential of dermatologic procedures (rarely performed by other medical specialists). Phototherapy refers to the use of artificial UV light to treat skin (and potentially systemic) disorders, a domain that for several decades was based largely on empirical evidence but over time has developed a stronger foundation in the scientific literature.

The major mechanisms of phototherapy in most inflammatory skin disorders, such as psoriasis and atopic dermatitis, are: (1) diminishing effector T cell responses, (2) promoting the development of regulatory immune cells, and (3) restoring normal barrier function, especially in atopic dermatitis [85]. Increasing knowledge and insight derived from photoimmunology have provided cellular and molecular mechanisms that account for phototherapy's beneficial effects (Table 10.3). Both UVB and UVA1 can induce

Table 10.2 Evaluation of photosensitivity disorders

	Phototesting		Photopatch testing	
Disorder	Useful?	Result?	Useful?	Result?
Polymorphic light eruption	Yes	Normal (usually) or decreased MEDs to UVB/UVA	No	N/A
Actinic prurigo	Yes	Low or normal MEDs to UVA > UVB	No	N/A
Solar urticaria	Yes[a]	Broad spectrum sensitivity (hives) to visible and UV light	No	N/A
Hydroa vacciniforme	Yes	Vesiculation/scarring following UVA exposure	No	N/A
Chronic actinic dermatitis	Yes	Delayed dermatitis or pseudolymphomatous response to UVB, UVA and visible light	Yes	Delayed dermatitis, often to sunscreen allergens, topical medicines and Compositae

MED minimal erythema dose

[a]Phototesting in solar urticaria should be performed with caution, by experienced clinicians and only on very small areas, due to the risk of anaphylaxis

apoptosis, and the threshold for causing programmed cell death is much lower for lymphocytes than for other cells. Apoptosis by UVB may be achieved via mutations or activation of death receptors (e.g., Fas/Fas ligand, TNF receptor, TRAIL [TNF-Related Apoptosis Inducing Ligand] receptor) [86]. By contrast, apoptosis by UVA may be mediated through reactive oxygen species, differential killing of Th1 over Th2 cells, or lysis of mast cells [86]. As cited previously, UVB light can induce secretion of cytokines including IL-1, IL-4, IL-6, IL-8, IL-10, and TNF-α, with the last two deemed responsible for immunosuppression [18–22]. UV light also leads to down-regulation of major histocompatibility complex (MHC) and co-stimulatory molecules on antigen presenting cells (APCs) in the skin, making these cells functionally impaired in their ability to stimulate T cell effector function [85]. As cited previously, ultraviolet B also generates regulatory immune cells, the most well-characterized being regulatory T cells which are critical to phototherapy-mediated immunosuppression [34–36]. Regulatory T cells are FoxP3+ and CD4+, and they secrete IL-10 and TGF-beta, which dampen effector T cell and APC function. By contrast, UVA (but not UVB) can inhibit collagen synthesis and metalloproteinase activity, leading to changes in extracellular matrix which makes UVA therapy useful in sclerotic skin diseases [86].

In general, UVB, UVA, and PUVA are useful for treating T cell–mediated inflammatory skin diseases because of the aforementioned mechanisms. PUVA tends to be the most effective but is also the most toxic since it is very mutagenic and thus a risk factor for both melanoma and nonmelanoma skin cancer. Indeed patients treated with PUVA for more than 250 sessions remain at risk for melanoma long after the photochemotherapy has ceased [87]. The commercial availability of light sources emitting high-energy, narrower-range wavelengths (i.e., UVA1 and narrow-band UVB) has led to phototherapy protocols that are less toxic and better matched to treat specific skin diseases. Thus the older modalities of broadband UVB and PUVA have given way to shorter-timed, more efficient narrow-band UVB and safer UVA1 treatments. With respect to HIV infection, there is no compelling proof that UVB or PUVA leads to systemic effects in seropositive patients, although UV has been shown to activate HIV gene expression in vitro [88, 89]. Clinically, NB-UVB and PUVA do not affect HIV viral loads, CD4 count, or risk of infection/malignancy and, therefore, phototherapy is recommended as a first line treatment for psoriasis in patients with moderate to severe HIV infection [90].

Specific therapeutic indications correlate with depth of penetration, which in turn is based on the nature of the absorbing chromophores (Table 10.4). Thus, UVB is most useful for eczematous disorders that primarily involve the epidermis and superficial dermis, such as psoriasis, atopic dermatitis, patch-stage cutaneous T-cell lymphoma, vitiligo, and pruritus due to various causes. For these diseases, narrow-band UVB is the phototherapy of choice since it

Table 10.3 Mechanisms of phototherapy

	UVB	UVA1
Apoptosis	Yes	Yes
Cytokine secretion	Yes	Probably
Regulatory T cell induction	Yes	?
Altered APC function	Yes	?
Extracellular matrix remodeling	?	Yes

Table 10.4 Selected therapeutic indications

NB-UVB	UVA (PUVA and/or UVA1)
Atopic dermatitis	Atopic dermatitis
Psoriasis	Localized cutaneous scleroderma
Vitiligo	Urticaria pigmentosa
Patch-stage cutaneous T-cell lymphoma	Plaque-stage cutaneous T-cell lymphoma
Polymorphic light eruption	Follicular mucinosis
Actinic prurigo	Polymorphic light eruption
Erythropoietic porphyria	Actinic Prurigo
Pityriasis lichenoides	Dyshidrotic eczema
Generalized pruritus	Disseminated granuloma annulare
	Pityriasis lichenoides
	Systemic lupus erythematosus

NB narrow band, *UV* ultraviolet, *PUVA* psoralens plus UVA

Table 10.5 Risks of phototherapy

Risks	UVB	PUVA
Skin aging	Yes	Yes
Skin cancer		
Melanoma	Probably	Probably (>200 sessions)
Non-melanoma	Yes	Yes
Drug photo-sensitivity	Rare	Yes
Ocular damage		
Cataracts	No	Yes
Keratitis	Yes	No
Immunosuppression	Yes	Yes
Internal malignancy	No	No

PUVA psoralens plus UVA

approximates, if not equals, the efficacy of PUVA, while avoiding the latter's risks of greater mutagenicity/carcinogenicity and cataracts (Table 10.5). The principal limitation to UVB-based protocols is erythema, which peaks a day after exposure; for this reason, UVB phototherapy is delivered on an every-other-day basis to avoid causing burns.

Unlike UVB, UVA-based protocols allow penetration deeper into the dermis, and thus are useful for treating diseases characterized by fibrosis (e.g., scleroderma and/or morphea, at least during the early inflammatory phase). Counterintuitively, despite the importance of lupus patients avoiding sunlight exposure, UVA1 phototherapy has also been found to have Level A data (randomized controlled trials and/or meta-analysis) supporting its efficacy in systemic lupus erythematosus, probably by reducing interferon production by Th1 cells and antibody production by pathogenic B cells [91]. UVA therapy has also been used for diseases for which UVB is indicated (Table 10.4). In this respect, PUVA may be more efficacious than UVA1, but the latter comes close enough to PUVA's efficacy while avoiding its toxicity. Erythema is generally not a problem with UVA1 treatment so it can be used to treat patients with erythroderma and acute flares of atopic dermatitis. The principal limitations to UVA1 are cost of the equipment and its installation (currently available in only a few centers in the United States) and hyperpigmentation. The risks of phototherapy are dose related, so the greatest frequency of adverse events is associated with high-exposure doses of treatment. Risks are similar to those resulting from chronic exposure to sunlight and are augmented by additional exposure to sunlight. Patients should be selected appropriately and counseled on both the benefits and risks of this treatment modality.

Aside from traditional phototherapy regimens described above, there are several other important light-based treatment modalities which are thought to work based on their effects on the immune system. One particularly important modality in dermatology is extracorporeal photopheresis (ECP), in which blood is cycled through a circuit outside the body and treated with ultraviolet light to induce apoptosis of malignant lymphocytes [92]. This technique is most often used in dermatology to treat erythrodermic cutaneous T cell lymphoma, but it has recently found use in some other disorders as well, including chronic graft versus host disease [93]. Photodynamic therapy (PDT), a commonly used method for treatment of actinic keratoses and some non-melanoma skin cancers, involves the application of a photosensitizer to the skin followed by exposure to light designed to target the photosensitizer. While there are many proposed mechanisms of PDT, its action may in part be related to induction of tumor immunity [94]. Finally, several lasers are being used in treatment of inflammatory skin disease, including the excimer laser (308 nm, within the UVB spectrum) to target recalcitrant lesions of psoriasis or vitiligo [92], and pulsed dye laser (PDL, 585 or 595 nm) to treat conditions such as acne and psoriasis [95].

Conclusion

Ultraviolet light is a major environmental factor that can have both acute and chronic effects on the skin. The study of the effects of UV light on the skin and its associated immune system has resulted in a better understanding of the effects of this physical modality on the skin. Ultraviolet light has an important pathogenic role in skin cancer development (by means of both photocarcinogenesis and photoimmunosuppression) and immune-mediated photosensitivity diseases. However, UV light can also be utilized as an effective therapy in the treatment of certain skin diseases, such as psoriasis and atopic dermatitis, and it shows promise for the treatment of some systemic autoimmune diseases.

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Questions

1. Which of the following wavelengths of light correctly describes the UVA1 spectrum?
   A. 200–290 nm
   B. 290–320 nm
   C. 320–340 nm
   D. 340–400 nm
   E. 400–800 nm

2. Which of the following cytokines is critical to UV-induced immunosuppression?
   A. IL-1
   B. IL-5
   C. IL-10
   D. TGF-beta
   E. Interferon-alpha

3. Phototesting of patients with polymorphic light eruption is most likely to demonstrate which of the following findings?
   A. Decreased MED-A and decreased MED-B
   B. Decreased MED-A and normal MED-B
   C. Normal MED-A and decreased MED-B
   D. Normal MED-A and normal MED-B
   E. Vesiculation and scarring in response to UVA exposure

4. Patch test positivity to para-pheylenediamene (PPD) is most likely in patients with which of the following immunologically medicated photodermatoses?
   A. Actinic Prurigo
   B. Chronic Actinic Dermatitis
   C. Hydroa Vacciniforme
   D. Polymorphic Light Eruption
   E. Solar Urticaria

5. Which of the following presentations is characteristic of actinic prurigo?
   A. A 5 year old boy with itchy papules and excoriations on the face and neck, conjunctivitis, and cheilitis lasting throughout the year
   B. An 8 year old boy with recurrent vesicles on the cheeks that umbilicate and heal with pox-like scars
   C. A 10 year old boy with itchy papules on the ears for 1–2 weeks every spring
   D. A 24 year old woman with itchy papules on the forearms for 1–2 weeks every spring
   E. A 60 year old man with itchy lichenified plaques on the hands, face, and neck

Answers

1. D
2. C
3. D
4. B
5. A

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Angiogenesis for the Clinician

11

Michael Y. Bonner and Jack L. Arbiser

Abstract

Angiogenesis, the development of a microvasculature to a neoplastic, inflammatory, or infectious disease process, is a promising therapeutic target that has not been fully exploited. Virtually all processes of therapy impinge on cutaneous angiogenesis. A proper understanding of cutaneous pathophysiology, with respect to angiogenesis, will lead to a more effective use of current therapies for dermatologic diseases, as well as development of novel therapies. With this knowledge, the clinician can make educated guesses on the effect of therapy on a process. The primary disorders of the skin are infectious, inflammatory, and neoplastic. All of these categories are capable of inducing angiogenesis through a limited and overlapping subset of mechanisms, and these mechanisms can be understood by the practicing dermatologist. This chapter discusses the primary mediators of angiogenesis and examples of common skin disorders in which they occur. Antiangiogenic therapy is also discussed. Factors that directly impact endothelium are called direct angiogenesis stimulators or inhibitors, while factors that stimulate nonendothelial cells to make stimulators or inhibitors are called indirect angiogenesis stimulators and inhibitors.

Keywords

Clinical Angiogenesis • Infectious disease • Microvasculature • Neoplastic • Inflammatory • Pathogenesis • Skin disease • Skin disorders • Acne • Psoriasis • Warts • Pre-cancer • Skin cancer • Skin abnormalities • Stem cell

Clinical Angiogenesis

Angiogenesis, the development of a microvasculature to a neoplastic, inflammatory, or infectious disease process, is a promising therapeutic target that has not been fully exploited [1]. Virtually all processes of therapy impinge on cutaneous angiogenesis. A proper understanding of cutaneous pathophysiology, with respect to angiogenesis, will lead to a more effective use of current therapies for dermatologic diseases, as well as development of novel therapies. With this knowledge, the clinician can make educated guesses on the effect of therapy on a process. The primary disorders of the skin are infectious, inflammatory, and neoplastic. All of these categories are capable of inducing angiogenesis through a limited and overlapping subset of mechanisms, and these mechanisms can be understood by the practicing dermatologist. This chapter discusses the primary mediators of angiogenesis and examples of common skin disorders in which they occur. Antiangiogenic therapy is also discussed. Factors that

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Key Points

- Angiogenesis is the development of microvasculature in response to an infectious, neoplastic, or inflammatory agent.
- Angiogenesis contributes to the pathogenesis of many common skin disorders, such as acne, psoriasis, photoaging, common warts, and a variety of precancers and skin cancers (basal cell carcinoma, squamous cell carcinoma, actinic keratosis, and melanomas), and wound healing.
- Angiogenesis in the skin is accomplished by recruitment of mesenchymal cells locally and by recruitment of the bone marrow–derived stem cells that can differentiate into endothelial cells.
- Important growth factors for endothelial cells include vascular endothelial growth factor, basic fibroblast growth factor, platelet-derived growth factor, cyclooxygenase-2, and prostaglandin E_2.
- There are a number of angiogenesis inhibitors, many of which are experimental. These agents have great potential as therapeutic interventions for a number of skin diseases.

directly impact endothelium are called direct angiogenesis stimulators or inhibitors, while factors that stimulate non-endothelial cells to make stimulators or inhibitors are called indirect angiogenesis stimulators and inhibitors (Table 11.1).

Infectious Processes

Neither acute nor chronic infections have traditionally been considered angiogenic processes, but the resolution of an acute infection and the maintenance of a chronic infection require an intact angiogenic system. Colonization and invasion of the skin by both gram-positive and gram-negative organisms activates the innate immune system, which results in the production of antimicrobial peptides, some of which also impact endothelial cells. While the activation of the innate immune system is sufficient to control and eliminate many bacterial colonizations and invasions, it is not sufficient to control high inocula of infection or particularly virulent bacterial or viral infections. Therefore, danger signals are transmitted systemically, which allow for cellular reinforcements. The arrival of cellular reinforcements requires an intact vascular system, which can respond to infection by activation of endothelial adhesion molecules (vascular cell adhesion molecule [VCAM], intercellular adhesion molecule [ICAM], E-selectin), allowing neutrophils, lymphocytes, and macrophages to proceed to the site of infection with selectivity (Fig. 11.1).

One of the most graphic demonstrations of the importance of this system is the genetic deficiency of CD11, a neutrophil adhesion molecule that is required for the exit of neutrophils from blood vessels. Patients lacking CD11 develop neutrophilia due to inflammatory stimuli, but these neutrophils are unable to leave the blood vessel, leading to "cold" staphylococcal abscesses and high neutrophil counts due to the inability of these neutrophils to reach the site of infection. In the absence of bone marrow transplantations, these patients succumb to staphylococcal sepsis. Acute infections like colonization of atopic dermatitis by gram-positive organisms e.g. Staphylococcus and Streptococcus cause exacerbation of Th2 type of inflammation. A major player in the vascular permeability of gram-positive infections is angiopoietin-2 (Ang2). Recently we described elevated levels of Ang2 in superinfected atopic dermatitis and demonstrated that the NADPH oxidase inhibitor, gentian violet, is useful in the treatment of atopic dermatitis, due to both downregulation of host Ang-2 and killing of gram-positive organisms [14].

Chronic infections are usually the result of bacteria capable of intracellular colonization (treponemes, mycobacterium, *Bartonella*), viral infections that are capable of latency, or viral oncogenes (human papillomavirus [HPV], herpes virus). Bacterial infections often colonize cells of endothelial or monocyte/macrophage origin, and in retrospect, this should not be surprising. These cells share a common precursor cell in the hemangioblast. As opposed to most extracellular bacteria, chronic bacterial infections of the skin often manifest after a systemic infection, perhaps due to infection of the hemangioblast, with preferential colonization of the infected endothelial cell or monocyte in the dermis. Of note, there are two major receptors for VEGF, the major chemotactic angiogenic factor, and these are differentially expressed in the descendants of the hemangioblasts. Endothelial cells express primarily VEGFR2, while monocytes/macrophages express VEGFR1. Blockade of each of these receptors with specific antibodies impairs angiogenesis, thus demonstrating a role of both endothelial cells and hematopoietic cells in angiogenesis. These blockades likely results in the highly impaired wound healing in patients who are neutropenic.

Recently, tissue hypoxia has been shown to be a factor favoring the persistence of intracellular organisms such as Leishmania. This may also hold true of cutaneous tuberculosis, in which tissue hypoxia may prevent access of bactericidal nitric oxide [21].

Outside of embryonic cells, angiogenesis is affected primarily by the endothelial cell in two ways: through recruitment of endothelial cells from local mesenchymal cells, and

Table 11.1 Description of angiogenesis inhibitors and stimulators

Angiogenesis inhibitors	
Angiostatin	Naturally occurring protein found in some animal species. Angiostatin is known to be cleaved by MMPs [2]. Angiostatin binds to endothelial cell surface adenosine triphosphate (ATP) synthase and angiomotin.
Bevacizumab	A humanized monoclonal antibody. The first commercially available angiogenesis inhibitor. Inhibits the actions of VEGF by binding directly to VEGF-A. Used primarily for colorectal cancer [3]. Usually used along with combination drug chemotherapy.
Celecoxib	A nonsteroidal anti-inflammatory drug (NSAID). Highly selective COX-2 inhibitor. This selectivity helps reduce stomach ulcers.
Curcumin	Anti-inflammatory properties are due to inhibition of eicosanoid biosynthesis. Interferes with the activity of the transcription factor NF-kB, which has been linked to a number of inflammatory diseases and tumor survival [4, 5].
Epigallocatechin gallate	A flavanoid class molecule that acts as a powerful antioxidant and protects against oxidative stress and free radical damage.
Endostatin	A C-terminal fragment derived from type 18 collagen. It is a broad-spectrum angiogenesis inhibitor that interferes with the proangiogenic action of growth factors, bFGF and VEGF.
Imatinib mesylate	Acts by inhibiting particular tyrosine kinase (TK) enzymes, instead of nonspecifically inhibiting rapidly dividing cells. It occupies the TK active site, leading to a decrease in bcr-abl transformation. Imatinib mesylate is especially useful in that it is one of the few tyrosine kinase inhibitors with appropriate selectivity and limited toxicity. Studies show that imatinib may be also useful in treating smallpox [6].
Honokiol	Melanoma therapy under development. Relevant in that IFN-α, the only widely prescribed adjuvant therapy for melanoma, has marked side effects. A biphenolic ring with an ortho-allyl moiety, honokiol induced caspase-dependent cell death in B-CLL cells [7] and a variety of melanoma cell lines. Mechanism of action is likely direct inhibition of GRP78 and activation of Sirt3 [8].
Silymarin	Silymarin consists of a family of flavonoids commonly found in the dried fruit of the milk thistle plant. Extensive research has shown that silymarin can suppress the proliferation of a variety of tumor cells. This is accomplished by inhibition of cell-survival kinases (AKT and MAPK) and inhibition of inflammatory transcription factors (NF-kβ). It can also downregulate gene products involved in the proliferation of tumor cells (COX-2), invasion (MMP-9), angiogenesis (VEGF), and metastasis [9].
Thalidomide	Thalidomide inhibits the release of TNF-α from monocytes and modulates other cytokine action. Thalidomide may act to heal aphthous ulcers by inhibiting angiogenesis and promoting reepithelialization [10]. Thalidomide is also useful in the treatment of multiple myelomas, autoimmune diseases, and leprosy [11].
Gentian violet	Occupies the chemical class of triphenylmethane dyes. Once used as a topical antiseptic, gentian violet is experiencing a reawakening as a nox4 inhibitor. Potentially useful in the treatment of melanoma, eczema, psoriasis, and verruca vulgaris [12–14].
Solenopsin	Alkaloidal component of fire-ant venom. Inhibitor of phosphatidylinositol-3–kinase signaling and angiogenesis [15–17].
Rapamycin	Macrocyclic triene antibiotic, also known as sirolimus, possessing immunosuppressant and antiproliferative properties. Works through inhibition of mTOR, leading to interruption of IL-2 signaling and a cell cycle arrest at G1–S [18]. In preliminary trials for treatment of angiomyolipomas and brain tumors associated with tuberous sclerosis [19]. Used in the treatment of Kaposi's sarcoma in patients receiving renal transplants [20].
Angiogenesis stimulators	
Vascular endothelial growth factor (VEGF)	The upregulation of VEGF is the main operator in the physiologic response during exercise. Muscle contraction increases the blood flow to the affected areas. This increased flow causes an increase in the mRNA production of VEGF receptors 1 and 2. This increase in receptors increases the signaling cascades related to angiogenesis.
Basic fibroblast growth factor (bFGF)	Basic fibroblast growth factor is a member of the fibroblast growth factor family. In normal tissue, basic fibroblast growth factor is present in basement membranes and in the subendothelial extracellular matrix of blood vessels. bFGF stays membrane-bound as long as there is no signal peptide. During both wound healing of normal tissues and tumor development, the action of heparan sulfate–degrading enzymes activates bFGF, thus mediating the formation of new blood vessels (angiogenesis).
Matrix metalloproteinase (MMP)	MMPs help degrade the proteins that keep the vessel walls solid. This proteolysis allows the endothelial cells to escape into the interstitial matrix as seen in sprouting angiogenesis. These enzymes are highly regulated during the vessel formation process because this destruction of the extracellular matrix would destroy the integrity of the microvasculature. MMP2 and MMP9 are the two proteinases linked to angiogenesis.
Cyclooxygenase-2 (COX2)– prostaglandin E_2 (PGE_2)	COX-2 inhibitors are a class of nonsteroidal anti-inflammatory drugs (NSAIDs) that selectively block the COX-2 enzyme. This action impedes the production of the chemical messengers (prostaglandins) that cause the pain and swelling of arthritis inflammation. Being that COX-2 inhibitors selectively block the COX-2 enzyme and not the COX-1 enzyme, these drugs are uniquely different from traditional NSAIDs.
Platelet-derived Growth factor (PDGF)	PDGF is a dimer that activates its signaling pathway by a ligand-induced receptor dimerization and autophosphorylation. PDGF has provided a market for protein receptor antagonists to treat disease. These antagonists include specific antibodies that target the molecule of interest.

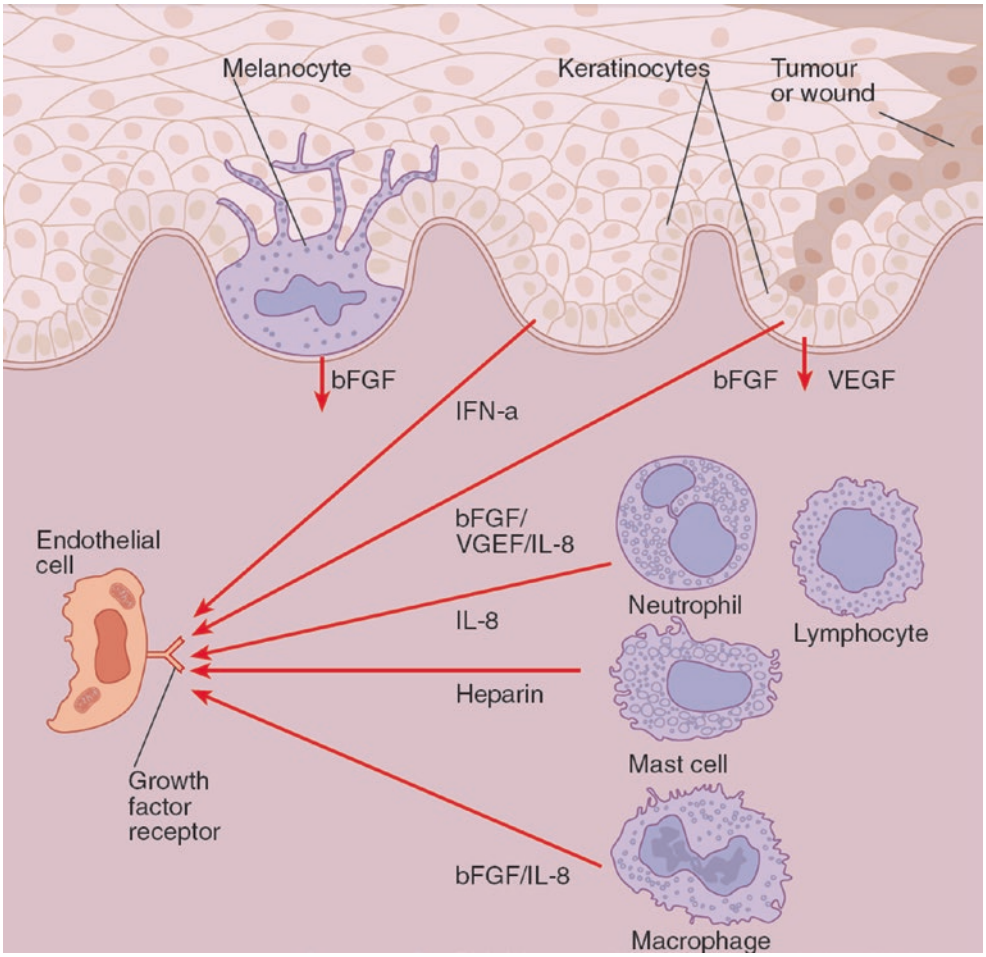

Fig. 11.1 Basement membrane-reservoir of acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (*bFGF*) bound to heparan sulfate proteoglycan leads to the disruption of basement membrane which leads to release of growth factor. Keratinocyte-derived interferon-α (IFN-α) directly inhibits endothelial growth. Upon activation of a growth factor receptor by a growth factor, the endothelial cell is stimulated to proliferate, produce proteases that migrate toward the source of growth factors, and form tubes, the precursors of capillaries

through recruitment and trans-differentiation of bone marrow-derived stem cells into endothelial cells. As cells may be derived from either of these sources, the efficacy of an antiangiogenic treatment is dependent on the source of cells in a particular lesion. For instance, in advanced malignancy, tumor cells can form vascular channels and phenotypically resemble endothelial cells; this process is known as vascular mimicry. Thus, for the development of an efficacious angiogenesis inhibitor, and for a proper understanding of the dynamic and unique nature of a lesion, the mechanisms of angiogenesis must be understood.

One of the cornerstones in the development of angiogenesis inhibitors is the angiogenic "switch." In a given cell, there is a balance between pro- and antiangiogenic signals. As cells become more malignant, a threshold is crossed as the balance shifts toward the proangiogenic state. It follows that cells produce angiogenesis stimulators and direct or indirect inhibitors. The direct inhibitors diminish endothelial cells' development of neovasculature. Indirect inhibitors block the angiogenic stimulatory pathway through inhibition of growth factors such as VEGF or the basic fibroblast growth factor (bFGF), or through blockade of releasing factors such as the corticotropin-releasing factor [22]. All of this functions, in the end, to impair cells' ability to promote angiogenesis.

The regulation of angiogenic growth factors is often complex, but the best elucidated is the regulatory pathways surrounding VEGF. Several stimuli regulate VEGF; on a transcriptional level there are AP1 and Sp1, and on a translational level there are hypoxia-inducible factor 2α (HIF-2α) and mitogen-activated protein kinase (MAPK) (Fig. 11.2). Though much research has focused on inhibition of HIF-1, selective HIF-2α inhibition is emerging as a more vital protein in VEGF transcription, or the inhibition thereof. The fact that HIF1-deficient cells are capable of forming aggressive tumors implicates HIF1-independent processes in VEGF upregulation. Though many specific and selective inhibitors of HIF, akt, and a variety of other VEGF signaling molecules are under development, it must be understood that, like many biologic systems, tumors are dynamic and capable of switching signaling pathways, for example from HIF1-dependence to HIF1-independence.

The major angiogenesis stimulators in the skin are VEGF and bFGF, and the major inhibitor is interferon-α (IFN-α), the latter being a widely prescribed adjuvant therapy for

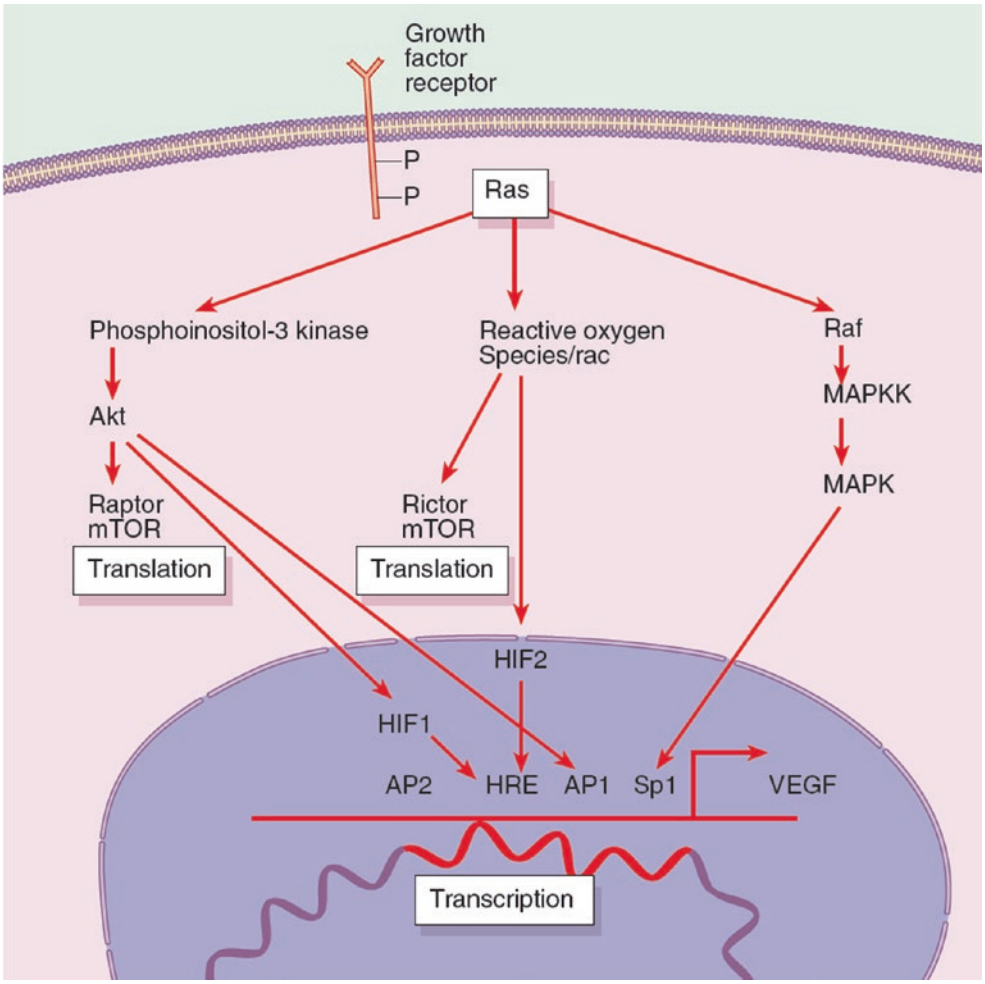

Fig. 11.2 Summary of molecular pathways that lead to vascular endothelial growth factor (*VEGF*) gene expression. As indicated, there are a number of signaling pathways that can activate *VEGF* transcriptions. *HIF*, hypoxia-inducible factor; *MAPK*, mitogen-activated protein kinase; *MAPKK*, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; *P*, phosphate

melanoma. Both the major stimulants and inhibitors are produced by keratinocytes, helping to provide a barrier between vasculature and the epidermis. When there is contact between the two (barrier disruption), VEGF is increased; when there is occlusion, VEGF is decreased. This lends credence to occlusional therapies.

The Importance of Stem Cell Recruitment in Angiogenesis

The reservoir of endothelial cells for neovascular development is of two sources: local recruitment of endothelial cells, and differentiation of quiescent mesenchymal cells. Exposure of cells to cytokines and other stimuli leads to upregulation of adhesion molecules, which bind immune effector cells; such cells can migrate to sites of infection and inflammation. Withdrawal of the stimulus will lead to apoptosis of the neovasculature. Persistent endothelial stimulation, either through angiogenic growth factors, loss of endogenous factors, or mutations, will cause cells to resist physiologic apoptosis. Recall that mutations involve activation of the phosphoinositol-3-kinase (PI3K)/akt pathway; such activation accounts for neovascular formation in humans.

The recruitment of bone marrow cells is imperative for the success of vascular formation. This notion explains some observations in cancer therapy. A large group of natural products, known as chemo-preventive agents, prevent cancer development but have little effect on advanced neoplasms. Xenograft models can help select for angiogenesis inhibitors that block mesenchymal recruitment, though tumors persist in recruiting local endothelial cells. While an effective treatment may require inhibition of both pathways, recognition of the tumor's dynamic ability to switch signaling pathways may even render recruitment-inhibiting therapies ineffective.

The ligands responsible for endothelial stem cell recruitment in ischemic tissues and tumors likely start with high expression of stromal cell-derived factor 1 (SFD-1). CXCR4 (also known as fusin is a CXC chemokine receptor 4) is the SDF1 receptor, and blockade of CXCR4 is a promising new

course of therapy that has been shown to block revascularization of ischemic tissue. Matrix metalloproteinase 9 (MMP-9) mobilizes the small kit ligand (sKITL). Mobilization of sKITL could be clinically useful in the treatment of acute myocardial infarction.

Recognition by the clinician and pathologist of certain signaling markers could be vital in the early detection of aberrant cells. Angiopoietin-1 (ang1) and angiopoietin-2 (ang2) have antagonistic effects: the former binds Tie-2 to inhibit vascular permeability, while the latter binds Tie-2 to stimulate vascular permeability. Hemangiomas of infancy and the hemangioma-like verruga peruana both show elevated levels of ang2. Work by Arbiser et al. suggests that hemangiomas arise from an unknown event, causing rapid stem cell recruitment from bone marrow (Fig. 11.3). A similar situation likely exists in high-malignancy tumors. Thus early detection of alterations in ang2 expression could be developed as a diagnostic tool for early detection of tumors.

Hemangiomas exhibit a characteristic natural involution after several years. This is likely due to a decreased VEGF expression leading to apoptosis. This parallels the apoptosis observed in endothelial cells during menstruation upon lessening of estrogen signaling. This fate of the hemangioma illustrates the potent response toward removal of proangiogenic, specifically VEGF, signaling.

The beta blocker propranolol was accidently discovered to cause hemangioma regression, and has become the treatment of choice for large hemangiomas of infancy [12, 23–25]. Thus, it is possible that hemangiomas can be inhibited by beta blocker independent modes of action, and that beta blockade per se is not essential to the activity of propranolol for hemangiomas.

Inflammatory Angiogenesis

Inflammatory skin conditions can be separated into two categories. Those expressing a predominance of interferon gamma (IFN-γ), interleukin-12 (IL-12), and interferon-inducible protein 12 (IP-12) are classified as Th1. Psoriasis is considered Th17+, with IL-17 being a mainstay of inflammation. Those expressing predominantly IL-4, -5, -6, and -10 are classified as Th2, such as systemic inflammatory

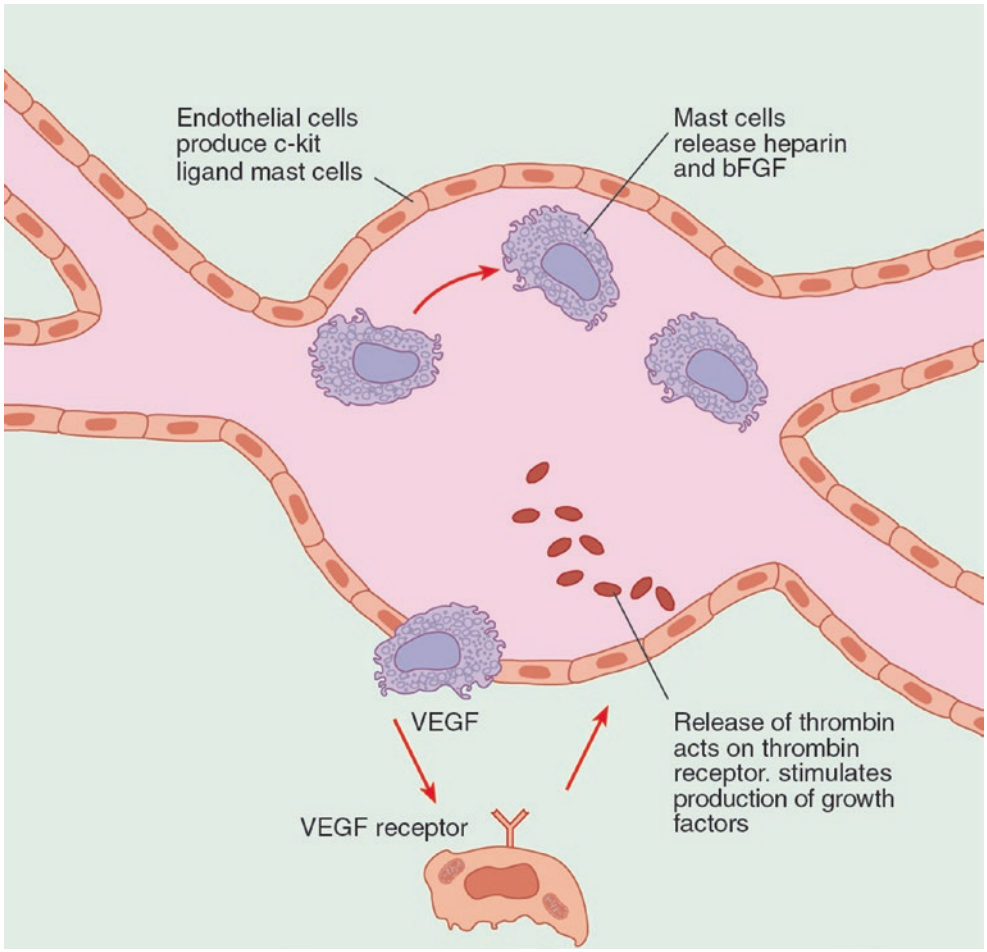

Fig. 11.3 A model for angiogenesis in hemangiomas. This model demonstrates interaction between endothelial cells. Most cells and stem cells that are recruited from bone marrow are induced to differentiate into endothelial cells

infiltrates of lymphocytes, mast cells, granulocytes, and macrophages. Both conditions show excess angiogenesis (despite the presence of antiangiogenic IL-12 in Th1 disorders), showing the potential utility of angiogenesis inhibitors for treatment, not merely of malignant neoplasms, but also in a wide range of dermatologic disorders.

The Role of Angiogenesis in Major Skin Disorders

Acne Vulgaris

Acne is the most common cutaneous disorder in the United States. This disorder accounts for over 10% of all patient encounters with a primary care physician. While the number of cases of acne vulgaris in adolescents has remained relatively stable over the past decade, the number of cases of adult-onset acne is increasing. The majority of debilitating effects of acne are psychological, with embarrassment and anxiety being among the top reported symptoms. Scarring is not uncommon and contributes to the lifelong effects of a moderate to severe case.

The role of MMPs in acne has been somewhat unclear in recent work, though it appears that they are involved in acne progression. The source of MMPs in acne appears to be keratinocytes [26] or neutrophils.

Treatment of acne includes both topical and systemic therapies. For treatment of noninflammatory comedones, topical retinoids such as tretinoin, adapalene, and tazarotene are often prescribed. Salicylic acid also has proven comedolytic activity. The most prominent therapy, both over-the-counter and prescription-based, is topical benzoyl peroxide. Some patients experience increased inflammation due to the presence of *Propionibacterium acnes* in sebaceous follicles. Treatment of such cases usually involves topical antibiotics such as clindamycin or erythromycin. It should be noted that to lessen the opportunities for the generation of antibiotic-resistant bacteria, treatments should be coupled with benzoyl peroxide [27, 28]. In severe cases of inflammatory acne, patients can be prescribed system isotretinoin. There are prescribing restrictions for this drug that are left to the discretion of the physician. Blue light and laser therapy are expensive and unproven treatments for acne should be avoided pending further data.

Psoriasis

Psoriasis is a common cutaneous disorder characterized by erythematous papules and silvery-scaled plaques. Though not entirely understood, the pathophysiology of clinical psoriasis tends to result from hyperproliferation and abnormal differentiation of epidermal keratinocytes, accompanied by inflammatory cell infiltration and vascularization. This remodeling resembles a prolonged wound response, with many reparative and remodeling processes being utilized [28] (Fig. 11.4).

To follow the wound healing analogy presented by Nickoloff, et al. [28], tumor necrosis factor (TNF), VEGF, IL-23, and transforming growth factor (TGF) are present in high levels in psoriatic tissue. Though these cytokines are

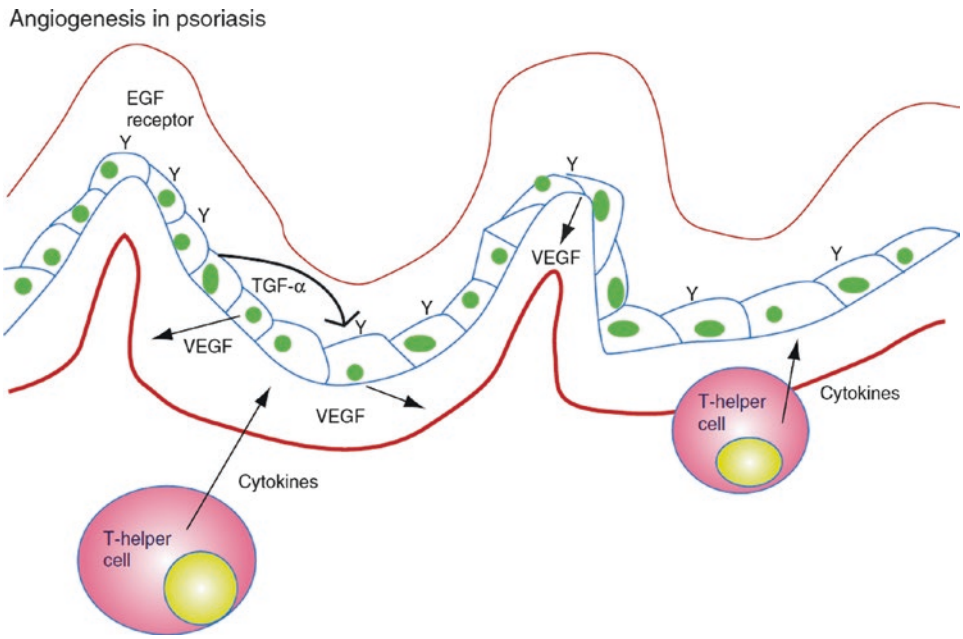

Fig. 11.4 Psoriasis is an example of chronic inflammation that drives excessive angiogenesis. It is a result of T cells and keratinocyte interactions, resulting in *VEGF* production, which then impacts endothelial cells

present in healthy skin, the exaggerated angiogenic response and epidermal thickening draws a definite line between healthy epidermal/dermal signaling and pathologic psoriatic skin. Genetic susceptibility plays a major role, as evidenced in the Koebner phenomenon, which describes an outbreak of psoriatic lesions on genetically susceptible patients upon mild trauma applied to the skin. Expression of TGF is a relevant early event in the formation of psoriatic lesions. Such expression leads to upregulation of VEGF, which promotes angiogenesis and changes in blood vessel morphology. In fact, elevated levels of VEGF are noted in lesional keratinocytes in psoriasis [29]. Accordingly, the angiopoietin (Ang)-Tie signaling pathway is activated in psoriasis, leading to vascular remodeling, formation, and invasion. To view the whole picture, VEGF expression leads to increased vascular permeability and capillary diameter. Once this angiogenic "switch" is turned, alterations in ang1/ang2 expression allow for increased vascular proliferation. Tumor necrosis factor provides differential direct regulation of the Tie2 signaling pathway. Tumor necrosis factor and ang1/ang2 allow for vascular survival and maintenance during this proliferative phase [30].

The increased angiogenesis in the early phases of psoriasis formation allows for the proinflammatory response that will follow. It is important to note that psoriasis is not simply an epithelial hyperplasia growth along the lines of carcinoma, but rather a metaplastic exaggerated wound response.

More recently, additional cytokines such as IL-17, IL-22, and IL-23 have been implicated in the pathogenesis of psoriasis. Based upon the involvement of IL-23 in psoriasis, ustekinumab a monoclonal antibody to the common p40 subunit of IL12- and IL-23 has been used extensively for psoriasis [31].

Treatments of psoriasis are varied, and include topical and systemic corticosteroids, immunomodulators such as cyclosporine and methotrexate, TNF blockers, and topical therapies such as calcipotriene. A long-trusted treatment has been topical coal tar treatment, and it is supposed by Arbiser that carbazole is the active ingredient in coal tar–mediated psoriasis treatment [32].

Ultraviolet light is one of the more popular therapeutic methods. Experimental treatments include topical applications of gentian violet, a triphenylmethane dye once used as a topical antiseptic that selectively kills gram-positive bacteria.

Warts (Verruca Vulgaris)

Verrucae are a common clinical manifestation of an HPV infection of epithelial tissue. The common wart or verruca vulgaris, derived from HPV-2, is a benign proliferative lesion manifesting as a raised hyperkeratotic papillomous lesion. There are many HPV subtypes, some seemingly more site-specific than others. Though all humans are susceptible, infections are more common in children and young adults. Infection occurs through skin-to-skin contact, with macerated sites predisposing one to infection. Human papillomavirus infections from HPV-16 or HPV-18 are considered high risk as HPV-16 and HPV-18 are carcinogenic. Accordingly, there exist in all HPV infections proangiogenic signaling. There is no consensus on which cytokines actually stimulate the angiogenesis and vasodilation seen in HPV^+ skin, as VEGF is commonly found in healthy uninfected human skin as well [33]. It is known that HPV-2 DNA is correlated with increases in angiogenesis [34], but it is not confirmed whether or not VEGF is responsible for verruca angiogenesis. However, VEGF is implicated in the angiogenesis of HPV-induced cervical tumors. Though it is unconfirmed at this time, VEGF overexpression is the likely initiator of wart microvascularization in the common HPV-2 infection (Fig. 11.5).

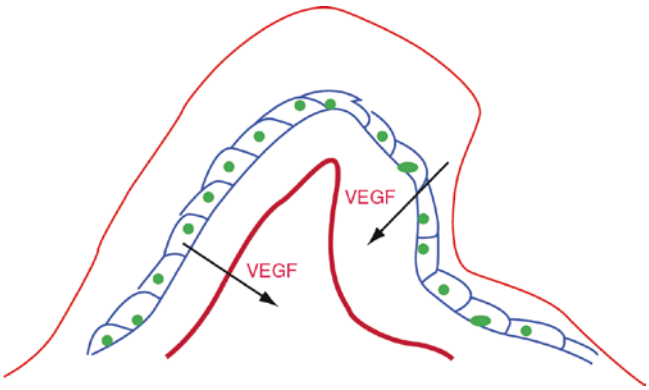

Fig. 11.5 Angiogenesis in common warts is the result of overproduction of *VEGF* by hyperproliferating epidermal keratinocytes

Current therapies for cutaneous nongenital warts include debridement accompanied by freezing with liquid nitrogen, treatment with salicylic acid, bichloracetic acid, or cantharidin. Flat warts are treated with cryotherapy, 5-fluorouracil, or tretinoin, while filiform warts are generally treated with a snip excision. Imiquimod is a common topical treatment for anogenital warts, working through local cytokine induction (including the antiangiogenic cytokine interferon-α). Currently, we combine debulking plus the combination of gentian violet and imiquimod for the treatment of warts. Debulking allows decreased tissue hypoxia, which is in itself immunosuppressive [13]. Gentian violet, which is used to inhibit angiogenesis, may promote dendritic cell maturation and effective presentation of viral antigen, while imiquimod promotes interferon alpha production through TLR7 agonism.

Basal Cell Carcinoma

Basal cell carcinomas (BCCs) occupy a classification of skin cancers known as the nonmelanoma skin cancers (NMSC). This classification extends to squamous cell carcinomas (SCCs) and BCCs almost exclusively. The causes of BCCs vary, but are largely attributed to overexposure to ultraviolet (UV) light, genetic predisposition (illustrated in an extreme case by xeroderma pigmentosum), and possibly HPV infection.

The main effector of angiogenesis in BCCs appears to be the basic fibroblast growth factor (bFGF), which occupies a small family of peptides including the acidic fibroblast growth factor (aFGF) and the Kaposi fibroblast growth factor (kFGF). The fibroblast growth factors (FGFs) are small molecules, ranging from 18 to 21 kd. These molecules bind heparin and interact with a family of four receptors [22]. In mice that were irradiated with UV light, upregulation of bFGF rather than VEGF was noted. Considering that UV overexposure is considered one of the primary causes of BCCs in humans, this finding may underlie the cutaneous angiogenesis behind UV-induced tumors. An introduction of signaling peptides that convert bFGF into an oncogene with the capability of malignant transformation. Basic FGF shows baseline limited expressivity in normal epidermis. However, studies have found diffuse and strong bFGF immunoreactivity in BCCs. It is known that SCCs produce a FGF-binding protein that localizes bFGF, and BCCs could work along a similar pathway.

Therapy and treatment modalities for BCCs vary, but surgical excision still remains the most reliable treatment for a primary or recurrent tumors. Among the surgeries, the most prevalent methods are cryosurgery, curettage and electrodissection, and Mohs' micrographic surgery (MMS). The latter has some marked advantages, especially in that it affords a much higher confidence in a clear border. Studies have shown that MMS does not provide significantly lower rates of recurrence, but the cosmetic sensitivity of MMS often tips the scales. It is most useful on tumors of the face, neck, head, and hands. As with all surgeries, the cosmetic and practical outcome of the procedure is largely influenced by the skill of the surgeon. Adjuvant therapy for BCCs includes radiation therapy (RT), or topical treatment with imiquimod or 5-fluorouracil.

Small molecules of sonic hedgehog have entered the clinic, including vismodegib, an antagonist of the receptor smoothened. Vismodegib has been used in both inoperable/metastatic BCC and for basal cell nevus syndrome [35].

Regression of both of these have been seen, although resistance is common in large BCCs, likely due to both intrinsic (mutant smoothened) and acquired resistance. A major side effect is altered taste (dysgusia), which may reflect a requirement for active sonic hedgehog signaling for taste bud activity [36].

Squamous Cell Carcinoma

Squamous cell carcinomas are the second member of the class of NMSCs, and they result from malignant proliferation of keratinocytes in the epidermis. The tumors present as a hyperkeratotic nodule, papule, or plaque, closely resembling actinic keratoses. The angiogenic pathophysiology of an SCC involves dual signaling for neovascularization, blood vessel permeability, and vasodilation by both VEGF and bFGF [1]. It is theorized that the NMSCs are the result of UVB radiation, while melanomas result from mostly UVA radiation. Despite the fact that SCCs overexpress both VEGF and bFGF, they are predominantly locally invasive. The most effective treatments are surgery, with reasonable effectiveness following a topical treatment with imiquimod of 5-fluorouracil. Patients must be given careful follow-up exams to ensure complete resection of all tumor tissue.

Photoaging

Photoaging is the overall process of skin aging due to UV exposure. It is characterized by wrinkle formation, blistering, and reduced recoil capacity. The cause of photoaging is likely matrix degradation or remodeling due to overexposure to UV light. The most common source of matrix degradation or remodeling in most people is chronic sun exposure. Matrix metalloproteinases secreted by dermal fibroblasts and keratinocytes are generally considered responsible for the matrix remodeling. The MMPs are zinc-dependent endopeptidases in a subclass of the metzincin superfamily of proteins. The effects of MMP can be inhibited by the tissue inhibitor of MMP (TIMP-1). Another effector of cutaneous photoaging is the serine protease granzyme B (GrB) [37]. Keratinocytes irradiated with UVA simultaneously produced GrB and MMP1. The result of this finding elucidates the complementary actions of MMPs and GrB in matrix remodeling upon prolonged UV irradiation. Therapy and treatment for photoaging is limited.

Actinic Keratosis

Actinic keratosis (AK) is a premalignant lesion occurring on sun-damaged skin. Over many years AKs can progress to SCCs, exemplifying tumorigenesis through loss of tumor suppressor genes. These tumors display increased angiogenesis as the tumor progresses [38]. Angiogenesis inhibitor thrombospondin-1 (TSP-1) is strongly expressed in most AKs [39]. It has been reported that patients treated with sorafenib resulted in inflammation of AK, which in some cases progressed to invasive squamous cell carcinoma [40].

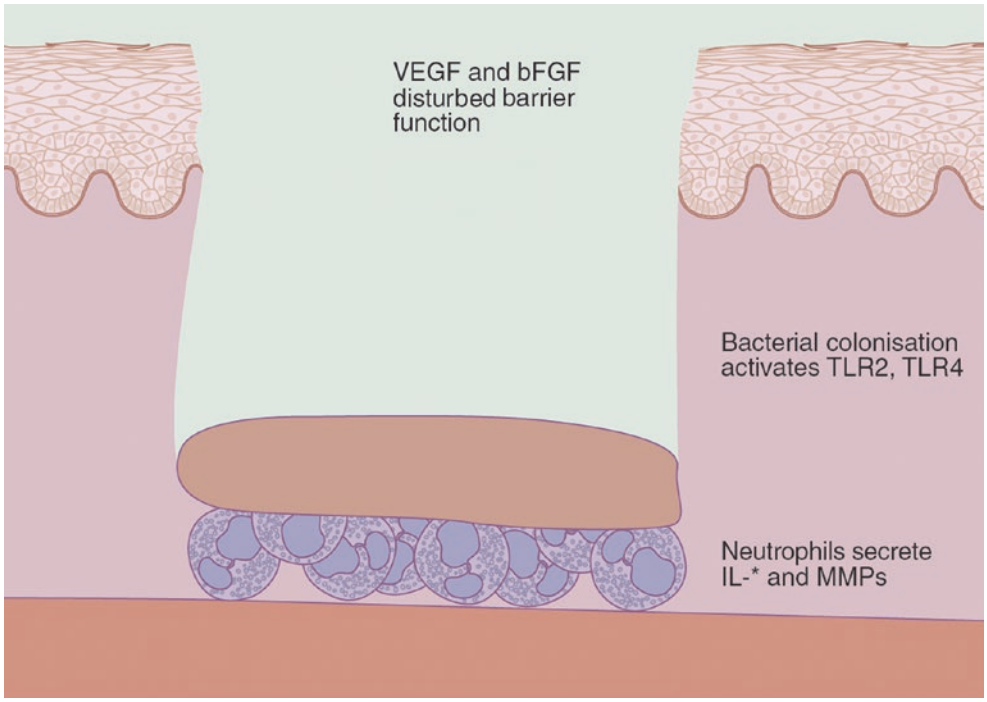

Fig. 11.6 A model for angiogenesis in venous ulcers. In chronic wounds, there is a proinflammatory process that drives angiogenesis. There is also imbalanced collagen production/degradation by fibroblasts, with the result being a chronic wound

The treatment for AK is cryotherapy and, in some cases, a topical treatment with imiquimod or 5-fluorouracil. Physicians should be vigilant in follow-up exams, because these lesions are a risk factor for NMSC.

Ulcers

Chronic skin ulcers occur in approximately 1 million people in the United States. Little is known about the pathways leading to degeneration of tissue and ulcer formation, though common theories connect degeneration to inadequate circulation and ischemia—elements in most dermal ulcers. Collagen is the primary component of mechanical strength in most tissues. Collagen stability is dependent on adequate oxygen supply. In ischemic skin, biochemical mechanisms of tissue repair are activated, with increases in lactate, TGF-β, VEGF, collagen synthesis, and MMP-1 and -2. The upregulation of VEGF and MMP (primary angiogenesis stimulators) are known to signal increased angiogenesis to the location. In some cases unstable collagen molecules are synthesized together with upregulated MMPs, resulting in collagen denaturation, defective angiogenesis, weaker skin, and predisposition to ulceration [41].

In chronic ulcers, slow and abnormal healing occurs due to biochemical and physiologic defects of the local tissue. The inflammatory phase may be prolonged, possibly with decreases in collagen synthesis by fibroblasts and increased levels of MMPs (Fig. 11.6). Epithelial cells and fibroblasts are senescent at the wound edges, and do not respond to growth factor signals such as platelet-derived growth factor (PDGF) or TGF-β. Nonhealing ulcers including diabetic, venous stasis, and pressure ulcers, can be characterized by inadequate wound granulation, and thus inadequate angiogenesis. In diabetic ulcers, glucose is antiproliferative, which causes loss of angiogenic stimulators, such as PDGF-BB, TGF-β, and ang-1. In pressure ulcers, tissue compression and vasoconstriction result in poor tissue perfusion. In venous stasis ulcers, fibrin cuffs around capillaries cause local hypoperfusion and also may sequester growth factors.

Ulcers are commonly treated through mechanical and compression therapy. Topical treatments vary, but include occlusive therapy, topical debriding agents, PDGF, and topical antibiotics. Gentian violet can be used topically to decrease inflammation in skin ulcers (JLA, 2010). In some cases, surgery (skin grafting or transposition skin) may be necessary. However, preventative care remains the best way to avoid development or exacerbation of skin ulcers.

Melanoma

Melanoma is the sixth most common cancer in the United States, with rates of occurrence increasing faster than those of any other cancer. Some risk factors for melanoma include overexposure to UVA radiation, sensitive skin type, immunosuppression, family history of melanoma, dysplastic mole syndrome, multiple common or atypical nevi, and exposure to positive mutagens.

Melanoma is infamous for its poor prognosis and resistance to treatment. Melanoma tends to metastasize via the lymphatic vessels to the areas surrounding the tumor and to draining lymph nodes. Overexpression of angiogenesis factors such as VEGF-A, FGF-2, IL-8, PDGF, and ang2 have been observed in human melanoma. VEGFA-transfected melanomas are

characterized by increased angiogenesis and tumor growth. Importantly, several receptors previously thought to be exclusively expressed on endothelial cells such as VEGFR-1, VEGFR-2, and Tie-2 are also expressed in different tumor cells. Recent experiments implicate the Tie-2 signaling pathway as an important angiogenesis modulator in melanoma. Ang1/ang2 are ligands that (de)activate the Tie-2 pathway. Dominant Ang-2 expression against ang-1 through Tie2 receptor in the presence of VEGF plays a critical role in initiating early neovascularization [42]. Akt/PI3K has emerged as a critical pathway downstream of Tie2 that is necessary for cell survival effects as well as for chemotaxis, activation of endothelial nitric oxide synthase, and perhaps for anti-inflammatory effects of Tie2 activation. Mitogen-activated protein kinase (MAPK) activation has also emerged as a pathway that may be responsible for the morphogenetic effects of Tie2 on endothelial cells.

Several experimental approaches to treat melanoma using angiogenesis inhibitors have been reported. Besides the surgical modalities for the removal of melanoma, the common medical treatment modalities include adjuvant therapy with IFN. However, due to the marked side effects of IFN, many patients do not complete the full course. Cheaper, more effective, and safer methods under study include endogenous inhibitors of TSP-1, TSP-1 fragments, TRAIL, TSP-2, IL-12, angiostatin, endostatin, and other compounds such as TNP-470, thalidomide, SU6668, SR25989, solenopsin, honokiol, and batimastat. Triphenylmethane dyes are suspected to suppress Ang2 via nox4 inhibition, and are promising medical therapies for melanoma [13].

Approximately 60 % of melanomas carry mutations in the Braf oncogene. Based upon this, Braf inhibitors and combination Braf/MEK inhibition have been used in the treatment of metastatic melanoma. The use of vermurafenib alone led to increased survival, albeit, with the presence of keratinocyte neoplasia, some with Hras mutations. The combination of dabrafenib and the MEK inhibitor trametinib showed superior results with decreased development of keratinocyte neoplasia [43]. However, blockade of ERK/BRaf does not address additional signaling mechanisms in melanoma, such as reactive oxygen/NFkB signaling. Perhaps combinations of reactive oxygen signaling inhibitors and Braf/MEK inhibitors may lead to enhanced survival and cure of melanoma. Reactive oxygen inhibition also leads to suppression of notch signaling, which may be of additional benefit in melanoma [44].

Conclusion

Angiogenesis, or the formation of microvasculature is a fundamental process responsible for tissue homeostasis, and is also a part of many disease processes. Angiogenesis can be manipulated by resetting the balance of pro- and antiangiogenic factors in endothelial cells. Because angiogenesis plays an important role in a number of common skin diseases, experimental agents (mostly angiogenesis inhibitors), have great potential as therapeutic agents.

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Questions

1. Which of the following is not an important growth factor for endothelial cells?
   A. angiopoietin-2
   B. basic fibroblast growth factor
   C. cyclooxygenase-2
   D. platelet-derived growth factor
   E. prostaglandin E-2

2. Which signaling pathway does not active vascular endothelial growth factor (VEGF)?
   A. hypoxia-inducible factor (HIF)
   B. interferon (IFN) alpha
   C. mammalian target of rapamycin (mTOR)
   D. mitogen-activated protein kinase (MAPK)
   E. mitogen-activated protein kinase kinase (MAPKK)

3. Overexpression of which of the following angiogenesis factors is not involved in melanoma progression?
   A. Ang-2
   B. FGF-2
   C. IL-8
   D. TRAIL
   E. VEGF-A

4. Mutations involving activation of which pathway accounts for most neovascular formation in humans?
   A. bFGF
   B. IFN alpha
   C. PI3K/akt
   D. reactive oxygen species/rac
   E. Raf

5. Which of the following is true of angiopoietin (ang)-1?
   A. it has antagonistic activity to ang-2
   B. it binds Tie-2 to stimulate vascular permeability
   C. it is stimulated by carbazole therapy
   D. it is stimulated by ultraviolet light therapy
   E. it is stimulated by methotrexate

Answers

1. A
2. B
3. D
4. C
5. A

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Cutaneous Neuroimmunology

12

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Abstract

Many observations suggest a functional relationship between the nervous system and cutaneous immunology. Experimental work has demonstrated the presence of receptors for nerve-derived factors on epidermal and dermal cells as well as an anatomic relationship between peripheral nerves and both immune and non-immune cells within the skin. The skin contains an extensive network of nerve fibers that both transmit afferent sensory signals to the central nervous system and are also capable of secreting various mediators in the periphery. These mediators include both peptide and non-peptide factors that regulate a variety of processes including blood flow, sensation, temperature regulation as well as other homeostatic processes. There is a large body of evidence that products of nerves regulate, in part, immunity, inflammation and wound healing. In support of a role for products of nerves in the pathophysiology of skin disease, much evidence exists showing a key role for innervation in psoriasis and there is some data indicating a role for nervous system influences in other inflammatory skin disorders. An improved understanding of the relationship between the nervous system and cutaneous immunity may have important therapeutic implications for inflammatory skin diseases including atopic dermatitis, psoriasis, rosacea, acne vulgaris and, perhaps, neoplastic and other disorders.

Keywords

Neuropeptide • Neurogenic inflammation • Cutaneous Immunity • Cutaneous Nerves • Substance P

Introduction

Human skin contains a sophisticated network of nerve fibers and specialized sensory structures that serve to transduce sensations of touch, vibration, temperature and pain. Nerve fibers have dual functions: to transmit afferent sensory impulses to the central nervous system and to secrete mediators into the local environment. While many of these mediators are polypeptides (called neuropeptides), others are non-peptide factors. These factors affect various biological processes including inflammation, immunity, wound healing and perhaps even aging.

Cutaneous neurobiology is an expanding field of research with increasingly appreciated clinical relevance. The presence of neuropeptide receptors on epidermal and dermal cells and the close anatomic relationship of nerve fibers with immune and non-immune cells demonstrate the link between the nervous system and the skin.

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Core Messages

- The skin is a complex organ system containing a sophisticated network of nerve fibers and specialized sensory structures.
- Cutaneous nerves release neuropeptides and neurohormones into the local milieu in response to internal and external stimuli. Many appear to serve an immunoregulatory role.
- Neuropeptides play a role in the pathophysiology of common skin diseases such as atopic dermatitis and psoriasis.
- Substance P and calcitonin gene-related peptide are among the most prevalent and multifunctional neuropeptides in the skin.

Background

The classic "triple response" of sensory nerves was demonstrated by Lewis in 1927 [1]. This is seen after the skin is stroked, with production of local erythema (capillary dilatation), followed by the axon-reflex flare to produce erythema (arteriolar dilatation) and a wheal (transudation of fluid). Vasodilatation has been shown to occur following dorsal nerve root stimulation and is inhibited by depleting sensory nerves of neuropeptides with capsaicin, demonstrating the role of nerves in cutaneous inflammation [2]. Furthermore, Bayliss and Bruce noted that patients with defective cutaneous sensory systems could not mount normal inflammatory responses to cutaneously applied inflammatory agents [3, 4]. Similarly, observations that patients with sensory disorders such as postherpetic neuralgia have defective responses to inflammatory stimuli suggest that the cutaneous nervous system modulates inflammation [5].

Anatomy

Highly specialized afferent sensory and efferent autonomic nerve branches innervate the skin [5–7]. The nervous system is split into two main divisions – the central and peripheral nervous systems. The peripheral nervous system (PNS) includes peripheral nerves, sensory nerves and the autonomic nervous system (ANS). The ANS is further divided into the sympathetic, parasympathetic and enteric nervous systems [8].

The sensory component of the peripheral nervous system conveys mechanical and chemical activity to the central nervous system (CNS). Stimuli include external and internal physiologic and mechanical triggers. Afferent unmyelinated C or myelinated Aδ fibers from the dorsal root ganglia innervate the epidermis as fine nerve fibers with free endings that converge in the dermis [5]. Sensory receptors include encapsulated structures such as Pacini, Rufini and Meissner's corpuscles, and nerve fibers with "free-ends." Free nerve endings are in contact with hair follicles, pilosebaceous units, glandular structures and the epidermis. The trunk, extremities, neck and posterior scalp are supplied by nerves derived from the dorsal root ganglia, whereas the upper anterior neck, face and the majority of the scalp are innervated by the trigeminal nerve [9].

Sensory fibers are generally classified into three groups based on their size and conduction velocity. C-fibers are unmyelinated, thin afferent fibers consisting of pain receptors called nociceptors and mechanoreceptors. A subpopulation termed C-polymodal nociceptors represent 70 % of all cutaneous C-fibers and participate in the release of neuropeptides [10]. Aδ-fibers are small, myelinated fibers that innervate the skin although to a much smaller extent than C-fibers. Autonomic nerves represent a minority of the cutaneous fibers. These nerves predominantly generate neurotransmitters such as acetylcholine and catecholamines; however, they also produce neuropeptides including neuropeptide Y (NPY), calcitonin gene related peptide (CGRP), vasoactive intestinal peptide (VIP) and atrial natriuretic peptide (ANP) [2].

Neuropeptides

Neuropeptides (NPs) are a heterogeneous group of polypeptides ranging from 2 to greater than 40 amino acids in size. There are over 50 identified NPs, 11 of which are found in human skin [11]. NPs are released in response to a range of stimuli including pain, temperature and irritation, in order to mediate diverse biologic processes related to injury, inflammation, infection and wound healing. NPs are synthesized in the nerve cell bodies. Their precursors are produced in the endoplasmic reticulum, and then processed and packaged in the Golgi apparatus for eventual transport to the nerve endings. The most abundant NPs in the skin include substance P (SP), CGRP, neurokinin A (NKA), neurotensin, pituitary adenylate cyclase activating polypeptide (PACAP), VIP, NPY, β-endorphin, enkelphalin, somatostatin, galanin, dynorphin, ANP, α or γ-melanocyte stimulating hormone (MSH), parathyroid hormone-related protein (PTHrp), urocortin and corticotrophin-releasing hormone (CRH) [2]. NPs are released predominantly from nerve fibers; however, evidence exists that epidermal and dermal cells also produce neuropeptides and neurohormones. These cells include fibroblasts, keratinocytes, Langerhans cells, macrophages, mast cells, melanocytes, endothelial cells, Merkel cells and leukocytes [2, 12]. Table 12.1 summarizes some of the well-characterized neuromediators found in the skin.

Table 12.1 Neuromediators in the skin

Neuromediator	Source	Receptor	Primary function
Substance P	Sensory nerve fibers Meissner corpuscles Perivascular nerves Mast cells Monocytes Eosinophils	Tachykinin (neurokinin) receptor	Mediates erythema and edema Increases histamine and TNF-α release from mast cells Upregulates cell adhesion molecule expression in dermal endothelial cells Stimulates release of proinflammatory cytokines from keratinocytes
VIP	Sensory nerve fibers Sweat glands Merkel cells PMNs	VPAC1 and VPAC2 receptors	Vasodilation Increases sweat secretion Histamine release from mast cells Keratinocyte proliferation and migration Inhibits Langerhans cell antigen presentation for Th1 responses while augmenting Th2 and Th17 responses
PACAP	Sensory nerve fibers Autonomic nerves Lymphocytes Endothelial cells	VPAC1 and VPAC2 receptors	Vasodilation Down-regulates proinflammatory cytokines in T cells Modulates mast cells Inhibits NF-κB Inhibits Langerhans cell antigen presentation for Th1 responses while augmenting Th2 and Th17 responses
CGRP	Sensory nerve fibers Perivascular nerves Meissner corpuscles	CGRP receptors	Vasodilation Keratinocyte proliferation Histamine release (mast cells) Inhibits NF-κB Inhibits Langerhans cell antigen presentation for Th1 responses while augmenting Th2 responses
POMC peptides	Keratinocytes Melanocytes Langerhans cells Fibroblasts Mast cells Monocytes Macrophages Endothelial cells PMNs Sensory nerves	Melanocortin receptors	Immunomodulation (upregulates IL-10 and antagonizes effects of proinflammatory cytokines) Inhibits NF-κB Melanogenesis
Catecholamines	Autonomic adrenergic nerves Keratinocytes Melanocytes	Adrenergic receptors	Inhibit keratinocyte migration Regulates natural killer and monocyte activity Inhibits Langerhans cell antigen presentation for Th1 responses
NPY	Perivascular nerves Langerhans cells	G protein-coupled Y1-Y5 rhodopsin-type receptors	Vasoconstriction Eccrine sweat production Antimicrobial properties
ATP	Numerous sources with intracellular and/or extracellular activity Keratinocytes Sympathetic nerve fibers	Ligand-gated purinergic P2X receptors G protein-coupled purinergic P2Y receptors	Regulation of cell growth and differentiation Immunomodulation [promotes dermal microvascular endothelial cell production of IL-6, GRO-α (CXCL1) and IL-8 (CXCL8), MCP-1 (CCL2) production; IL-1 release by macrophages] Enhance Langerhans cell antigen presentation
Catestatin	Sensory nerve fibers Adrenal chromaffin cells Adrenergic nerve fibers Immune cells	Nicotinic acetylcholine receptors G protein-coupled receptors	Keratinocyte migration Stimulation of monocyte and PMN chemotaxis Mast cell degranulation Pro-inflammatory cyto- and chemokine production Antimicrobial properties

Source: Data from Scholzen et al. (1998) [10], Steinhoff et al. (2003) [2], Kodali et al, (2004) [13], Kodali et al. (2003) [14], and Inoue et al. (2007) [15]

The activities of various neuromediators in the skin account for the ways in which nerves influence cutaneous immunology

Abbreviations: *CGRP* calcitonin gene-related peptide, *IL* interleukin, *PACAP* pituitary adenylate cyclase activating polypeptide, *NF-κB* nuclear factor κB, *POMC* pro-opiomelanocortin, *TNF-α* tumor necrosis factor-alpha, *VIP* vasoactive intestinal peptide, *NPY* neuropeptide Y, *ATP* adenosine triphosphate

The distribution of NPs varies depending on the body site. High levels of SP, NKA and CGRP are found in areas with the greatest tactile sensation. Intermediate levels are found in the neck and face, whereas the lowest levels are present in the groin, arm and thigh [16]. Levels of VIP and peptide histidine methionine (PHM) are also highest in axillary skin, suggesting their role in axillary eccrine sweat production. The location of NPs also varies based upon the layer of the skin in question. SP, NKA and CGRP are found in nerves penetrating the epidermis and the neuropeptides SP, CGRP, VIP and NKA are in nerves innervating dermal structures [9]. NPs bind to specific receptors on nerves and other cell types and, in turn, activate intracellular signaling cascades. NPs are then inactivated by peptidases such as neutral endopeptidase or angiotensin-converting enzyme (ACE) [17, 18].

Delineating the myriad functions of neuropeptides and neurohormones has led to the emergence and explosive growth of a field investigating the intimate relationship between the nervous, immune and endocrine systems and the skin. Terms such as the neuro-immune-cutaneous system are used to imply a relationship between immune and non-immune cells in the skin and between the immune system and the nervous system within the skin [19].

The skin is an ideal organ in which to investigate these relationships due to its location, size and function. As the interface between the external and internal environments the skin has a unique role in modulating and transmitting outside stimuli while maintaining internal homeostasis. As a result, there is increasing attention and focus on the skin as a site for unraveling these connections, applying them to other organ systems and ultimately, using them as a model for therapeutic interventions in several disease states. In this chapter we highlight several NPs and other neural signals, discuss their functions and describe their role in clinical skin disorders (Table 12.1).

Receptors

There are numerous neuropeptide receptors. For example, SP has three receptors, NK_1, NK_2, NK_3. Two receptors have been identified for CGRP (1,2) and NPY has receptors referred to as $Y_{1,2,3,4,5}$ [20]. Most cutaneous cells express several receptor types; to discuss each in detail is beyond the scope of this chapter, and more comprehensive reviews are available [9]. Rather, the receptors most relevant to the skin will be discussed. These include NK_1, preferentially expressed on human keratinocytes [2], VPAC-1R (vasoactive intestinal polypeptide/pituitary adenylate cyclase activating polypeptide 1 receptor) on dermal endothelial cells and Langerhans cells [21], VPAC-2R on keratinocytes and Langerhans cells [21] and melanocortin 1 receptor (MC-1R) on melanocytes, keratinocytes, monocytes, fibroblasts and Langerhans cells [9]. Additionally, CGRP has effects on many cellular components of skin that can be blocked by competitive inhibitors of the type I CGRP receptor (see below) suggesting the presence of CGRP receptors on various cell types within the skin.

Substance P (SP)

SP is the best-characterized neuropeptide. An undecapeptide, it is a member of the tachykinin family along with NKA and neurokinin B (NKB). SP is released from sensory nerves that are in contact with endothelial cells, mast cells, hair follicles and epidermal cells [22]. Its biological effects in the skin are mediated predominantly by the NK_1 receptor. SP receptor-binding leads to activation of phospholipase C, an increase in intracellular calcium and subsequent activation of nuclear factor-kappa B (NF-kB) [23].

SP is a potent vasodilator, accounting for its role in the wheal and flare response in neurogenic inflammation. SP directly acts on vascular smooth muscle and indirectly on the endothelium to enhance the production of nitric oxide, which contributes to vasodilatation and increased vascular permeability [24]. In human skin, SP is also released by free nerve endings in the dermal papilla and epidermis of human fingers, in Meissner corpuscles, and near sweat gland ducts and blood vessels [12]. Receptors for SP are found on mast cells, lymphocytes, leukocytes and macrophages. SP-stimulated macrophages generate prostaglandin E2, thromboxane B2 and superoxide ion, and SP-stimulated keratinocytes release proinflammatory cytokines IL-1α, IL-1β and IL-8 [2]. Furthermore, SP induces histamine release from mast cells, lymphocyte proliferation and chemotaxis, immunoglobulin production and the release of cytokines IL-1, IL-6 and TNF-α [25]. An increase in SP-expressing nerve fibers is seen in inflammatory skin diseases such as atopic dermatitis [26]. SP increases the release of proinflammatory cytokines from keratinocytes and TNF-α, histamine, leukotriene B4 and prostaglandin D2 from mast cells. SP also upregulates IL-2 production to promote T-cell proliferation and induces the expression of the adhesion molecules P-selectin, intercellular adhesion molecule (ICAM) 1 and vascular cell adhesion molecule (VCAM) 1 [2, 26]. Recent evidence suggests that SP also plays a role in regulating cutaneous microflora [27]. SP-treated bacteria populations including *B. cereus* were found to have increased biofilm formation activity. Furthermore, SP enhanced *B. cereus*-induced cytotoxic activity against keratinocytes, evidenced by increased caspase 1 production and morphological changes characteristic of necrosis [27]. The effect of SP on the cutaneous microbiome may influence a variety of skin disorders [27].

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) and Vasoactive Intestinal Peptide (VIP)

PACAP exists in two forms, PACAP-38 and a truncated form, PACAP-27 [28]. PACAP belongs to the glucagon-secretin family that includes VIP and growth hormone-releasing hormone. PACAP binds to two types of receptors. PAC1 binds PACAP to activate adenylate cyclase and phospholipase C, whereas a second type of receptors, VPAC1 and VPAC2, bind both PACAP and VIP to activate adenylate cyclase [14]. PACAP has the highest immunoreactivity around blood vessels, hair follicles and sweat glands, and has been shown to modulate inflammatory responses in the skin [2].

VIP is a 28-amino-acid peptide that is localized in the deeper dermis in nerve fibers situated around eccrine sweat glands, superficial and deep vascular plexuses and hair follicles [24]. VIP is a potent vasodilator that contributes to the development of pruritus, erythema and edema. VIP also plays a role in the regulation of cutaneous blood flow, the promotion of nitric oxide synthesis, keratinocyte proliferation and sweat production. VPAC-1R (vasoactive intestinal polypeptide/pituitary adenylate cyclase activating polypeptide 1 receptor) is the dominant receptor on human dermal endothelial cells, whereas VPAC-2R is expressed on keratinocytes [2]. Both receptor types are found on Langerhans cells [21].

In a murine model, intradermal administration of PACAP suppressed the induction of contact hypersensitivity (CHS) at the injected site and *in vitro* treatment of epidermal antigen presenting cells inhibits their ability to present antigen for elicitation of delayed-type hypersensitivity (DTH) in previously immunized mice [14]. PACAP also inhibited the ability of Langerhans cells to present antigen in wholly *in vitro* assay systems for a Th1 response [14] . *In vitro* studies suggest this may be due to PACAP-induced suppression of IL-1β release and augmentation of IL-10 production [14]. In a mouse model, VIP also inhibited the ability of epidermal cells enriched for Langerhans cell content to present antigen for elicitation of delayed-type hypersensitivity in previously immunized mice [13]. Furthermore, VIP inhibited Langerhans cell antigen presenting capability in *in vitro* assays of antigen presentation for Th1 responses [13].

The inhibitory effects of PACAP, VIP and CGRP on antigen presentation may occur through suppression of NF-kB, which is known to be involved in regulating transcription of the TNF-α gene [29], as well as CD86 and other pro-inflammatory cytokines [14].

While VIP and PACAP inhibit Langerhans cell antigen presentation for Th1 immune responses, these NPs enhance antigen presentation to generate Th2 cells [30]. *In vivo* promotion of Th2 responses by VIP and PACAP signaling has also been demonstrated through manipulation of VPAC2 expression in a transgenic murine model; overexpression of VPAC2 in $CD4^+$ T cells biased toward Th2 immunity, whereas mice deficient in VPAC2 demonstrated predominance of the Th1 response [31].

Th17 cells are a subset of $CD4^+$ T cells that produce a variety of cytokines of the IL-17 family, as well as IL-21 and IL-22. IL-21 production may lead to further differentiation of more Th17 cells, while IL-22 plays a role in keratinocyte proliferation and epidermal hyperplasia [32]. Some Th17 cells produce IL-17A but only very little IL-22 [33].

Th22 cells are $CD4^+$ T cells that produce IL-22 but not IL-17A. IL-22 is known to act on epithelial cells such as keratinocytes to trigger antimicrobial peptide production [30]. VIP and PACAP have been shown *in vitro* to bias Langerhans cell antigen presentation toward Th17, in addition to Th2 immune responses. Exposure of Langerhans cells to PACAP or VIP followed by antigen presentation to $CD4^+$ T cells led to enhanced production of IL-17A and IL-4 by $CD4^+$ T cells with suppressed production of IL-22 and IFN-γ [30]. Furthermore, after antigen presentation the $CD4^+$ T cell population had enhanced number of cells expressing intracellular IL-17A or IL-4 with decreased numbers of cells expressing IFNγ [30]. In accordance with these findings, the Th17 cell-associated transcription factor RORγT and the Th2-associated transcription factor GATA3 were elevated while T-bet (Th1-associated) was decreased [30].

In vivo studies in which PACAP was applied topically to human skin revealed that PACAP induces marked edema in a concentration-dependent manner [34]. Investigators also injected PACAP intravenously and observed a concentration-dependent vascular response (flushing, erythema and edema) as well as a 2.7 °C rise in body temperature [34]. Together, this evidence suggests a role for PACAP in the vasoactive component of neurogenic inflammation found in skin disorders such as rosacea, urticaria and atopic dermatitis [34].

Recent experiments demonstrated that human keratinocytes significantly upregulated vascular endothelial growth factor (VEGF) production in response to VIP stimulation in a dose- and time-dependent manner [35]. Maximum enhancement of VEGF production was observed with the combination of VIP and IFN-γ stimulation [35]. This mechanism might underlie the angiogenesis and vasodilatation characteristic of lesional psoriasis skin [35].

Calcitonin Gene Related Peptide (CGRP)

CGRP is a 37-amino-acid neuropeptide discovered in 1982 [36]. There are two forms, CGRP-α, or CGRP-1, and CGRP-β, or CGRP-2. In human skin, CGRP is co-localized with SP in nerves in the dermal papillae and free nerve endings of glabrous skin; however, when co-localized with somatostatin, it is found in nerve fibers associated with the

epidermis and perivascular space [24]. CGRP is among the most prevalent cutaneous NPs and is found in association with mast cells, melanocytes, keratinocytes, Langerhans cells and Merkel cells [2, 22].

CGRP inhibits antigen presentation by macrophages [37] and blood-derived dendritic cells [38, 39] and upregulates melanocyte proliferation, dendricity and melanogenesis [19]. Early observations of CGRP-containing unmyelinated nerve fibers in direct contact with the surface of epidermal Langerhans cells suggested an intimate relationship [40] (Fig. 12.1). While CGRP inhibits Langerhans cell antigen presentation in Th1 responses [40], it enhances Langerhans cell antigen presentation for Th2 responses, as evidenced by increased IL-4 production [41]. It also stimulates Langerhans cell production of the Th2 chemokines CXCL17 and CXCL22 while inhibiting the stimulated production of the Th1 chemokines CXCL9 and CXCL10 [40]. CGRP effects on LC antigen presentation for Th1 responses can be inhibited by $CGRP_{8–37}$, a competitive inhibitor of the type I CGRP receptor [42]. Recent *in vivo* studies have corroborated CGRP as a mediator of Th2-type immune responses [43]. The significance of CGRP's role in shifting toward a Th2 immune response is not fully understood.

CGRP is a mediator of neurogenic vasodilatation and a modulator of keratinocyte proliferation and cytokine production [2]. Human primary dermal microvascular endothelial cells (pDMECs) express mRNA for CGRP and adrenomedullin receptors, and recent investigations found that CGRP inhibits lipopolysaccharide-induced production of various chemokines, including IL-8, MCP-1 and GRO-α by pDMECs, as well as by human microvascular endothelial 1 cells (HMEC-1) [44]. These cells were found to express all components of the CGRP receptors at the mRNA level and the effects on chemokine release could be blocked by the type I CGRP receptor antagonist $CGRP_{8–37}$ [44]. CGRP inhibits NF-kB activation in the HMEC-1 cell line [44], and inhibition of NF-kB signaling was found to suppress the production of these chemokines [44]. Accordingly, CGRP treatment also suppresses neutrophil and monocyte chemoattraction by LPS-stimulated HMEC-1 cells [44]. These experiments suggest a role for CGRP in endogenous anti-inflammatory pathways, in addition to its effects on the adaptive immune response [31]. There is also some *in vitro* and *in vivo* experimental evidence that CGRP inhibits inflammation via a pathway that involves rapid upregulation of the inducible cAMP early repressor (ICER) [45]. ICER is a repressor protein that, in dendritic cells, suppresses LPS-induced transcription of genes encoding the inflammatory molecules TNF-α and CCL4, both of which share the quality of having a CRE-kB promoter element [45]. CGRP did not attenuate the expression of genes such as the one that encodes CXCL1 that lack this element [45]. These findings were found to occur in the absence of IL-10, a well-known suppressor of inflammation, suggesting that CGRP suppresses immune responses by cAMP-induced activation of intracellular kinases such as protein kinase A (PKA), providing an alternative to the NF-kB and MAPK pathways as a route by which interference with the downstream effects of toll-like receptor (TLR) signaling ultimately attenuates inflammation [45].

CGRP is involved in ultraviolet radiation (UVR)-induced immunosuppression. In the low dose model of UVB-induced immunosuppression (sensitization to a hapten is impaired at the irradiated site) UVR releases CGRP from sensory neurons to locally impair contact hypersensitivity responses [46]. The release of CGRP after UVR may be triggering mast cells to release stored TNF-α, which in turn downregulates Langerhans cell density and function to impair CH induction [47]. CGRP also contributes to the high-dose or systemic model of UVR-induced immunosuppression (sensitization at a non-irradiated site is impaired) as suppression of CHS responses are inhibited when mice are pretreated with CGRP antagonists [48].

Proopiomelanocortin Peptides

The skin is a source of neuroendocrine hormones of the proopiomelanocortin (POMC) family, termed melanocortins (MCs). POMC hormones include α, β, and γ-melanocyte stimulating hormone (MSH). These are derived from POMC along with adrenocorticotrophic hormone (ACTH) and endorphins. POMC is predominantly synthesized in the pituitary gland; however, it is also present in the skin, expressed by melanocytes, keratinocytes, Langerhans cells, endothelial cells, mast cells and fibroblasts [10]. The cutaneous melanocortin system is well characterized [49] with melanocortin receptor (MC-R) expression in nearly all cell types, including fibroblasts, adipocytes, endothelial cells and mast cells [50]. MC-1R is the most prevalent amongst the five melanocortin receptors, with high affinity for α-MSH and ACTH.

POMC-derived peptides influence several processes including melanogenesis and skin immunity, and are also suggested to have a role in the hair cycle, sebum and eccrine gland function and epidermal proliferation [50]. The known functions of melancortins in cutaneous biology continue to expand with potential clinical applications. Human sebocytes increase lipid droplet formation in response to MCs, suggesting acne vulgaris may be affected. Evidence suggests MCs are involved in keratinocyte proliferation and differentiation, with potential repercussions in regenerative processes [49]. In addition, although the mechanism is not completely understood, it is believed that MSH induces melanocyte proliferation and melanogenesis through engagement of the MC1-R, serving as a substrate or promoter of the enzyme tyrosinase [19].

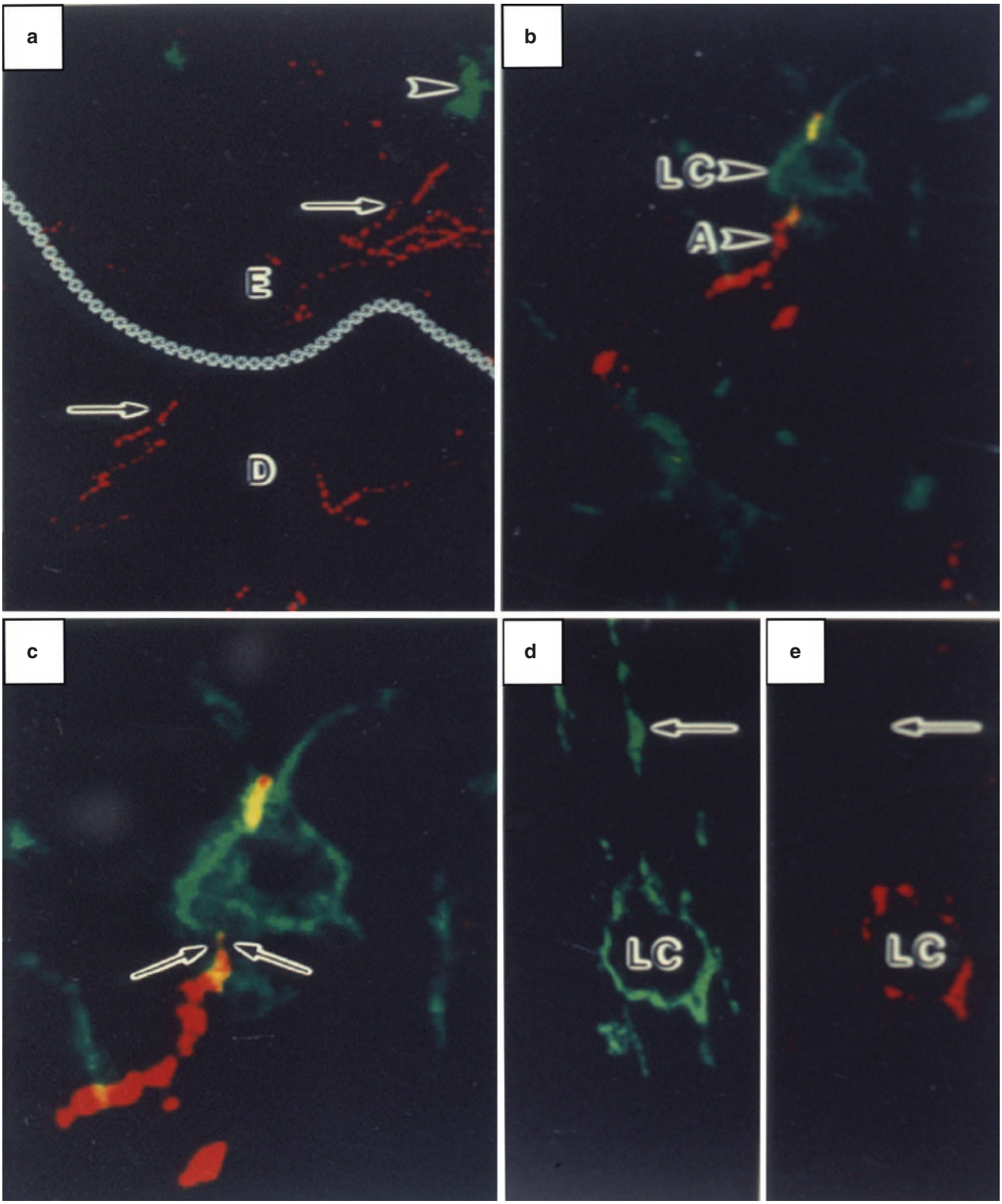

Fig. 12.1 Laser confocal scanning microscopy illustrating the anatomic association between Langerhans cells (LCs) and CGRP-containing nerve axons (*A*; *arrows*) in the dermis (*D*) and epidermis (*E*) (dermal-epidermal junction demarcated by *asterisks*). LCs, shown in *green*, were stained with an antibody against CD1a. Nerves, shown in *red*, were stained with an antibody against CGRP. (**a**) CGRP-positive axons in the epidermis and dermis (*arrows*) and CD1a-positive LCs (*arrowheads*). (**b**, **c**) Apparent contact of CGRP-positive axon and a LC. (**d**, **e**) Single channel analysese of LC CD1a reactivity (*D*) and CGRP staining (*E*). (Reprinted with permission from Hosoi et al. [40])

POMC mRNA and related peptides such as α-MSH and ACTH are upregulated by UVR. Mid-range UVR [UVB radiation (280–320 nm)] upregulates ACTH, α-MSH and MC-1R in epidermal melanocytes and increases MC-1R expression in cultured normal and malignant melanocytes and keratinocytes, the latter associated with increased melanogenesis in cell culture and *in vivo* [9, 49]. The melanogenic and dendritogenic effects of α-MSH and ACTH on the skin and follicular melanocytes may correlate with tanning and hair color, respectively [49].

POMC-derived peptides play a role in immunity and inflammation, especially α-MSH. α-MSH is anti-inflammatory and may be involved in host defense. More specifically, it has been shown to inhibit neutrophil migration and block the production of inflammatory cytokines, including IFN-γ, TNF-α, IL-1 and IL-6, by macrophages [51]. In studies of human microvascular endothelial cells, α-MSH has been found to suppress lymphocyte adhesion and inhibit lipopolysaccharide-mediated upregulation of ICAM-1, CAM and E-selectin receptors [51]. α-MSH also inhibits contact hypersensitivity and induces hapten-specific tolerance through IL-10 upregulation in a murine model [52]. Recent experiments demonstrated that α-MSH encourages dendritic cells to induce a T regulatory cell immune response, which in turn suppressed the proliferation of and cytokine secretion from Th17 cells obtained from individuals with psoriasis [53].

α-MSH has also been found in a murine model to augment antitumoral immunity by enhancing $CD8^+$ T cell cytolytic activity via interactions at the MC-1R [54]. These studies demonstrated that α-MSH-stimulated $CD8^+$ T cells were associated with significant *in vitro* as well as *in vivo* anti-tumor effects [54]. In addition, the anti-TNF and antimicrobial effects of α-MSH suggest it may reduce replication of the human immunodeficiency virus (HIV), because TNF promotes HIV replication [55]. Furthermore, its anti-inflammatory properties in animal models of hepatic inflammation and arthritic processes suggest that it may be an important anti-inflammatory agent in the treatment of inflammatory diseases in humans [56–58].

Catecholamines

Sympathetic fibers of the autonomic nervous system travel with sensory nerve fibers and as single fibers to innervate blood vessels, hair follicles and sweat glands [5]. Keratinocytes also synthesize catecholamines [9, 59]. Catecholamines inhibit antigen presentation in Langerhans cells through the β2 adrenergic receptor [60]. The sympathetic neurotransmitter norepinephrine and the co-transmitter adenosine triphosphate (ATP) have been shown to act synergistically to induce IL-6 production by human dermal microvascular endothelial cells [61]. As IL-6 is important in the differentiation of Th17 cells, this finding suggests a possible link between stress-associated activation of the sympathetic nervous system and Th17-cell-mediated immune responses [61]. This might help to explain the role of stress in exacerbating psoriasis and other skin disorders in which Th17 mechanisms play a role [61, 62].

Interestingly, evidence exists that norepinephrine plays a role *in vivo* in enhancing CHS through involvement in the trafficking of skin dendritic cells to draining lymph nodes, possibly through effects on α-adrenergic receptors [63]. Thus, the effects of catecholamines on skin immunity may be complex, with the outcome dependent upon factors such as the timing of exposure and the presence of other regulatory factors.

Adenosine 5′-Triphosphate (ATP)

The nucleotide ATP is a ubiquitous carrier of energy involved in countless cellular processes. Several lines of evidence suggest extracellular ATP participates in inflammatory and regenerative responses in the skin and affects melanocyte and Langerhans cell function [64]. ATP plays a role in the nociceptive signaling pathways following cutaneous cell injury [65] and may contribute to the pathophysiology of inflammatory skin conditions such as rosacea by augmenting the production of inflammatory mediators by endothelial cells [66]. The human microvascular endothelial cell line HMEC-1 expresses a variety of ligand-gated purinergic P2X receptors and G protein-coupled purinergic P2Y receptors which, when activated with ATP or the long-lived ATP analog ATPγS, was associated with increased production of the inflammatory cytokine IL-6 and the chemokines IL-8, MCP-1 and GRO-α [31, 66] Furthermore, *in vitro* studies revealed that tetracycline inhibited ATPγS- and TNFα-induced release of GRO-α and IL-8 by HMEC-1 cells and human dermal microvascular endothelial cells, a finding that may account, in part, for the beneficial effects of these agents in treating various inflammatory disorders [67].

Purinergic agonists also appear to enhance Langerhans cell antigen presenting function when exposure occurs along with activation by an additional signal (lipopolysaccharide) [68]. In this regard an ATP analog and $P2X_7R$ agonist 2′(3′)-*O*-(4-benzoylbenzoyl) ATP (BzATP) was shown to induce Th17-type immune responses in lesional and non-lesional skin from patients with psoriasis [69]. The authors interpreted this as due to action at the $P2X_7$ receptor, which is coupled to G_S-dependent intracellular changes in cAMP and its downstream effectors; however, BzATP also has high potency at $P2X_1R$ [70] as well as activity at other P2 receptors [15]. Investigators observed that this response was carried out via antigen-presenting-cell-dependent mechanisms

and was mediated by the pro-inflammatory cytokines IL-6, TNF-α and IL-1β and IL-23 [69]. Among other pro-inflammatory activities, these cytokines promote Th17 cell differentiation, supporting the apparent role of Th17 cells in the pathogenesis of psoriasis [69]. Furthermore, IL-6 secretion from endothelial cells is upregulated synergistically in response to ATP and norepinephrine signaling [61], and may contribute to the pathogenesis of psoriasis by inhibiting regulatory T cell functions [69]. These data may better explain the relationship between sympathetic nervous system activity and psoriasis flares. The function of ATP in the neuropeptide milieu of epidermal and dermal cell types and sensory nerves in the skin remains the subject of ongoing investigations.

Neuropeptide Y (NPY)

NPY is a 36-amino-acid peptide identified predominantly in periarteriolar nerve fibers in the dermal plexuses and, epidermal basal cells as well as sweat glands, sebaceous glands and hair follicles. It also appears to be produced by Langerhans cells [71]. NPY causes vasoconstriction and may also play a role in eccrine sweat production. It also reportedly has antimicrobial properties [72].

Catestatin

Catestatin is a 21-amino-acid neuropeptide derived from chromogranin A, which is a neuroendocrine pro-hormone and a member of the granin family of peptides [31]. This family of proteins is found within secretory vesicles of nerve, endocrine and immune cells that release antimicrobial peptides derived from chromogranin A [73]. Catestatin is upregulated in response to injury [74]. It displays antimicrobial properties against cutaneous pathogens including gram-positive and negative bacteria, yeast, and fungi [74, 75], and also acts as a chemoattractant for monocytes [76]. Furthermore, catestatin was observed to stimulate keratinocytes to migrate and produce IL-8, suggesting a role in immunomodulation [77]. Additionally, catestatin has been shown to induce human mast cell migration, degranulation, and pro-inflammatory cytokine and chemokine production [75]. This may contribute to the known role that mast cells play in cutaneous neurogenic inflammation.

Neurogenic Inflammation

Cutaneous neurogenic inflammation implies the role of nerves in cutaneous inflammation. The neuropeptides secreted by sensory nerves evoke an inflammatory response referred to as neurogenic inflammation. The axon-reflex model implies that tissue injury triggers a signal to the dorsal root ganglion toward the central nervous system (orthodromic response) with return of the signal from branch points in the reverse direction (antidromic signaling) to exert effects at the local level. The orthodromic response transmits pain whereas the antidromic response leads to the release of neuropeptides in the innervated tissue [12].

Various inflammatory cells are known to express neuropeptide receptors. Neutrophils express receptors for SP including NK_1, NK_2 and NK_3 [78]. SP reportedly upregulates neutrophil expression of COX-2 and PGE_2 [78]. Furthermore, mast cells express receptors for various neuropeptides including VIP, NYP, SP, CGRP, POMC, galanin, neuromedin U, PACAP, neurotensin and CRH [78]. Although located throughout the body, mast cells localize near blood vessels, nerve fibers and lymphatic vessels [31]. More specifically, mast cells and nerves engage in a bidirectional interaction in which degranulation provokes membrane depolarization of sympathetic neurons [78]. Pre-treatment of mast cells with selective antagonists of substance P and CGRP in a murine model was observed to decrease neuropeptide-associated degranulation, supporting the role of this interaction in neurogenic inflammation [78].

The most prominent neuropeptides in UVR-induced neuroinflammation are SP and CGRP. SP and CGRP mediate vasodilatation and SP and NKA are responsible for plasma extravasation [22]. SP is well known to provoke erythema and edema by mast cell-dependent and independent pathways [10]. The flare is the result of the antidromic arm of the axon reflex leading to the local release of neuropeptides such as SP and CGRP [20]. Both H1 receptor antagonism and local anesthetic injection have been shown to reduce the spread of the flare response to SP in human skin, suggesting a prominent role of histamine in neurogenic inflammation [79].

Cutaneous/Dermatologic Diseases

The relationship between neuropeptides and dermatologic diseases has been explored for decades. In inflammatory processes, the neuro-immuno-endocrine-cutaneous nexus participates in the trigger and maintenance of inflammation in healthy and pathologic skin. Understanding the role of neurotransmitters and their receptors may lead to the identification of novel therapeutic targets for the treatment of several common cutaneous diseases.

There are several lines of evidence suggesting a neurogenic component in dermatologic disease. Neuropeptides may induce or alleviate urticarial symptoms, hypersensitivity reactions and rosacea, and may play a role in the pathophysiology of pruritus, psoriasis, atopic dermatitis, alopecia areata, vitiligo and nodular prurigo [2, 12]. Several of these conditions are discussed below.

Urticaria

Urticaria presents as transient swellings or wheals caused by plasma leakage. The primary effector cell in the pathogenesis of urticaria is the mast cell [5]. Neuropeptides such as SP, VIP and somatostatin active mast cells to secrete histamine and other mediators that induce urticaria and mediate the late-phase response [5, 80]. SP and CGRP exert effects in cases of chronic urticaria and ACTH is present in the cutaneous mast cells of patients with urticaria pigmentosa [81, 82]. The expression of the POMC gene in mast cells suggests that α-MSH may be contributing to the cutaneous hyperpigmentation seen in patients with urticaria pigmentosa [83]. These studies suggest an interrelationship between the cutaneous nervous system and mast cells in the pathophysiology of urticaria.

Pruritus

Pruritus is one of the most common symptoms encountered in dermatology and significantly impacts quality of life. Itch may be peripheral (dermal or neuropathic) or central (neuropathic, neurogenic or psychogenic) in origin. Neuropathic itch originates at any point along the afferent pathway and is the result of damage to the nervous system whereas neurogenic itch is induced centrally. In the skin, itch is induced by the stimulation of specialized C-fibers by various pruritogens. This is followed by the release of neuropeptides from the cleavage of type-2 proteinase-activated receptors (PAR-2) by tryptase to release histamine, CGRP and SP [84].

It is important to understand the underlying mechanisms to provide effective management and treatment. Multiple cutaneous mediators such as histamine, prostanoids, cytokines and kinins induce pruritus [80]. Pruritus-inducing mechanisms of these mediators include nerve fiber sensitization and receptor stimulation, direct pruritogenic effects and mast cell activation [7]. Histamine is well known for its pruritic effects, especially in urticaria. However, in conditions such as atopic dermatitis the inability of antihistamines to eliminate itch suggest that other mediators are involved as well. Intradermal injection of SP, VIP and somatostatin evokes pruritus; however, CGRP does not have pruritogenic effects in humans [80]. SP is released from C-fiber nerve terminals by the action of mast cell tryptase on PAR-2 in the terminals to directly cause itching and by inducing mast cells to release histamine. Lesional skin in chronic pruritus demonstrates a predominance of dermal SP-containing nerve fibers relative to sympathetic nerve fibers [85]. Additional studies have shown that the pruritic lesional skin of patients with psoriasis contained an increased number of both SP-positive nerves and NK_2 receptor-positive cells. These data suggest that antagonizing the SP receptors NK_1 and NK_2 may be useful in treating patients with pruritic skin disorders [86].

Experimental evidence suggests that the itch circuitry responsible for chronic pruritus may be under tonic inhibitory control by specific types of sensory neurons that are sensitive to mechanical stimulation [87]. These effects are thought to be mediated by members of the TRPV1 family of ion channels [87]. In a murine model, toxin-mediated ablation of GRP- and neuromedin B receptors was associated with abolition of scratching behavior after intradermal application of various pruritogens [88, 89].

There are continuing new developments surfacing in the understanding of the pathogenesis of pruritus including the identification of IL-31 as a role-player in inflammation and pruritus [90]. IL-31 is a known product of mast cells as well as Th2 cells [88]. Interestingly, blood levels of IL-31, CGRP, and SP were recently found to be significantly higher in psoriasis patients than in a control group while the level of CRH was reduced [82]. After 20 treatments with narrow-band UVB radiation, the IL-31 level was significantly reduced, suggesting a possible role in some manifestations of psoriasis [91]. IL-31 levels are also reportedly increased in the serum of patients with chronic urticaria and atopic dermatitis [88]. IL-31 receptors have been found on DRG neurons, suggesting that IL-31 may directly activate these sensory nerve fibers, and perhaps offering an explanation for its role in inducing the sensation of itch [88].

Atopic Dermatitis

Atopic dermatitis (AD) is characterized by cutaneous hyperreactivity to nonspecific stimuli leading to a cycle of pruritus, scratching and further worsening of skin lesions [92]. AD is characterized by increased amounts of Th2-derived cytokines [93], as well as an increased density of nerve fibers and neuropeptide levels in the various stages of skin lesions seen in this condition [94]. The diameter of these fibers are larger than those in nonatopic control subjects possibly due to keratinocyte-derived, nerve growth factor (NGF) [95]. The free nerve endings in the skin of atopics also lack a surrounding sheath of Schwann cells suggesting an active state of excitation [94].

SP is a potent pruritogenic neuropeptide in AD that, along with CGRP, is down regulated by phototherapy [96, 97]. An increase in nerve fibers containing SP and CGRP is often observed although Fantini et al. demonstrated a decrease in SP and an increase in VIP levels in chronic lesions of AD [98, 99]. In lesional skin of AD and nummular dermatitis patients, SP and CGRP, but not VIP, fibers in the dermis are elevated compared to nonlesional controls, and are likely maintained by an increased number of mast cells [100]. Intradermal SP increases nitric oxide and enhances

SP-induced pruritus while acute stress triggers SP-induced cutaneous mast cell degranulation [7]. Elevated VIP was found to be present in eczema [101].

Plasma levels of NGF and VIP are reportedly higher in patients with AD than in psoriatic patients and healthy controls [102]. SP plasma concentrations were higher in a specific sub-group of AD patients with "extrinsic AD," a definition that referred to patients who presented with IgE-mediated sensitization to common food and/or aeroallergens based on skin prick tests and/or elevated total IgE serum levels [102]. Investigators using a skin model based on the coculture of human dermal fibroblasts, keratinocytes and porcine DRG neurons found that neural-derived CGRP enhanced both keratinocyte proliferation and epidermal thickness, phenotypic changes consistent with the atopic phenotype. This isolate skin cell model reportedly allowed investigators to study the interaction of nerve and skin cells under defined conditions in which the effects of porcine neurons, which are most similar to their human counterparts, could be assessed. Interestingly, a thicker epidermis was seen when keratinocytes came from atopic skin [103]. These results were interpreted as demonstrating that epidermal nerve endings enhance the morphologic changes associated with the atopic phenotype [103]. Pharmacologic inhibition of CGRP signaling, but not SP signaling, reversed the atopic phenotypic changes. The strong effects of neuronal integration appeared to be mediated by upregulation of CGRP receptor mRNA components in both healthy and atopic models. These data may suggest a relationship between the skin barrier dysfunction characteristic of AD and abnormal CGRP signaling [103].

Allergen-induced late-phase reactions in patients with AD sensitive to a known allergen have been associated with infiltrates of inflammatory cells expressing CGRP and VEGF, suggesting a role of these compounds in mediating the erythema and inflammation that may be associated with allergic inflammation [104]. In situ hybridization revealed that neutrophils and T cells were the predominant CGRP-producing cells in late-phase edema [104]. Studies comparing these results with patients that have allergic diatheses, but not AD, do not appear to have been performed.

Evidence suggests that there may be a relationship between atopic dermatitis and its associated psychological symptoms, including anxiety and depression, which may be accounted for by cutaneous neuropeptide activity. One study demonstrated that SP and NGF are useful serum markers of disease activity in patients with AD, which was evaluated clinically using three different scoring systems [105]. Indeed, in another study NGF-reactive cells were detected more abundantly in epidermal and dermal AD-involved skin [106]. NPY-positive cells were also detected in greater abundance in the epidermis of patients with AD compared to healthy controls. Investigators reported that pruritus in patients with AD was positively correlated with state and trait levels of anxiety, and hypothesized that anxiety may induce pruritus through NPY- and NGF-dependent mechanisms in the skin that are believed to involve mast cells, fibroblasts, lymphocytes and eosinophils [106].

Recently, inherited mutations in filaggrin have been appreciated for the role they may play in AD pathogenesis. Filaggrin (filament-aggregating protein) and its precursor profilaggrin are among the main components of the F-type keratohyaline granules that comprise the stratum granulosum, and as such, filaggrin plays an integral role in maintaining skin hydration [107]. The relationship of filaggrin to AD appears to involve the role it plays in maintaining epidermal barrier function, the integrity of which is known to be lost in many patients with AD. There are more than 45 known mutations in the filaggrin-encoding gene, FLG, at least four of which are believed to contribute to AD pathogenesis [107, 108]. The most common loss-of-function mutation in FLG is found in almost one-third of European Caucasian patients [109]. However, the four recurrent FLG mutations associated with AD only have an estimated combined allele frequency of approximately 7–10 % [108], and at least 50 % of AD patients have no FLG defects [107]. Furthermore, patients with FLG mutations may develop other conditions, such as ichthyosis vulgaris, without developing AD [107]. Recent evidence suggests that the relationship of preexisting FLG mutations and AD development may be related to the expression of immune modulators such as thymic stromal lymphopoietin (TSLP), which is known to be involved in initiating allergic inflammatory responses [110]. Interestingly, keratinocytes that differentiate in the presence of Th2 cytokines show a lower filaggrin protein expression, independent of underlying FLG mutations [109]. The established role of neuropeptides in regulating the aberrant cutaneous immune responses seen in AD suggests that the pathogenesis of this disorder is multi-faceted and involves a complex interaction between nerve-derived signaling molecules, Th2-type immune responses and abnormal skin barrier function.

Psoriasis

Psoriasis is a multifactorial disease characterized by symmetric plaque lesions. The possibility of a neurogenic component is supported by the temporal onset or exacerbation of lesions with emotional stress, the appearance of lesions at sites of injury or trauma (Koebner phenomenon - believed to be initiated by the release of proinflammatory neuropeptides in traumatized skin) and by observations that lesions resolve in areas of denervation or following central nervous system lesions such as ischemic stroke [80, 111].

NPs and sensory nerve density are increased in lesional skin [80]; however, levels of SP and VIP vary in psoriasis. Elevated levels of both SP and VIP [112], normal SP and elevated VIP [101] and increased SP and normal VIP levels [113] have all been demonstrated in psoriatic lesions. There are conflicting reports of CGRP expression in lesional and nonlesional skin compared to normal controls. Moreover, VIP expression is reported to be increased, yet a statistical difference is not consistently observed between lesional and normal skin [111]. Serum CGRP and VIP are both reportedly elevated in psoriatic individuals. Treatment with anthralin or psoralen plus long-wave UVR [UVA radiation (320–400 nm)] (PUVA) led to a decrease in serum VIP while CGRP remained elevated [114]. Below we discuss evidence that VIP may be involved in biasing immune responses toward the Th17 pole, which suggests the potential relevance of these findings to enhancing the current understanding of psoriasis pathogenesis. Compared to psoriatic lesions, a lower level of SP is seen in lichen planus and lichen simplex chronicus while higher levels are observed in spongiotic dermatoses [113]. Serum beta-endorphin is elevated in psoriatic patients and is likely produced by inflammatory cells within psoriatic plaques [115]. PACAP-38 is also increased in lesional psoriatic skin and recent evidence suggests NGF is involved in the pathogenesis of psoriasis [28, 80]. Keratinocyte hyperproliferation is a histologic feature in psoriasis and NGF is suggested to induce keratinocyte proliferation and prevent apoptosis [111]. SP reportedly upregulates keratinocyte-derived IL-32 and mast-cell-derived tryptase in psoriatic lesional skin [116]. Additional evidence for neuropeptide-mediated mast cell involvement in psoriasis pathogenesis comes from investigations revealing that SP stimulates mast cells to increase CRH receptor (CRHR-1) expression, which in turn enhances NK_1 gene transcription, mediating SP signaling [117]. Overstimulation with CRH, as in chronic stress, reportedly leads to downregulation of CRHR-1 on the surface of mast cells, which may account for previous observations of decreased CRHR-1 gene expression in psoriatic lesional skin [117]. Together, these data may account for the relationship of stress-induced activation of the hypothalamic-pituitary-adrenal axis to psoriasis flares.

Novel murine models of psoriasis have enabled further studies of the role of neurocutaneous immunity in the pathogenesis of this disorder. The KC-Tie2 murine model is engineered to overexpress the angiopoietin receptor Tie2 in keratinocytes [32]. Tie2 receptor activation is associated with reactive oxygen species generation, which may mediate keratinocyte proliferation via VEGF production and angiogenesis [118]. KC-Tie2 mice spontaneously develop features characteristic of human psoriasis including increased numbers of dermal $CD4^+$ T cells, epidermal CD8+ T cell infiltrates and pro-inflammatory cytokines such as IFN-γ, TNF-α, IL-1α, IL-6, IL-12, IL-22, IL-23 and IL-17 [118]. In this model, systemic administration of the T-cell calcineurin inhibitor cyclosporin A was associated with significant improvement in the psoriasiform phenotype and a reduction in lesional T cells and their associated pro-inflammatory cytokines, an observation consistent with the role that T lymphocytes are believed to play in psoriasis pathogenesis [118]. In these experiments, antigen presenting cells remained high in lesional skin, which may have accounted for incomplete resolution of the disease phenotype [118]. In fact, clodronate-liposome-mediated depletion of antigen presenting cells in KC-Tie2 mouse skin was associated with resolution of the acanthotic skin phenotype, decreased dermal angiogenesis and normalization of the levels of multiple pro-inflammatory cytokines.

Additional investigations using the KC-Tie2 model revealed that denervation of cutaneous sensory nerves led to significant drops in lesional dendritic and $CD4^+$ T cells, reminiscent of denervation in human patients with diseases of the nervous system. However, administration of an SP agonist prevented decreases in $CD11c^+$ and $CD4^+$ cells, but had no effect on acanthosis [119]. Interestingly, administration of CGRP reversed the improvement in acanthosis and prevented the denervation-mediated decrease in numbers of $CD4^+$ cells [119]. Perhaps the relationship of sensory nerve activation to disease activity, i.e. secondary to external stimulation such as pressure, may partially contribute to the aforementioned Koebner phenomenon [119].

The imiquimod mouse model of psoriasis uses a toll-like receptor (TLR) 7 agonist to induce a psoriatic phenotype [120]. Investigators using this model to study the pathogenetic mechanisms of psoriasis found that IL-23-mediated activation of IL-17 and IL-22 cells was requisite in triggering the psoriasiform phenotypic changes characteristic of this model [120]. Additional investigations found that IL-22, a cytokine produced by Th1, Th17 and Th22 cell populations as well as by innate immune cells, is required for the imiquimod-induced psoriasiform inflammatory changes characteristic of this murine model, supporting the known role of these novel T cell populations in triggering psoriasis [121]. Recent evidence suggests that nociceptive sensory neurons expressing specific ion channels including TRPV1 are essential for driving this inflammatory through stimulating dermal dendritic cell to produce IL-23 which, in turn, induces dermal gamma-delta T cells to produces IL-23-dependent inflammatory cytokines [122]. Accordingly nociceptive sensory neuron loss was associated with a reduction in both local swelling and inflammatory infiltrates [122]. Furthermore, imiquimod treatment was associated with increased levels of IL-17A, IL-17F and IL-22, consistent with previous reports demonstrating the role of these cytokines in psoriasiform inflammation [122]. Finally, recent experiments have demonstrated that cutaneous denervation results in the loss of imiquimod's ability to

induce the psoriatic phenotype, highlighting the essential role of cutaneous nerves and the neuropeptides they secrete in triggering this skin disorder [123].

Vitiligo

Vitiligo is a depigmenting disorder often presenting in a symmetric or segmental distribution. The segmental or unilateral form of the disease is characterized by a neural-like distribution, suggesting that nerve-derived mediators may contribute to pigment loss [124]. Early studies reported a lack of depigmentation in vitiligo patients below regions of sympathectomy or nerve injury, further supporting the connection between cutaneous innervation and vitiligo pathogenesis [125].

There are changes in neuropeptide distribution in vitiligo-affected skin. Experimental examination of vitiliginous skin found reduced expression of α-MSH and the pro-convertases PC1 and PC2, which are responsible for cleaving POMC to α-MSH [126]. This may simply represent the decreased number of melanocytes characteristic of this condition [126]. However, reduced α-MSH expression may also contribute to the pathogenesis of vitiligo [126]. *In vivo* studies of plasma α-MSH levels in patients with vitiligo found that the median plasma level of this neuropeptide was significantly lower than in control persons, although no difference was observed between patients with active and stable vitiligo lesions [127]. The clinical efficacy of α-MSH analogues, with or without adjuvant narrow-band UVB phototherapy, in treating vitiligo provides further evidence that this neuropeptide is involved in the disease process [128, 129]. Apart from abnormal α-MSH levels, an increase in NPY, CGRP and SP has been demonstrated, whereas no changes in VIP were observed, supporting the concept that neuropeptides are involved in the pathogenesis of vitiligo [85, 130–134].

Alopecia Areata

Alopecia areata (AA) is characterized by non-scarring patches of hair loss. The pathogenesis is complex and unclear, with immunologic, genetic, environmental and psychological mechanisms implicated. Stressful life events may trigger or exacerbate the disease [135]. Enhanced expression of corticotrophin related hormone (CRH), ACTH and α-MSH in sites of AA suggest that the local stress response contributes to normal hair cycling [136]. SP contributes to down-regulation of stress-associated NGF receptors (TrkA) in murine hair follicles, promoting the apoptotic and catagenic effects of NGF [137]. SP has also been shown to induce mast cell degranulation and CD8+ T lymphocyte activation by Langerhans cells [138].

AA has been proposed to be a condition involving Langerhans cell antigen presentation and neuropeptides that trigger an inflammatory response in the vicinity of hair follicles with mechanisms that include mast cell degranulation [139]. An *in vivo* study of lesional and non-lesional skin in patients with alopecia areata reported that $CD8^+$ cells predominate in lesional skin; these authors hypothesized that Langerhans cells stimulated the $CD8^+$ T lymphocytes [139]. CGRP is reportedly decreased in alopecia areata lesions [140]. Recent studies of human scalp specimens found that CGRP down-regulates IFN-γ-induced MHC class I antigen expression but does not affect MHC class II expression, suggesting that CGRP may play a role in influencing immune privilege of human hair follicles [141]. Other investigations have elucidated immunomodulatory roles of proopiomalenocortin-derived peptides and TGF-β1 in anagen hair follicle epithelial cells [142, 143]. The full nature of the role that NPs play in regulating hair follicle immune privilege and contributing to the pathogenesis of alopecia areata remains unknown.

Allergic Contact Dermatitis (ACD)

In a murine model, topical application of SP, CGRP and somatostatin reportedly enhances allergic and irritant contact dermatitis [144]. SP acts as an adjuvant to raise the immunogenicity of cutaneously applied haptens to promote the induction of CHS, and when it is inhibited, decreases the CHS and DTH responses in humans [80]. Furthermore, the inhibition of SP-degrading peptidases leads to an exaggerated ACD response suggesting SP may be capable of boosting both the sensitization and elicitation phase of ACD [18, 144]. The effects of SP signaling in ACD appear to be mediated by transient receptor potential A1 (TRPA1) ion channels found on cutaneous sensory neurons [145]. Genetic ablation and/or pharmacologic inhibition of these channels in a murine model was associated with a decrease in findings characteristic of the ACD phenotype including skin edema, nerve growth, leukocyte infiltration, keratinocyte hyperplasia and anti-histamine-resistant pruritus [145].

In contrast to the effects of SP in eliciting the ACD phenotype, intradermal administration of CGRP suppresses the induction of contact hypersensitivity at the injected site [146]. Furthermore, treatment of epidermal antigen presenting cells with CGRP *in vitro* inhibits their ability to present antigen for immunization of naïve mice [146] or elicitation of DTH in previously sensitized mice by subcutaneous injection [40]. More recently, CGRP was shown *in vitro* to inhibit Th1-type CHS while promoting Th2-type CHS [43]. The difference between these results and those seen with topical application may represent concentration-dependent effects, the effects of secondary mediators induced in unknown

third-party cell targets or other factors peculiar to the route of administration. α-MSH inhibits both the induction and elicitation of CHS responses in mice [52]. These data support the conclusion that NPs have a modulatory role in the pathogenesis of ACD [147].

Rosacea

Rosacea encompasses a broad category of disorders characterized by cutaneous inflammation and abnormal vasoregulation in response to a variety of external stimuli. The direct interaction between cutaneous nerves and the inflammatory cells involved in rosacea pathogenesis is supported anecdotally by the role of spicy food, temperature extremes, pH changes, exercise and alcohol in triggering disease flares. Some of these effects have recently been shown to be mediated by the neurovascular transient receptor potential vanilloid receptor 1 (TRPV1) [148]. The TRPV1 family consists of 28 membrane channels that are expressed on neuronal and nonneuronal channels, including keratinocytes and endothelial cells [149]. These channels are thought to display greater sensitivity at baseline in patients with rosacea [149].

Recent studies have shown an intimate relationship between cutaneous sensory nerves, blood vessels and mast cells [150]. Mast cell density is upregulated in all subtypes of rosacea [150], and various NPs are believed to contribute to the pathogenesis of this disorder. CGRP release mediates potent vasodilatory effects on facial arterioles, producing clinical symptoms of flushing [149]. SP is believed to contribute to rosacea through various mechanisms [149, 151, 152]. SP produces local edema via its activity at the NK_1 receptor on postcapillary venules [149]. SP also induces mast cells to produce TNF-α, IL-3, and a variety of other chemokines that regulate antigen presentation, account for Th1 cell infiltrates in lesional skin and recruit neutrophils to the perifollicular region, contributing to pustule formation [152]. Neuropeptides may also alter antigen presentation to $CD8^+$ T lymphocytes [148]. Sustained, chronic inflammation in patients with rosacea is believed to be related mainly to the activity of Th1 cells, macrophages and mast cells [148].

Recent molecular studies revealed that PACAP and VIP are significantly enhanced in lesional skin of patients with rosacea. PACAP may mediate vasodilatation, and both PACAP and VIP are known to induce mast cell degranulation [153]. The role of catecholamines in inducing cutaneous flushing and telangiectasia indicates that the condition may respond to pharmacologic therapies that antagonize adrenergic receptors [154]. Further elucidating the role of these and other neuropeptides in rosacea pathogenesis is crucial, particularly because current treatment of this condition focuses on alleviating symptoms rather than targeting underlying disease mechanisms [149].

Acne Vulgaris

Acne vulgaris is among the most common chronic inflammatory disorders of the skin. Its multifactorial pathogenesis involves abnormal follicular development, aberrant hormonal regulation of sebaceous gland activity and a dysregulated inflammatory response to local pathogens.

Facial lesional skin from patients with acne is reportedly characterized by an increased number of SP-containing nerves and mast cells [155]. Sebaceous glands express receptors for a variety of neuropeptides, including VIP, CGRP and NPY [156]. Sebocytes also reportedly respond strongly to CRH and α-MSH [157]. The neuropeptide α-MSH enhances lipidogenesis and dose-dependently inhibits the secretion of IL-8, a signaling molecule believed to be involved in the pathogenesis of acne vulgaris [158]. Immunohistochemical studies have revealed that MC-1R expression is markedly increased in the sebocytes and keratinocytes of acne-involved and non-involved skin in patients with acne vulgaris, compared to normal individuals, suggesting a prominent role for α-MSH in acne pathogenesis [159]. CRHR-1 expression is also reportedly higher in lesional skin of acne patients [157]. CRH induces lipid synthesis and enhances the expression of local enzymes involved in testosterone production [158].

Neuropeptide receptor binding may therefore influence the clinical course of acne by affecting local proliferation, differentiation, and androgen metabolism within sebaceous glands [157]. This may partially account for anecdotal evidence suggesting a relationship of acne flares to physical or psychological stress [157].

Ultraviolet Radiation

UVR produces changes in the skin such as erythema and has immunosuppressive and carcinogenic effects as well [5]. UVR acts on keratinocytes, mast cells, Langerhans cells, dermal fibroblasts and endothelial cells to induce the release of various cytokines, neurohormones and growth factors [22]. Afferent sensory nerves are a source of inflammatory mediators, notably neuropeptides following UVR exposure. Ultraviolet B radiation (290–320 nm) induces CGRP, NKA and SP release from cutaneous sensory nerves and NGF release from epidermal keratinocytes [9]. Langerhans cells are also a source of NGF that, along with NGF from keratinocytes, leads to an increase in nerve fibers in sun-exposed skin. CGRP and α-MSH appear to be immunosuppressive in the skin, at least partially through IL-10 production, and SP is involved in the healing of photodamaged skin [19]. CGRP also inhibits the upregulation of IL-12 p40, IL-1β and CD86 [160].

UVR-induced immune suppression of CHS and DTH is believed to be partially mediated by IL-10, TNF-α and the histidine metabolite cis-urocanic acid. Urocanic acid exists

in the epidermis primarily as the trans isomer. UVB (and UVC) radiation induces a trans-cis isomerization and considerable evidence supports a role for cis-urocanic acid in UVR-induced immune suppression [161].

Experimental observations indicate that mast cell products such as histamine are important in downstream systemic immunosuppression [31], effects that may be carried out via neural mechanisms. Evidence suggests sensory C-fibers and mast cells form a functional unit with bidirectional effects [162]. Cis-urocanic acid can activate mast cells by its effects on release of neuropeptides by afferent sensory nerves [163]. Furthermore cis-urocanic acid induction of the release of neuropeptides such as CGRP may participate in the regulation of UVB-induced inflammation and Langerhans cell function [164].

UVR leads to the release of both pro and anti-inflammatory mediators and it is the balance of these factors that ultimately determine the host response and clinical outcome. Pro-inflammatory neuropeptides include SP, NKA and CGRP, whereas immunosuppressive or anti-inflammatory neuropeptides include CGRP and α-MSH [22, 165]. CGRP can be pro or anti-inflammatory depending on the experimental system. As mentioned previously, CGRP immunosuppression has been observed in low dose and high dose models of UVB-induced immunosuppression. CGRP release in response to low dose UVR exposure locally impairs contact hypersensivity responses [46], a phenomenon that may be related to the ability of CGRP to trigger mast cells to release stored TNF-α, which in turn down-regulates Langerhans cell density and function [47]. In the high-dose or systemic model of UVR-induced immunosuppression (sensitization at a non-irradiated site is impaired) CHS responses were inhibited when mice were pretreated with CGRP antagonists [48].

Wound Healing

Studies suggest that the nervous system is important in wound healing and tissue repair. Patients with sensory defects due to injury or a disease process such as diabetic neuropathy, spinal cord injury or lepromatous leprosy have non-healing ulcers [22]. Neuropeptides participate in wound repair by initially evoking vascular responses and then influencing the proliferation and differentiation of target cells in the healing process [80]. CGRP promotes human keratinocyte and endothelial cell proliferation [166] and together with SP, has proliferative effects on culture fibroblasts [80]. The role of SP is further supported by the finding of elevated neutral endopeptidase expression in wounds and in the skin and ulcers of diabetics, indicating that it may contribute to deficient neuroinflammatory signaling and impaired wound healing [167, 168]. In recent experiments, sensory-neuron derived SP was found to promote wound healing in a tissue-engineered murine model [169]. These effects were reproduced by adding SP to a deinnervated model, and were completely blocked by antagonism of the SP NK-1 receptor on keratinocytes, suggesting that SP plays an important role in, but is not essential for, proper wound healing [169].

Hair Cycling

The cyclical activity of the hair follicle appears to be regulated by a "biological clock" that also affects local neuropeptide expression. The pattern of sensory innervation is also hair cycle-dependent with an increase in nerve fibers seen during growth (anagen), followed by a decrease during regression (catagen) and persistently low levels during the resting stage (telogen) [153].

The corticotrophin-releasing hormone (CRH)/POMC system may regulate the hair follicle pigmentary unit [170]. The expression and processing of POMC and its melanocortin derivatives (α-MSH, ACTH, β-endorphin) vary in the hair follicle as a function of their anatomic location and melanogenic activity [170]. The POMC system appears to be most expressed during early stages of melanocyte differentiation and becomes down-regulated in mature melanocytes suggesting additional systems are involved in maintaining melanogenesis [171].

Stress leads to premature termination or arrest of hair in telogen in a NGF- and mast-cell-dependent manner, suggesting that interference with neuropeptide signaling may be an effective measure in the management of stress-induced hair loss [153]. In a murine model of depilation-induced hair cycling, a rapid surge in skin NGF content occurred within one day of depilation, followed by elevated SP-containing nerve fiber count 2 days later [172]. This evidence supports the role of these neuropeptides in contributing to the anagen phase of hair cycling [172]. Additionally, recent evidence points to a potential role for exogenous α-MSH administration in mitigating chemotherapy-induced alopecia [173].

Photoaging

Chronic, excessive exposure to sunlight results in skin changes termed photoaging. Mast cell mediators participate in the dermal changes associated with photoaging [174] and the correlation between the degree of epidermal innervation and chronic photodamage suggests a possible role of neural influences on photodamaged skin [175]. Toyoda et al. demonstrated an increase in dermal nerve fibers, notably CGRP-positive fibers and increased tissue levels of SP, CGRP and NGF in sun-exposed skin compared to sun-protected skin [176]. Furthermore, mast cells were intimately associated with fibroblasts and contained larger amounts of SP when compared to controls [176]. These findings support a role for

cutaneous neurogenic factors and mast cells in chronic ultraviolet injury and potential target for future therapeutic options [176].

Melanoma

The role of α-MSH in experimental models of melanoma is controversial [177]. In the human metastatic melanoma cell lines HBL and A375SM, α-MSH inhibits invasion through a layer of human fibronectin. Furthermore, immunohistochemical analysis has demonstrated that there is enhanced expression of CRH, ACTH and α-MSH in human melanoma, squamous cell carcinoma and basal cell carcinoma tumors. This suggests a possible role of the stress response in the pathogenesis of skin malignancies [178]. These findings suggest melanocortins, notably α-MSH may offer new insight into the understanding of the biology of skin cancers, notably melanoma, and potential therapeutic interventions.

Melanocortin receptors are widely distributed in the human body and of the five receptor types, the MC-1 receptor (MCR1) is expressed in cutaneous cells (keratinocytes, fibroblasts, melanocytes) and melanoma cells. Previous data demonstrate MCR1 variants predispose to cutaneous melanoma independent of skin type and hair color, and the Asp84Glu variant confers the highest risk [179]. MCR1 gene variants are shown to be independent risk factors for nonmelanoma skin cancer [180]. However, recent analyses show the association of MC1R variants and constitutive pigmentation phenotypes is less than previously reported and has a low rather than high penetrance susceptibility locus for melanoma [181].

A recent observational study of 184 lesions found that NPY expression is significantly enhanced in cutaneous melanoma, particularly the nodular variety [182]. Furthermore, NPY expression was associated with invasiveness independent of other proliferative markers [182]. The relationship of this neuropeptide to the pathogenesis of melanoma is not clearly understood.

Conclusion

The skin is part of an active neuro-immuno-endocrine network with influential connections to both the local and central levels of the immune system [50]. There are complex interactions between nerve fibers, neuropeptides, target cells and proteases that are now beginning to be understood. Research in this highly sophisticated system has significantly increased our understanding of neuropeptides and their activities in the skin. The relevance of understanding this intimate relationship is clear as the pathogenesis of several dermatologic conditions involve the neuro-immune-endocrine network described herein. A further understanding of these mechanisms may promote novel approaches to the treatment of skin disorders, with potential therapeutic targets that may include neuropeptides, receptors and proteases, suggesting a promising future for the implications of this growing focus of investigation [20].

Questions

1. Neuropeptides known to be in the skin include:
   A. Norepinephrine
   B. Acetylcholine
   C. Adenosine Triphosphate
   D. Calcitonin gene-rated peptide
   E. Tryptase

2. Clinical and experimental evidence exists that nerves in the skin play a role in:
   A. Regulating the expression of psoriasis.
   B. Regulating vasomotor tone.
   C. Biasing Langerhans cell antigen presentation toward IL-17A responses.
   D. Regulating release of chemotactic polypeptides by endothelial cells.
   E. All of the above

3. Adenosine triphosphate is:
   A. A sensory neuropeptide
   B. A sympathetic nerve co-transmitter
   C. A carrier of energy
   D. a & b
   E. b & c

4. Substance P does each of the following except:
   A. Stimulates macrophages to generate prostaglandin E2
   B. Induces release of histamine from mast cells
   C. Increases release of proinflammatory cytokines from keratinocytes
   D. Inhibits expression of the adhesion molecules P-selectin, intracellular adhesion molecule-1 and vascular cell adhesion molecule-1.
   E. Induces nitric oxide production by endothelium

5. The following are all true of proopiomelanocortin peptides except:
   A. Proopiomelanocortin is only synthesized in the pituitary gland
   B. Proopiomelanocortin-derived peptides regulate melanogenesis
   C. Proopiomelanocortin-derived peptides regulate immunity and inflammation

D. Administration of alpha-melanocyte-stimulating hormone inhibits the induction of contact hypersensitivity in mice.
E. Alpha-melanocyte-stimulating hormone biases dendritic cells towards inducing a T regulatory cell immune response.

Answers

1. D
2. E
3. E
4. D
5. A

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Cell Death and Skin Disease

13

Erin Harberts, Kerry Heitmiller, and Anthony A. Gaspari

Abstract

Cell death is a necessary part of the life of an organism. In order to grow and maintain health at an organismal level, tissues and cells must be able to withstand injury, die, or recover. The skin is perhaps the most dramatic example of this paradigm. Skin cells are constantly exposed to the external environment and the associated stressors. One of the critical mechanisms by which epithelial tissue is able to adapt to external injury is by undergoing programmed cell death. The process by which a cell dies has become recognized as a critical factor in determining the type of immune response that it stimulates locally and systemically. Some modes of cell death are considered pro-inflammatory, *e.g.*, pyroptosis and necrosis, and are mostly associated with microbial infections and pathogenic conditions that lead to significant tissue damage. Other modes of cell death are considered to be immunologically silent, such as apoptosis and autophagy, and are usually associated with homeostatic cell death and maintenance of healthy, mutation-free tissue. All of the aforementioned cell death pathways have been, and continue to be, extensively studied and have specific characteristics, molecular markers, and signaling molecules associated with them. This chapter focuses on the mechanisms which epithelial cells use to die, the consequences of these different types of death, and some of the skin diseases associated with dysfunctional programmed cell death.

Keywords

Cell death • Epithelial cell • Apoptosis • Skin barrier • Necrosis • Pyroptosis • Nod-like Receptors • Hypersensitivity Syndromes • Inflammation • Autophagy • NLR • Immune signaling

Mechanisms of Cell Death

Cell death is a necessary part of the life of an organism. In order to grow and maintain health at an organismal level, tissues and cells must be able to withstand injury, die, or recover. The skin is perhaps the most dramatic example of this paradigm. Skin cells are constantly exposed to the external environment and the associated stressors. One of the critical mechanisms by which epithelial tissue is able to adapt to external injury is by undergoing programmed cell

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Table 13.1 Summary of canonical cell death pathways

Type of death	Characteristics	Molecular markers	Critical signaling molecules
Apoptosis	Can be induced by extracellular (TNF-α, FasL, TLRs…) or intracellular (ROS, cytochrome-c) stimuli Initiated by formation of the apoptosome Considered 'anti-inflammatory'	Ladder; Blebbing; TUNEL positive; extracellular Annexin-V; Cleavage of: caspase-3, Rip1, iCAD, PARP; Cytoplasmic cytochrome-c; Membrane integrity maintained	TNFR, Fas, TRAIL, TLR4, TLR2, Bax, Bak ↓ FADD, TRADD, MyD88, APAF, other DD mlcls ↓ Caspase-8, −9, −6, −7, −3
Necrosis/ Necroptosis	Classical inflammatory cell death Originally thought to be 'unordered', now specific signaling pathways have been identified	Release of PAMPS/DAMPS; Increased TNF-α; Cell swelling; Upregulation of Rip3; Phosphorylation of MLKL	Any apoptotic initiator in presence of caspase inhibitor, DNA damage ↓ Rip1, Rip3 ↓ MLKL
Autophagy	Homeostatic process Can lead to cell death by destruction of cellular components Involves formation of autophagosome which fuses with lysosome Is regulated by status of other cell death pathways	Covalent bonding of Atg5 with Atg12; Formation of LC3-II	Beclin, PI3K ↓ LC3-1, Atg family proteins ↓ Lysosomal fusion
Pyroptosis	Initiated by activation of the caspase-1 inflammasome Dependent on intracellular PRR, many of which require a 'first hit' to be upregulated Leads to inflammatory cell death	Release of PAMPs/DAMPs; Increased IL-1β and IL-18; Cell swelling; Cleavage of caspase-1; TUNEL positive	NLRP1, NLRC4, Aim2, other intracellular PRR ↓ ASC, CARD domain containing adaptors ↓ Caspase-1

death. The process by which a cell dies has become recognized as a critical factor in determining the type of immune response that it stimulates locally and systemically [1–4]. Some modes of cell death are considered pro-inflammatory, *e.g.*, pyroptosis and necrosis, and are mostly associated with microbial infections and pathogenic conditions that lead to significant tissue damage [5]. Other modes of cell death are considered to be immunologically silent, such as apoptosis and autophagy, and are usually associated with homeostatic cell death and maintenance of healthy, mutation-free tissue [6]. All of the aforementioned cell death pathways have been, and continue to be, extensively studied and have specific characteristics, molecular markers, and signaling molecules associated with them (Table 13.1) [7–15]. This chapter focuses on the mechanisms which epithelial cells use to die, the consequences of these different types of death, and some of the skin diseases associated with dysfunctional programmed cell death.

Apoptosis

Keratinocyte apoptosis is a classic example of how programmed cell death is utilized to maintain a healthy epidermal skin barrier. As a keratinocyte matures it moves from the basal side of the epidermis towards the apical interface with the environment. During this migration it halts proliferation, undergoes keratinization, and ultimately begins the process of apoptotic cell death so that it can be sloughed off once it reaches the apical surface. This apoptotic cell death can be acutely activated by environmental stress, such as UV-radiation, or can be slowly achieved for homeostatic maintenance of the epidermal tissue. The apoptotic death of these important barrier cells is inevitable and crucial to their function.

There are two classical pathways that, upon activation, lead to an immunologically silent apoptotic cell death, the extrinsic and the intrinsic pathways [16]. The extrinsic pathway is initiated by engagement of cell surface death receptors, most notably TNFR and Fas, that associate with the scaffolding proteins, TRADD and FADD, respectively, to create a death-inducing signaling complex (DISC) [17]. The intrinsic pathway is initiated by release of cytochrome-c from the mitochondria, followed by formation of the apoptosome [18]. Many varied conditions can cause the release of cytochrome-c from the mitochondria, including the build-up of reactive oxygen species (ROS), genomic instability, metabolic instability, and the recognition of any other condition in which it makes more teleologic sense for the cell to die rather than live. The apoptosome is initiated by the binding of cytochrome-c to APAF-1 which is then able to oligimerize to create a circular structure that activates the apoptosis execution proteins named caspases (Fig. 13.1).

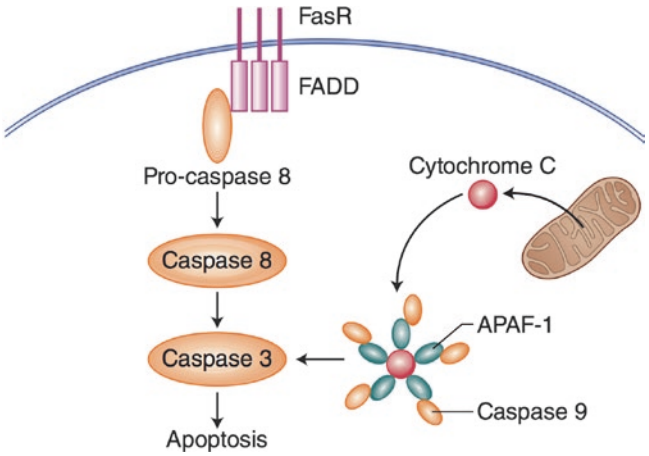

Fig. 13.1 Extrinsic and intrinsic apoptosis. For extrinsic apoptosis, engagement of Fas Receptor by Fas triggers a signal cascade that involves FADD, pro-caspase 8 activation, and programmed cell death by apoptosis. Intrinsic apoptosis can be triggered by intracellular ROS, resulting in the release of mitochondrial cytochrome c, which binds to APAF-1, resulting in executioner caspases that trigger apoptosis

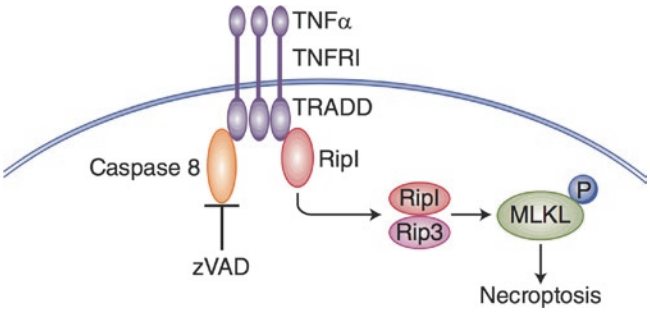

Fig. 13.2 Necroptosis. This form of cell death was originally identified using an extrinsic death pathway TNF-a receptor and its ligand TNF-a. When the activities of executioner caspases are blocked by a chemical such as z-VAD, an alternate, default death pathway becomes active (necroptosis). In this form of inflammatory cell death MLKL is phosphorylated, and migrates to the plasma membrane, resulting in membrane leakiness, and cell swelling and inflammatory death. Necroptotic cell death can occur in naturally occurring circumstances, such as reperfusion injury

Both pathways, either through a DISC or the apoptosome, result in cleavage of caspase-3 that, in turn, cleaves and activates caspase-activated DNase (CAD). Once activated, CAD translocates into the nucleus where it starts digesting genomic DNA, among other cellular components, which is released from the cell in apoptotic vesicles. This digestion process results in the "DNA laddering" that is a hallmark of apoptotic cells [19]. DNA laddering is the result of DNA cleavage by CAD between nucleosomes, which results in DNA fragments increasing in size consistently by about 180 base pairs, which is the amount of DNA wrapped around a histone. In addition to active cleavage of DNA, apoptotic cells undergo a loss of survival signals and homeostatic processes. Experiments using caspase inhibitors show that without the ability to activate the apoptotic pathway, cell death can be skewed to an inflammatory, caspase-independent cell death that is dependent on the formation of specific signaling complexes and is termed necroptosis [20].

Additionally, ER stress and activation of the associated unfolded protein response is another cause for the initiation of apoptosis. When secretory proteins are not able to fold correctly and emerge from the ER, the unfolded protein response is activated and can lead to activation of caspase-3 and subsequent apoptosis [21]. This is just one example of an instance where a cell cannot survive in its current state and so apoptotic death is initiated so that further damage to surrounding cells is not propagated, or survival of a terminally damaged cell is not prolonged [22]. Apoptosis is an elegant way that organismal homeostasis is able to be maintained at the cellular level.

Necrosis

The term necrosis is pathologically described as irreversible cell damage that leads to the extracellular release of intracellular material and destruction of surrounding tissue [23]. This definition originated mostly from gross observation of what was deemed to be 'necrotic' tissue. Such tissue can be separated into five main distinct categories that are based on morphological patterns: coagulative, liquefactive, caseous, fat, and fibrinoid necrosis [24–28]. As molecular biology techniques advanced, the definition of necrosis has evolved to include more biochemically defining characteristics. These include swelling of the cell followed by the release of specific damage-associated molecular patterns (DAMPs), and other pro-inflammatory molecules from dying cells [29]. Necrotic cell death was originally viewed as a 'back-up' or default way for a cell to die that simply involved the non-specific swelling and lysis of a cell. More recently several specific signaling pathways that result in necrosis have been identified, making necrosis an umbrella category for ordered cell death that results in a pro-inflammatory lytic cell death. Of the recently described necrotic signaling pathways, a process termed necroptosis has been the most thoroughly described.

Necroptosis is initiated by the Rip1-Rip3 protein complex and leads to inflammatory cell death [30]. The many consequences of and cellular mechanisms leading to the Rip1-Rip3 protein complex, and subsequently necroptosis, is still a widespread active area of investigation. However, to date, the necroptotic pathway has been most completely described downstream of extracellular death receptors, such as TNFR1 (Fig. 13.2). In this scenario, TNF-α engages TNFR1 inhibition of caspase-8 activation by zVAD has been shown to activate the necrosome which leads to a

necroptotic cell death. A limited number of markers of necroptosis have been identified, among them an increase in Rip3, and maintenance of intact Rip1, and phosphorylation of Mixed lineage kinase (MLKL) [31]. This is in contrast to Rip1 cleavage which is associated with caspase-8 activation and apoptosis [32, 33]. The recently reported ability of Rip1 to mediate production of TNF-α during necroptosis helps to describe the inflammatory nature of necroptotic cell death and may prove to also be a hallmark of necroptosis [34]. Many inflammatory and necrotic phenotypes are reported as a result of inhibiting the apoptosis pathway components. For example, mice in which caspase 8 is conditionally knocked-out in hepatic cells die of necrosis of the liver [35]. Also, FADD conditional knockout in intestinal epithelial cells leads to a spontaneous colitis phenotype that is rescued when Rip3 is also knocked out [36]. In a MyD88 deficient model it has also been found that necroptosis, rather than apoptosis, is activated in UV-radiated KC [37], suggesting an unexpected role for innate signaling pathways in dictating cell death outcomes. Previously described inflammatory phenotypes may now be better explained as a consequence of using necroptosis as a default death pathway when apoptosis is inhibited.

Pyroptosis

Pyroptosis is a pro-inflammatory cell death that is the result of the activation of an inflammasome. During pyroptotic cell death many pro-inflammatory zymogens are cleaved and activated by the pyroptosis executer protein, caspase-1 [38]. These zymogens include the extensively studied pro-inflammatory cytokines, IL-1β and IL-18, which lead to the wide spread inflammation associated with this type of programmed cell death [39]. Pyroptotic cell death is classically thought to require a priming step which initiates the production of the aforementioned zymogens and necessary machinery which can subsequently be activated to form an inflammasome [40]. However, more recently it has been reported that inflammasome activation can occur independently of an initial priming step, this is an active area of current investigation (Fig. 13.3) [41].

The inflammasome, is a cytosolic signaling complex that is dependent on the activation of intracellular pattern recognition receptors (PRR) and is anecdotally referred to as the 'wheel of death'. To initiate an inflammasome cytoplasmic PRRs identify an agonist and then begin to cluster into a wheel shaped aggregate [42, 43]. This signaling complex continues to recruit adaptor proteins, including apoptosis-associated speck-like protein containing a CARD domain (ASC), that scaffold to eventually include a CARD-domain containing protein [39]. The cluster of Caspase activation and recruitment domain (CARD)-domain containing proteins are

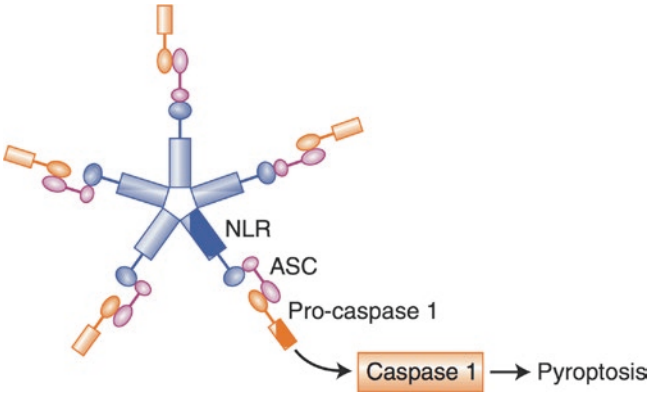

Fig. 13.3 Pyroptosis. This form of cell death is pro-inflammatory, because the inflammasome is activated by NLR, ASC and activation of caspase 1, resulting in the cleavage (activation) of IL-1beta and IL-18, two pro-inflammatory molecules. This form of cell death is activated by intracellular pattern recognition receptors (Nod receptors), which are typically mobilized by PAMPS forming a "wheel of death", but can also be mobilized by DAMPS (such as extracellular ATP)

then able to activate caspase-1, which directly activates the cell death and severe inflammation associated with pyroptosis. Inflammasome activation is also able to mobilize the adaptive arm of an immune response against the microbes it initially sensed [44]. Mobilization of both the innate and adaptive arms of the immune system combined with the death of the infected cell allows pyroptosis to be an effective way for our body to rid itself of pathogenic microbes.

Autophagy

Autophagy is a homeostatic cell process that can be, but is not always, associated with cell death [45]. This process is initiated when a cytoplasmic fraction is recognized as needing to be destroyed or recycled. The damaged/infected fraction of the cytosol is engulfed into a double membrane organelle termed the autophagasome which then fuses with a lysosome. The lysosomal enzymes then have access to the contents of the autophagosome which are promptly digested. If too much of the cytosolic fraction has been labeled for destruction by autophagasome, the cell can die by autophagy. This autophagic cell death is considered non-inflammatory and almost as 'silent' as apoptotic cell death (Fig. 13.4) [46].

Many different conditions can lead to the activation of autophagy all of which have certainly not yet been appreciated. The anti-inflammatory nature of autophagic cell death has been thought to serve several different purposes [47]. It is an inconsequential way of surveying the cytosol of a cell without initiating a robust immune response [48]. It has also been labeled as a way for cells to regulate and dampen inflammatory cell death that would otherwise run rampant

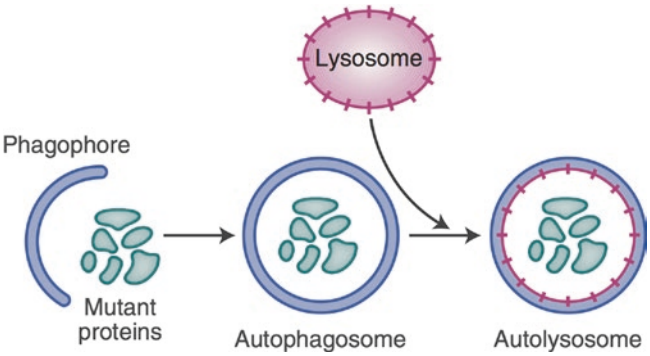

Fig. 13.4 Autophagy. This is a homeostatic process that may or may not result in cell death. Damaged intracellular organelles are engulfed by a membrane termed the autophagosome, which then fuses with a lysosome, resulting in the digestion of the damaged organelle. Limited autophagy does not result in cell death. Extensive organelle damage can lead to cell death: an autophagic, silent, non-inflammatory cell death

during a microbial infection. Activation of autophagy following disease-related oxidative stress can regulate the inflammatory response and widespread inflammation that would normally result from inflammasome activation by ROS [49].

Immune Signaling Resulting in Cell Death

Nod-Like Receptors (NLRs)

NLR signaling is most classically associated with activation of the inflammasome and subsequent pyroptotic cell death. However, in the years following their initial discovery it has been shown that signaling downstream of NLRs can lead to many more types of cell death than simply pyroptosis. For example, interferon-inducible protein AIM2 and NLRP3 inflammasomes have been reported to activate both pyroptotic and apoptotic cell death [50]. In fact, recent research has gone so far as to call NLRs the 'master regulators of inflammation' [51]. This title, while perhaps overstated, has some truth to it. NLRs have, to date, been associated with almost all reported types of programmed cell death. NLRs are also able to stimulate an autophagic response upon activation [52–55]. Furthermore, phagocytosis of autophagic cells has been found to activate the inflammasome and cause the production of many pro-inflammatory cytokines including TNF-α, IL-6, IL-8, and IL-1β [56].

However, activation of NLRs seems to be exclusive from necrosis; if a cell has activated inflammasomes, it will not die by necrosis. Lysosome rupture seems to initiate necrosis and control NLR signaling [57]. Importantly, the two main pathways leading to inflammatory cell death do not appear to overlap, which may allow for the organism to be able to recover from inflammation rather than compound it.

Non-classical Signaling Pathways and Mechanisms Controlling Cell Death

As members of the IL-1 family receptors, toll-like receptors (TLR) are classically known to induce inflammation and, similarly to NLRs, lead to the clearance of the pathogen that they sense. However, as more research is done on these extremely dynamic signaling pathways, it is becoming clear that they are capable of producing many more outcomes than initially thought. For example, extracellular TLRs primed during pyroptotic cell death causes different isoforms of damage associated molecular patterns (DAMPs) to be released, which skews the resulting immune response [58]. A pathway in which engagement of TLR2 and TLR4 signaling stabilizes the autophagy promoting protein Beclin-1 has also recently been described [59]. In this example of a non-classical cell death control mechanism, activation of a pro-inflammatory signaling pathway leads to immunologically silent autophagic cell death. This new association could represent an internal check for inflammation, which does not allow TLR-induced inflammatory responses to go out of control.

As previously mentioned, a novel MyD88-dependent TLR4 signaling pathway that directly activates apoptosis in response to UV has also recently been reported. In the absence of a functional TLR4/MyD88 signaling pathway, cell death after UV-irradiation is skewed to a more inflammatory ordered necrotic cell death termed necroptosis. Because TLR4/MyD88-deficient cells are dying in a more inflammatory and immunogenic way, $MyD88^{-/-}$ and $TLR4^{-/-}$ animals are resistant to systemic immune suppression caused by UV-irradiation, which plays a critical role in the development of UV-induced skin cancers. Activated apoptotic mechanisms previously thought to be a less damaging, less inflammatory, and therefore more desirable outcome during infection, may prove to facilitate the propagation of long-term consequences, such as carcinogenesis.

It is now clear that there is much more cross-talk between apoptosis, necrosis, and autophagic signaling pathways than previously appreciated. Signaling proteins once thought to be mutally exclusive are now being shown to play a role in multiple programmed cell death pathways [60]. If we can identify non-classical cell death pathways activated during tissue damaging circumstances, and determine the consequences of them, we may be able to skew toward alternative cell deaths that will allow for a more immunogenic environment, propagation of fewer potentially cancerous mutations, and an overall more favorable outcome.

Table 13.2 Diseases and therapies associated with different types of cell death

Disease/therapy	Pathophysiology	Related cell death	References
Psoriasis	Hyperproliferation of the epidermis T-lymphocyte mediated disorder Associated with comorbid chronic systemic inflammatory diseases (Crohn's Disease)	Decreased apoptosis	[61–70]
Systemic Lupus Erythematosus (SLE)	Antibodies against nuclear autoantigens Malar or discoid rashes, oral ulcers and photosensitivity	Increased apoptosis and decreased clearance of apoptotic bodies; secondary necrosis; increased apoptosis to UV light (photosensitivity)	[71–79]
Keloids	Overgrowth of granulation/scar tissue Increased fibroblast activity	Decreased apoptosis	[80–102]
SJS-TEN spectrum	Drug hypersensitivity reaction Blistering and widespread epidermal detachment	Increased KC apoptosis; evidence of necroptosis	[103–122]
Skin cancer	Tumor formation after chronic UVR exposure	Decreased apoptosis	[63, 123–145]
Crohn's Disease	Chronic inflammation of terminal ileum Abnormal immune response to commensal bacteria of the gut	Necroptosis, Necrosis	[36, 146–151]
Ischemic Reperfusion (IR) injury	Tissue ischemia followed by reperfusion that initiates an inflammatory response that may exacerbate local injury and lead to impaired remote organ function MPT-induced necrosis involving cyclophilin D and RIP kinase mediated necroptosis	Necrosis, Necroptosis	[152–169]
Graft vs. host disease (GVHD)	Most common complication of BMT Donor T cells recognize host tissue (APC) as foreign	Increased apoptosis	[107, 108, 170–188]
Photodynamic therapy (PDT)	Cell death induced by light after sensitizing the tissue with a photosensitizing agent ROS formation after photosensitizing agent exposed to light induces cell death	Apoptosis; necrosis/necroptosis	[189–195]
Ingenol Mebutate (Picato gel)	Rapid necrosis of cells in the lesion/tumor followed by neutrophil-mediated, antibody-dependent cellular cytotoxicity of the residual tumor cells	Necrosis	[196–205]
Cryosurgery	Direct cell injury followed by vascular stasis and tissue ischemia	Necrosis	[206–224]

Cell Death in Skin Diseases

Dysfunctional or altered cell death can result in a variety of different dermatological diseases with varying clinical presentations and characteristics. In many of these diseases, inflammatory responses of the immune system are altered in conjunction with impaired cell death leading to chronic inflammation or immune suppression. Not only is abnormal cell death involved in the pathogenesis of dermatological diseases, but cell death can also be induced using therapeutic techniques to treat a wide range of dermatological diseases (Table 13.2).

Conditions Associated with Decreased Apoptosis

There are several common skin disorders during which decreased apoptotic cell death causes a wide variety of pathogenic symptoms. These conditions include psoriasis, keloids, and skin cancer.

Psoriasis

Psoriasis is a chronic immune-mediated inflammatory skin disease usually manifesting as erythematous plaques with silvery scales, most often on the elbows, knees, hands, feet and lower back. While skin lesions are the most common manifestation, individuals can also develop psoriatic arthritis and nail dystrophy [61]. During psoriasis pathogenesis, epidermal KC associated with lesions become hyperproliferative and are resistant to apoptotic cell death. This resistance is due to both the overexpression of intracellular anti-apoptotic molecules and extracellular cytokines which influence cell fate [62, 63].

While psoriasis is classically viewed as a T-lymphocyte mediated inflammatory skin disease, macrophages have also been implicated as having a role in its pathogenesis [64]. Research using a mouse model of psoriasis has shown that elimination of macrophages attenuated psoriatic symptoms, suggesting that psoriasis development depended on macrophages and not necessarily T-lymphocytes. Macrophages are

the major source of TNF-α in the skin [65]. Previous studies have demonstrated that TNF and TNFR1 signaling is critical in the development of a psoriasis-like inflammatory skin disease in mice models [66]. Treatment with IL-11 or IL-4, which inhibit macrophage production of TNF-α [67, 68], has shown to improve symptoms in patients with psoriasis [69, 70]. Cells in psoriatic lesions may exhibit abnormal signaling and contribute to the inappropriate activation of the immune system. This deregulation of immune signaling alters the homeostatic apoptosis which is critical to maintaining a healthy epithelial layer.

Keloids

Keloids develop due to abnormal wound healing leading to overgrowth of granulation tissue that enlarges and often extends beyond the margins of the original wound. Keloids do not spontaneously regress and often recur after excision [80]. Keloid formation is believed to be attributed to increased proliferation and decreased apoptosis of fibroblasts [81–84]. Fibroblasts are important for producing and depositing ECM proteins during wound healing. Fibroblasts of normal skin tend to be quiescent with very low activity; however, keloid-derived fibroblasts have higher densities and proliferative activity [85]. Many studies have shown that keloid fibroblasts exhibit reduced apoptotic rates compared to normal fibroblasts [81–84]. These reduced rates in apoptosis have been attributed to alterations in gene expression in both the fibroblasts themselves and the surrounding KC [83, 84, 86–95].

Various immune cells such as T lymphocytes and macrophages have also been implicated in keloid formation since they are involved in coordinating fibroblast activity during wound healing [96–100]. Failure to clear the inflammatory cells at the wound site due to decreased apoptosis may lead to the imbalance of inflammatory cell populations observed in keloid tissue and the increased activity of fibroblasts contributing to keloid formation [101, 102]. Therefore, the decrease in apoptosis observed in keloid tissue seems to account for the persistence of cellular infiltrate and the accumulation and enlargement of granulation tissue at and beyond the wound site.

Skin Cancer

Skin cancer results when there is hyperproliferation and/or decreased apoptosis of mutated cells that form a tumor which progresses to a malignant state, usually after chronic ultraviolet radiation (UVR) exposure. Actinic keratoses (AK), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) have higher proliferation rates and decreased apoptotic rates compared to normal skin [63]. These differences were most likely associated with increased expression of anti-apoptotic molecules and decreased expression of death receptors that induce apoptosis [225–230]. Mutations in P53, a tumor suppressor gene, are also commonly associated with skin cancers [123–127] and is often induced by chronic UVR exposure, preventing cell cycle arrest or DNA damage-induced apoptosis [128–130]. Consequently, mutated cells are able to survive and clonally expand outside their original compartment eventually forming a tumor [131–133]. Clonal expansion not only requires a decrease in apoptosis of the mutant cells, but also apoptosis of the surrounding cells. Apoptosis of KC adjacent to the mutant cells allows the mutant clones to expand and develop into a tumor [133]. Therefore, it appears that both apoptosis and decreased apoptosis are critical at specific stages in skin carcinogenesis.

In addition to impaired apoptosis, UV-induced immune suppression plays an important role in skin carcinogenesis. Individuals on immunosuppressants have greater incidences of skin cancer on sun-exposed areas compared to immunocompetent individuals [134–136], further supporting the notion that UV-induced immune suppression is critical for the development of skin cancer. UV-induced DNA damage seems to trigger immunosuppression [137–141] by promoting the production of cytokines and apoptosis-inducing ligands like TNF and FasL [130, 142, 143], without which UV-induced immunosuppression does not occur and skin cancer incidence is decreased [144, 145]. Based on this evidence, skin cancer formation involves a state of immunosuppression and decreased inflammatory responses in combination with dysregulated apoptosis to allow the mutated cells to persist and progress into a tumor.

Conditions Associated with Increased Apoptosis

Just as there are several common skin disorders associated with decreased apoptotic cell death, there are many skin conditions with pathogenic symptoms due to increased apoptotic cell death. These conditions include SLE, SJS-TEN spectrum, and GVHD.

Cutaneous and Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects multiple organs including the skin. Individuals with SLE can develop malar or discoid rashes, oral ulcers and photosensitivity [71]. SLE is characterized by the presence of autoantibodies against nuclear antigens. The pathogenesis of SLE is believed to be due to an increase in apoptosis and a decrease in the clearance of apoptotic cells [72–75]. Many studies have shown an increase in KC apoptosis in LE lesions compared to normal controls [76, 77], which may be mediated by increased Fas expression and decreased Bcl2 expression [77]. While the mechanism remains unclear, it appears that clearance of

apoptotic cells by phagocytes is impaired in lupus patients and mouse models. Accumulating apoptotic cells that would normally be cleared by phagocytes undergo secondary necrosis, releasing their intracellular contents and exposing their apoptosis-induced autoantigens to the immune system, which present as danger signals to APCs [78]. The antigens are taken up by DC leading to IFN-α production and activation of T and B cells [79]. Antibodies are produced against the autoantigens resulting in chronic inflammation and the development of SLE [79]. Therefore, while there is increased apoptosis which may be associated with immunosuppressive effects, the decreased clearance of the apoptotic cells results in secondary necrosis and the release of intracellular material, and exposure of the immune system to otherwise cryptic self-antigens. This activates the immune system leading to the chronic inflammatory state of SLE.

Severe Drug Hypersensitivity Syndromes

Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolylsis (TEN) are life-threatening skin diseases caused by drugs or infections characterized by blistering lesions and widespread epidermal detachment [103]. SJS involves less than 10 % of the body surface area (BSA) and TEN involves greater than 30 % of the BSA [104]. The pathogenesis of SJS-TEN involves widespread KC apoptosis mediated by cytotoxic T lymphocytes (CTLs) [105]. CTLs may induce KC apoptosis via the perforin/granzyme pathway and/or the Fas/FasL pathway [106–108]. Increased levels of these apoptotic molecules have been found in epidermal KC, blister fluid and serum of TEN patients [106, 109–111]. While FasL on KC have been implicated in inducing KC apoptosis seen in SJS-TEN, soluble FasL (sFasL) from PBMC, not KC, has been suggested as the main mediator of KC apoptosis in SJS-TEN [112]. Whether FasL on KC or sFasL from PBMCs is responsible, evidence suggests that the Fas/FasL pathway is important in KC apoptosis and the pathogenesis of SJS-TEN. This is corroborated by the favorable outcomes observed in vitro and in vivo with administration of anti-Fas antibodies or IVIG, which contains naturally occurring anti-Fas antibodies [111, 113–120]. Along with Fas/FasL and perforin/granzyme pathways, granulysin has also been implicated in the pathogenesis of SJS-TEN [121]. Granulysin, a molecule demonstrated to be cytotoxic in vitro, showed the greatest staining in TEN skin biopsies compared to any other SJS-TEN-associated molecule and granulysin protein levels correlated with clinical severity [121]. This suggests that granulysin may contribute to epidermal destruction in SJS-TEN and may better explain the widespread KC apoptosis since granulysin can induce apoptosis without direct cell contact, unlike the Fas/FasL and perforin/granzyme pathways [121]. While most of the evidence points to apoptosis as the main mechanism of KC death and the development of SJS-TEN, recent studies have suggested that necroptosis may also be involved in the pathogenesis of SJS-TEN. It has recently been reported that severe adverse blistering reactions to topical therapies may be the result of secreted Annexin-V which initiates a necroptotic, rather than apoptotic, cell death [122]. Although the pathogenic mechanisms are still being elucidated, it appears that these drug sensitivity reactions involve an inflammatory response leading to inappropriate KC death and widespread epidermal detachment.

Graft Versus Host Disease

Graft vs. host disease (GVHD) is the main complication following a bone marrow transplant (BMT) [170]. GVHD is an immunological disorder affecting multiple organ systems mainly the skin, liver and gastrointestinal tract. The skin is the most frequently affected and usually the first organ involved. A pruritic, maculopapular rash develops that can spread all over the body typically sparing the scalp. GVHD is thought to progress in three stages. First, the host tissue is damaged as a result of the BMT conditioning regimen before the transplantation, usually with chemotherapy. The damaged host tissue produces proinflammatory cytokines which activate host APCs and upregulate MHC antigens on the APCs [171–182]. Second, donor T cells recognize the antigens on host APC leading to T cell activation, differentiation and migration. This recognition and activation can occur in MHC-mismatched and MHC-matched transplantations [183, 184]. Activated T cells then produce Th1 cytokines (IFN gamma, IL-2, TNF-α) [185, 186], which stimulate CTL. Third, CTL and inflammatory cytokines promote inflammation and tissue injury by inducing apoptosis. CTLs induce apoptosis mainly via the Fas/FasL or perforin/granzyme pathways [107, 108]. In the skin, Fas-mediated apoptosis by CTLs and TNF-α-mediated apoptosis seem to contribute the most to KC apoptosis and tissue damage [187]. Anti-Fas or anti-TNF-α antibodies were shown to diminish GVHD skin lesions [188] and using both anti-Fas and anti-TNF-α antibodies together completely blocked the skin lesions, suggesting that Fas and TNF-α signaling contribute to tissue injury in GVHD [188]. Based on current evidence, GVHD pathogenesis involves an inappropriate inflammatory response resulting in excessive apoptosis and tissue destruction that can affect a variety of organs, including the skin.

Conditions Associated with Necrosis/Necroptosis

Not only are there skin disorders associated with impaired apoptosis, there are many common dermatological conditions linked to necrosis or necroptosis. These conditions include ischemia reperfusion (IR) injury and Crohn's Disease.

IR injury involves tissue ischemia followed by reperfusion that initiates an inflammatory response that may exacerbate local injury and lead to impaired remote organ function [152]. While it is not well studied in the skin, IR injury has been well studied in the central nervous system and cardiovascular system and the pathogenesis of such injury involves fundamental processes that occur in the skin. IR injury can result after acute vascular occlusions followed by their respective reperfusion strategies, surgical procedures and shock or trauma [153]. Systemic effects of IR injury can include serious conditions like systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). Majority of the cell death in IR injury is believed to be attributed to necrosis [154, 155]. Various types of necrosis have been linked to the cell death seen in IR injury such as MPT-induced necrosis involving cyclophilin D [156], RIP kinase-mediated necroptosis [156] and PARP-mediated necrosis [157–161]. Necroptosis/necrosis leads to the release of intracellular molecules that can promote inflammation via interactions with TLRs and other receptors on immune cells [162, 163]. The postischemic organ also releases proinflammatory cytokines, which promotes the production of inflammatory mediators and inflammation at remote organs [163, 164]. Therefore, the damage that occurs promotes the widespread inflammatory response seen in IR injury. Necrostatin-1 (Nec-1), an inhibitor of RIPK1 and necroptosis, has been shown to be protective against IR injury in the heart, brain and kidney [165–169]; however, caspase inhibitors do not appear to offer any protective benefit against kidney IR injury [165]. This supports the notion that necroptosis/necrosis is principally involved in IR injury. It appears that the effects of tissue ischemia and subsequent reperfusion lead to activation of a variety of necrotic pathways resulting in cell death and an inflammatory response. Although not well studied, this phenomenon is likely to be highly relevant to dermatologic surgery, particularly survival of skin grafts and flaps used during reconstructive surgery after Mohs surgery for skin cancer.

Crohn's disease is an inflammatory bowel disease involving chronic inflammation primarily of the terminal ileum. Individuals with Crohn's disease often develop bloody diarrhea and crampy abdominal pain. The inflammation is thought to be triggered by an abnormal immune response to the commensal bacteria of the gut possibly as a result of a disruption of the intestinal epithelial barrier allowing the bacteria to translocate across the bowel wall and stimulate TLR signaling [36, 146–149]. Increased epithelial cell death is a hallmark of intestinal inflammation and believed to be a possible mechanism driving Crohn's disease [147]. Many studies have shown that the intestinal epithelial cells die by necrosis, specifically necroptosis [36, 149]. This necroptotic death of epithelial cells could be induced by TNF-α in vitro and was associated with increased expression of RIPK3, a key mediator of necroptosis [36, 149]. Studies of mice deficient in caspase 8 or FADD observed spontaneous development of epithelial cell necrosis, intestinal inflammation and loss of Paneth cells similar to that seen in Crohn's disease [36, 149]. Caspase 8 and FADD are known to be important mediators of apoptosis and regulators of necroptosis, normally keeping the necroptotic pathway silent. Treatment with Nec-1 was protective against the cell death and intestinal inflammation in murine models of Crohn's disease [36, 149]. Therefore, Crohn's disease appears to be driven by a disrupted intestinal barrier and inappropriately activated immune response leading to necroptosis of intestinal epithelial cells causing a chronic inflammatory condition.

Crohn's disease is associated with other chronic inflammatory diseases like psoriasis [150, 151]. The increased incidence of Crohn's disease among psoriatic patients may be related to the changes in cell death seen in these diseases. The decreased apoptosis seen in psoriasis may be linked to the increased necroptosis observed in Crohn's disease as apoptotic molecules have been implicated in negatively regulating necroptosis. Further studies are needed to investigate the role of necroptosis in psoriasis.

Therapies Associated with Cell Death

While impaired or abnormal cell death can lead to various dermatological diseases, cell death can also be manipulated for therapeutic purposes commonly used in the field of dermatology. These treatments include PDT, ingenol mebutate, and cryosurgery.

Photodynamic therapy (PDT) is a type of phototherapy in which cell death is induced by light after application of a photosensitizing agent. PDT is important in the treatment of many dermatological conditions such as psoriasis, eczema, non-melanoma skin cancer (NMSC) and melanoma [189]. When the photosensitizing agent is exposed to light, ROS formation results which induces cell death [190–192]. Many studies support the idea that PDT triggers necrotic cell death [192, 193]. However, PDT has also been shown to induce multiple modes of cell death including apoptosis, necrosis and autophagic cell death [192]. PDT using UVA and hypericin, a photosensitizing agent and major component of St. John's Wort, induced apoptosis in unpigmented cells (KC and non-pigmented melanoma cells) and induced necrosis in pigmented cells (MC and pigmented melanoma cells) [194]. It is likely that the presence of melanin and the site of hypericin localization after UVA exposure contributed to the induced mode of cell death. Hypericin disrupted the melanosome membrane in melanocytes and pigmented melanoma cells causing melanin to leak into the cytoplasm and act as an oxidant to induce cell necrosis. Hypericin localized to the mitochondria in unpigmented cells, leading to mitochondrial

damage, cytochrome c release and apoptosis. In pigmented cells, hypericin localized to the ER/Golgi which likely led to cell stress and necrosis. The presence or absence of RIPK3 has also been implicated as a determinant of the modality of cell death induced by PDT, suggesting that PDT may induce necroptosis depending on the cellular context [195]. Therefore, PDT induces and manipulates cell death processes to treat various skin diseases.

Ingenol mebutate is the active ingredient in the sap from the *Euphorbia peplus* plant [196–198] that has been used to treat a variety of skin lesions [199, 200] including warts, AKs [196, 199, 201, 202], and NMSC [203, 204]. Recent clinical trials have demonstrated that two or three applications of ingenol mebutate is effective in clearing AKs and BCCs. The efficacy of the treatment increased in a dose dependent manner [196, 201, 204] with only mild dose dependent dermatological side effects, confirming that short term use of ingenol mebutate is safe when treating a variety of skin lesions.

Ingenol mebutate has been shown to have a dual mode of action in treating skin lesions. First, rapid necrosis of cells in the targeted lesion is induced [198], which leads to the release of proinflammatory cytokines and activation of the immune system. Second, the activated immune system promotes an inflammatory response and neutrophil-mediated, antibody-dependent cellular cytotoxicity [198, 205], which destroy any residual cells from the lesion. This second mode of action is especially important in completely eradicating the abnormal cells as neutrophil deficient mice treated with ingenol mebutate exhibited tumor regrowth after 25 days compared to neutrophil replete mice who did not exhibit tumor regrowth [205]. Therefore, the inflammatory response and neutrophil-mediated, antibody-dependent cellular cytotoxicity are important to prevent tumor regrowth after the initial necrotic cell death, making ingenol mebutate an effective short term treatment for various skin lesions.

Cryosurgery is a therapeutic technique that involves tissue destruction by freezing, typically using liquid nitrogen [206]. It is widely used in dermatology to treat a variety of benign, premalignant, and malignant skin lesions [207–209]. Cryosurgery treats these lesions by ultimately inducing tissue necrosis [210]. Tissue injury is first induced by freezing, causing direct cell injury [210]. Cell freezing with low cooling rates leads to extracellular ice formation which increases the extracellular solute concentration causing osmotic dehydration of the cell and cell shrinkage [211, 212]. Cell shrinkage and the resulting increased intracellular solute concentration damage the plasma membrane and cellular constituents [213]. The extracellular ice crystals that form can also apply mechanical pressure on the weakened cell, contributing to the plasma membrane damage [212, 214]. Another consequence of freezing is intracellular ice formation, seen at higher cooling rates, which disrupts the cell membrane and cellular organelles leading to cell death [211, 215–220]. Thawing allows recrystallization to occur during which ice crystals can fuse and form larger crystals that are more likely to cause damage to the cellular membrane [211, 221–223]. In addition, as the ice melts, the extracellular fluid becomes briefly hypotonic, which drives water into the cell resulting in cell rupture due to the damaged plasma membrane [224]. Therefore, thawing further contributes to the cell death that was first induced by freezing.

In combination with direct cell injury, vascular stasis plays a major role in the tissue destruction after cryosurgery. Freezing completely terminates circulation in the vessels within the treated region of the tissue [223, 231, 232]. After thawing, there is vasodilation and an increase in blood flow followed by increased vascular leakage due to endothelial cell damage [232], edema, platelet aggregation and blood flow cessation, particularly in the microvasculature [233]. The occlusion of blood flow leads to tissue ischemia and subsequent tissue necrosis [224, 234]. Vascular stasis is believed to be the major mechanism of tissue necrosis induced by cryosurgery.

Some studies suggest that further damage occurs after vascular stasis and tissue necrosis due to an immune response. After the tissue undergoes a necrotic cell death, intracellular contents are exposed which trigger an immune response [235, 236] against the targeted tissue [237]. This suggests that an immune response after cryosurgery contributes to the death of cells that were not immediately killed by freezing and thawing. Edema of and around the treated tissue is observed after cryosurgery, providing further evidence of the activation of the immune system and inflammatory response after tissue necrosis [212, 224]. However, this mechanism is still being elucidated and requires further study. Overall, cryosurgery is an effective therapeutic technique for targeted tissue destruction that induces cell death and an inflammatory response to eliminate unwanted tissue.

Conclusion

As evidenced by the dermatological diseases discussed, cell death can play a significant role in the pathogenic symptoms of a variety of disorders and can significantly influence inflammation. Therefore, it appears that necrosis promotes inflammatory responses while apoptosis has anti-inflammatory effects. However, SJS-TEN spectrum, SLE and GVHD exhibited an increase in apoptosis and an increased inflammatory response, which seems contradictory to the findings that apoptosis is anti-inflammatory/immunosuppressive. These contradictions may be explained by additional factors related to the pathogenesis of these diseases. SJS-TEN spectrum, as recent studies have suggested, may involve necroptosis, which would better explain the increased inflammatory response. SLE

not only involves increased apoptosis but also decreased clearance of apoptotic cells which leads to secondary necrosis and drives the inflammatory response/state seen in individuals with SLE. GVHD, like SJS-TEN spectrum and SLE, exhibits an increase in apoptosis and inflammatory response; yet, further studies are needed to explain this discrepancy by looking for necrotic/necroptotic markers in patients with GVHD. Overall, changes in cell death significantly influence inflammation in a variety of dermatological diseases. In particular, this inflammatory response seems to be related to a decrease in apoptosis and/or an increase in necrosis. Understanding this concept has helped and will continue to help develop more advanced techniques to treat these inflammatory dermatological diseases.

Questions

1. How can a keratinocyte undergoing apoptotic cell death be differentiated from one that is dying by necrosis/necroptosis?
   A. DNA "laddering"
   B. Cellular "blebbing" on electron microscopy
   C. Lack of phosphorylation of MLKL
   D. Cleavage of RIP1
   E. All of the above
   F. None of the above

Correct Answer: (E) All of the above cellular and biochemical events described above are hallmarks of apoptosis, and distinguish this form of cell death from necroptosis

2. What type of cell death may be advantageous to promote in a patient presenting with symptoms of hyper-immune cell activation and several areas of inflammatory skin lesions?
   A. Necroptosis
   B. Pyroroptosis
   C. Apoptosis
   D. Autophagy

Correct Answer: (C) Apoptosis is a non-inflammatory type of cell death, and can actually suppress antigen presenting cell function, and thus dampen immune cell activation. This is not true for the other types of cell death or autophagy

3. Crohn's disease is associated most strongly with which mode of cell death?
   A. Apoptosis
   B. Pyroptosis
   C. Autophagy
   D. Necroptosis

Correct Answer: (D) Crohn's disease has been associated with necroptotic cell death, which is thought to contribute to the pathogenesis of the gut-associated inflammation

4. What conditions/symptoms would be present in a patient in which the skin is undergoing extensive necroptosis?
   A. Fever
   B. Inflammation
   C. Elevated TNF-alpha
   D. Sloughing of dead skin
   E. None of the above
   F. All of the above

Correct Answer: (F) All of the above symptoms are associated with necroptosis

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Adipose Tissue and Cutaneous Inflammation

14

Anna Balato and Matteo Megna

Abstract

Adipose tissue (AT) is a highly dynamic and interactive organ with both immune and endocrine roles. It plays a major role in inflammation through the production of a huge class of bioactive mediators termed adipokines. Since adipocytes, macrophages and other cell types resident in AT are a non neglectable source of these mediators, together they might perpetuate a vicious cycle of macrophage recruitment and production of pro-inflammatory cytokines, playing a major role in the development and sustainment of inflammatory conditions. Therefore, given AT proprieties and widespread localization in human body which is also responsible for the strict and extensive contact between skin and subcutaneous fat, AT is able to significantly influence the immune responses, the immune skin system and consequently the pathogenesis of several cutaneous inflammatory disorders.

Keywords

Skin tissue • Adipose tissue • Cutaneous inflammation • Psoriasis • Inflammatory cytokines • Tissue structure • Adipogenesis • Leptin • Immune system • Adiponectin • Hidradenitis suppurativa • Skin diseases • Atopic dermatitis

Abbreviations

AD	Atopic dermatitis
ANA	Anti nuclear antibody
aP2	Adipocyte fatty acid binding protein
apoE	Apolipoprotein E
ASP	Acylation stimulating protein
AT	Adipose tissue
BAT	Brown adipose tissue
BMI	Body mass index
CCL	Chemokine (C-C motif) ligand
CMKLR1	Chemokine-like receptor-1
CORS-26	Collagenous repeat containing sequence of 26 kDa protein
CRP	C-reactive protein
CSF	Colony stimulating factor
DCs	Dendritic cells
dcSSc	Diffuse cutaneous SSc
FFAs	Free fatty acids
FGF	Fibroblast growth factors
GH	Growth hormone
hBD2	Human βdefensin-2
HGF	Hepatocyte growth factor
HS	Hidradenitis suppurativa
ICAM-1	Intercellular adhesion molecule-1
IGF-1	Insulin like growth factor-1
IFN	Interferon
IL	Interleukin
IL-1RA	Interleukin-1 receptor antagonist
IP-10	Interferon-gamma inducible protein 10
LPS	Lipopolysaccharide
MCP-1	Monocyte chemotactic protein-1
MIF	Macrophage migration inhibitory factor
MIP-1α	Macrophage inhibitory protein-1 alpha
MMP	Matrix metalloproteinase
NGF	Nerve growth factor

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NK cells	Natural killer cells
PAI-1	Plasminogen activator inhibitor-1
PBMCs	Peripheral blood mononuclear cells
PGE2	Prostaglandin E2
PGI2	Prostacyclin
pDCs	Plasmacytoid dendritic cells
PPAR	Peroxisome proliferator-activated receptor
ROS	Reactive oxygen species
SDF-1	Stromal derived factor-1
SLE	Systemic lupus erythematosus
SSc	Systemic sclerosis
TGs	Triglycerides
TGF	Transforming growth factor
TNF-α	Tumor necrosis factor-α
Tregs	Regulatory T cells
VCAM-1	Vascular cell-adhesion molecule
VEGF	Vascular endothelial growth factor
VLA	Very Late Antigen
WAT	White adipose tissue

Introduction

Adipose tissue (AT) has long been neglected and misunderstood, merely considered as an inert energy storage organ providing thermal and mechanical insulation. This stereotype has been completely abolished in the last two decades with the discovery of its ability to act as an endocrine organ and to produce several cytokines as well as many different mediators, being also a resource of stem cells. AT is now seen as a highly dynamic organ, being involved in a wide range of physiological and metabolic processes. The growing knowledge of genetics, growth factors and cytokines as well as the role of AT in their production and regulation, almost daily turns our attention to the intrinsic connection of increased AT mass with different diseases, such as metabolic and cardiovascular ones (atherosclerosis, diabetes, dyslipidemia, obesity, etc.) [1]. Modern society is characterized by an epidemic of obesity highlighting the importance of increased fat mass in the development of different pathologic conditions: adipocytes are a critical component of metabolic control and endocrine organs, possibly having both good and bad effects [2]. In particular, several studies show that obesity is characterized by a state of chronic low-grade inflammation in which AT plays a key role [3–6]. Indeed, increasing evidences show a potential pro-inflammatory role of AT which may also exert its effects in the skin system influencing the pathophysiology of several cutaneous diseases. The aim of this chapter is to review the main features of AT focusing on the relationship with skin inflammation and cutaneous diseases.

Adipose Tissue Structure

In humans, AT is traditionally classified as white adipose tissue (WAT) and brown adipose tissue (BAT), two histologically and functionally distinct types of AT. WAT is the predominant form of AT found in adults whereas BAT is principally found in neonates, having largely disappeared within the first years after birth [7, 8]. However, since WAT is involved in many different functions being able to act as an endocrine/immune organ whereas BAT is mainly implied in the production of heat [7, 8], for this chapter we will focus on WAT as regarding AT. AT represents a loose connective tissue structured in lobules of adipocytes, held in place by fibrous septa and surrounded by a rich capillary and innervation network. It is a heterogeneous tissue with mature adipocytes representing the most common type of cells (around 40 %) [9]. Mature white adipocytes are spherical and can vary enormously in size with a diameter ranging from 20 μm to >200 μm due to their unlimited capacity for growth. Lipids within these adipocytes are organized in a large unilocular droplet, mainly containing triglycerides (TGs) (up to 95 %). The lipid droplet in mature adipocytes occupies the majority of the cell volume, stretching the nucleus and the cytoplasm to a small edge around the droplet [7]. Although the adipose depot can increase from 20 % of total body weight in lean individuals to >50 % in morbidly obese individuals, the number of adipocytes appears to remain fairly constant in lean and obese individuals, once adulthood is reached [10]. In contrast, the adipocyte turnover is highly dynamic, with approximately 10 % of the cells renewed annually [10]. Apart from adipocytes, AT is also characterized by the presence of preadipocytes (immature adipocytes) and the stromal-vascular fraction [9]. This cell population is known to consist of various cell types, including endothelial cells and fibroblasts as well as a large proportion of immune/hematopoietic cells including macrophages and lymphocytes which have been largely involved in the inflammatory process linked to the onset of obesity [11–13]. Particularly, it has been reported that macrophages represent about 10 % of the total AT cell population [14] with their number being directly correlated with adiposity and adipocyte size without showing significant differences between subcutaneous and visceral AT [15–17]. Notably, both the adipocytes and the non-adipocyte cells of AT are able to secrete adipokines, bioactive proteins which participate in a wide variety of physiological or physiopathological processes, including immunity and inflammation [14, 18, 19]. Each cell population present in AT has its own secretion profile and specific regulation, accounting for the different proprieties and processes in which AT could be involved: it is also AT multifarious composition which renders itself an important and central mediator of metabolism and inflammation [20].

Adipose Tissue: Development and Adipogenesis

The development of AT occurs to a large extent postnatally and continues throughout life. The acquisition of fat cells appears to be an irreversible process, as apoptosis has not been shown to be significant under physiological conditions [21]. Like all tissues, organogenesis of AT takes place in early life during gestation (between 14 and 16 weeks of fetal life with a cranial to caudal and proximal to distal gradient) [22]. Adipocytes differentiate from a pluripotent mesenchymal stem cell whose commitment into the adipocyte lineage is triggered by mechanisms yet to be identified [23]. This process creates an unipotent adipoblast which subsequently gives rise to an early preadipocyte of first order which expresses early genes such as α2 chain of collagen 6, insulin-like growth factor (IGF)-1, and lipoprotein lipase [24, 25]. After mitosis and clonal expansion, the preadipocyte of second order arises and undergoes growth arrest. Only these preadipocytes can differentiate into mature adipocytes by reentering into the cell cycle and the subsequent clonal expansion. The ability of growth-arrested preadipocytes for further differentiation depends on the expression of early and intermediate markers of differentiation [26]. Among them, the peroxisome proliferator-activated receptor (PPAR) γ appears as the central regulator of adipogenesis playing a dominant role in fat tissue development [27]. As a consequence of all these transcriptional main events, immature adipocytes begin to accumulate lipid droplets and to express late markers of differentiation (glucose transporter-4, perilipin, and lipogenic and lipolytic enzymes) whereas mature adipocytes are characterized by the expression and secretion of highly specific and very late markers of differentiation such as leptin [28], adiponectin [29], resistin [30], visfatin [31], omentin [32], adipsin [33], and collagenous repeat containing sequence of 26 kDa protein (CORS-26) [34]. These molecules not only regulate metabolism [35] but also represent pro- and anti-inflammatory mediators of the AT [18, 36]. After birth, adipocytes can undergo hyperplasia and hypertrophy. In growth stages (childhood and adolescence), adiposity is increased mainly through hyperplasia [37], whereas in adult life adipocytes mostly increase in size [10]. Indeed, the number of adipocytes is usually constant in adults in which the capacity of preadipocytes to become fully functional mature adipocytes declines [10, 38]. Moreover, it has been demonstrated that the key regulator of adipogenesis PPARγ2 is more highly expressed in younger patients than in older patients [39]. However a late hyperplastic development of AT still remains possible. Indeed, a significant proportion of stromal-vascular cells from the subcutaneous AT of elderly subjects is able to differentiate into adipose cells [40] indicating that these adipose precursor cells should be responsible for the formation of new fat cells known to continue to take place in severely obese adult patients [41].

Adipose Tissue: General Details

AT has a generalized distribution throughout the body, surrounding and infiltrating the subcutaneous region, visceral organs, and a variety of muscle groups [42]. Particularly, subcutaneous AT is located underneath the skin and is responsible for the distinct body compositions of human males and females, accounting for temperature regulation and thermal isolation whereas visceral AT fills in space gaps between organs and maintains them in the adequate position [43]. In humans, abdominal fat mass is predominant in males whereas the subcutaneous fat mass is more important in females. The features of fat distribution are fundamental: it has been recognized that the cardiovascular risk of obesity and increased body weight are more related to body fat distribution rather than total body fat. Individuals with upper abdominal, central or android obesity are at a greater risk than those with gluteofemoral, peripheral or gynoid obesity [44, 45]. Indeed, subcutaneous fat accumulation represents the normal physiological buffer for excess energy intake with limited energy expenditure. It acts as a metabolic sink where excess free fatty acids (FFAs) and glycerol are stored as TGs in adipocytes [46]. When the storage capacity of subcutaneous AT is exceeded or its ability to generate new adipocytes is impaired, fat begins to accumulate in areas outside the subcutaneous tissue. About 80 % of all body fat is in the subcutaneous area [47, 48] with the femerogluteal regions, back and anterior abdominal wall being the most represented, whereas visceral fat is mainly present intra-abdominally tending to increase with age in both genders [48]. The endocrine function and features of adipocytes differ between subcutaneous AT and visceral AT. For example, large adipocytes, which are insulin-resistant, are present in a greater number in visceral AT, whereas small adipocytes, which are insulin-sensitive and have high avidity for FFAs and TGs uptake, are more common in subcutaneous AT [49, 50]. Adipokines, factors which can influence the immune system, are differently produced by these two types of AT with adiponectin [46, 51], C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin (IL)-6 [16, 52, 53] and monocyte chemotactic protein (MCP)-1 [2, 54] being more expressed in visceral AT whereas leptin is more expressed in subcutaneous AT [48]. These findings may be explained by the fact that inflammatory cells (especially macrophages) tend to be more prevalent in visceral compared with subcutaneous fat [55, 56]. Moreover, because of its anatomical position, visceral fat seems to be more involved in immune and inflammatory processes. Indeed, visceral AT venous blood is drained directly to the liver

through the portal vein allowing direct hepatic access to FFAs and adipokines secreted by visceral adipocytes which can activate hepatic immune mechanisms with production of inflammatory mediators such as CRP, IL-6, etc. [50, 57] Thus it is apparent that differential regional deposition of AT can impact on disease outcome, perhaps mediated by their altered adipokines repertoire.

Adipose Tissue as an Endocrine Organ

The identification and characterization of leptin in 1994 firstly and firmly established AT as an endocrine organ as well as initiated a more intense research in this field [58]. Leptin is a hormone with structural homology to cytokines that regulates growth, metabolism, and behavior being secreted primarily from adipocytes in direct proportion to AT mass and nutritional status [59, 60]. It shows many paracrine effects including stimulation of anorexigenic peptides in the hypothalamus, improvement of insulin sensitivity, alteration of several sex and growth hormones, as well as regulation of immune functions and angiogenesis [61]. Accounting for its numerous endocrine activities, AT is able to release other hormones such as IGF-1 which is expressed by stromal-vascular cells and is strictly interrelated with the growth hormone (GH) creating a system termed the GH-IGF axis with known effects on metabolism and growth [62, 63] being also a major site for metabolism of sex steroids [64] and for numerous receptors that allow AT to respond to afferent signals from traditional hormone systems as well as the central nervous system [65]. Thus, besides the biological repertoire necessary for storing and releasing energy, AT contains the metabolic machinery to permit communication with distant organs. Through this interactive network, AT is integrally involved in coordinating a variety of biological processes including energy metabolism, neuroendocrine and immune function. Indeed, other secretory products of AT, the so-called adipocytokines or adipokines (interchangeable terms used to identify cytokines/bioactive peptides produced by AT) (Table 14.1), are able to clearly regulate energy homeostasis, appetite/satiety, and insulin sensitivity as well as influence neuroendocrine, endothelial, immunological, hematological, angiogenetic, and vascular functions in a systemic (endocrine) and/or local (paracrine and autocrine) manner [59, 65, 66]. The diversity of the adipokines, both in terms of protein structure and of putative function, is considerable [35, 67]. Therefore, we will use the term "adipokines" because it seems rather more satisfactory than "adipocytokines" since it does not imply that the proteins belong to a particular functional group. Indeed, adipokines includes different factors such as cytokines, chemokines, growth factors, acute phase proteins, factors directly affecting metabolism, etc. (Table 14.1). Moreover, the important endocrine function of AT is further emphasized by the adverse metabolic consequences of both AT excess and deficiency. Both AT excess or obesity, particularly in the visceral compartment, and AT deficiency or lipodystrophy are associated with features of metabolic syndrome and pro-inflammatory states [68, 69]. Although adipocytes express and secrete several factors such as leptin and adiponectin, many secreted and bioactive proteins are derived from the non-adipocyte fraction (stromal-vascular cells) of AT [59] together functioning as an integrated unit, making AT a true, complex and highly active metabolic and endocrine organ [70, 71].

Adipose Tissue and Immune System

In the last decade, AT has been recognized to be an active source of several pro-inflammatory cytokines, chemokines, growth factors and complement proteins [74, 75]. Indeed, it is well known that apart from adipocytes, AT contains numerous mature immune cells including macrophages and lymphocytes [11–14] which are important source of inflammatory cytokines and factors as also adipocytes can be. Monocyte infiltration and differentiation in AT has been shown to correlate with adipocyte hypertrophy, as well as body mass [16] and the expansion of AT in obesity is associated with an increased infiltration with macrophages of the M1 or "classically activated" phenotype from the circulation [76]. These macrophages are usually recruited to sites of tissue damage and have been reported to be in a pro-inflammatory state with increased expression of TNF-α [77]. The cellular mechanisms responsible for this enhanced macrophage recruitment remain largely unknown, but it has been suggested that dysregulated adipokines production and increased adipocyte size might contribute to this phenomenon in a crosstalk between adipocytes and macrophages [78]. More recently, different surveys have shown that AT harbors mast lineage cells that are able to home to organs such as intestine and skin where they fulfill their role. The role of AT as a reservoir of mast cell precursors is interesting according to its strategic location (disseminated all over the body, in the vicinity of visceral organs or skin) and rich vascularization (allowing efficient precursor emigration). In normal physiological states, AT contains only a few mature mast cells whereas their number is increased in obesity [79–81]. Since mast cells produce a large panel of multifunctional molecules including cytokines, growth factors, or enzymes [82, 83] they may contribute to the secretory potential of AT. Expansion of AT, which occurs in obesity, is characterized by chronic low-grade inflammation (persistent and elevated levels of inflammatory factors, including IL-1β, IL-6, and TNF-α) [84]. The consequences of this inflammatory state are also especially dire at epithelial tissues where intraepithelial γδ T lymphocytes normally help maintain barrier function and protect from pathogens [85–88]. However, γδ T cells are sensitive to inflammation and their function

Table 14.1 Adipokines [8, 14, 18, 20, 65, 66, 72, 73]

Acute phase proteins, cytokines and chemokines	**Adhesion molecules and ECM components**	**Factors directly affecting metabolism**
α1-acid-glycoprotein	α2-macroglobulin	Adipocyte fatty acid binding protein (aP2)
Chemerin	Collagen I, III, IV, VI	Adiponectin
Chemokine (C-C motif) ligand 5 (CCL-5)	Fibronectin	Adipsin
CRP	Gelsolin	Apelin
Haptoglobin	Intercellular adhesion molecule-1 (ICAM-1)	Apolipoprotein E (apoE)
Interferon (IFN)-β and IFN-γ	Lysyl oxidase	Cholesteryl ester transfer protein
IL-1, 4, 6, 8, 10, 15, 17D, 18	Matrix metalloproteinase (MMP)-1,2,7,9,10,11,14,15	Leptin
IL-1Ra, sIL-1R, IL-1RII, sTNFR	Vascular cell adhesion molecule-1 (VCAM-1)	Lipoprotein lipase
IFN-γ inducible protein 10 (IP-10)		Omentin
Macrophage inhibitory protein-1 alpha (MIP-1α)		Resistin
Macrophage migration inhibitory factor (MIF)		Retinol binding protein 4
MCP-1		Vaspin
Plasminogen activator inhibitor (PAI)-1		Visfatin
Serum amyloid A3		
TNF-α		
Growth and angiogenic factors	**Other factors**	
Angiopoietin 1 and 2, angiopoietin-like proteins	Acylation stimulating protein (ASP)	
Fibroblast growth factors (FGF)	Angiotensinogen	
IGF-1	CORS-26	
Hepatocyte growth factor (HGF)	Complement-like factors	
Nerve growth factor (NGF)	Hepcidin	
Stromal derived factor-1 (SDF-1)	Nitric oxide	
Transforming growth factor (TGF)-α and β	Prostaglandin E2 (PGE2) and prostacyclin (PGI2)	
Tissue factor	Tissue inhibitor of metalloproteinases	
Vascular endothelial growth factor (VEGF)		

becomes impaired by obesity-associated inflammation. AT expansion results in the recruitment of cytotoxic CD8+ T cells and macrophages with increased release of TNF-α, IL-6, and IL-1β as well as depletion of regulatory T cells (Tregs). These events contribute to an increase in systemic inflammation that results in the dysregulation of γδ T cell function and subsequently a decline in barrier homeostasis which may lead to impaired wound healing, increased infections, and susceptibility to inflammatory disease [89]. Moreover, the strict relationship between AT and immune system has always been highlighted by the anatomical close contact between lymph nodes and AT (e.g. lymph nodes are generally surrounded by pericapsular AT). Histological examination of the outer capsule of lymph nodes reveals a fairly thin, loose layer of collagenous material, with numerous very fine lymph vessels that branch from the main vessel and enter the node over almost its entire surface [90]. Such tiny vessels are permeable to large molecules and certain small cells [91] and their arrangement increase the area of vessels passing through the AT immediately surrounding the node, where they may take up lipolytic products released by adjacent adipocytes into the extracellular space. Other sites where lymphoid tissues are in similar intimate contact with adipocytes include the omentum [91, 92] and bone marrow: [93] AT around lymph nodes is a specialized tissue whose function is strictly correlated with lymph node cell populations (e.g. lymph node lymphocytes and tissue dendritic cells (DCs) that acquire their fatty acids from the contiguous adipocytes potentially being influenced by them [94]. Therefore, lymphocytes are often found in close proximity to the adipocytes surrounding the lymph node; consequently, there may be paracrine relationships between the lymphocytes and adipocytes, allowing the exchange of information between the two [17]. However, despite the significative and non negligible presence of immune cells in AT as well as the close contact between lymph nodes and AT, researches into the functional association between AT and the immune system only began in the early 1990s, when adipsin secreted from adipocytes was shown to be identical to complement factor D produced in the immune system [95, 96]. Since

then, many more protein secretions and/or cytokine receptors have been described [97]. In most cases, cytokines such as TNF-α were isolated first from the immune system and later found to be secreted by and/or taken up by adipocytes, but others, notably leptin [98], were identified first in AT and later shown to modulate immune function.

Adipokines and Other Mediators: Role in Inflammation

AT is highly dynamic and interactive, playing a central signaling role in the regulation of energy homeostasis, appetite, inflammation, and insulin sensitivity. However in contrast to numerous and excellent publications describing its role in metabolic syndrome and atherosclerosis [99, 100], reviews on the inflammatory role of AT outside the field of metabolism are rare [18, 78, 101]. AT plays a major role in inflammation through the production of a huge class of mediators, the adipokines. Since both adipocytes, macrophages and other cell types present in AT are a non neglectable source of bioactive mediators, they might together perpetuate a vicious cycle of macrophage recruitment and production of pro-inflammatory cytokines, playing a major role in the development and sustainment of inflammatory conditions, especially including skin diseases also due to the anatomical and strict relationship between skin system and AT.

Adiponectin

Adiponectin, also known as Acrp30, AdipoQ, apM1, and GBP28, is a 30-kDa polypeptide highly and specifically expressed in differentiated adipocytes which circulates at high levels in the bloodstream [102]. It is mainly expressed in adipocytes, being higher in subcutaneous than visceral AT [103]. Adiponectin shows anti-inflammatory effects on endothelial cells through the inhibition of TNF-α induced adhesion-molecule expression [104], interferes with the function of macrophages [104, 105], induces the production of important anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonist (IL-1RA), by human monocytes, macrophages and DCs, hampers IL-6 production, inhibits the actions of TNF-α and suppresses the production of interferon (IFN)-γ by lipopolysaccharide (LPS) stimulated human macrophages [105, 106]. Moreover, the presence of adiponectin in T-cell proliferation assays results in a decreased ability to evoke an allogeneic T-cell response, and adiponectin also markedly reduces the phagocytic capacity of macrophages as well as TNF-α production by macrophages [106]. Adiponectin is also able to decrease endothelial vascular cell-adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1 and selectin expression [20] and, by decreasing reactive oxygen species (ROS), is an antioxidant [107]. Adiponectin, acting on natural killer (NK) cells, a key component of innate immune system, suppresses the IL-2-enhanced cytotoxic activity of NK cells without affecting their basal cytotoxicity [108] and is also able to inhibit Toll-receptor activation and its consequences [109]. In addition, it increases nitric oxide production in endothelial cells and stimulates angiogenesis. A strong and consistent inverse association between adiponectin and both insulin resistance and inflammatory states has been established [102, 110]. Taken together, these studies suggest that adiponectin is a unique adipocyte-derived hormone with anti-diabetic, anti-inflammatory, and anti-atherogenic effects. Indeed, adiponectin expression is decreased by pro-inflammatory cytokines such as TNF-α and IL-6 [111, 112] and, in obesity, due to the elevated level of these pro-inflammatory cytokines, serum adiponectin is reduced and negatively correlated with Body Mass Index (BMI) [113, 114].

Leptin

Leptin, a 16-kDa nonglycosylated peptide containing 167 amino acids, is mainly produced by adipocytes presenting as an important mediator of immune-mediated diseases and inflammatory processes [115]. Its synthesis is greater in subcutaneous AT rather than visceral AT [116]. Leptin is considered to be a pro-inflammatory cytokine and it has structural similarity to other pro-inflammatory cytokines such as IL-6, IL-12 and granulocyte-CSF [117]. It promotes proliferation and differentiation of hematopoietic cells, alters cytokine production by immune cells, stimulates endothelial cell growth and angiogenesis, and accelerates wound healing [28, 118]. In monocytes and macrophages, leptin increases the production of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-12 [119]. Leptin is able to stimulate the proliferation of human circulating monocytes in vitro and up-regulates the expression of activation markers such as CD25 (also known as IL-2Rα) and CD71 (the transferrin receptor) on these cells [120]. Furthermore, leptin also activates neutrophils, as assessed by increased expression of CD11b, stimulates neutrophil chemotaxis and the production of ROS by these cells, all of which are very important in innate immune responses and regulation of pathogen colonization of the skin and mucosa. This protein also regulates NK cells differentiation, proliferation, activation and cytotoxicity [121]. Further known direct actions of leptin on immune responses include the promotion of phagocyte function [122], the induction of the synthesis of eicosanoids [123] and nitric oxide [124], the protection of DCs from apoptosis and promotion of their LPS-induced maturation and of a cytokine production profile featuring low levels of IL-10 and high levels of IL-12, TNF-α and costimulatory molecules, which favours the proliferation of allogeneic CD4+ T cells [125]. Other major actions of leptin appear to occur on the level of adaptive immune responses, mainly in T cells regulation. Leptin induces cytokine producing capacity switch towards TNF-α and IL-2 [98, 126], particularly by increasing IFN-γ. Moreover, leptin causes generation, maturation, and survival of T cells, protecting them from apoptosis. It should however be noted that in models of severe inflamma-

tion, leptin appears to exert suppressive effects which are contrary to those described above leading to decrease of Th1 type cytokines, increase of Th2 cytokines and reduction in T cells proliferation. Thus leptin effects on the immune system appear to depend not only on the leptin concentrations, but also on the status of the immune system [127]. The complexity of the picture is increased by findings that leptin deficient mice show resistance to certain autoimmune diseases and the susceptibility is recovered by leptin administration [128, 129]. Indeed, leptin may lead to enhancement of autoimmune reactions, in part by reducing Tregs; in addition, its levels are reported to be increased in patients with autoimmune diseases [126]. Moreover, inflammatory cytokines, including TNF-α, and IL-1 are able to induce leptin production [130] with leptin deficiency itself being a known cause of impaired T cell-mediated immunity [131].

Resistin

Resistin, also known as ADSF and FIZZ3, is a 12.5 kDa protein secreted from adipocytes and macrophages that mostly circulates as a high-molecular-weight hexamer, appearing to have many features of an inflammatory cytokine [132]. Greater levels of resistin expression in AT are displayed by monocytes and macrophages versus adipocytes [133]. In humans, resistin appears to play a role in inflammation regulated by pro-inflammatory cytokines, including TNF-α and IL-6 which are also produced by AT. Indeed, resistin stimulates the production by various cell types of inflammatory cytokines such as IL-1, IL-6, IL-12 and TNF-α through a NF-kB-dependent pathway [134–136]. Particularly, even if resistin is minimally expressed in primary adipocytes, these may be target cells for resistin itself: it has been demonstrated that resistin could induce the expression of IL-6, IL-8, and TNF-α by AT [137]. In addition, resistin is also able to up-regulate endothelial cell production of ICAM-1, VCAM-1, endothelin-1 and MCP-1 [138, 139], thus producing a biochemical profile of dysfunctional endothelium. Moreover, resistin mRNA has been also found in human peripheral blood mononuclear cells (PBMCs) and it was reported that pro-inflammatory mediators such as IL-6, TNF-α, IL-1β, IL-12 or LPS can strongly increase the expression of resistin in PBMCs, and that resistin itself is able to stimulate its own production and the secretion of TNF-α, IL-1β, IL-6 and IL-8 in PBMCs as well as IL-12 in macrophages, creating a vicious cycle and suggesting a role in the process of inflammation [134–136].

Visfatin

Visfatin, also known as PBEF, has recently been identified as an adipocytokine that is secreted by adipocytes [140]. However, visfatin is not only produced by AT, but also by endotoxin-challenged neutrophils, in which it prevents apoptosis through a mechanism mediated by caspases 3 and 8 [141]. Circulating visfatin levels are closely correlated with AT accumulation. Visfatin mRNA levels increase in the course of adipocyte differentiation, and its synthesis is regulated by several factors, including glucocorticoids, TNF-α, IL-6 and GH [142]. This molecule binds to and activates the insulin receptor but does not compete with insulin, which indicates that the two proteins bind different sites on the insulin receptor. Visfatin was originally identified more than 10 years ago and since then, it has been linked to several inflammatory disease states [143, 144]. Furthermore, expression of visfatin has been shown to be up-regulated in activated neutrophils and to inhibit their apoptosis [141]. It has been shown that visfatin is able to induce chemotaxis and the production of IL1-β, TNF-α, IL-6 and co-stimulatory molecules by CD14+ monocytes, and to increase their ability to induce alloproliferative responses in lymphocytes [145]. By induction of co-stimulatory molecules such as CD80, CD40 and ICAM-1 visfatin promotes the activation of T cells [146]. Visfatin was also identified in inflammatory cells (lymphocytes) and its levels were reported to be increased in various inflammatory conditions [140]. However, future studies of the cell biology of this natural insulin mimetic and potential inflammation-regulating adipokine should help to define its role in insulin resistance and associated inflammatory disorders.

Chemerin

Chemerin is a 18 kDa chemokine, also known as TIG2 and RARRES2, that was found to be expressed primarily by mature adipocytes and found in ng/mL ranges in human plasma [147]. Various cell types involved in innate and adaptive immunity [plasmacytoid DCs (pDCs), myeloid DCs, macrophages and NK cells] express the orphan G protein-coupled receptor chemokine-like receptor-1 (CMKLR1), and chemerin is now known to function as a chemoattractant that promotes the recruitment of these cells to lymphoid organs and sites of tissue injury acting hence as a pro-inflammatory agent [148–151]. For example, it was found to promote the clustering of the Very Late Antigen (VLA)-4 and VLA-5 integrins at the cell surface, leading to adhesion of macrophages to VCAM-1 and fibronectin [152]. Serum chemerin levels correlate with levels of the pro-inflammatory cytokines TNF-α, IL-6 and CRP [153, 154]. Moreover, chemerin, whose synthesis is increased by TNF-α and Il-1β [155], seems to be able to decrease adiponectin and leptin expression [147]. While initial studies suggested that chemerin might modulate adipogenesis [156, 157] and that functional chemerin receptors were present on both immune cells and AT, little is known of its endocrine or paracrine roles.

Adispin, Apelin, Hepcidin and Vaspin

Adipsin, also known as complement factor D, is a serine protease mainly produced by adipocytes as well as monocytes and macrophages resident in fat [158, 159]. It presents high

levels of expression in AT and its circulating concentrations tends to correlate positively with the degree of adiposity, being elevated in obesity and reduced in individuals with total lipoatrophy, AIDS related cachexia and anorexia nervosa [160]. Adipsin mediates the rate-limiting step in the complement activation alternative pathway, underlying the role of AT in immune system biology [158, 159].

Apelin, a recently identified adipokine, which acts thorough the binding to a specific G-protein-coupled receptor named API, present on endothelial cells, vascular smooth myocytes, and cardiomyocytes [161, 162]. It is synthesized in adipocytes and strongly up-regulated by insulin; high plasma apelin levels were found in obese humans [163]. TNF-α may act as a key player in the up-regulation of apelin expression in adipocytes both in obese and lean humans [164]. It has been reported that apelin has a regulatory effect on neoangiogenesis, lymphangiogenesis and fibrogenesis [165, 166].

Hepcidin, first described as a small antimicrobial factor, is a peptide of 25 amino acids which regulates iron homeostasis inhibiting iron absorption by enterocytes, iron release from macrophages, and iron transport across the placenta [167, 168]. Even if it is mainly produced by the liver, AT is able to express hepcidin at both mRNA and protein levels and this expression is reported to be enhanced in obese patients [169]. Levels of hepcidin correlate with levels of CRP and IL-6 [168, 169] and they are reported to be associated with obesity but not liver disease [170]. Indeed, hepcidin expression in AT appears to be stimulated rather by inflammatory stimuli than by iron, particularly through IL-6/STAT3 pathway [171, 172].

Vaspin (visceral adipose-tissue derived serine protease inhibitor), also known as serpinA12, has similarities to adiponectin in that it improves insulin sensitivity. It is a serine protease inhibitor mainly but not exclusively produced by AT (e.g. it can be secreted by skin, stomach, hypothalamus, etc.) which is able to reduce levels of leptin, resistin, and TNF-α; [173, 174] it was also reported to be able to protect endothelial cells from pro-inflammatory cytokines induced inflammation and expression of adhesion molecules [175, 176] showing its multiple potential anti-inflammatory effects.

TNF-α

TNF-α is a cytokine present as either a 26 kDa transmembrane monomer or a 17 kDa soluble molecule. The TNF-α from AT is thought to be primarily produced by macrophages though isolated adipocytes are also known to produce this cytokine [59, 177]. Adipocytes also express both types of TNF-α receptors as membrane bound and soluble forms [178]. TNF-α is a powerful local regulator within AT, acting in both an autocrine and a paracrine manner to influence a range of processes, including apoptosis [97]. Moreover, TNF-α changes the expression of several adipocyte secreted factors, being also the key regulator of several pro-inflammatory compounds; particularly, it enhances the production of IL-6, MCP-1, resistin and visfatin whereas it is able to decrease adiponectin and leptin concentrations [179–181]. TNF-α stimulates adhesion of monocytes to the surface of endothelial cells by enhancing the expression of adhesion molecules (ICAM-1, VCAM-1) being a key cytokine of inflammatory processes [182]. AT expression of TNF-α is increased in obese humans and is positively correlated with adiposity and insulin resistance [178, 183] so that it appears as one of the key links between metabolic disorders and TNF-α mediated skin diseases [e.g. psoriasis and the development of the so-called psoriatic march [184] as well as hidradenitis suppurativa (HS) [185, 186]; indeed, both skin disorders are linked with obesity and metabolic syndrome].

IL-6

Approximately 20–30 % of IL-6 in the circulation is produced by AT [187]. However, IL-6 can be secreted by numerous cell types present in AT with macrophages contributing up to 50 % of AT-derived IL-6 [16] with around 10 % of circulating IL-6 being attributed to synthesis by adipocytes [61]. Within AT, IL-6 and IL-6R are expressed by adipocytes and AT matrix [59]. Like leptin, production of IL-6 by AT increases with increasing adiposity, and circulating IL-6 concentrations are highly correlated with percentage of body fat [188]. Expression and secretion of IL-6 are 2–3 times greater in visceral relative to subcutaneous AT [48, 59]. IL-6 synthesis and secretion by adipocytes is thought to be constitutive but is also stimulated by numerous factors including β-adrenergic agonists [187] and IL-1β [189]. Initial studies also indicated that endothelin-1 may induce adipocyte secretion of IL-6 [190]. Several studies have associated IL-6 polymorphisms with obesity [191] but clear mechanisms have yet to be identified. Circulating IL-6 is the single most important factor controlling the hepatic acute-phase response, the rapid, coordinated physiologic reaction to tissue damage or infection designed to recruit host defense mechanisms, eliminate damaged cells, contain pathogens, and begin tissue repair [57]. Indeed, IL-6 is known to increase the secretion of CRP, a clinically used marker of inflammation, as well as the synthesis of other acute phase response proteins including fibrinogen, serum amyloid-A and α-1 antichymotrypsin [192]. Moreover, IL-6 is also able to inhibit adipogenesis and decrease secretion of anti-inflammatory factors such as adiponectin.

Other Cytokines

Numerous other cytokines are present in AT. For example, IL-15 is secreted from AT, has several functional similarities to IL-2, and it is thought that these two interleukins negatively regulate one another. IL-10 secretion from human AT

has also been described [20]. The expression of IL-17D has been showed in adipocytes; [193] it seems that this cytokine is able to stimulate the production of IL-6 and IL-8 from endothelial cells [193]. IL-8 is secreted from both AT explants and cultured adipocytes [194] though others have suggested that IL-8 expression and production is primarily from non-adipocyte cells found in AT [195]. Production and concentrations of IL-8 appear to be AT depot dependent and stimulated by TNF-α [194, 196], differentially regulated in lean and obese men [197] as well as women [198] and influenced by plasma non-esterified fatty acids in overweight men and women [199]. IL-8 production in AT may provide another link between AT and obesity associated diseases. In addition, several reports indicate that circulating levels of IL-18 are elevated in obesity and that they fall following weight loss [200, 201], raising the possibility that AT may be an important direct source of this cytokine in plasma. Indeed, adipocytes and the stromal-vascular fraction of AT are also able to produce IL-18, raising the possibility of cross talk between adipocytes and other cellular components within the tissue [202]. IL-18 acts as a pleiotrophic pro-inflammatory cytokine inducing the expression of chemokines, cytokines, angiogenesis-related, and adhesion molecules such as IL-8, TNF-α, vascular endothelial growth factor (VEGF), and ICAM-1 [203–205]. Even if TNF-α stimulation is able to significantly increase IL-18 production in adipocytes [202] they seem unlikely, however, to contribute significantly to the circulating levels of this cytokine and to the increased levels associated with obesity; therefore, in contrast to other inflammation related factors such as IL-6, TNF-α, MCP-1, and adiponectin, IL-18 cannot be considered a major adipokine.

MCP-1

MCP-1, also referred to as chemokine (C–C motif) ligand (CCL)2, is a chemokine that recruits monocytes to sites of inflammation and has been shown to induce insulin resistance and macrophage infiltration in AT [206]. MCP-1 is expressed and secreted by both adipocytes and stromal-vascular cells [207]. Increased circulating MCP-1 is associated with increased circulating monocytes [208] and MCP-1 expression appears to be highly regulated by TNF-α [209] and, to a lesser extent, by IL-6 and GH [210]. MCP-1 exhibits depot-dependant differences in expression [55] and appears to respond to surgically induced weight loss with a decrease in gene expression [211]. Obesity is associated with increased AT infiltration by macrophages [16, 207] and activated macrophages are able to secrete inflammatory factors, including TNF-α and IL-6. MCP-1 is increased by leptin, obesity, and insulin-resistance-inducing hormones [212].

CRP

The possibility that AT directly contributes to the circulating pool of CRP is suggested by a study which showed that the gene encoding CRP is expressed in AT [213]. However, it is not clear whether CRP expression in adipocytes is significant. Indeed, it seems that AT is not so able to produce significant amounts of CRP [18]. However, IL-6 is highly secreted by AT in obesity and this is the major cytokine regulating the hepatic production of CRP [57, 214]. Thus, AT may be a major player in the raised circulating levels of CRP in obesity, but through the indirect route of adipocyte-derived IL-6. The circulating level of CRP rises with BMI [215, 216], and elevated levels of this inflammatory marker have been associated with both obesity and diabetes, falling with weight loss [217].

As showed above, AT is able to produce plenty of factors so that it could deeply influence the inflammatory response and immune system. Even if it is important to note that these mediators are not all exclusively derived from AT, the factors mainly produced by adipocytes (e.g. adiponectin, leptin and to a less extent visfatin and resistin), can circulate at high concentrations making AT an active and complex actor in inflammation and immunity (Table 14.2).

Adipose Tissue in Dermatologic Inflammatory Diseases

As the outermost protective barrier of our body, the skin consists of three layers: epidermis, dermis and a subcutaneous layer which is mainly composed of AT. Given that AT is able to secrete various bioactive proteins, it is not surprising that it may have dynamic functions in skin physiology and pathophysiology. For example, by producing various interleukins, subcutaneous AT may regulate B- and T-lymphocytes in concert with the epidermal keratinocytes, deeply influencing cutaneous inflammation and the development of several skin diseases [1]. Without any doubts, psoriasis represents the dermatologic inflammatory disorder which has been most intensively investigated regarding relationships with increased fat mass and adipokines. However, the literature is also constantly enriched with studies which try to elucidate the role of AT and adipokines in different skin diseases, such as atopic dermatitis (AD), HS, etc., highlighting the growing interest in this topic.

Psoriasis

Psoriasis is a chronic skin inflammatory disease which is now considered a systemic immune mediated disorder. It is well established that obesity is a risk factor for psoriasis [218, 219]. Obesity is one of the most common psoriasis comorbidities. Indeed, obesity prevalence is significantly higher than in general population [218, 220] and it appears to be associated with increased morbidity of psoriasis [221, 222]. Psoriasis is positively correlated with higher BMI, which is

Table 14.2 The role of the main distinctive adipokines in inflammation and immunity [8, 117, 127]

Adipocytokines	Inflammatory effect	Effects on Innate immunity	Effects on adaptive immunity
Adiponectin	Anti-inflammatory	↓Endothelial adhesion molecules ↓ NF-kB ↓ TNF-α ↓ IL-6 ↓ IFNγ ↑ IL-10 ↑ IL-1RA ↓ Phagocytosis	↓ B-cell lymphopoiesis ↓ T-cell responses, activation and proliferation ↓ T-cell activation and proliferation
Leptin	Pro-inflammatory	↑ TNF-α ↑ IL-6 ↑ IL-12 ↑ Neutrophil activation ↑ ROS ↑ Chemotaxis ↑ NK-cell function	↑Lymphopoiesis ↑ Thymocyte survival ↑ T-cell proliferation ↑ T-cell activation ↑ TH1 response (IL-2 and IFNγ) ↓ TH2 response (IL-4)
Resistin	Pro-inflammatory	↑ TNF-α ↑ IL-1β ↑ IL-6 ↑ IL-12 ↑ NF-kB ↑ Endothelial adhesion molecules	–
Visfatin	Pro-inflammatory	↑ IL-6 ↑ IL-8 ↓ Apoptosis of neutrophils	–

also associated with more severe psoriasis and negatively impacts long-term treatment options [223–225]. Moreover, there is increasing evidence that progressive weight loss can produce significant improvements in the severity of psoriasis [226–228] and another direct evidence that obesity may be causal in psoriasis is the fact that bariatric surgery can produce rapid remission from psoriasis [227, 229, 230]. Obesity, with its low-grade systemic inflammation state, and in particular AT, with its immune and endocrine roles, are able to influence the pathogenesis and the development of psoriasis in numerous different ways. For example, the consequences of obesity induced chronic inflammatory state are also especially dire at epithelial tissues with the resulting dysregulation of γδ T cells which may contribute to susceptibility to psoriasis in obese subjects. Indeed, a reduction of a subset of γδ T cells (Vγ9Vδ2) in peripheral blood and their subsequent increase in the epidermis has been documented in psoriasis patients [231]. These cells were able to produce inflammatory cytokines and mediate crosstalk with keratinocytes, suggesting a role for these T cells in the development and the exacerbation of the dermatosis [231]. However, a crucial role in the connection between obesity and psoriasis is indubitably played by the adipokines secreted by AT. Various cells in AT are able to produce TNF-α, as well as other cytokines involved in psoriasis, such as IL-1, IL-6, IL-17 and IFN-γ [20, 66, 97, 232]. The abnormal adipokine levels reported in psoriasis suggest that the systemic inflammation associated with the disease may be linked with AT inflammation, similar to that seen in obesity. Central obesity is associated with greater amounts of inflammatory visceral fat, which is more hypertrophied, contains more macrophage infiltration, has an increased presence of activated T cell populations and expresses a more pro-inflammatory cytokine profile, marked by increased TNF-α, IL-6 and IL-17, and decreased adiponectin [16, 77, 233]. Interestingly, a similar inflammatory state of activated T cells and increased pro-inflammatory cytokines has been described in psoriasis, and is thought to be responsible for the induction of psoriatic plaque formation [234]. All the above mentioned factors, as well as other adipokines such as leptin, are recruited and stimulated in obesity and may have an autocrine and paracrine effect on nearby skin [232, 235]. In particular, leptin levels (both serum and tissue leptin) are enhanced in psoriasis also due to possible functional polymorphism of its gene [236–238] and their concentrations have been also shown to correlate with psoriasis severity [237]. In addition, tissue leptin receptor expression is reported to be significantly higher in patients with severe psoriasis than in patients with mild–moderate psoriasis and controls [237]. Leptin decreases T cells autoregulation, is potentially involved in inflammatory processes stimulating cytokine release and its raised levels may mediate proliferative and antiapoptotic activities in a number of cell types including T cells as well as the increased production of pro-inflammatory cytokines such as IL-6 and TNF-α, some of the major cytokines acting in psoriasis pathogenesis [126, 239, 240]. Moreover, leptin synergistically with IL-1β enhances

the production of the antimicrobial peptide human βdefensin-2 (hBD2), whose levels are elevated in psoriasis [241, 242]. In particular, hBD2 seems to be involved in the development of skin inflammation by binding to the CC chemokine receptor 6, consequently inducing chemotaxis of memory T cells or immature DCs and the production of IL-6, CCL20, and CCL5 in keratinocytes [243, 244]. The released hBD-2 may in turn act on keratinocytes, inducing their proliferation and production of pro-inflammatory cytokines/chemokines in an autocrine/paracrine manner, sustaining the dysregulations observed in psoriasis [243]. Thanks to all these proprieties, leptin is able to deeply regulate immune response, being involved in psoriasis pathogenesis, especially its effects on T cells differentiation to Th1 phenotype, induction of pro-inflammatory cytokines by keratinocytes and promotion of keratinocyte proliferation as well as angiogenesis [126, 245–248]. However, there are other several adipokines which are both dysregulated in obesity and psoriasis. For example, resistin leads to up-regulation of inflammatory processes, including TNF-α secretion, is increased in serum of patients with psoriasis, correlating with obesity and increased severity of psoriasis [249, 250]. These evidences support the view that resistin may be involved in the pathogenesis of psoriasis, especially in overweight individuals, possibly by augmenting cytokine expression by the inflammatory infiltrate. On the other hand, levels of adiponectin are lower in psoriatic skin compared with controls (also independently of cardiometabolic risk factors) and in obese psoriatic patients versus normal weight psoriasis subjects [251, 252]. In addition, it has been shown that patients with psoriasis, plasma adiponectin negatively correlated with psoriasis severity and that successful anti-inflammatory treatments may elevate adiponectin levels [253, 254]. All these observations constitute some indirect evidence that the immunological and metabolic alterations associated with obesity may be linked with the pathophysiology of psoriasis. Further data which highlight the complexity of the biologic relationship between AT and psoriasis came from Albanesi et al. who reported elevated serum chemerin levels in psoriatic patients and expression of chemerin in early psoriatic lesions as well as pre-psoriatic skin adjacent to active lesions, in parallel to infiltration by pDCs and neutrophils [255]. However, in chronic plaques low chemerin levels and low pDCs infiltration were found whereas immunoreactive chemerin was present in fibroblasts, mast cells and endothelial cells in early lesions [255, 256]. Taken together all these evidences suggested a role for chemerin in the early phases of psoriasis development. Conversely, the relationship between increased fat mass, psoriasis and adipokines alteration is not always so clear and linear as is highlighted by few papers which show controversial results compared with the above mentioned [250, 257]. In this context the case of an adipokine, such as vaspin, is paradigmatic since it is reported to be elevated in serum of obese subjects [258] whereas serum vaspin is not enhanced in psoriasis patients, being also entirely independent of BMI. Moreover, its expression is decreased in lesional psoriatic skin [259, 260]. However, the literature is full of evidences regarding the strict link between obesity and psoriasis; nevertheless, a causal relationship between them has not been fully established yet. Obesity may occur as a consequence of psoriasis or the obese state may well exacerbate the severity of the disease.

Atopic Dermatitis

AD is a chronic, recurrent, pruritic inflammatory disorder of the skin that has reached epidemic proportions throughout the world, especially in children and adolescents who also showed significantly increased rates of obesity versus previous decades [261–265]. Several studies reported that obesity, particularly when it begins early in life, is associated with increased risk and severity of AD in children whereas other investigations provided evidence of an association between obesity and increased AD also in adult individuals, proposing AD as yet another harmful consequence of obesity [266–270]. To date, the mechanism behind the association of obesity and AD remains unclear. However, few studies indicated that the immunomodulatory effects of obesity might be most profound during the first 2 years of life suggesting a possible effect on the immature immune system. Indeed, obesity is associated with a variety of immune sequelae, including accumulation of macrophages and cytotoxic T cells in AT, production of TNF-α and IL-6, and increased secretion of leptin. This has numerous pro-inflammatory effects on the immune system, including proliferation and activation of circulating monocytes and CD41 and CD81 T cells, polarization of T cells toward a Th1 response, activation of NK cells, promotion of neutrophil chemotaxis and upregulation of numerous cytokines [16, 98, 121, 129, 271–273]. Indeed, the prevalence of AD is reported to be somewhat increased in children with high leptin levels [274]. In addition, obesity is associated with low levels of adiponectin, whose reduced expression has also been detected in AD subjects [274]. Moreover, recently Machura et al. reported that children with AD showed elevated apelin concentrations, apelin/BMI ratio and resistin levels versus healthy children despite similar body weight whereas Suga et al. observed that visfatin levels were increased in both serum and skin lesions of AD patients suggesting that these adipokines may be implicated in AD immunopathogenesis [275, 276]. However, studies on visfatin in AD children are controversial since another survey reported lower serum concentration versus healthy controls [275]. Finally, it is also reported that non obese children with AD show an increased prevalence of fatty liver, a known risk factor for lifestyle-related diseases such as obesity, versus non obese healthy controls [277]. The reasons for this association is not well elucidated but it is speculated that AD subjects may present an enhanced permeability of the small intestine which

leads to fatty acid dysregulation which is involved in the pathogenesis of both AD and fatty liver [278, 279].

Hidradenitis Suppurativa

HS is a chronic inflammatory skin disorder characterized by recurrent, painful nodules, abscesses, and draining sinuses, with resultant scarring. Obesity has been associated with HS in up to 75% of cases and, compared to controls, HS was significantly associated with higher BMI [280–282]. In addition there seems to be a relationship between obesity and the severity of HS so that obesity is considered as a HS severity risk factor [283, 284]. Moreover, weight loss of more than 15% is reported to be associated with a significant reduction of disease severity [285]. In general, it seems that obesity is able to confer a 1.2-times greater risk for developing HS [282]. Consequently, obesity is considered to participate in the pathophysiology of this skin disorder [280, 281]. A disturbance of the cutaneous innate immune system has been implicated in the key pathogenic steps of HS such as follicular occlusion and chronic inflammation [281]. Moreover, HS has been associated with abnormal expression of antimicrobial proteins, deficiency of IL-20 and IL-22, and elevated TNF-α, IL-1β, and IL-8 levels leading to sustained inflammation and chronic pro-inflammatory state [186, 286–289]. Since AT is a significant source of different pro-inflammatory molecules, which levels are up-regulated when AT is excessively present such as in obesity, it is not unreasonable to hypothesize that AT and its secretory products may participate in the pathomechanisms of HS and the related cutaneous immune system dysregulations. Indeed, obesity is causally linked to a systemic low-grade inflammatory state with increased levels of pro-inflammatory cytokines in the blood (e.g. IL-1, IL-6, IL-8, TNF-α, CRP) [97, 290, 291] and many of these factors are enhanced and involved also in HS pathogenesis. Furthermore, PBMCs in the obese are in a constant pro-inflammatory mode and demonstrated a significant increase in NF-kB binding and an increase in the transcription of pro-inflammatory genes regulated by NF-kB in these cells [292]. Therefore, even if obesity may not be the primary causative factor in HS, the above mentioned data suggest how it may contribute to HS pathogenesis. However, since a pro-inflammatory state has been also thought to be the driving force for an increase in obesity and metabolic syndrome, to date it is not known if the HS triggers these pathologies or if obesity and metabolic disorders predispose the individual to the development of the inflammatory skin disorder.

Other Skin Diseases

Several links between adipokine dysregulation and skin inflammatory diseases, pathologies which are characterized during their natural course by cutaneous inflammatory phases and/or a more widespread alteration of the immune system are reported in literature. For example, laboratory assessments of patients with systemic lupus erythematosus (SLE) indicated elevated leptin levels which might contribute to systemic inflammation even if to date the clinical and pathogenic significance of this elevation remains unknown [293–296]. However, Yu et al. showed that leptin was able to promote Th17 responses in normal human CD4+ T cells, both in vitro and in vivo, by inducing RORγt transcription. Since Th17 cells play an important role in the development and maintenance of inflammation and autoimmunity, the authors hypothesized the involvement of leptin in autoimmunity and in the connection between metabolism/nutrition and susceptibility to autoimmunity [297]. Several other adipokines are reported to be altered in SLE. Indeed, SLE was independently associated with higher resistin levels and the presence of an association between resistin and inflammation, low complement levels, bone mineral density, and renal function is suggested [298, 299]. In addition, lupus erythematous skin lesions significantly express chemerin (in the endothelium of dermal blood vessels and venules of secondary lymphoid organs) and harbor CMKLR-expressing pDCs, suggesting that chemerin is involved in the recruitment of these cells and other leukocyte populations in this disease [148, 300]. Moreover, visfatin concentrations are reported to be significantly higher in patients with SLE which might reflect inflammation in this systemic disease [293]. However, few other surveys showed controversial data investigating adipokines profile in SLE patients (e.g. lower or unchanged levels of leptin and no significant differences for resistin) [301, 302].

In regard to other disorders, serum leptin concentrations were reported to be significantly increased in systemic sclerosis (SSc) patients versus healthy subjects with similar BMI, however without being significantly correlated or associated with disease duration, clinical activity score, skin score, CRP and antinuclear antibody (ANA) test results [303]. The possible involvement of adipokines in SSc development (where inflammation occurs prior to fibrotic response) is highlighted also by Masui et al. who reported that adiponectin serum levels where significantly lower in patients with diffuse cutaneous SSc (dcSSc) versus those with limited cutaneous SSc and that these levels inversely correlated with the activity of progressive skin sclerosis in dcSSc patients, suggesting that adiponectin serum concentrations may serve as a useful marker to evaluate the activity of progressive skin sclerosis in dcSSc [304]. Consequently, the authors hypothesized that decreased serum levels of an anti-inflammatory factor such as adiponectin may be associated with increased IL-6 levels through modulating the TNF-α action, which promotes the inflammatory process in early dcSSc leading to resultant fibrosis. Indeed, other authors investigating adiponectin, IL-6, IL-2, CRP, ANA and antibodies to extractable nuclear antigens levels in 39 SSc patients speculated that adiponectin could play a protective role in skin related changes during SSc by

observing that lower adiponectin serum levels were associated with an advanced stage of skin fibrosis and also because adiponectin is reported to exert anti-fibrotic proprieties [305, 306]. All these findings are reinforced by the study of Arakawa et al. who showed that adiponectin mRNA levels were reduced in skin tissues from patients with dcSSc [307]. Finally, another example could be represented by sarcoidosis. Indeed, very recently Harpsøe et al. conducted a study on 75,008 women who were followed during a median time of 11 years in order to investigate a possible aetiological link between obesity and certain autoimmune diseases [308]. In the sub-group of obese women a high risk was observed for sarcoidosis. Nevertheless its aetiology remains unknown; the disease involves immunological changes similar to those seen in obesity, including TNF-α production [309, 310] and its development may depend on a combination of genetic factors, the triggering antigen itself and immune system status in which the immunologic alterations caused by obesity could play a role [311].

Conclusion

AT has both immune and endocrine roles producing numerous molecules including cytokines, chemokines, growth factors, hormones etc. Therefore, given its proprieties and widespread localization in the human body which is also responsible for the strict and extensive contact between skin and subcutaneous AT, it is indisputable that AT is able to significantly influence the immune responses, the immune skin system and consequently the pathogenesis of several cutaneous inflammatory disorders. We have just shown above the main examples of this interesting relationship trying also to highlight its numerous different molecular and biological basis. Without any doubts, further investigations are needed to deeply investigate AT functions and its ability to regulate skin immune system and cutaneous inflammation.

Questions

1. What cell type does NOT constitute normal adipose tissue?
   A. Adipocytes and pre-adipocytes
   B. Vascular cells
   C. Stromal cells
   D. Lymphoid/hematopoietic cells
   E. Neutrophils

Correct answer: (E) Neutrophils do not normally constitute adipose tissue. This cell type can migrate into adipose tissue during inflammatory responses.

2. How can the adipose tissue act as an endocrine organ?
   A. Secretion of hormone s such as leptin and IGF-1
   B. Producing GH
   C. Producing insulin
   D. Modulating TSH

Correct answer (A) Secretion of hormones such as Leptin and IGF-1. Adipose tissue does not produce GH, Insulin or modulate TSH.

3. What is the principal adipokine and its relative functions?
   A. Insulin, which controls appetite.
   B. Leptin, which regulates growth, metabolism, and behavior
   C. Growth hormone, which repairs tissue damage
   D. Testosterone, which controls sex drive.

Correct answer: (B) Leptin controls growth, metatolism and behavior. None of the other hormones are derived from adipose tissue, and are thus not considered to be adipokines.

4. What is the role of adipose tissue in a chronic inflammatory skin disease such as psoriasis?
   A. Metabolize drugs used to treat psoriasis
   B. Enhances the absorption of lipophilic drugs such as acitretin
   C. Plays a role in suppressing immune responses
   D. Cells in adipose tissue produce inflammatory cytokines involved in pathogenesis of psoriasis

Correct answer: (D) Inflammatory cells that reside in adipose tissue can produce a number of cytokines involved in psoriasis, such as IL-1, IL-6, IL-17 and IFN-γ.

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Cytokines and Chemokines

15

Matthew J. Turner

Abstract

Cytokines are small proteins used for intercellular communication in the immune system and beyond. They are subclassified based on membership in tumor necrosis factor, interleukin, interferon, transforming growth factor beta and chemokine superfamilies. Readily observed and easily accessible for manipulation and isolation, the skin is an excellent model system for studying the biology of cytokines. Important insights into mechanisms of cutaneous inflammation and immunity in health and disease have been and continue to be translated into the practice of dermatology and other areas of medicine. Cytokine-targeted therapies are now commonplace in the practice of dermatology, with many cytokine or cytokine receptor-target therapies in clinical trials for dermatologic diseases. As the list of available therapeutics grows, it is incumbent upon physicians to understand when and how to use these medications for safe and effective outcomes. To achieve this goal, a sound understanding of mechanisms of cutaneous inflammation and immunology mediated by cytokines is important.

Keywords

Cytokines • Chemokines • JAK-STAT signaling • Macrophages • Dendritic cells • Keratinocytes • Interleukins • Interferon family • T cell trafficking

Introduction

Cytokines are structurally and functionally heterogeneous soluble proteins that, following active or passive release from cells, bind and signal through ligand-specific receptors on target cells. While it may be evident that signals transduced by cytokines are dependent on the cytokine and its receptor (s), other key determinants dictating functional consequences of cytokine receptor engagement include the cell type expressing the receptor and the niche where it resides. In this way, the human body uses a relatively small number of soluble molecules to orchestrate an almost incomprehensible number of developmental and effector functions. The influence of cytokines on T helper (Th) cell development is an excellent example of this concept and will be referred to throughout this chapter (Fig. 15.1). For example Th9 cell development requires a dendritic cell to present antigen and provide proper co-stimulation to a naïve T cell in the lymph node in the presence of the cytokines, interleukin 4 (IL-4) and transforming growth factor beta (TGF-β). In contrast, omitting TGF-β leads to Th2 cell development (Fig. 15.1). The ensuing sections attempt to focus on those aspects of cytokine production and function involved in normal and pathologic aspects of inflammation and immunity in the skin. After a brief overview of JAK/STAT signaling, this chapter will describe the biology of individual cytokines in the context of the superfamilies to which they belong; these superfamilies are defined as tumor necrosis factor (TNF), interleukin, interferon, transforming growth factor beta (TGFβ) and chemokine. To illustrate how cytokines contribute to cutaneous inflammation in

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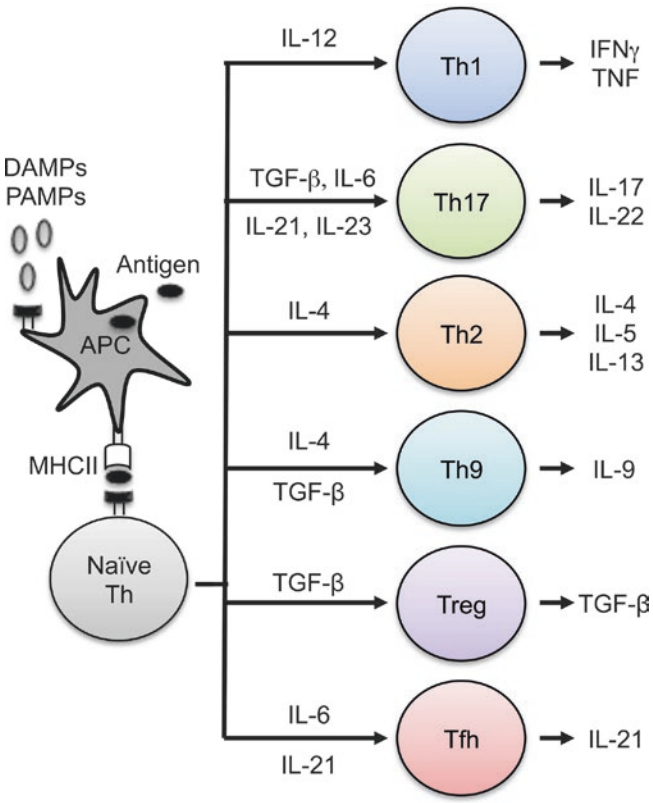

Fig. 15.1 Effect of cytokines on T helper (*Th*) cell development. Schematic depicts a mature dendritic cell (*APC*) in the lymph node presenting antigen (*black oval*) in the context of an MHC class II molecule to a naïve Th cell. Damage- or pathogen-associated molecular patterns (*DAMPs and PAMPs*) promote maturation and cytokine production by the APC. Development of Th subsets (*Th1, Th2, Th9, Th17*), $CD4^{+}FoxP3^{+}$ regulatory T cells (*Treg*) and follicular helper cells (*Tfh*) is dictated by interaction with the APC and specific cytokines or combinations of cytokines including interleukins (*IL*) and transforming growth factor beta (*TGF-β*). These Th subsets in turn produce specific cytokines including interleukins, TGF-β and interferon gamma (*IFNγ*) (Adapted from figure courtesy of Mark H. Kaplan, Ph.D.)

dermatologic disease and summarize much of the information presented in this chapter, Table 15.1 synthesizes many of the important characteristics of cytokines implicated in psoriasis or atopic dermatitis (AD) pathogenesis.

JAK-STAT Signaling

Cells use cytokines to communicate with themselves and other cells via cytokine-specific receptors. Receptor engagement activates intracellular signaling pathways that dictate target cell responses. Regulation of target gene expression is an important effect of cytokine stimulation. Many cytokines, namely interleukins and interferons, use receptors that activate Janus kinases (JAK1, JAK2, JAK3 and Tyk2) to initiate signal transduction; Janus kinases in turn phosphorylate signal transducer and activator of transcription (STAT) proteins which include STAT1, STAT2, STAT3, STAT4, STAT5 and STAT6. Phosphorylated STAT proteins then homo- or heterodimerize and translocate to the nucleus where they regulate cytokine-dependent target gene expression (Fig. 15.2). Janus kinase inhibitors are currently in clinical trials for psoriasis and atopic dermatitis. Thus, understanding the concepts of how JAK and STAT proteins function will likely become more important to the practice of dermatology. The specific JAK and STAT proteins used by individual cytokines will be discussed in the coming sections.

TNF Family

The tumor necrosis factor superfamily (TNFSF) originally consisted of TNF and lymphotoxin; at least 16 more members have been identified [1]. In addition to immunomodulation, some TNFSF members also regulate processes like organogenesis, epithelial cell development and bone remodeling [2]. Some of the other family members are linked to pathogenesis of rare diseases like X-linked hypohidrotic ectodermal dysplasia, Stevens-Johnson Syndrome/Toxic epidermal necrosis and hyper IgM syndrome, but the most important by far to the current understanding of cutaneous immunology, pathogenesis and therapy for dermatologic diseases is TNF [3–5].

The TNFSF is composed of cell surface-bound and/or soluble proteins, most of which are synthesized as heterotrimeric transmembrane proteins [6, 7]. The trimeric TNF homology domain of many TNFSF members can be liberated from the cell surface (i.e. solubilized) by specific proteases [6]. Surface bound and/or soluble forms of the trimeric TNF homology domains act as ligands for their cognate receptor(s) [7]. In humans, the TNF receptor superfamily (TNFRSF) includes at least 29 members [8]. Most are synthesized as transmembrane proteins; some undergo proteolysis to generate soluble receptors, while others are synthesized *de novo* as soluble receptors. The extracellular domain or soluble forms of these receptors bind TNFSF members, while the intracellular portions of the transmembrane forms mediate signal transduction. The TNFRSF are divided into activating and death receptors. Activating receptors trigger nuclear factor kappa B (NF-kB) and mitogen-activated protein kinase (MAPK) signaling, promoting cellular survival, proliferation and differentiation. Death receptors initiate apoptosis or necroptosis [8].

Table 15.1 Role of selected cytokines in psoriasis or atopic dermatitis pathogenesis

Disease	Cytokine	Sources	Stimuli	Target	Effect	Tx
Psoriasis	TNF	DC, M, K Th17, Th22	TNF, IL-1, GM-CSF, DAMP, PAMP	K, DC, T	Chem; N recruit; K prolif; AMP; IL-1,-6,-20R,-23,-36, DC mig/mat, Th17/Th22 dev	Y
	IL-1	K, M, T, DC, Ma	TNF, IL-1, -36, DAMP, PAMP	K, DC, M, N	Chem; TNF, AMP, IL-6, -17, -36; N act, recruit, survival; APC act	N
	IL-6	K, M, T, E	TNF, IL-1, -17, PAMP	T, B, M, N	Chem; IL-21; Th17 dev; N recruit	N
	IL-12	DC, M, N	PAMP	T, NK	IFNγ, IL-9	Y
	IL-17	Th17, ILC3, Tc17, γδT, NKT	TNF, IL-1, -6, -23	K, T, M	Chem; TNF, IL-1, -6, G(M)-CSF; AMP; recruit T, DC, N	CL
	IL-20R	K, M	IL-1, -17, -22 TNF, wound, UVB, GM-CSF	K	↓ IL-1, ↓ IL-17	N
	IL-22	Th17, γδT, Th22, NK, ILC3	IL-23	K	K prolif/↓ K diff; AMP	CL
	IL-23	DC, M	PAMP, TNF, IL-12, CD40L	Th17, γδT, ILC3	IL-17, -22; Th17 act	Y
	IL-28 or 29	DC, M, Th17	PAMP	K, M, T	AVP	N
	IL-36	K	TNF, IL-1, -17	K, M, DC	Chem; N, T, M recruit; IL-1, -6; APC act	CL
	IFN-α/β	pDC	PAMP	mDC	IL-12, -23	
AD	IL-4	Th2, Ma, Ba, Eo	TCR act; FcεRI x-link; IL-33, TSLP, IL-25	K, T, B, My	Th2 dev: BCR, MHCII, IgE in B; DC mat; Eo recruit; Ma prolif/survival; IL-10, -12; ↓ K diff/prolif → ↓barrier fxn	CL
	IL-5	Th2, ILC2, Ma, NK, Eo	TCR act; FcεRI x-link: PAMP, IL-33, TSLP, IL-25	Eo, B, Ma, Ba	Eo recruit, survival, act, B cell diff	N
	IL-13	Th2, ILC2, Ma, Ba, Eo, NKT	TCR act; FcεRI x-link: IL-33, TSLP, IL-25	T, B, My	IgE, DC mat, Eo recruit, Ma act, FcεR and MHC II on B and M,	CL
	IL-25	Th2, Ma, NKT, K, DC, Eo,	TCR act; PAMPs	T, M, K, ILC2	Th2 cytokines (IL-4, -5, -13); IgE, ↓ K diff/AMP	N
	IL-31	Th2, Ma	IL-4, -33, AMP	Neur, K, DC, M	Itch; ↓ K diff/prolif → ↓barrier fxn, Chem,	CL
	IL-33	K, Ma, E	IFNγ, IL-1, TNF, cell damage or death	ILC2, Th2, Ma, M, Eo, Ba, DC	Th2 cytokines (IL-4, -5, -13; -31); Th2 dev; Ma act, M2 dev; Eo recruit,	N
	TSLP	K, DC	Epid injury, PAR2, PAMP	DC, Ma, Eo, Th2, ILC2, Treg	Itch, IL-4, -5, -13; Chem → Th2 recruit	CL

Arrows pointing down indicate reduction; all other effects reflect increases or promotion or an action

M monocyte/macrophage, *DC* dendritic cell, *K* keratinocyte, *Ma* mast cell, *Eo* eosinophil, *T(h)* T (helper) cell, *B* B cell, *E* endothelial cell, *ILC(2 or 3)* type 2 or 3 innate lymphoid cell, *NK(T)* natural killer (T) cell, *Ba* basophil my-myeloid cell, *N* neutrophil, *Neur* neuron, *M2-M2* polarized macrophages, *DAMP/PAMP* damage- or pathogen-associated molecular pattern, *act* activation, *x-link* cross-link, *Epid* epidermal, *PAR2* protease activated receptor 2, *AMP* antimicrobial peptides, *AVP* antiviral proteins, *Recruit* recruitment, *Chem* chemokine, *dev* development, *diff* differentiation, *prolif* proliferation, *fxn* function, *mig* migration, *mat* maturation, *Tx* cytokine-targeted therapy for this disease?, *Y* Yes, *N* No, *CL* in clinical trials

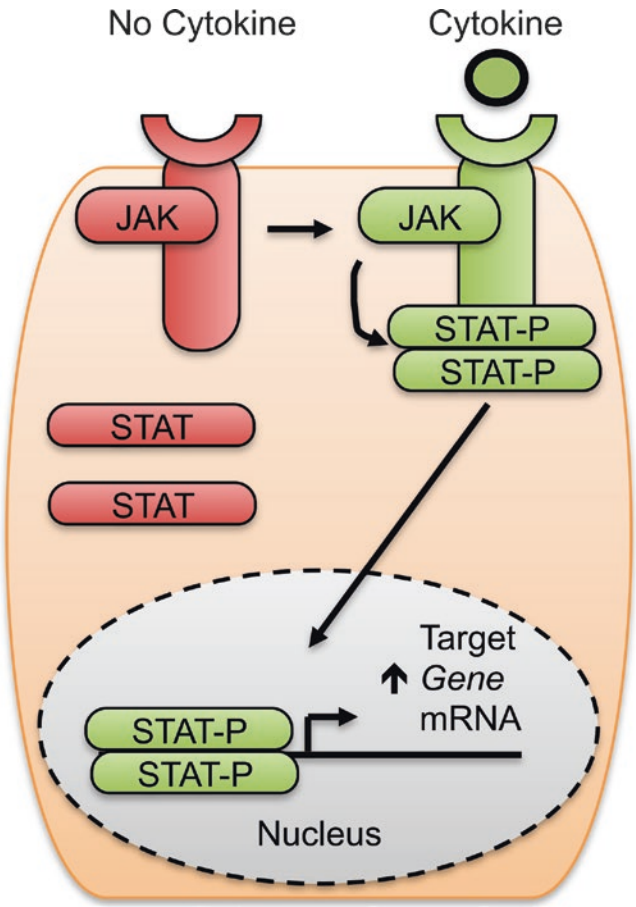

Fig. 15.2 Overview of Janus kinase (*JAK*) and signal transducer and activator (*STAT*) signaling pathway utilized by many cytokines. In the absence of cytokine, the cytokine receptor, JAKs and STATs are inactive (*red shading*). Cytokine (*green circle*) activates the pathway (*green shading*). Activated JAKs phosphorylate STAT proteins (designated STAT-P). STAT-P then dimerizes and translocates to the nucleus to regulate target gene expression

Tumor Necrosis Factor

Multiple cell types including macrophages, dendritic cells and keratinocytes produce TNF [9–11]. Production is regulated at transcriptional and post-transcriptional levels and is induced by cytokines (e.g. GM-CSF, IL-1 & TNF) and danger signals, the latter of which are subclassified as pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) such as lipopolysaccharide and the host protein HMGB1, respectively [12–15].

After synthesis, TNF is expressed on the cell surface as a homotrimer, which can activate TNF receptors directly or undergo site-specific cleavage by the proteases ADAM10 and 17 releasing a homotrimeric soluble form [13, 16, 17]. While the membrane-bound form is important for secondary lymphoid tissue development, defense against intracellular pathogens and limiting chronic inflammation and autoimmunity, the soluble form promotes acute and chronic inflammation [18, 19]. These differential effects are in part explained by the fact that the membrane-bound form of TNF can activate both TNF receptors (TNFR1 and TNFR2), while the soluble form can only engage TNFR2 [13, 20]. While TNFR1 is widely expressed on many cells types, TNFR2 expression is more restricted to leukocytes, endothelial cells, keratinocytes and several other cell types [21]. TNFR2 is an activating receptor, while TNFR1 is both a death and activating receptor.

Given the pleiotropic functions and numerous contexts in which TNF acts, it is beyond the scope of this chapter to discuss all that this molecule does in the skin. Instead its role in psoriasis will be presented as an example of how TNF regulates cutaneous inflammation (see also Chap. 21). Increased quantities of TNF mRNA and protein are detected in psoriatic lesions [22]. Potential sources of TNF in psoriatic lesions include myeloid cells (e.g. TNF/iNOS-producing dendritic cells), T helper cell subsets (Th17 and Th22) and keratinocytes [23, 24]. In response to TNF, keratinocytes can proliferate and produce chemokines, cytokines and antimicrobial peptides; chemokines produced by TNF-stimulated keratinocytes recruit myeloid dendritic cells, Th17 cells and neutrophils, promoting inflammation [23]. Conversely, TNF acts on immature dendritic cells in the skin to promote their maturation and migration out of the skin and into draining lymph nodes where they present antigen to naïve T cells [23]. In the lymph node, TNF can promote differentiation of Th17 and Th22 cells from naïve T cells [25]. The Th17 and Th22 subsets can then return to the skin where they produce effector cytokines including IL-17A, IL-17F and/or IL-22, that can stimulate keratinocytes, leading to epidermal hyperplasia, production of antimicrobial peptides, chemokines and other cytokines, further promoting inflammation and disease [23]. The pleiotropic functions of TNF in psoriasis pathogenesis demonstrate how this cytokine is a central mediator of disease, which helps explain why TNF inhibitors are so efficacious for treatment of psoriasis and other dermatologic conditions [26, 27].

Interleukins

The term *Interleukin* (meaning between leukocytes) was originally proposed in 1979 in an effort to distinguish between two biochemically distinct lymphocyte activating proteins, interleukin 1 (IL-1) and interleukin 2 (IL-2) [28]. Since then, the list of interleukins has grown, now ending at IL-38 [29]. Some interleukin designations are further subdivided (i.e. IL-1α and IL-1β or IL-36α, β, γ) as a result of identifying cytokines (encoded by different genes) that signal via the same receptor. Based on similarities in structure, function and/or receptor usage, interleukins can be subdivided into families. In this section, the defining characteristics

of a given interleukin family will be presented followed by a more detailed discussion of the family members most relevant to understanding cutaneous immunity, inflammation and disease. The families discussed are, IL-1, common gamma chain (γ_c), beta chain, IL-6, IL-10, IL-12, and IL-17.

Interleukin 1 Family

The IL-1 family consists of structurally similar but functionally diverse cytokines with a number of critical pro- and anti-inflammatory actions that govern cutaneous inflammation, immunity and disease. In humans, there are at least 11 members of the IL-1 family, most of which are receptor agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ and IL-37), with the remainder being receptor antagonists (IL-1Ra, IL-36Ra and IL-38) [29]. With the exception of IL-37, which can bind to IL-18α alone, IL-1 family members that act as receptor agonists bind heterodimeric receptors [29]. The receptor complexes for IL-1α, IL-1β, IL-33 and IL-36α, IL-36β and IL-36γ are composed of a ligand-selective subunit bound to IL-1 receptor accessory protein (IL-1RAcP), while the IL-18 receptor is composed of two unique subunits, IL-18Rα and IL-18Rβ. The IL-1 receptor family members consist of an extracellular immunoglobulin-like domain and an intracellular portion that includes a TIR domain, the latter of which is also present in toll-like receptors (TLR). The TIR domain recruits the adaptor protein MyD88 to initiate downstream signaling events leading to MAPK and NF-kB activation and subsequently, target gene expression [30]. Unique aspects of IL-1 family member synthesis, processing and release will be discussed in the coming sections.

Interleukin 1

The designation IL-1 refers to two structurally distinct proteins, IL-1α and IL-1β that are encoded by different genes but share common receptors (i.e. IL-1R1/IL-1RAcP or IL-1R2/IL-1RAcP receptor complexes) [30]. There is also an endogenous IL-1 receptor antagonist, IL-1Ra, a recombinant form of which has been used therapeutically to treat IL-1-mediated inflammatory diseases [31]. Similar to TNF, IL-1α and IL-1β are considered innate cytokines, playing a key role in promoting local and in some cases, systemic inflammation and immunity. In addition to contributing to homeostatic aspects of cutaneous immunity and inflammation, IL-1 is implicated in the pathogenesis of Still's disease, pyoderma gangrenosum (PG), Behçet's disease, hidradenitis suppurativa (HS) and a number of autoinflammatory diseases. Importantly, IL-1 blockade can be an effective therapy for these diseases [32–34]. Host defense against bacterial pathogens is also mediated by IL-1, as are various aspects of keratinocyte proliferation, differentiation and function (see also Chap. 16) [35–37].

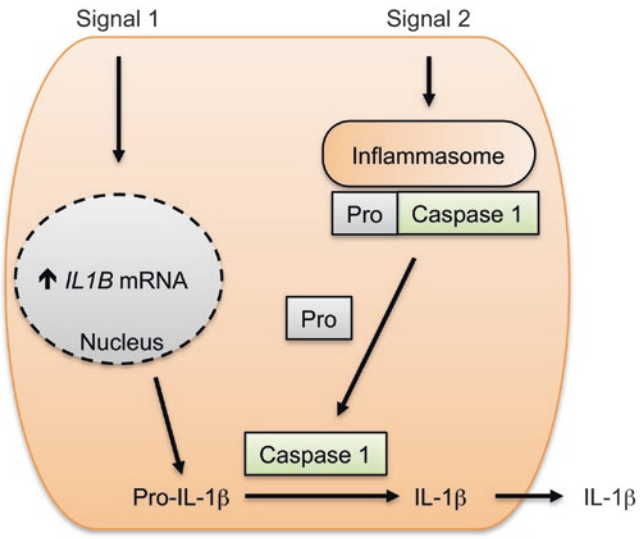

Fig. 15.3 Two-signal model for production of IL-1β in host cells. Signal 1 (e.g. cytokine) induces expression of *IL1B* mRNA, which is then translated in an inactive protein (Pro-IL-1β). Signal 2 (e.g. ATP) stimulates the same cell activating the inflammasome, which then generates Caspase 1 from its inactive precursor, Pro-Caspase 1. Caspase 1 then cleaves Pro-IL-1β, generating IL-1β that is then secreted from the cell

Keratinocytes and other epithelial cell types constitutively produce IL-1α [38]. Unlike IL-1β and most other cytokines, IL-1α is synthesized as a precursor containing a nuclear localization sequence allowing IL-1α to traffic into the nucleus, bind chromatin and regulate transcription [29]. Cellular damage or necrosis promotes release of IL-1α into the local milieu thereby promoting local (neutrophil-mediated) inflammation; as such IL-1α is classified as an alarmin. In contrast, IL-1α remains tightly bound to chromatin during apoptosis, thus restricting inflammation [29].

The disparate effects of IL-1β, compared to IL-1α, are largely a function of differences in cellular processing and cellular sources rather than differences in signaling pathways used by these cytokines [29, 38]. Sources of IL-1β include monocytes, macrophages and dendritic cells and keratinocytes [30]. Like IL-1α, IL-1β is synthesized as a precursor. Unlike IL-1α, production and release of the active form of IL-1β is a highly regulated process mediated by an intracellular complex known as the inflammasome (Fig. 15.3). Production of IL-1β begins with transcription of the *IL1B* gene and synthesis of Pro-IL-1β. Multiple stimuli (referred to as signal 1) promote *IL1B* transcription (e.g. TNF, TLR ligands and IL-1) [29]. Unlike IL-1α, the IL-1β precursor (Pro-IL-1β) does not activate IL-1 receptors. Instead, Pro-IL-1β must first undergo proteolysis to generate mature IL-1β [39]. Proteolysis of Pro-IL-1β is performed by caspase-1, which itself must first undergo proteolytic cleavage by the inflammasome. Stimuli that trigger inflammasome activation (referred to here as signal 2) include DAMPs like ATP, pore forming bacterial toxins and others [36]. Upon

activation, the inflammasome cleaves procaspase-1 to caspase-1; caspase-1 in turn cleaves Pro-IL-1β generating IL-1β, which is then secreted (Fig. 15.3).

Gain of function mutations in genes encoding proteins in inflammasome underlie a number of autoinflammatory diseases with cutaneous manifestations including: pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome as well as the periodic fever syndromes, familial Mediterranean fever (FMF) and cold induced autoinflammatory syndromes-1 (CIAS-1), like Muckle-Wells syndrome, familial cold urticaria and neonatal-onset multisystem inflammatory disease [40]. In patients with CIAS-1 spectrum disease, periodic fevers result from increased circulating levels of IL-1β acting as a pyrogen, while urticarial eruptions may be due to skin-resident mast cell-derived IL-1β stimulating increased permeability of cutaneous blood vessels [41, 42]. The critical role of IL-1β in autoinflammatory diseases is highlighted by their responsiveness to IL-1-blocking therapeutics [40].

Several key aspects of host defense against cutaneous infections with *Staphylococcus aureus* are dependent on IL-1 [36]. In this setting, cell wall components from *S. aureus* (e.g. lipotechoic acid and bacterial peptidoglycan) activate TLR2 on keratinocytes and macrophages stimulating IL-1 production [43]. Keratinocytes in turn produce IL-1α, and lesser amounts of IL-1β, while macrophages primarily produce IL-1β [36]. Activation of IL-1 receptors for IL-1α or IL-1β, then stimulates keratinocyte production of antimicrobial peptides, neutrophil-attracting chemokines and other cytokines, like IL-6. Antimicrobial peptides can directly combat the infection, while chemokines and IL-6 can recruit neutrophils into the skin; notably, IL-1 can also prolong neutrophil survival, further promoting neutrophil-mediated clearance of the infection [36, 44].

Interleukin 33

Interleukin 33 (IL-33) is a dual function protein that acts as a cytokine and a nuclear factor that may regulate NF-kB-dependent genes [45, 46]. Cellular damage or necrosis releases IL-33 promoting local inflammation; thus IL-33 is an alarmin like IL-1α [47]. Conversely, proteolytic cleavage inactivates IL-33 during apoptosis preventing IL-33-mediated inflammation [48]. The IL-33 receptor is expressed on Th2 cells, innate lymphoid type 2 cells (ILC2), macrophages, mast cells, eosinophils and other cells. Effects of receptor engagement are cell type specific explaining the pleiotropic functions of IL-33 [49]. For example, IL-33 promotes Th2 cell development and cytokine production, mast cell maturation and activation and polarization of macrophage development towards an M2 phenotype [50, 51]. Paradoxically, IL-33 can promote Th1-polarized inflammation via NK and $CD8^+$ T cell stimulation [50]. Expression of IL-33 is increased in lesional skin keratinocytes in atopic dermatitis (AD), psoriasis and lichen planus [52, 53]. In AD, IL-33 concentrations are reportedly increased in the peripheral blood of patients with moderate to severe disease and are reduced following effective topical therapy with steroids or calcineurin inhibitors [52, 54]. These results suggest keratinocyte damage or death in AD lesional skin (possibly secondary to scratching the skin) release IL-33 into the circulation, the consequences of which are not defined in this context.

Interleukin 36

The IL-36 family includes receptor agonists IL-36α, IL-36β and IL-36γ, hereafter referred to as IL-36. The family also includes IL-36Ra, a receptor antagonist [55]. The IL-36 receptor is composed of IL-36R and IL-1RAcP and utilizes signal transduction pathways like that described above for other IL-1 family members. Loss of function mutations in the *IL36RN* gene (encodes IL-36Ra) cause some familial and sporadic cases of generalized pustular psoriasis and Acrodermatitis Continua of Hallopeau [56, 57]. Loss of IL-36Ra function is thought to lead to unopposed activation of IL-36 signaling.

The IL-36 receptor is expressed on keratinocytes, monocytes and some dendritic cells [55, 58, 59]. Keratinocytes can produce IL-36α, IL-36β, IL-36γ and IL-36Ra [55]. Transcripts encoding IL-36 family, in low abundance under non-inflammatory conditions, are increased under inflammatory conditions as seen in psoriatic lesions or keratinocytes stimulated with TNF, IL-17, or IL-1 [55]. In human keratinocytes, IL-36 stimulates production of chemokines known to attract T cells, neutrophils and macrophages [58]. In contrast, stimulation of monocytes and myeloid dendritic cells with IL-36 triggers IL-1β and IL-6 production and increased proportions of cells expressing the co-stimulatory molecule CD86 and MHC class II [58]. These observations suggest IL-36 family members are important mediators of inflammation in the skin. Consistent with results from genetic studies suggesting a role for IL-36 in psoriasis pathogenesis, IL-36 is reported to promote psoriasiform inflammation in mice [55–59]. Integrating our current understanding of psoriasis pathogenesis and IL-36 biology, the literature suggests the following role for IL-36 in disease: (1) innate cytokines like IL-1β and TNF as well as IL-17A can stimulate IL-36 production by keratinocytes; (2) IL-36 stimulates keratinocytes to produce multiple chemokines promoting migration of neutrophils, macrophages and T cells in and into the skin; (3) IL-36 stimulates some dendritic cell subsets promoting antigen presentation to T cells and production of innate cytokines like IL-1β and IL-6; (4) IL-1β, TNF, and IL-17A promote various aspects of inflammation in psoriatic lesions and further production of IL-36 by keratinocytes leading to a vicious cycle and persistent disease. Thus, IL-36 or its receptor may become viable therapeutic targets for psoriasis and

potentially other dermatologic conditions. Future investigation is still needed to clarify the role of IL-36 in other aspects of normal or pathologic inflammation and immunity in the skin.

Common Gamma Chain Receptor Family

The common gamma chain (γ_c) receptor family includes IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 [60]. The γ_c receptor subunit that defines the family is a transmembrane protein that transduces signals in part through JAK3 [61]. Receptor signaling is initiated by heterodimerization or heterotrimerization with ligand-specific receptor subunits [62]. These receptors signal via JAK-STAT, PI3K-Akt and RAS/MAPK pathways promoting cell survival, proliferation and regulation of target gene expression [63]. The JAK-STAT family members activated by these cytokines is ligand-specific. Recently, a secreted form of γ_c generated by alternative splicing has been identified in mouse and human T cells and appears to act as a decoy receptor for some γ_c family cytokines [64].

Cytokines in the γ_c family regulate lymphoid cell development, survival and/or function (see also Chap. 8) [65]. Additional targets include mast cells, myeloid cells and other hematopoietic cells. Genetic deficiency of functional γ_c chains or JAK3, leads to severe combined immunodeficiency (SCID), highlighting the importance of this receptor family in host immunity and inflammation [66]. Cutaneous manifestations of these forms of SCID include eczematous dermatitis and infections with *S. aureus*, HPV or *Candida spp*. and graft versus host disease (GVHD) [67]. The diverse cutaneous and systemic manifestations seen in these forms of SCID reflect broad defects in IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 function.

In the coming sections, the roles of IL-2, IL-4 and IL-9 in cutaneous immunity and inflammation will be presented. The functions of IL-7, -15 and -21 in the skin are less well defined and will be discussed only briefly. Owing to overlap in cytokine receptor usage with some γ_c family members, IL-13 and TSLP will also be discussed in this section.

Interleukin 2

Interleukin 2 (IL-2) is the founding member of this family. Activated T cells produce IL-2, as do B cells and some dendritic cells [68–70]. There are two receptor complexes for IL-2, a heterodimer of the β and γ_c chains and a heterotrimer of the high affinity subunit IL-2Rα (i.e. CD25) bound to the β and γ_c chains [71]. While IL-2Rα increases binding affinity for IL-2, the β and γ_c chains regulate signal transduction. Shared usage of β and γ_c chains explains similarities in biologic effects of IL-2 and IL-15, while use of distinct high affinity receptor subunits (i.e. IL-2Rα vs. IL-15Rα) helps explain differences between these cytokines [72].

One or both forms of the IL-2 receptor are expressed on T cells, B cells, NK cells, monocytes and macrophages [71, 73, 74]. In addition to PI3K-Akt and RAS/MAPK signaling, receptor engagement activates JAK1 and JAK3 triggering phosphorylation of STAT1, STAT3 and STAT5. T cell survival and proliferation are important effects of IL-2 during antigen-specific immune responses, as in allergic contact dermatitis [75]. Based on the ability of IL-2 to promote T cell survival and proliferation, recombinant human IL-2 became one of the first FDA-approved immunotherapies for metastatic melanoma [76, 77]. In contrast, an IL-2-diphtheria toxin fusion protein is used to trigger death in cells expressing IL-2Rα; this agent is used to treat some forms of cutaneous T cell lymphoma (CTCL) [78]. Interleukin 2 also regulates peripheral tolerance through effects on regulatory T cells, promotion of Fas-mediated activation-induced cell death of Th cells, and by restraining $CD8^+$ T cell responses [79, 80]. Some of the aforementioned immunostimulatory and immunosuppressive effects of IL-2 are lacking in IL-2Rα deficient patients, leading to features of autoimmunity and immunodeficiency as well as cutaneous manifestations including psoriasiform dermatitis and alopecia universalis [81].

Interleukin 4 and 13

Interleukin 4 (IL-4) is a canonical Th2 cytokine produced by Th2 cells, mast cells, basophils and eosinophils [82]. There are two functionally distinct heterodimeric IL-4 receptor complexes. The first (Type I receptor) is specific for IL-4 and is composed of IL-4Rα and γ_c; the second (Type II receptor) binds IL-4 or IL-13 and is composed of IL-4Rα and the IL-13Rα1 [83]. The Type I receptor is expressed on T and B cells, myeloid cells and fibroblasts and acts through JAK1 and JAK3 leading to phosphorylation of STAT3 and STAT6. The Type II receptor is also expressed by myeloid cells and fibroblasts and is selectively expressed on smooth muscle and epithelial cells, including keratinocytes and acts through JAK1, JAK2, JAK3 and TYK2 and activation of STAT3, STAT5 and STAT6 [82–84].

Effects of IL-4 are pleiotropic and cell-type dependent. Depending on the presence or absence of TGF-β, IL-4 can polarize naïve $CD4^+$ Th cell differentiation towards Th2 or Th9 lineages; IL-4 can also promote T cell survival (Fig. 15.1) [85, 86]. In B cells, IL-4 promotes survival, B cell receptor and MHC class II expression, and IgE class switching [87–89]. Mast cell survival and proliferation are also promoted by IL-4 [90]. In keratinocytes, IL-4 impairs differentiation, which correlates with impaired epidermal barrier function in vivo [91–93]. Dendritic cell maturation and eosinophil chemotaxis are also stimulated by IL-4 [94, 95].

Interleukin 13 is another Th2 cytokine and is produced by Th2 cells, ILC2, mast cells, eosinophils, basophils and NKT cells [96, 97]. The Type II receptor (shared with IL-4), mediates many of the overlapping biologic effects of IL-4 and IL-13 including IgE class switching, dendritic cell maturation, mast cell activation and eosinophil recruitment [83]. A second receptor, IL-13Rα2, is IL-13-specific and is generally regarded as a decoy receptor; though some IL-13Rα2-specific functions have been demonstrated in mouse models of allergic lung inflammation [83, 98].

The aforementioned functions of IL-4 and IL-13 explain why they are key mediators of allergic inflammation in multiple tissues and are implicated in the pathogenesis of various aspects of atopy, including AD [99]. Both cytokines are detectable in acute and chronic AD lesions and mediate key aspects of AD pathogenesis, epidermal barrier dysfunction and Th2-polarized inflammation. A neutralizing antibody against IL-4Rα that prevents IL-4 and IL-13 signaling is in clinical trials for AD [100].

Interleukin 9

Interleukin 9 is produced by several Th subsets, most notably Th9 cells, and mast cells; of note Th9 cell development is dependent on IL-4 and TGF-β (Fig. 15.1) [86, 101]. Type I interferons, IL-1, IL-6, IL-10, IL-12, IL-21, IL-25 and LPS promote IL-9 production in relevant cells types [86, 102, 103]. The IL-9 receptor is a heterodimer of IL-9R and γ_c that signals via JAK1 and JAK3 and then, STAT1, STAT3 and STAT5 [86, 104]. This receptor can be expressed on mast cells, Th2 cells, Th17 cells, ILC2, B cells, smooth muscle cells and some epithelial cells [86, 105]. Stimulation with IL-9 promotes mast cell proliferation and production of proteases and a variety of cytokines including IL-1β, IL-5, IL-6, IL-13, TGF-β [106, 107]. This cytokine also promotes Th17 development, ILC2 survival and production of IgE and IgG by B cells [86, 105].

Recently, IL-9 was implicated in the pathogenesis of psoriasis and mouse models of melanoma [108–110]. With respect to psoriasis, Th9 cells were more abundant in psoriatic lesions, suggesting a role in disease [108, 111]. In mouse models of melanoma, IL-9 can exert anti-tumor effects; although the proposed mechanisms underlying these observations varied with one report demonstrating mast cell-dependent anti-tumor effects of IL-9 and another suggesting that IL-9 indirectly promotes effector $CD8^+$ T cell recruitment into tumors [109, 110].

Thymic Stromal Lymphopoietin

Thymic stromal lymphopoietin (TSLP) is included in this section because its receptor is composed of IL-7Rα and a γ_c-like receptor-subunit, TSLPR. In contrast to IL-7, which regulates development, proliferation and/or survival of lymphoid cells, TSLP promotes allergic inflammation in skin and other epithelial surfaces [112–114]. Upon stimulation with TSLP, its receptor activates JAK1 and JAK2 and subsequently, STAT1, STAT3, STAT4, STAT5, and STAT6, which then regulate TSLP target genes [74].

Keratinocytes and dendritic cells can produce TSLP [115]. Production of TSLP by keratinocytes is increased in lesional skin of AD and Netherton syndrome, both of which are characterized by epidermal barrier dysfunction and Th2-polarized inflammation [115, 116] (see also Chap. 22). Interestingly, experimentally induced epidermal barrier disruption and/or injury trigger TSLP production by keratinocytes in normal human skin [117]. In Netherton syndrome and AD, excessive serine protease activity can also stimulate the protease activated receptor 2 and consequently, TSLP production by keratinocytes. Activation of TLR2 signaling in keratinocytes by membrane fragments of *S. aureus*, a common skin pathogen that also colonizes the skin of patients with AD, can stimulate TSLP production [118]. Inflammatory cytokines can also promote TSLP production by keratinocytes and dendritic cells [114]. Thus, there are multiple potential stimuli to explain the increased production of TSLP by keratinocytes in lesional skin of patients with AD and Netherton syndrome, the downstream consequences of which will be described below.

Dendritic cells, Th2 cells, regulatory T cells, ILC2, mast cells, eosinophils, B cells and NKT cells express the TSLP receptor [114, 119]. Stimulation of dendritic cells with TSLP polarizes naïve T cell differentiation towards Th2 cells and promotes production of the chemokines CCL17 and CCL22, which can act as chemoattractants for Th2 cells. Moreover, stimulation of Th2, ILC2 and mast cells promotes Th2 cytokine production. Several mouse models of AD-like inflammation demonstrate that keratinocyte-derived TSLP production is sufficient to drive allergic inflammation, even in the absence of T cells [120]. Recently, TSLP was shown to directly stimulate sensory neurons in the skin leading to pruritus (itch) in mice injected with recombinant TSLP [121]. These findings indicate TSLP can directly promote allergic inflammation (via actions on leukocytes) and itch (via actions on neurons); both properties of TSLP are likely to be germane to AD pathogenesis. A phase I clinical trial with a humanized TSLP neutralizing antibody has been completed with healthy patients and patients with AD; however the results of these studies are not available at *ClinicalTrials.gov*.

Beta Chain Family: Interleukin 5

This family includes IL-3, IL-5 and granulocyte monocyte colony stimulating factor (GM-CSF) and is defined by use of the common β chain receptor (different from IL-2 receptor β chain) [122]. The common β chain receptor is widely

expressed in hematopoietic cells, while expression of ligand-specific subunits is more restricted [123]. Functional receptors are heterodimers of the ligand-specific subunit and the common β chain [122]. Sources of IL-5 include Th2 cells, ILC2, mast cells, NK cells, $CD8^+$ T cells, eosinophils and endothelial cells [96, 123]. The IL-5 receptor is composed of IL-5Rα and common β chain subunits; receptor engagement activates JAK1, JAK2, STAT1, STAT3 and STAT5 as well as RAS-MAPK and PI3K signaling [123]. As in other tissues, IL-5 promotes infiltration, survival and effector functions of eosinophils in the skin [123]. Like other Th2 cytokines, IL-5 is implicated in AD pathogenesis. However, treatment of moderate to severe disease with two weekly doses of an IL-5 neutralizing antibody failed to achieve the desired clinical endpoint suggesting IL-5 neutralization may not be sufficient to treat AD [124]. Interestingly, peripheral eosinophilia rapidly improved in patients treated with this antibody. While tissue eosinophilia was not assessed and the treatment course was short, these findings suggest eosinophils may not be required for persistence of AD lesions [124].

Interleukin 6 Family

This family is defined by shared receptor subunit usage amongst all or some of its members. Interleukins in this family include IL-6, IL-11, IL-30 and IL-31. Members that are not interleukins include oncostatin M, leukemia inhibitory factor and others [125, 126]. Most family members use a common receptor subunit named glycoprotein 130 (gp130), while other members (e.g. IL-31) are included in this family due to structural similarities with IL-6 or shared usage of receptor subunits with other family members and/or usage of gp130-receptor subunits [125, 126]. This discussion will focus on IL-6 and IL-31, both of which are implicated in important aspects of cutaneous immunity and inflammation in health and/or disease [125, 126].

Interleukin 6

A variety of cells produce IL-6 including lymphocytes, monocytes, macrophages, fibroblasts, keratinocytes, endothelial cells and others [125]. Stimuli for IL-6 production include PAMPs (e.g. LPS), cytokines (e.g. IL-1, TNF, IL-17) and others. The functional repertoire of IL-6 is extensive with effects on the acute phase response, thermal regulation, angiogenesis, pathologic bone resorption, lipid metabolism and many other physiologic and pathophysiologic processes. However, this section will focus on functions of IL-6 relevant to dermatologic diseases [125].

The IL-6 receptor is composed of gp130 and IL-6R. While gp130 is widely expressed, IL-6R is primarily restricted to lymphocytes, monocytes and neutrophils [125]. Receptor engagement activates JAK3, STAT3, MAPK and PI3K/Akt pathways [127]. A soluble form of the IL-6R subunit (similar to IL-15Rα) can also initiate signaling but the details of this are beyond the scope of this discussion [128]. Effects of IL-6 receptor activation are cell type specific. In B cells, IL-6 stimulates immunoglobulin production and plasma cell differentiation; the role of IL-6 in plasma cell differentiation may be an indirect effect mediated by IL-21 produced by IL-6 stimulated T cells [129]. Other effects of IL-6 on the T cell compartment are promotion of Th17 and follicular helper T cell (Tfh) cell development and suppression of regulatory T cell (Treg) development and function (Fig. 15.1) [130, 131]. As described below, IL-6 also promotes neutrophil accumulation in the skin [125].

While IL-6 can contribute to many normal (e.g. promotion of sterile inflammation after wounding) and pathologic processes in the skin, this section will focus on the roles of IL-6 in cutaneous bacterial infections and psoriasis. As noted earlier, IL-1 is a central mediator of host defense following cutaneous infections with *S. aureus*. One of the downstream mediators of this host defense mechanism is IL-6, which is produced by keratinocytes and myeloid cells in response to IL-1 [36]. Like IL-1, IL-6 stimulates production of chemokines that attract neutrophils; moreover, IL-6 stimulates endothelial cells to induce expression of adhesion molecules needed for neutrophil migration into the skin from the blood. The Th17 cell subset is thought to be an important source of IL-17A and IL-17F during cutaneous infections and in psoriatic lesions. Differentiation of Th17 cells from naïve T cells is promoted by IL-6, suggesting another, albeit indirect, mechanism whereby IL-6 can contribute to host defense against cutaneous bacterial infections and psoriasis pathogenesis (Fig. 15.1) [36]. For completeness sake, it should be noted that IL-17 producing cell types other than Th17 cells are also implicated in pathogenesis of cutaneous infections and psoriasis pathogenesis and that these populations do not appear to be IL-6 dependent [36, 132].

Interleukin 31

Like other Th2 cytokines, IL-31 is an important mediator of allergic inflammation and has been implicated in AD pathogenesis (see also Chap. 22) [126]. The Th2 cell subset produces IL-31 in response to IL-4 or IL-33, and mast cells can produce IL-31 upon stimulation with the antimicrobial peptides LL-37 and β-defensins [133, 134]. The IL-31 receptor is expressed on neurons, keratinocytes and some dendritic cell subsets, monocytes and macrophages [126, 135–137]. In contrast to most IL-6 family members, the IL-31 receptor does not utilize gp130; instead it is composed of a gp130-like subunit, IL-31Rα, and the oncostatin M receptor [138]. Receptor engagement activates JAK1, JAK2, STAT1, STAT3, STAT5, PI3K/Akt and MAPK signaling [138]. Like TSLP, IL-31 can directly stimulate neurons and provoke scratching of the skin in mice, suggesting IL-31 can directly induce

pruritus (see also Chap. 12) [126, 139]. In vitro studies also suggest IL-31 impairs keratinocyte proliferation and differentiation, which may impair epidermal barrier function [135]. Finally, stimulation of $CD1c^+$ dendritic cells with IL-31 promotes production of inflammatory cytokines and chemokines [137].

Pruritus is a characteristic feature of AD; IL-31 and TSLP are proposed to be important triggers for pruritus in this disease (i.e. they are pruritogens). In support of this, IL-31 expression is increased in lesional and nonlesional skin of patients with AD and in lesions of prurigo nodularis [126]. Serum levels of IL-31 are also elevated in AD and correlate with disease severity [140]. Based on the functions mentioned above, it is also possible that IL-31 contributes to pathogenesis via impairment of epidermal barrier function and promotion of cytokine and chemokine production. According to *ClinicalTrials.gov*, patients are currently being recruited for a phase I clinical trial of an IL-31 blocking antibody in healthy subjects and patients with AD.

Interleukin 10 Family

The IL-10 family includes IL-10, the IL-20 subfamily, which includes IL-19, IL-20, IL-22, IL-24, IL-26 and the type III interferon subfamily consisting of IL-28A, IL-28B and IL-29 [141]. This cytokine family is defined by common usage of type 2 cytokine receptors and structural similarities, the details of which will not be discussed in this chapter [141]. Despite structural similarities between these cytokines and similarities in receptor usage, the functions of many IL-10 family members are quite varied. This section will discuss IL-10 and IL-22 individually and then the IL-20 receptor cytokines, IL-19, IL-20 and IL-24. Though the details of type III interferons biology will not be reviewed here, these cytokines have recently been implicated in psoriasis pathogenesis and host defense against cutaneous viral infections; the reader is referred to the cited references for further information on these cytokines [141–147].

Interleukin 10

Interleukin 10 is one of the prototypic anti-inflammatory cytokines [141]. Multiple cells can produce IL-10 including lymphocytes (T, B & NK cells), macrophages, dendritic cells, mast cells, eosinophils, neutrophils and keratinocytes [141, 148]. The inductive stimuli regulating IL-10 production vary by cell type. In Th2 cells, IL-4 and IL-27 can stimulate IL-10 production; IL-6 and IL-27 can also stimulate IL-10 production by Th1 cells and Th17 cells [149, 150]. In addition to Th cells, several regulatory T cell subsets can produce IL-10; for instance, $CD4^+FoxP3^+$ regulatory T (Treg) cells produce IL-10 in response to IL-2 and IL-4 [151, 152]. Regulatory B cells also produce IL-10 [153]. In macrophages and dendritic cells, TLR (e.g. TLR2, TLR3, TLR4 and TLR9) engagement can also stimulate IL-10 production [154, 155].

The IL-10 receptor is primarily expressed on lymphoid and myeloid cell lineage cells and is composed of IL-10R1 and IL-10R2 [141, 156]. Receptor engagement by an IL-10 homodimer activates JAK1, TYK2, STAT1, STAT3 and STAT5 [157]. Two anti-inflammatory mechanisms by which IL-10 acts include the inhibition of various aspects of antigen presentation by macrophages and dendritic cells and attenuation of cytokine production by T cells [157]. Interestingly, multiple viruses, including Epstein Barr virus and cytomegalovirus produce IL-10 homologs, which are thought to attenuate antiviral immune responses [158]. With respect to dermatologic disease, the anti-inflammatory effects of IL-10 are likely to play a role in regulating many aspects of cutaneous inflammation and immunity. The list of dermatologic diseases in which IL-10 is implicated as a mediator of pathogenesis or a potential therapy is long including multiple infectious, inflammatory, autoimmune and neoplastic conditions [159]. Notably, clinical trials with recombinant IL-10 for psoriasis or wound healing did not show significant benefit [160].

Interleukin 22

Cellular sources of IL-22 include T cells (e.g. Th17, Th22, γδT cells), NK cells, and a subset of ILC3 [161–165]. Development of IL-22 producing cells and/or production of IL-22 are regulated by cytokines in a cell-type dependent manner. Development of Th17 cells, which produce IL-17 and IL-22, is driven by IL-6, IL-21 and TGF-β; whereas, development of Th22 cells, which produce IL-22 but not IL-17, is stimulated by IL-6 and TNF (Fig. 15.1) [25, 166–169]. Stimulation with IL-23 can induce IL-22 production by some T cell subsets and ILC3 [170–172]. While TGF-β promotes Th17 development, it inhibits IL-22 production by Th17 and Th22 cells [25, 173].

The IL-22 receptor, composed of IL-10R2 and IL-22R, is expressed on keratinocytes and other epithelial cells [141, 156, 174]. Receptor activation signals through JAK1, TYK2, STAT1, STAT3 and STAT5 and MAPK and Akt pathways [141]. In keratinocytes, IL-22 impairs differentiation and promotes proliferation (and thus epidermal hyperplasia) and increases expression of β-defensin 2, β-defensin 3, S100A8 and S100A9, all of which are molecular, cellular, or histologic features of psoriatic lesions [173, 175–177].

Psoriasis, AD and other diseases are mediated, in part, by IL-22 (see also Chap. 21) [23, 178]. In psoriasis and AD, increased levels of IL-22 and IL-22 producing cell subsets

are described in lesional skin, and lesion resolution is associated with reduced IL-22 expression [23, 162, 178–186]. In both diseases, IL-22 is thought to act on keratinocytes leading to acanthosis and increased expression of S100A8, S100A9 and other proteins. While IL-22 can promote production of β-defensin 2 and β-defensin 3, expression is higher in psoriatic lesions than in AD; this may be due to Th2 cytokines attenuating expression of these β-defensins in AD [177, 187–189]. According to *ClinicalTrials.gov* AD patients are being enrolled for a clinical trial using an antibody to IL-22.

Interleukins 19, 20 & 24 (IL-20 Receptor Cytokines)

Interleukins 19, 20 and 24, hereafter referred to as IL-20 receptor cytokines, are discussed together due to overlapping receptor usage and biologic effects. Keratinocytes and other epithelial cells, monocytes, some T cells and melanocytes can produce one or more IL-20 receptor cytokines; myeloid dendritic cells expressing IL-20 are also present in psoriatic lesions [190–196]. In keratinocytes, IL-1β, IL-17A, IL-22, UVB light and wounding promote IL-20 receptor cytokine production [191, 197–199]. In monocytes, LPS, GM-CSF and TNF stimulate production [141, 196, 200, 201]. Both receptors for these cytokines are expressed by keratinocytes [176, 191]. Receptor engagement activates STAT3 driving keratinocyte proliferation and epidermal hyperplasia [176, 191]. Other effects of one or more of these cytokines on keratinocytes include increased expression of S100A7, as well as *TNFA* and *MRP14* transcripts [176, 202].

Similar to IL-22, IL-20 receptor cytokines are implicated in psoriasis pathogenesis with expression of all three cytokines being increased in lesional skin [191, 196]. These cytokines are thought to contribute to epidermal hyperplasia in psoriatic lesions [176]. Consistent with this idea, local or systemic therapy for psoriasis reduces both epidermal hyperplasia and expression of these cytokines [203, 204]. It is uncertain whether targeting IL-20 receptor cytokines is a viable therapeutic option for psoriasis. According to *ClinicalTrials*.gov, one clinical trial with an anti-IL-20 blocking antibody was discontinued due to apparent lack of response; however, it is possible that IL-19 or IL-24 activities are sufficiently redundant to compensate despite IL-20 blockade. Paradoxically, IL-20 receptor cytokines were recently shown to exacerbate cutaneous infections with *S. aureus* in mice [205]. This is attributed to IL-20 receptor-mediated attenuation of IL-1β production, thereby decreasing the frequency of IL-17A producing T cells, which are important for host defense against infections with *S. aureus* [36, 205, 206]. This suggests the IL-20 receptor may be a viable therapeutic target for management of cutaneous infections with *S. aureus*.

Interleukin 12 Family

This family includes IL-12, IL-23, IL-27 and IL-35, all of which are heterodimers generated from the following subunits: p19, p28 (i.e. Ebi3) p35 (i.e. IL-12p35) and p40 (i.e. IL-12p40) [207]. Interleukins 12 and 23 are important pro-inflammatory cytokines in psoriasis and other diseases, and neutralizing antibodies against these cytokines are used to treat several of these diseases including psoriasis [208–211]. Understanding the roles of IL-12 and IL-23 in psoriasis and host defense against mycobacterial infections helps the clinician use these biologics effectively and safely. The other members of this family, IL-27 and IL-35, are primarily anti-inflammatory cytokines, but their roles in cutaneous immunology are less well defined and will not be discussed further in this chapter [207].

Interleukin 12

Interleukin 12 (IL-12p70) is composed of p40 and p35 and expressed by dendritic cells, monocytes, macrophages, B cells and neutrophils [212]. Stimulation of macrophages and dendritic cells with a variety of PAMPs causes IL-12 production [213, 214]. Cytokines (i.e. IFNγ, IL-4 and IL-13) and physical contact with T cells can also promote IL-12 production [215–217]. The IL-12 receptor, composed of IL-12Rβ1 and IL-12Rβ2, is expressed on T and NK cells [207]. Receptor engagement activates JAK2, Tyk2 and STAT4, triggering IFNγ production by T and NK cells [207, 218]. Stimulation of dendritic cells and monocytes with IFNγ can in turn stimulate additional IL-12 production resulting in a positive feedback loop [216]. Interleukin 12 also orchestrates differentiation of naïve T helper cells into Th1 cells, which are key producers of IFNγ (Fig. 15.1) [219, 220]. The ability of microbial products to trigger IL-12 production by dendritic cells and IL-12 to in turn dictate naïve T cell differentiation to Th1 cells is an example how cytokines link innate and adaptive immune responses.

Multiple dermatologic conditions are mediated by IL-12, some of which have been successfully treated with a p40 neutralizing antibody, which blocks both IL-12 and IL-23 [221–227]. As noted above, multiple clinical trials have established the efficacy of this antibody for psoriasis; there are case reports of therapeutic benefit in AD, HS and Behçet's disease as well [228–230]. Host defense against mycobacterial infections is also critically dependent on IL-12-driven production of IFNγ, as discussed later in this chapter.

Interleukin 23

Interleukin 23 is a heterodimer of p19 and p40, the latter of which is shared with IL-12 [231]. Dendritic cells and macrophages produce IL-23 following activation of various pattern recognition receptors (e.g. TLR4), cytokine stimulation (e.g. TNF and IL-12) and contact-dependent interactions with T

cells [212, 231, 232]. The IL-23 receptor is expressed on Th17 cells, some γδ T cells and ILC3 and is composed of IL-23R, and IL-12Rβ1; receptor engagement signals through JAK2, Tyk2, STAT3 and STAT4 [166, 231, 233–235]. Activation of STAT3 in naïve T cells with specific cytokines (e.g. IL-6) drives Th17 differentiation and IL-23 receptor expression; moreover, IL-23 stimulation further promotes IL-23R expression in these cells [166, 236]. In contrast, the IL-23 receptor is stably expressed on a subset of γδ T cells and is expressed on a subset of ILC3 [235, 237]. In Th17 cells and IL-23R$^+$ γδ T cells, IL-23 can promote expansion of these subsets and IL-17 production [166, 235, 236]. Moreover, IL-23 stimulates IL-22 production by IL-23R$^+$ ILC3 [237].

Interleukin 23 is implicated in the pathogenesis of psoriasis and many other dermatologic diseases [223, 224, 226, 227, 238–242]. Expression of both IL-23 subunits is increased in psoriatic lesions [238]. Moreover, several IL-23 responsive subsets of IL-17 producing cells are more abundant in psoriatic lesions; IL-17 and IL-22 expression are also increased in lesional skin [235, 237]. As noted, neutralization of p40 and thus IL-12 and IL-23 is an effective therapy for psoriasis. While, both cytokines likely contribute to disease, IL-23 is probably more important as demonstrated by the therapeutic efficacy of an IL-23 neutralizing antibody for psoriasis [243, 244]. In psoriasis, IL-23 is thought to drive IL-17 production; the therapeutic efficacy of IL-17 and IL-17 receptor blocking antibodies in clinical trials (discussed below) for psoriasis further supports the conclusion that IL-23 plays a more dominant role in psoriasis pathogenesis than does IL-12 (see also Chap. 43).

Interleukin 17 Family

The 6 members of this family are designated IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F; IL-17E is also known as IL-25 [245]. Emerging data suggest IL-25 can promote allergic inflammation in the skin; the reader is referred to the cited references for further information [120, 245–252]. The roles of IL-17B thru D in cutaneous immunology are not well established and will not be discussed further in this chapter. Family members IL-17A and IL-17F are important regulators of cutaneous immunity, inflammation and disease and will be discussed jointly due to shared receptor usage and biologic functions.

Interleukins 17A & 17F

Interleukin 17A, also known as IL-17, activates the same receptor as IL-17F, explaining the similar biologic functions of these cytokines. These cytokines can exist as homodimers or heterodimers of IL-17A and F [245]. Cells that produce IL-17A and/or F include several T cells subsets (i.e. Th17, Tc17 and γδTcells), NKT cells and a subset of ILC3 [165, 253–258] Investigations of IL-17 biology identified Th17 cells, a novel developmentally and functionally distinct population of T helper cells differentiated from naïve T cells stimulated with IL-1β, IL-6, IL-21, IL-23 and/or TGF-β; Th17 cell can produce IL-17A and F (Fig. 15.1) [253, 259]. Activation of the TCR and/or stimulation with IL-1 or IL23 can stimulate IL-17 production by Th17 cells, NKT cells and γδTcells [257, 260–262].

The receptor for IL-17A and F, referred to here as the IL-17 receptor, is a heterodimer of IL-17RA and IL-17RC [263]. T cells, B cells, macrophages, endothelial cells, fibroblasts and epithelial cells, including keratinocytes, express the IL-17 receptor [245]. Receptor engagement in keratinocytes recruits the adaptor protein Act1 and TRAF6 stimulating TAK1-mediated activation of NF-kB and C/EBP and MAPK-mediated activation of AP-1, culminating in transcriptional changes in IL-17 target genes [264–266]. Target genes induced by IL-17 receptor activation include those encoding pro-inflammatory cytokines (TNF, IL-1, IL-6, G-CSF, GM-CSF), chemokines (CXCL1, 3, 5, 6 and 8; CCL2, 7 and 20), antimicrobial peptides/proteins and matrix metalloproteinases [62, 245, 267–269]. Many chemokines produced by IL-17-stimulated keratinocytes recruit neutrophils into the skin, while keratinocyte-derived CCL20 can recruit dendritic cells and CCR6$^+$ T cell subsets, including Th17 cells [268].

While IL-17A and F are implicated in the pathogenesis of other dermatologic conditions, this section will focus on the role of these cytokines in psoriasis and cutaneous infections [23, 36, 224, 270–273]. In psoriasis, IL-17 is thought to stimulate keratinocyte-derived chemokine and growth factor production (i.e. G-CSF & GM-CSF) leading to recruitment of neutrophils, T cells (e.g. Th17 cells) and dendritic cells into lesional skin [23]. Cellular sources of IL-17 in psoriasis include Th17 cells, γδT cells, NKT cells and ILC3 [23, 274]. Data from clinical trials suggest neutralizing antibodies to IL-17A and the IL-17 receptor (anti-IL-17RA) will be effective therapies for psoriasis (see also Chap. 43) [210, 275–277].

With respect to cutaneous infections, IL-17A and/or F play important roles in host defense against *S. aureus* and *Candida spp*. [36, 273]. As noted, neutrophil influx into the skin, a critical aspect of host defense against *S. aureus* infections, is regulated by chemokines and growth factors produced by IL-17 stimulated keratinocytes [36]. Host defense against cutaneous *Candida spp*. infections is also regulated by IL-17A and IL-17F as defective IL-17 signaling can cause chronic mucocutaneous candidiasis (CMC) [273]. In CMC, mutations in IL-17RA and IL-17F have been identified in some patients, while others have mutations that impair development of IL-17 producing T cells or mutations that lead to development of IL-17 neutralizing antibodies [273].

Interferon Family

Interferons were originally described over 50 years ago as soluble cell/tissue-derived factors that *interfered* with the ability of viruses to infect other cells or tissues [278]. Since that time, over a dozen interferons have been identified in humans. This section will focus on the two canonical classes of interferons, types I and II, which are distinguished by receptor usage and partially divergent downstream signaling pathways. There are over a dozen type I interferons in humans; all use the same receptor. In contrast, there is only one type II interferon, IFNγ, which uses a unique receptor. Interferons are important for various aspects of host defense against pathogens and are involved in pathogenesis of multiple inflammatory diseases. As such, the following sections will provide more detail on the biology of type I and type II interferons and the dermatologic diseases associated with each type.

Type I Interferons

Within this family are multiple interferon alpha (IFN-α) proteins and IFN-β, as well as IFN-k and -ω [279]. Virtually all nucleated cells can produce at least one type I interferon, affording a means of defense against viral infections and other pathogens; however, some cell types, namely plasmacytoid dendritic cells, produce larger quantities of type I interferons [280, 281]. Type I interferon production is triggered by various PAMPs, which are not limited to viral products; for example, activation of TLR4 by LPS can trigger type I interferon production by macrophages. The toll-like receptors, TLR3, TLR4, TLR7 (target of imiquimod), TLR8 and TLR9 are important inducers of type I interferon production [282].

The type I interferon receptor (IFNAR) is widely expressed on nucleated cells and is composed of IFNAR1 and IFNAR2 subunits and signals via JAK1, Tyk2, STAT1, STAT2 and interferon response factor (IRF) 9 [283]. Following IFNAR activation, a STAT1/STAT2 heterodimer forms and binds to the protein IRF9; this protein complex then translocates to the nucleus to regulate hundreds of interferon target genes. Upregulated interferon target genes include those encoding antiviral proteins with multiple mechanisms of action as well as MHC class I molecules, chemokines and other proteins. Activation of IFNAR has anti-proliferative and in some cases (e.g. keratinocytes), pro-differentiation effects [284]. Thus interferons limit propagation of virus by directly interfering with viral replication, limiting host cell proliferation and promoting presentation of viral antigens in the context of MHC class I to T cells [284]. Other effects of type I interferons include T cell, NK cell, macrophage and myeloid dendritic cell activation and promotion of T cells survival [23, 281].

The local and systemic actions of type I interferons have also been exploited therapeutically for dermatologic disease. For example, recombinant type I interferons are used to treat some cases of malignant melanoma and CTCL [285, 286]. Moreover, imiquimod, a TLR7 agonist, stimulates type I interferon production and is used topically to treat verruca vulgaris and molluscum contagiosum [287, 288]. Type I interferons are also implicated in the pathogenesis of psoriasis, lichen planus, cutaneous and systemic lupus, dermatomyositis, systemic sclerosis and sarcoidosis [23, 289–291]. In psoriasis, type I interferons derived from plasmacytoid dendritic cells and/or other cell types may initiate lesion formation through activation of myeloid dendritic cells that in turn produce IL-12 and IL-23 [23]. In support of this idea, psoriasis can develop or flare in patients treated systemically with type I interferons or topically with imiquimod [292, 293].

Type II Interferon (IFNγ)

Interferon gamma is a key mediator of many aspects of cutaneous and systemic immunity. In contrast to type I interferons, IFNγ production is limited to NK cells, T cell subsets (Th1 and $CD8^+$ T cells) and ILC1 [294, 295]. Myeloid cell-derived cytokines including IL-12, IL-15 and/or IL-18 as well as IL-2 can stimulate production of IFNγ by T and NK cells [296, 297]. The IFNγ receptor, IFNGR, is a heterodimer of IFNGR1 and IFNGR1. In contrast to its ligand (IFNγ), IFNGR is widely expressed on a variety of cell types including keratinocytes, macrophages and dendritic cells [295]. Activation of IFNGR activates JAK1 and JAK2, and then, STAT1, which in turn regulates IFNγ target genes [295].

In contrast to type I interferons, IFNγ promotes MHC class II expression, is less critical for host defense against viruses and is more important for host defense against pathogens that target macrophages like *Mycobacterial spp*. [284, 295]. One of the critical functions of IFNγ in host defense is macrophage activation, a process in which activation of IFNGR leads to production of cytokines, nitric oxide and reactive oxygen species, which all contribute to clearance of intracellular pathogens that infect macrophages (i.e. *Mycobacterial spp*., as well as some fungi, parasites and other bacterial species) [298]. The critical role of IFNγ (and IL-12 which triggers IFNγ production) and macrophage activation in host defense against *Mycobacterial spp*. is highlighted by the fact that that human immunodeficiency disorders limited to type II interferon and/or IL-12 signaling (e.g. mutations in IFNGAR1, IFNGAR2, STAT1) greatly increase susceptibility to developing mycobacterial infections [298]. Other diseases in which IFNγ is implicated in pathogenesis include vitilgo, alopecia areata, allergic contact dermatitis, psoriasis and AD [23, 299–302].

A final note on IFNγ and its relevance to dermatology-interferon gamma release assays (IGRAs) are used to identify patients at risk for latent or active infections with *Mycobacterium tuberculosis* [303]. These tests involve incubating whole blood or PBMC with purified antigens from *M tuberculosis*; some T cells in the blood samples of patients with prior exposure to, or latent/active infection with *M tuberculosis*, can recognize these antigens and respond by producing IFNγ which is quantified [303]. Sufficiently high levels of IFNγ production in this assay alert the clinician to the possibility of latent or active infection. In addition to helping identify and treat patients at risk for infection, the results of this test help guide decisions regarding use of immunosuppressive medications for dermatologic and other disease indications.

Transforming Growth Factor β (TGF-β) Family

This family includes three isoforms of TGF-β (encoded by different genes) [304]. The isoform of TGF-β whose role in cutaneous immunology and disease is best understood is TGF-β1; hereafter though, all isoforms will be referred to collectively as TGF-β. In contrast to many cytokines described in this chapter, TGF-β and one or more of its receptors are expressed by most nucleated cells; sources of TGF-β in the skin include fibroblasts, macrophages and keratinocytes [305–308].

Like IL-1β and IL-18, TGF-β is synthesized in an inactive form and requires proteolytic cleavage to generate an active cytokine [309]. Unlike IL-1β and IL-18, maturation of TGF-β requires intracellular and extracellular processing (Fig. 15.4). Initially, the *TGFB* mRNA is translated into Pre-pro-TGF-β, which then undergoes proteolysis, removing the signal peptide and generating two proteins, a monomer of latency associated peptide (LAP) and a monomer of TGF-β; two LAP molecules form a homodimer, as do two TGF-β molecules, which then bind to each other (Fig. 15.4). Homodimers of LAP and TGF-β then bind another protein called latent TGF-β binding protein (LTBP) [309]. This complex is then secreted and can bind to the surface of cells bearing receptors for LAP or via binding of LTPB to extracellular matrix (ECM) components like collagen [310]. In this complex, TGF-β is inactive; depending on its location (i.e. ECM versus cell surface), proteolytic cleavage by a variety of different proteases or LAP receptor-mediated conformational changes liberate TGF-β, which can then bind and activate its receptors (Fig. 15.4) [310].

There are three receptors for TGF-β designated I, II and III [304, 311]. The type III receptor binds to TGF-β but does not activate downstream signaling cascades [311]. Binding of TGF-β to the type II receptor recruits the type I receptor, triggering phosphorylation of the proteins SMAD2 and 3, which then recruit SMAD4 (Fig. 15.4).

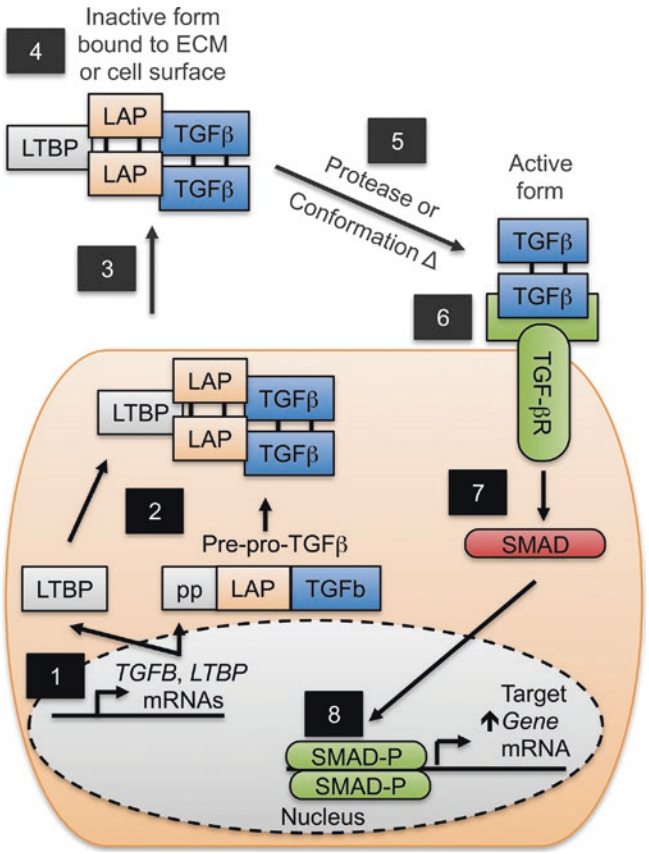

Fig. 15.4 Sequence of TGF-β production and receptor activation (*TGF-βR*): (*1*) *TGFB* and latent TGF-β binding protein *LTBP* mRNA is translated into Pre-pro-TGF-β and LTBP. (*2*) Pre-pro-TGF-β is cleaved into latency associated protein (*LAP*) and TGF-β followed by formation of a multi-protein complex of LAP, TGF-β and LTBP. (*3*) The complex is secreted. (*4*) It then binds extracellular matrix (*ECM*) components or LAP receptors on cells. (*5*) Proteolysis or conformational changes in the complex liberate TGF-β. (*6*) TGF-β then binds TGF-βR. (*7*) The TGF-βR then converts inactive SMAD proteins (*SMAD*) into an active phosphorylated form (*SMAD-P*). (*8*) Complexes of SMAD-P then translocate to the nucleus to regulate TGF-β target genes

This complex in turn binds to and regulates the expression of TGF-β target genes [312].

While TGF-β has many functions, the focus here will be on wound healing and cutaneous immunity. In the skin and other organs, TGF-β regulates extracellular matrix (ECM) production by stimulating synthesis of collagen, fibronectin and integrins by fibroblasts; ECM degradation is also suppressed by TGF-β via reduced metalloproteinases production and increased synthesis of protease inhibitors [313]. These effects of TGF-β are thought to be important for ECM formation and preservation during the wound healing processes including scar formation. Excessive TGF-β activity is thought to contribute to hypertrophic and keloid scar formation and sclerotic or fibrotic disorders like systemic sclerosis (scleroderma) and nephrogenic systemic fibrosis [313–315]. Immunoregulatory effects of TGF-β include promotion of Treg, Th9 and Th17

cell development and inhibition of Th1 and Th2 cell differentiation (Fig. 15.1) [309]. In addition, TGF-β (along with IL-10 and IL-35) is an important effector molecule that regulatory T cells use to suppress effector T cells [316].

Chemokines

Chemokines are an entity unto themselves in structure, function and signaling mechanisms. They are critical regulators of leukocyte recruitment directing cell migration between secondary lymphoid tissues, the blood and peripheral tissues [317, 318]. The two largest subgroups of chemokines are designated CXC and CC, so named for the configuration of four conserved cysteine residues in these molecules. Chemokines activate G protein coupled receptors designated as CCR for CC chemokines and CXCR for CXC chemokines. Receptor engagement activates multiple signaling pathways that culminate in cytoskeletal reorganization, integrin activation and chemotaxis along a chemokine gradient [319]. Given similar mechanisms by which chemokines and their receptors act, this section will not discuss all chemokine and chemokine receptors used to recruit different leukocyte subsets to and from the skin, blood and lymph nodes; instead the role of chemokines in dendritic and T cell trafficking from and to the skin, respectively, will be presented as examples of how chemokines regulate leukocyte movement. In addition, Table 15.2 lists chemokines and chemokine receptors that promote chemotaxis of specific leukocyte subsets.

Dendritic Cell Trafficking from the Skin to Lymph Nodes

Sensitization to antigens encountered in the skin is dependent on acquisition of antigen by immature dendritic cells (see also Chap. 9) [317, 318]. Immature dendritic cells in the skin (and other sites) are adept at antigen uptake. Keratinocyte-derived CCL20 binding to CCR6 on Langerhans cells helps maintain this population of dendritic cells in the epidermis [318, 320, 321]. Antigen uptake and inflammation promote Langerhans cell maturation and migration to the skin from draining lymph nodes [318]. Inflammatory stimuli like TNF promote Langerhans cell migration out of the epidermis and into the dermis via downregulation of CCR6 and upregulation of CXCR4, the latter of which is engaged by the chemokine CXCL12 [318]. In the dermis, Langerhans cells and other dendritic cell subsets upregulate CCR7 that directs migration towards CCL19 and CCL21 produced in the lymph node [318, 322, 323]. In lymph nodes, mature dendritic cells present antigen to naïve T cells, promoting differentiation into effector memory T cells that mediate various aspects of host defense (Fig. 15.1) [317].

Table 15.2 Representative chemokines and receptors used by leukocyte subsets

Target cell	Chemokine receptor	Chemokine
T cell	CCR4	CCL17 (K, DC, E, M); CCL22 (K)
	CCR6	CCL20 (K, E, M)
	CCR8	CCL1 (E, Ma)
	CCR10	CCL27 (K)
	CXCR3	CXCL9, 10, 11 (K, F, E, M)
	CXCR4	CXCL12
B cell	CXCR4	CXCL12
Monocyte	CCR1	CCL3
	CCR2	CCL2
	CCR5	CCL5, 8, 13
	CCR6	CCL20 (K, E, M)
	CXCR1	CXCL6, 8
	CXCR2	CXCL1
	CXCR3	CXCL9, 10, 11 (K, F, E, M)
	CXCR4	CXCL12
	CX3CR1	CX3CL1
Eosinophil	CCR3	CCL24, 26
Neutrophil	CCR1	CCL3
	CXCR1	CXCL6, 8
	CXCR2	CXCL1, 2, 3, 5, 6, 8

K keratinocyte, *DC* dendritic cell, *M* monocyte, *E* endothelial cell

T Cell Trafficking from the Blood to Skin

The entry of leukocytes into the skin from the blood begins in postcapillary venules with rolling on selectins expressed on the luminal side of the endothelium (see also Chap. 8) [324]. Rolling is followed by firm adhesion of leukocytes to the endothelium via integrins, a process promoted by stimulation of rolling leukocytes with chemokines [324].

Chemokines regulate T cell trafficking into the skin [317, 318]. Chemokine receptors important for directing T cells from the blood and into the skin include CCR4, CCR6, CCR8, CCR10 and CXCR3 [318]. An important marker of skin-homing (and skin resident) T cells is cutaneous lymphocyte antigen (CLA) which contributes to T cell rolling in postcapillary venules in the skin [325, 326]. The majority of CLA^+ T cells express CCR4, one of the ligands for which CCL17 is produced by keratinocytes, dendritic cells and endothelial cells [318, 325]. Activation of CCR4 by CCL17 promotes T cell entry into the skin from postcapillary venules [318]. Ligands for CCR6, CCR10 and CXCR3 include CCL20, CCL27, and CXCL10, produced by keratinocytes. Multiple cell types other than keratinocytes also produce chemokines mediating recruitment of leukocytes into the skin.

Chemokines and chemokine receptors are implicated in the pathogenesis of numerous dermatologic diseases; in fact,

it would be challenging to conceive of an inflammatory, infectious or autoimmune disease in which chemokines are not involved. Notable examples include CTCL, GVHD, vitiligo and alopecia areata [300, 327, 328]. According to *ClinicalTrials*.gov, clinical trials are ongoing for a CCR4 blocking antibody in CTCL and a CCR5 antibody for GVHD. Thus, knowledge about chemokine and chemokine receptor biology is on the verge of impacting how dermatologists and other clinicians manage disease in their patients.

Concluding Remarks

Much has changed in dermatology and cutaneous immunology since the term *cytokine* was coined 40 years ago. Even since the last edition of this text, a number of novel cytokine or chemokine-targeted therapies have entered clinical trials and/or routine clinical practice for dermatologic diseases. These are certainly exciting times, but the employment of novel immunomodulators will require an evermore sophisticated and nuanced approach to know how and when to safely and effectively use these therapies. In order to achieve these goals a sound understanding of cytokine and chemokine biology will be important.

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Questions

1. "Drug X" inhibits IL-12, TNF and IFNγ. Which of the following is correct regarding potential uses and risks of using this drug?
   A. Drug X is likely to trigger a flare of psoriasis
   B. Drug X is unlikely to be effective for sarcoidsosis
   C. Drug X may trigger reactivation of tuberculosis
   D. Drug X is likely to trigger a flare of hidradenitis suppurativa
   E. Drug X is unlikely to have any efficacy in treating psoriasis

2. The cytokines IL-4, IL-5, TSLP and IL-31 are implicated in atopic dermatitis pathogenesis. Which of the following is correct regarding these cytokines?
   A. TSLP activates keratinocytes
   B. Neuron-derived IL-31 causes pruritus
   C. IL-4 promotes keratinocyte differentiation
   D. IL-5 promotes eosinophil infiltration into skin
   E. TSLP is made by neurons

3. Type I interferons (IFN), IL-12 and IL-23 are implicated in psoriasis pathogenesis. Which of the following is correct?
   A. IL-12 promotes IFNγ production by macrophages
   B. IFNγ promotes IL-12 production by T cells
   C. Plasmacytoid dendritic cells produce large quantities of Type I IFN
   D. The p19 subunit is shared between IL-12 and IL-23
   E. Blockade of Type I IFN signaling can trigger psoriasis flares

4. Cytokines in the IL-1 family regulate many inflammatory pathways and dermatologic diseases. Which of the following is correct?
   A. Production of IL-1β is decreased in autoinflammatory diseases
   B. The TIR domain is shared between IL-1 family and toll-like receptors
   C. Inhibition of IL-36 activity may trigger a flare of psoriasis
   D. IL-1α and IL-1β signal via different receptors
   E. Caspase 1 cleaves and inactivates IL-1β

5. What is the best answer regarding the role of cytokines in host defense against microbial pathogens?
   A. Mutations in *TNF* cause chronic mucocutaneous candidiasis
   B. Blockade of IL-17A and F can prevent chronic mucocutaneous candidiasis
   C. Defective IL-4 and 13 signaling increase the risk of mycobacterial infections
   D. IL-1α and β regulate host defense against cutaneous *S. aureus* infections
   E. Inhibition of IL-17A and IL-17 F can prevent cutaneous *S. aureus* infections

Answers

1. C
2. D
3. C
4. B
5. D

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Bacterial Infections

16

Lloyd S. Miller

Abstract

Bacterial skin infections are a major cause of morbidity and mortality and consequently exert a significant medical and economic burden on healthcare resources. In an era of declining antimicrobial drug development and rising antibiotic resistance among clinical bacterial isolates, there are serious concerns that current antibiotic therapy will not provide a durable solution to this public health threat. If future immune-based therapies are to provide an alternative or complementary therapy to antibiotics, a greater understanding of the key innate and adaptive cutaneous immune responses provide host defense bacterial skin infections is essential. This chapter focuses on the host defense mechanisms against two of the most common bacterial skin pathogens, *Staphylococcus aureus* (*S. aureus*) and group A *Streptococcus*.

Keywords

Staphylococcus aureus • *Streptococcus pyogenes* • Group A *Streptococcus* • Antimicrobial peptides • Pattern recognition receptors • Toll-like receptors • Inflammasome • Innate immunity • Adaptive immunity

Staphylococcus aureus

S. aureus is a Gram-positive extracellular bacterium that is the most common cause of skin and soft tissue infections in humans such as impetigo, folliculitis/furunculosis, cellulitis and infected wounds and ulcers [1–3]. *S. aureus* skin infections result in 11–14 million outpatient visits and nearly 500,000 hospital admissions annually in the U.S. [4, 5]. In addition, *S. aureus* infections can become more invasive and lead to life-threatening infections such as lymphangitis, septic arthritis, osteomyelitis, pneumonia, abscesses of various organs, meningitis, bacteremia, endocarditis and sepsis [6, 7]. *S. aureus* colonization of the skin and mucosa is found in ~30 % of the general population and is a major risk factor for infection [8–10]. Patients with atopic dermatitis, diabetes mellitus, renal disease (hemodialysis), HIV infection, a history of intravenous drug abuse and preexisting tissue injury or inflammation (e.g. ulcer, surgical wound) are predisposed to *S. aureus* infections [3]. In addition, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains are becoming increasing resistant to multiple antibiotics and are causing severe skin infections in healthy people outside of hospital settings and without known risk factors for infection [6, 11, 12]. In particular, USA300 isolates are the most common CA-MRSA strains that cause the majority of skin infections presenting to emergency rooms in the U.S. [13, 14].

Clinical Manifestations

S. aureus cutaneous infections result in a variably pyogenic immune response characterized by purulent lesions with surrounding erythema, warmth and induration [1–3].

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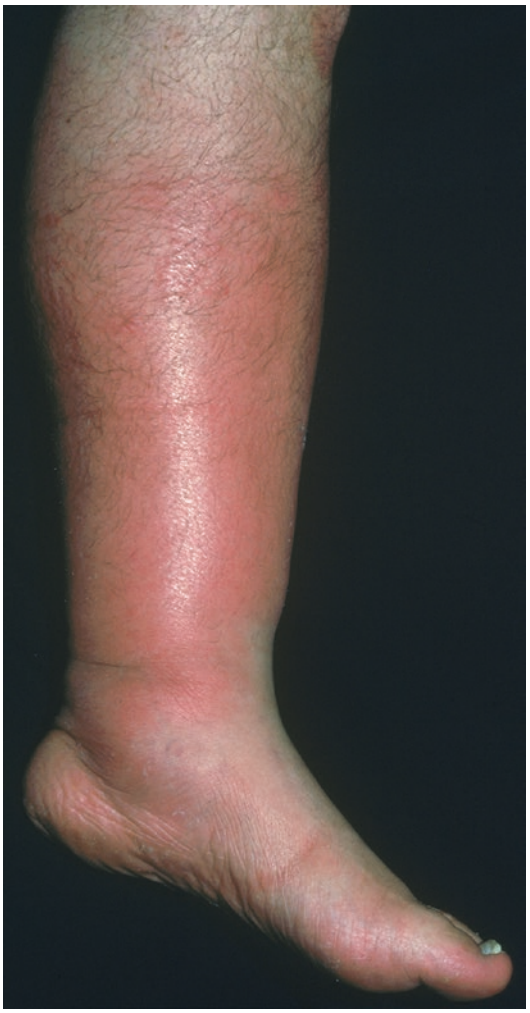

Fig. 16.1 *S. aureus* cellulitis on the lower leg. The involved skin characteristically shows signs of inflammation, including erythema, warmth, edema, and pain (Photograph is courtesy of the Victor D. Newcomer collection at UCLA and Logical Images, Inc.)

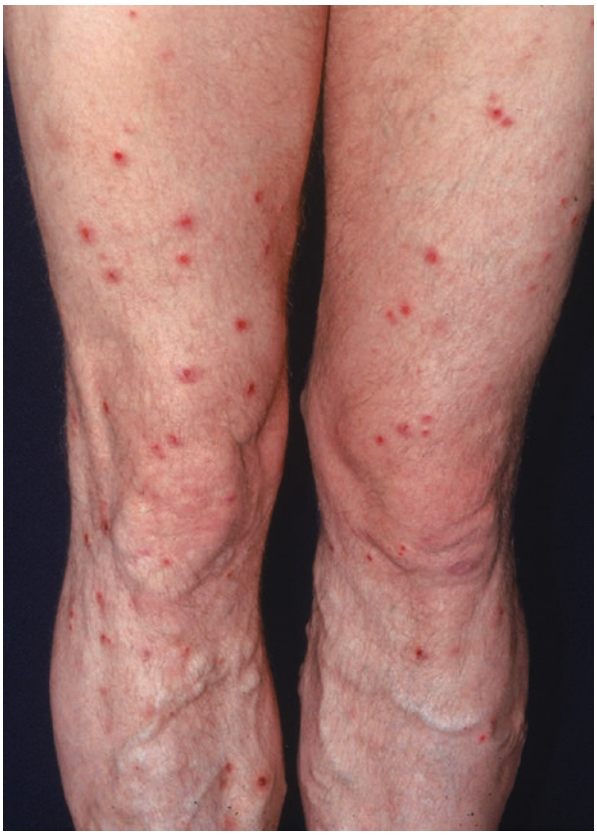

Fig. 16.2 *S. aureus* folliculitis/furuncles (boils). Typically, infected hair follicles present as follicularly-based erythematous, warm, edematous, and pus-filled papules and nodules (Photograph is courtesy of the Victor D. Newcomer collection at UCLA and Logical Images, Inc.)

Microscopically, these lesions are comprised of collections of neutrophils, a hallmark of *S. aureus* infections [15, 16]. Superficial *S. aureus* infections result in impetigo, an infection confined to the upper layers of the epidermis [1–3]. However, *S. aureus* skin infections can become more invasive and involve dermal planes and subcutaneous tissues as seen in cellulitis (Fig. 16.1) or deep ulcerative lesions as seen in ecthyma [1–3]. *S. aureus* also infects individual hair follicles leading to folliculitis, furunculosis and carbuncle formation when multiple follicles become involved (Fig. 16.2) [1–3]. Lastly, *S. aureus* can cause superinfection of inflammatory skin diseases such as atopic dermatitis and intertrigo [17].

Innate Immune Responses Against *S. aureus*

Innate immune responses are directed against conserved components of microorganisms called pathogen associated molecular patterns (PAMPs) [18, 19]. The host cellular receptors that recognize different PAMPs are called pattern recognition receptors (PRRs) [18, 19]. PAMPs are predominantly expressed by microorganisms and not by host cells, thus enabling the host's immune system to recognize the pathogen rather than self [18, 19]. In this section, soluble factors of the innate immune system such as antimicrobial peptides and complement will be discussed, followed by a review of the known PRRs, inflammasomes and the role of innate immune system cells such as neutrophils and monocytes/macrophages in host defense against *S. aureus*.

Soluble Mediators of Innate Immunity Against *S. aureus*

Antimicrobial Peptides

Antimicrobial peptides are polypeptides that have antimicrobial activity at physiologic conditions and are believed to function by disrupting bacterial membranes [20–23]. There are several human antimicrobial peptides involved in skin host defense, including defensins (α and β), cathelicidin, RNase 7, dermcidin and REG3A (Table 16.1) [20–24].

Alpha-defensins (also called human neutrophil peptides, HNPs) are produced by neutrophils, whereas β-defensins are produced predominantly by epithelial cells (including keratinocytes) and are also produced by macrophages and dendritic cells [25]. Cathelicidin is produced constitutively by neutrophils and can be induced in epithelial cells, including keratinocytes [20, 26, 27].

There are six known human α-defensins (HNP1-6), which constitute ~50 % of the peptides found within neutrophil granules [28]. Notably, HNPs 2 and 5 (and not HNPs 1, 3, 4, and 6) have antimicrobial activity against *S. aureus in vitro* [29]. The antimicrobial activity of α-defensins is likely important in host defense since *S. aureus* produces proteins such as staphylokinase, which directly binds to α-defensins to inhibit their activity [30]. In addition, MprF and products of the *dlt*ABCD operon reduce the negative charge of the bacterial membrane via lysinylating phosphatidylglycerol [31] and alanylating teichoic acids [32] in the bacterial cell envelope, respectively, to reduce the activity of α-defensins.

Table 16.1 Soluble mediators and pattern recognition receptors that contribute to host defense against *S. aureus* skin infections and colonization

	Cellular expression in skin	*S. aureus* evasion mechanisms	Mechanisms of action
Antimicrobial peptides			
α-defensins (human neutrophil peptides [HNPs])	Neutrophils	Staphylokinase, MprF, *dlt*ABCD operon	Antimicrobial activity, chemotaxis of T cells and immature dendritic cells
hBD2	Keratinocytes macrophages DCs	IsdA, *dlt*ABCD operon	Antimicrobial activity, chemotaxis of immature dendritic cells and memory CD4+ T cells via CCR6
hBD3	Keratinocytes	*dlt*ABCD operon	
LL-37	Keratinocytes macrophages neutrophils	IsdA, aureolysin, MprF, *dlt*ABCD operon	Antimicrobial activity, chemotaxis of neutrophils, monocytes and T cells via FPRL1
Dermcidin	Eccrine glands	Extracellular proteases, *dlt*ABCD operon	Antimicrobial activity
RNase 7	Keratinocytes	*dlt*ABCD operon	Antimicrobial activity
REG3A	Keratinocytes		Antimicrobial activity
Complement			
C3a, C5a	Serum	SCIN	Chemotaxis of neutrophils
C3b	Serum	SCIN, Efb, C4BP, staphylokinase	Opsonophagocytosis
Mannose-binding lectin (MBL)	Serum	SCIN, Efb, C4BP, staphylokinase	Activation of the lectin complement pathway, opsonophagocytosis,
Toll-like receptors (TLRs)			
TLR2	Multiple	SSL3	Recognize *S. aureus* lipopeptides, LTA, PGN
TLR9	Multiple		Recognize *S. aureus* DNA in endosomes
NOD2	Multiple		Recognize cytosolic muramyl-dipeptide (a breakdown product of *S. aureus* PGN)
Inflammasomes			
NLRP3/ASC	Multiple		Activated by *S. aureus* toxins, ATP & K+ efflux to induce pro-IL-1β processing
AIM2/ASC	Multiple		Activated by cytosolic *S. aureus* DNA to induce pro-IL-1β processing
Formyl peptide receptors			
FPR1, FPR2, FPR3	Macrophages Neutrophils	CHIPS	Recognize formylated peptides to promote chemotaxis, phagocytosis and oxidative burst
Tumor necrosis factor-α receptor 1 (TNFR1)			
TNFR1	Multiple		BINDS *S. aureus* protein A to promote inflammation
Peptidoglycan recognition proteins (PGLYRP1)			
PGLYRP1	Neutrophils		BINDS *S. aureus* PGN to induce antimicrobial activity
PGLYRP2	Keratinocytes		BINDS *S. aureus* PGN
PGLYRP3,4	Keratinocytes Hair follicles Sebaceous glands Sweat glands		BINDS *S. aureus* PGN

There are four well-characterized human β-defensins (HBD1-4), which are expressed by various epithelial cells, including keratinocytes, as well as by activated monocytes/macrophages and dendritic cells. HBDs 1, 2, and 4 have been shown to have only a weak bacteriostatic effect against *S. aureus in vitro* [33, 34]. In contrast, HBD3 has strong *in vitro* bactericidal activity against *S. aureus* [35]. hCAP-18 is the only known member of the human cathelicidin family and is the precursor to the active cleaved C-terminal peptide LL-37 [36–38]. Like HBD3, LL-37 has been shown to have potent antimicrobial activity against *S. aureus* [36–38].

Keratinocyte production of HBD2, HBD3, and LL-37 can be induced by live or heat-killed *S. aureus* and by *S. aureus* components, including lipopeptides and lipoteichoic acid (LTA) via activation of TLR2 [39–42]. Thus, human keratinocytes can upregulate the production of HBD2 and HBD3 in response to *S. aureus* or its components, thereby increasing the innate immune response in the skin. Interestingly, activation of the epidermal growth factor receptor (EGF-R) by wounding of human skin *in* vitro resulted in increased production of HBD3 and antimicrobial activity against *S. aureus* [43, 44]. Thus, wounded skin may resist infection by *S. aureus* via production of HBD3 [43, 44]. In addition, vitamin D has been shown to increase LL-37 production by keratinocytes, neutrophils and monocytes/macrophages [45–47], suggesting vitamin D may also promote host defense against *S. aureus* [48, 49]. Of note, the important role of HBDs and LL-37 in host defense against *S. aureus* in skin is illustrated by the expression of the surface protein, iron surface determinant A (IsdA), by *S. aureus*, which renders the bacteria resistant to hBD2 and LL-37 by decreasing the bacterial membrane hydrophobicity [50]. *S. aureus* also produces aureolysin, a protease that cleaves LL-37 to inactivate its activity [51]. Moreover, MprF is not only effective against α-defensins (see above) but can also neutralize the activity of LL-37 [52].

Newer antimicrobial peptides have been identified in skin that have activity against *S. aureus*, including RNase 7, which is produced by keratinocytes and can prevent skin colonization with *S. aureus* [53], dermcidin, which is produced by eccrine glands and secreted in human sweat [54, 55], and REG3A [24], which is produced by keratinocytes and the mouse homolog, REG3γ, has activity against *S. aureus* pneumonia [56]. Of note, *S. aureus* secretes extracellular proteases that degrade and neutralize the activity of dermcidin [57]. It should be highlighted that the *S. aureus*-derived products of the *dlt*ABCD operon appear to have a global effect on inhibiting antimicrobial peptide activity by decreasing the negative charge on the bacterial surface, since a mutant *S. aureus* strain deficient in D-alanylated teichoic acids (*dltA* mutant) was more susceptible to killing by HBD2, HBD3, LL-37, RNase 7 and dermcidin [58].

Antimicrobial peptides may also be involved in the pathogenesis of certain inflammatory skin diseases. HBD2 and HBD3 are expressed at increased levels in the hyperproliferative and inflammatory skin disease, psoriasis, which has been associated with a Th1 and Th17 cytokine profile [33, 35, 59, 60]. In contrast, levels of HBD2, HBD3, and LL-37 are expressed at significantly lower levels in atopic dermatitis, another inflammatory skin disease that is associated with a Th2 cytokine profile [59, 60]. These findings provide an explanation of why *S. aureus* colonization and superinfection is more frequently seen in atopic dermatitis and rarely in psoriasis.

Lastly, antimicrobial peptides not only have microbicidal activity, but also promote the recruitment of immune system cells to the site of infection. For example, HNPs promote chemotaxis of T cells and immature dendritic cells, HBDs promote chemotaxis of immature dendritic cells and memory CD4 T cells, and LL-37 promotes chemotaxis of neutrophils, monocytes, and T cells [61–63]. The chemotactic activity of HBDs and LL-37 is mediated by the chemokine receptor CCR6 and the formyl peptide receptor-like 1 (FPRL1), respectively [61–63].

Complement

The complement system includes a family of serum proteins, proteolytic fragments and cell surface receptors [64, 65]. There are three main functions of complement activation: (1) direct killing of bacteria via formation of the membrane attack complex (MAC), which perforates cell membranes; (2) promotion of phagocytosis by opsonizing the bacterial surface with complement components such as C3b; and (3) recruitment of immune system cells to the site of infection by generating the complement chemoattractant peptides C3a and C5a [64, 65]. There are three pathways of complement activation: the classical, alternative and lectin pathways [64, 65]. Each pathway requires generation of an enzyme complex called the C3 convertase [64, 65]. In the classical pathway, generation of the C3 convertase involves the interaction of complement components with natural IgM antibody or antigen specific IgG antibody that is bound to antigens of the pathogen [64, 65]. In the alternative pathway, there are low levels of direct activation of the C3 convertase by components of the pathogen [64, 65]. In the lectin pathway, the C3 convertase is activated via recognition of carbohydrate groups on the surface of the bacteria via mannose-binding lectin (MBL) or via H-, L-, or M-ficolin and MBL-associated serine proteases (MASP1, 2 and 3) [66].

There have been several reports demonstrating the key role of complement in host defense against *S. aureus* (Table 16.1). First, in a mouse model of *S. aureus*-induced arthritis and bacteremia, depletion of complement components resulted in higher mortality and significantly decreased neutrophil recruitment and impairment of phagocytosis [67]. In another study, complement mediated opsonization by C3b as

well as activation of complement receptor 1 (CD35) (the primary receptor for C3b) were more critical in controlling *S. aureus* bacteremia than C5a or generation of the MAC [68].

MBL is also important in host defense against *S. aureus* infections [69–71]. For example, individuals with an MBL gene mutation, who have impaired MBL-dependent opsonization, suffer from recurrent *S. aureus* infections [72]. MBL as well as IgG directed against glycoepitopes on teichoic acid facilitates complement-mediated opsonophagocytosis of *S. aureus* [73, 74]. In addition, MBL-deficient mice had only a slightly decreased survival whereas C3-deficient mice and mice deficient in both MBL and C3 had markedly decreased survival compared with wildtype mice after an intravenous challenge of *S. aureus* [69, 70]. Thus, C3 and complement activation may play a more important role than MBL in host defense against *S. aureus* [69, 70]. Finally, MBL can bind to *S. aureus in vitro*, resulting in increased phagocytosis [71]. Taken together, MBL is involved in activation of the lectin complement pathway and in opsonization of *S. aureus*.

S. aureus has several mechanisms to counteract complement activity. *S. aureus* produces a protein called staphylococcus complement inhibitor (SCIN), which blocks activation of the complement cascade by inhibiting the C3 convertase [75]. In addition, *S. aureus* secretes extracellular fibrinogen-binding protein (Efb) and extracellular complement-binding (Ecb) proteins, which bind C3 and blocks C3 opsonization [76–78]. *S. aureus* also produces C4b-binding protein (C4BP) that cleaves CD4b into inactive forms iC4b and C4d, which decreases C3b-mediated opsonization [79]. Lastly, staphylokinase (SAK) inhibits opsonization of *S. aureus* and subsequent phagocytosis by converting plasminogen into plasmin on the bacterial surface, which leads to removal of the anti-staphylococcal opsonins IgG and C3b [80].

Pattern Recognition Receptors that Recognize Components of *S. aureus*

Toll-Like Receptors

Toll-like receptors (TLRs) are important PRRs involved in host defense against a variety of pathogenic microorganisms, including *S. aureus* [18, 19]. Activation of TLRs initiates several signaling cascades including NF-kB activation, ultimately leading to production of cytokines, chemokines, antimicrobial peptides and up-regulation of costimulatory and adhesion molecules involved in innate and adaptive immune responses [18, 19].

Of the known human TLRs (1-10), TLR2 has been the most implicated in host defense against *S. aureus* (Fig. 16.3, Table 16.1). TLR2 is expressed on the cell surface of numerous cell types in the skin, including keratinocytes, Langerhans cells, monocytes/macrophages, dendritic cells, mast cells, endothelial cells, fibroblasts and adipocytes [81–89]. TLR2 can be activated by live or heat-killed *S. aureus* as well as the *S. aureus* components, lipopeptides, lipoteichoic acid (LTA) and peptidoglycan (PGN) [90].

With regard to *S. aureus* skin infections, TLR2-deficient mice develop larger skin lesions than wildtype mice after *S. aureus* skin inoculation [91–93]. Human keratinocytes also express TLR2 and can be activated by live or heat-killed *S. aureus* and *S. aureus* components, resulting in increased production of cytokines such as IL-1β, IL-8, TNFα and production of HBD2 and HBD3 [82, 85, 86, 94]. Polymorphisms in TLR2 have been linked to increased severity of atopic dermatitis [95–98], which is frequently associated with superinfection by *S. aureus* [99, 100]. In addition, TLR2 was found to enhance barrier repair in human skin and the reduced expression of TLR2 in atopic dermatitis may contribute to the impaired skin barrier in this disease [101]. Most recently, a study in mice found that activation of TLR2 promoted the shift from acute Th2-mediated dermatitis to chronic cutaneous inflammation in a mechanism that involved IL-4 suppression of IL-10 [102]. This study provides additional rationale for the therapeutic targeting of IL-4, which is currently under investigation against atopic dermatitis in human trials [103].

Since *S. aureus* lipopeptides, LTA and PGN have distinct biochemical structures, it was unclear how one receptor could recognize such a broad spectrum of molecules [104]. However, TLR2 interacts with other TLRs and additional co-receptors, which enables TLR2 to recognize these different ligands. TLR2 heterodimerizes with TLR1 or TLR6 to recognize tri-acyl and di-acyl lipopeptides, respectively [105, 106]. Therefore, the ability of the host to recognize certain lipopeptides depends on the formation of TLR2 heterodimers. CD14, a membrane protein that lacks an intracellular signaling domain, was initially characterized as a TLR4 co-receptor for LPS of Gram-negative bacteria [104]. However, CD14 also acts as a TLR2 co-receptor to recognize *S. aureus* LTA and PGN [107, 108]. CD14 is likely involved in cutaneous immunity against *S. aureus* since studies have demonstrated that increased CD14 expression in keratinocytes inhibited growth of *S. aureus* [49, 109]. In addition, CD36 represents another TLR2 co-receptor involved in the recognition of *S. aureus* LTA (which is di-acylated) and in the activation of signaling via the TLR2/6 heterodimer [92, 110]. The importance of TLR2 is exemplified by the existence of superantigen-like protein 3 (SSL3), a *S. aureus*-derived factor that binds and inhibits the function of TLR2 [111, 112].

TLR9 is an intracellular TLR that is found spanning the membranes of endosomes that has been shown to recognize hypomethylated CpG (cytosine-phosphate-guanosine) motifs of bacterial DNA and is involved in promoting type I interferon responses [113–115]. Although the role of TLR9 against *S. aureus* skin infections is not entirely clear,

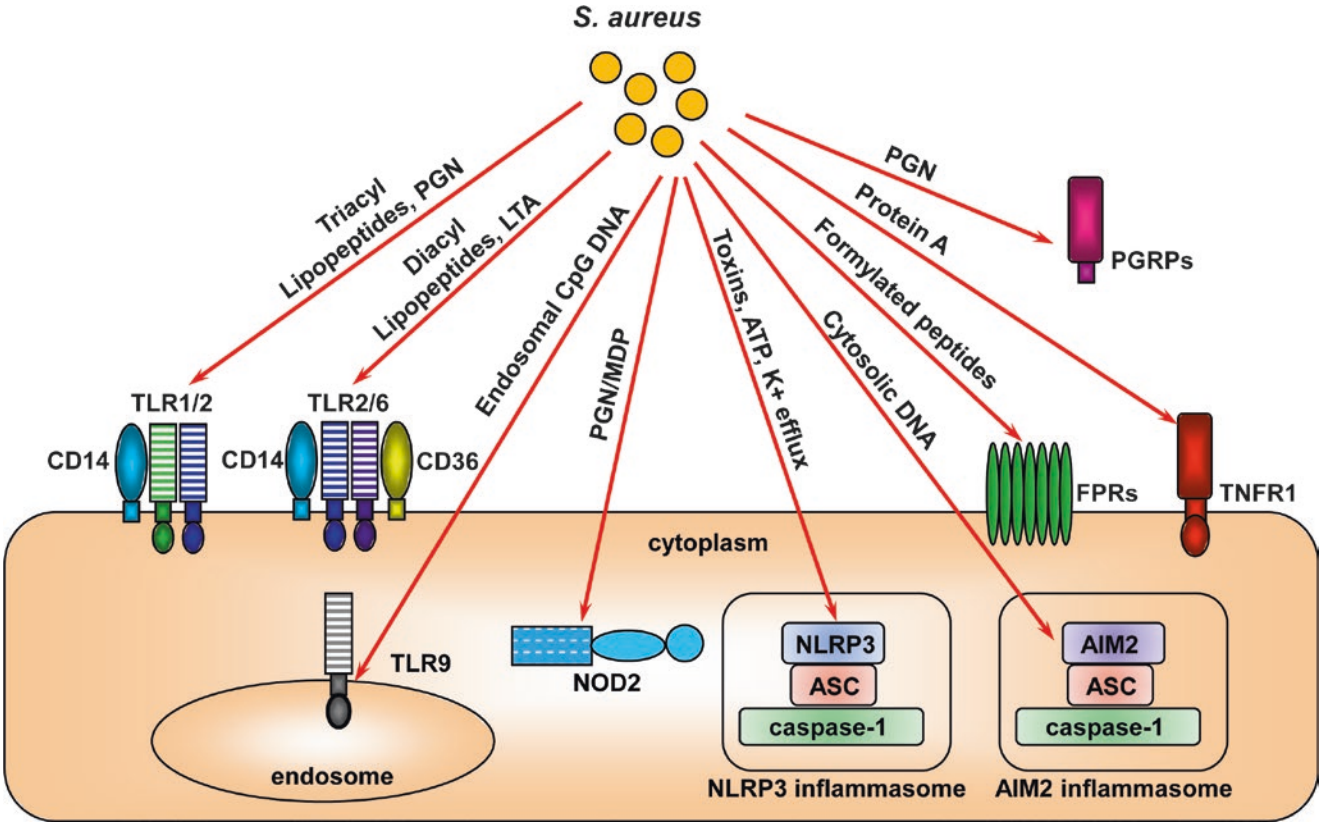

Fig. 16.3 Pattern recognition receptors (PRRs) of host cells involved in recognizing components of *S. aureus* and initiating immune responses. The *S. aureus* components recognized by these PRRs and the cellular localization of these PRRs are shown

TLR9-deficient mice have been found to have enhanced clearance against a *S. aureus* pneumonia infection, suggesting that TLR9 responses may not be associated with protection against *S. aureus* infections [116].

Nucleotide-Binding Oligomerization Domain Proteins (NOD1 and NOD2)

In contrast to TLR2, nucleotide-binding oligomerization domain proteins (NOD1 and NOD2) are found in the cytosol and detect breakdown products of PGN [117]. NOD1 recognizes breakdown products of Gram-negative PGN [117]. In contrast, NOD2 recognizes muramyl dipeptide (MDP), which is a breakdown product of both Gram-positive and Gram-negative PGN [117], and has been shown to recognize MDP-derived from *S. aureus* PGN (Fig. 16.3, Table 16.1) [118]. After ligand detection, NODs activate a signaling pathway that results in NF-kB activation and transcription of host genes involved in innate and acquired immune responses [117].

Since NOD2 is a cytoplasmic receptor, this calls into question whether an intracellular PRR could be involved in recognition of a *S. aureus* infection, since *S. aureus* has classically been considered an extracellular pathogen. However, several studies have found that *S. aureus* can invade the cytoplasm of various cells, including keratinocytes, epithelial cells, fibroblasts, endothelial cells, osteoblasts and neutrophils [119]. Once *S. aureus* enters the cytoplasm, host and/or bacterial enzymes may break down *S. aureus* PGN into MDP that can be recognized by NOD2 [119]. A few studies have found that NOD2 is involved in host defense against *S. aureus* skin infections in mice [91, 120]. Moreover, NOD2 expressed by keratinocytes can induce inflammatory cytokines such as IL-1β, IL-6 and IL-17C [121, 122], which promote antimicrobial mechanisms (e.g., neutrophil recruitment and activation and expression of antimicrobial peptides) against *S. aureus* [91, 120, 122]. Finally, NOD2 may also play a role against human *S. aureus* infections because polymorphisms in NOD2 (like TLR2) were identified in patients with atopic dermatitis [123, 124].

Inflammasomes

IL-1β plays a critical role in the recruitment of neutrophils to the site of *S. aureus* infection in the skin [91, 93, 125, 126]. Although, PRRs such as TLRs and NODs can induce transcription and translation of pro-IL-1β (as well as

pro-IL-18) [127], a second signal is required to induce cleavage of pro-IL-1β (and pro-IL-18) into its active and secreted form. This process is typically mediated by inflammasomes, which are cytoplasmic protein complexes that regulate caspase-1-mediated proteolytic processing of pro-IL-1β into active IL-1β (Fig. 16.3, Table 16.1) [128–130]. There are several known inflammasome complexes, which are reviewed elsewhere [128–130]. However, the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome has been the primary inflammasome complex implicated in IL-1β processing in the context of *S. aureus* infections (Fig. 16.3). In *S. aureus*-infected macrophages *in vitro*, the NLRP3-inflammasome processes IL-1β in a mechanism that involves activation of this inflammasome by *S. aureus* toxins (α-, β-, and γ-toxins and Panton-Valentine leukocidin [PVL]), ATP/$P2X_7R$ activation and phagosomal rupture [131–136]. This may be relevant to *S. aureus* skin infections *in vivo*, since mice deficient in ASC (apoptosis-associated speck-like protein containing a CARD), which is a critical component of the NLRP3 inflammasome, has the same impaired immune response against *S. aureus* skin infections as IL-1β-deficient mice [126]. In addition, the AIM2 (absent in melanoma 2) inflammasome, which like NLRP3 requires interaction with ASC [137–139], was recently shown to be important for host defense against a *S. aureus* brain abscess infection [140]. AIM2 recognizes cytosolic bacterial DNA [137–139] and the AIM2/ASC inflammasome may recognize *S. aureus* DNA in the cytosol to promote host defense. Interestingly, in certain cell types, such as neutrophils, processing of IL-1β can be mediated in an inflammasome-independent manner via the activity of other proteases, such as elastase, proteinase 3, cathepsins and matrix metalloproteinases (MMPs) [141–145]. In addition, neutrophils have been shown to be an important source of IL-1β during *S. aureus* skin infections in mice and it is likely that both inflammasome and non-inflammasome mechanisms contribute to IL-1β processing and release in neutrophils [91].

Formyl Peptide Receptors (FPRs)

Bacteria but not mammalian cells produce peptides and proteins containing formylated methionine [146]. These formylated non-self peptides and proteins can be recognized by formyl peptide receptors (FPRs) on host cells (including macrophages and neutrophils) (Fig. 16.3, Table 16.1) [146]. There are three known human FPRs, FPR1, FPR2 and FPR3 [146]. In particular, human FPR1 and the mouse ortholog mFPR1 as well as human FPR2 have been implicated in promoting neutrophil chemotaxis, phagocytosis and oxidative burst against *S. aureus* infections [147–149]. The importance of FPRs in host defense against *S. aureus* is demonstrated by the activity of the chemotaxis inhibitory protein of staphylococci (CHIPS), which blocks the chemotactic activity of FPR1, FPR2 and FPR-like 1 inhibitory proteins (FLIPr and FLIPr-like) [150, 151].

Tumor Necrosis Factor-α Receptor 1 (TNFR1)

TNF-α receptor 1 (TNFR1) is a receptor for TNF-α that is expressed on many different cell types. *S. aureus* protein A, which is known to bind the Fc portion of antibody, was found to activate TNFR1 to trigger production of proinflammatory cytokines and chemokines and contributed to virulence in a mouse model of *S. aureus* pneumonia (Fig. 16.3, Table 16.1) [152, 153]. A similar mechanism of protein A and TNFR1 interaction has been shown to occur in human keratinocytes, which induces production of proinflammatory cytokines and chemokines [154]. However the relevance of TNFR1 in contributing to the pathogenesis of *S. aureus* skin infections *in vivo* is unclear since TNFR1-deficient mice had similar lesion sizes and bacteria clearance as wildtype mice during an *in vivo S. aureus* skin infection [93].

Peptidoglycan Recognition Proteins (PGRPs)

In humans, there are four PGRP genes (PGLYRP1, 2, 3, 4, formally named PGRP-S, -L, -Iα, and -Iβ based on their short [S], long [L] and intermediate [I] transcript lengths) [155]. All of these gene products are secreted proteins (Fig. 16.3, Table 16.1) [155]. PGLYRP1 is expressed within tertiary granules of neutrophils and it has been shown to bind *S. aureus* PGN to promote antimicrobial activity [156, 157]. PGLYRP2 is expressed in keratinocytes and has an active amidase that cleaves *S. aureus* PGN [158]. PGLYRP3 and PGLYRP4 are also expressed in the kertainocytes as well hair follicles, sebaceous glands and sweat glands [159]. It is unclear whether these PGRPs play an important host defense role against *S. aureus* skin infections. However, PGLYRP2-deficient mice had no immune impairment against a systemic challenge with *S. aureus* [160].

Cellular Innate Immune Responses Against *S. aureus*

Neutrophils

Neutrophils are first responders of the innate immune system and are recruited to sites of *S. aureus* infection [15, 16]. Neutrophilic responses are thought to be crucial for immunity against both primary and recurrent *S. aureus* infections since patients with congenital or acquired defects in neutrophil number, recruitment or function (e.g., congenital conditions such as chronic granulomatous disease and acquired conditions such as neutropenic chemotherapy

patients or patients with impaired neutrophil function in diabetes or renal insufficiency) are highly susceptible to *S. aureus* infections in many tissues and organs, including the skin [127]. Keratinocytes and other resident skin cells produce neutrophil-attracting chemokines such as neutrophil chemotactic factor IL-8 (CXCL8), growth-related oncogene-α, -β, -γ (GRO-α, -β, -γ), neutrophil-activating peptide-2 (NAP-2; CXCL7) and epithelial cell-derived neutrophil-activating peptide-78 (ENA-78, CXCL5) [161]. All of these chemokines contain glutamic acid-leucine-arginine (ELR) residues preceding the first cysteine and activate the receptors CXCR1 and CXCR2 on neutrophils to promote chemotaxis and are thus called ELR+ chemokines [161]. The antimicrobial peptide LL-37 and the complement components C3a and C5a are also strong neutrophil chemoattractants [161, 162]. In addition, neutrophils themselves release leukotrienes, which are proinflammatory molecules that are chemoattractant for most leukocytes [161].

One of the main neutrophil functions is to engulf microbes into a phagosome, which fuses with a lysosome to form a phagolysosome (Fig. 16.4) [15, 16]. In the phagosome, reactive oxygen species (ROS) are produced such as superoxide (O_2–), hydrogen peroxide (H_2O_2), and hyperchlorous acid (HOCl) by the enzymes NADPH oxidase, superoxide dismutase and myeloperoxidase (MPO), respectively. These ROS are toxic to certain bacterial pathogens, but *S. aureus* is somewhat resistant to ROS-mediated killing alone [15, 163]. However, ROS also promote killing of bacteria such as *S. aureus* by producing a charge across the phagocytic vacuole membrane, resulting in K^+ influx and release of proteases and antimicrobial peptides from neutrophil granules into the vacuole [16]. Some of the components of neutrophil granules that are important in bacterial killing include proteinases (e.g. cathepsin G, elastase, and proteinase 3), α-defensins, lysozyme, acid hydrolases, lactoferrin (which sequesters iron and copper), transcobalamin II (which binds cyanocobalamin [vitamin

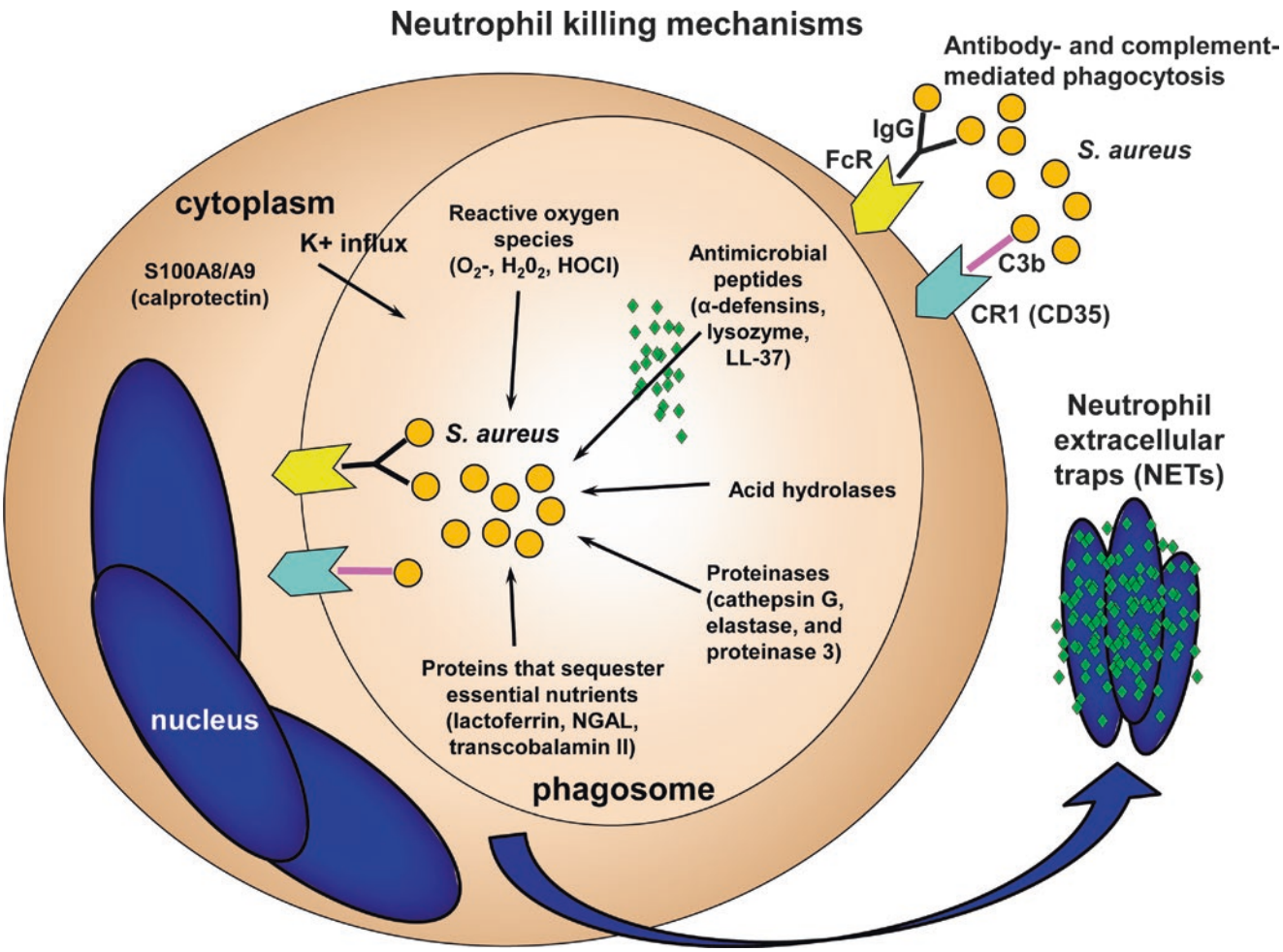

Fig. 16.4 Neutrophil antimicrobial mechanisms against *S. aureus*

B12]), and neutrophil gelatinase-associated lipocalin (NGAL) [164]. NGAL is an antimicrobial protein that binds to bacterial siderophores and blocks their ability to extract iron needed for bacterial growth [164]. If neutrophils escape the phagosome and enter the cytoplasm, approximately 40–50 % of the protein found in the cytoplasm is comprised of calprotection, a complex of S100A8 and S100A9 proteins, which chelates $Mn2^{+}$ and $Zn2^{+}$ and sequesters these essential nutrients to prevent bacterial growth [164]. Finally, neutrophils can release antimicrobial peptides, proteases and chromatin through NETs (neutrophil extracellular traps), which trap and promote antimicrobial activity against *S. aureus* [165, 166].

Recently, a host defense circuit has been uncovered that triggers neutrophil recruitment to a site of *S. aureus* skin infection in mice (Fig. 16.5). This process begins with recognition of the *S. aureus* skin infection by PRRs such as TLR2, NOD2 and FPRs, which leads to production and secretion of IL-1β in a mechanism involving activation of the NLRP3/ASC inflammasome [91, 93, 126]. IL-1β then acts on IL-1R-expressing resident skin cells and subsequent signaling via MyD88 leads to production of neutrophil-attracting chemokines (KC, MIP2) and granulopoiesis factors (G-CSF and GM-CSF) to mediate neutrophil recruitment [91, 93, 126]. Interestingly, a major cellular source of IL-1β at the site of *S. aureus* infection in the skin were neutrophils, which amplified and sustained the neutrophilic response for optimal abscess formation and bacterial clearance [91]. It should also be mentioned that IL-1α also contributed to IL-1R/MyD88-mediated host defense against a superficial *S. aureus* skin infection in mice [125], which is consistent with the identification of an IL-1α autocrine signaling loop in keratinocytes to continuously produce neutrophil-attracting chemokines [167, 168]. A similar mechanism involving IL-1α/β in host defense may exist in humans since pediatric

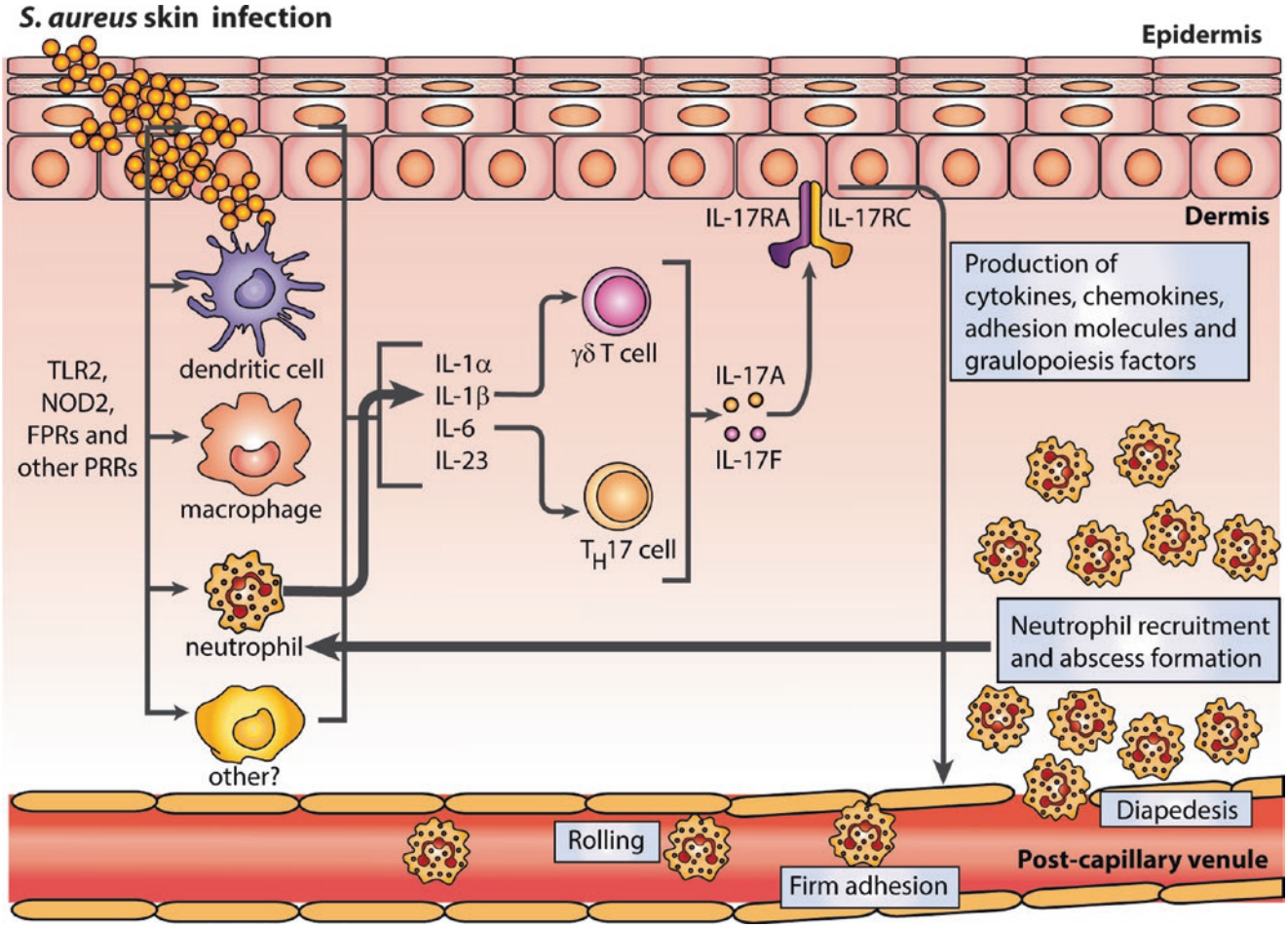

Fig. 16.5 Proposed host defense circuit for neutrophil recruitment to a site of *S. aureus* infection in the skin. Recognition of *S. aureus* components by PRRs (TLR2, NOD2, FPR1) and activation of cells in the skin to produce IL-1α, IL-1β, IL-6 and IL-23, which are required to activate γδ T cells and/or Th17 cells to produce IL-17A and IL-17 F. IL-17A/F subsequently activates IL-17 receptors, expressed primarily on keratinocytes, to produce cytokines, chemokines, adhesion molecules and granulopoiesis factor to promote neutrophil recruitment from the circulation to the site of infection. Finally, neutrophils, themselves, are important sources of IL-1β, which can amplify and sustain the neutrophil recruitment response for optimal abscess formation

patients with a deficiency in MyD88 or IRAK4 (signaling molecules downstream of IL-1Rs and TLRs) are highly susceptible to pyogenic bacterial infections, including *S. pneumoniae*, *S. aureus* and *P. aeruginosa* [169–171]. In these patients, the *S. aureus* infections were limited to the skin in most cases (84 %) [169–171], which is different than patients with impaired neutrophilic responses because they have a systemic susceptibility to *S. aureus* infections [127]. Furthermore, the MyD88/IRAK4-deficient patients developed pus at sites of infection in the absence of neutrophilia, suggesting an impaired ability to sustain the neutrophilic response [170], which is consistent with the findings in mice [91, 93, 126].

The critical role of neutrophils in host defense against *S. aureus* is further illustrated by the existence of mechanisms that *S. aureus* possesses to inhibit neutrophil recruitment and function [172]. For example, *S. aureus* inhibits neutrophil extravasation by producing staphylococcal superantigen-like 5 (SSL5) that blocks P-selectin-mediated neutrophil rolling, and extracellular adherence protein (Eap) that binds to ICAM-1 to inhibit adherence to endothelium [76]. *S. aureus* also inhibits neutrophil chemotaxis via production of Staphopain A, which blocks activity of CXCR2 chemokines [173], and CHIPS that inhibits neutrophil chemotaxis by blocking CD5aR, FPR1, FPR2, FPRL1 and FLIPr and FLIPr-like receptors [150, 151]. *S. aureus* also produces factors that inhibit neutrophil function. For example, the yellow carotenoid pigment of *S. aureus*, staphyloxanthin, which is responsible for its golden color, is an antioxidant that blocks ROS-mediated killing of *S. aureus* [174]. In addition, *S. aureus* produces catalase and alkyl hydroperoxide reductase, which converts hydrogen peroxide to water, and two superoxide dismutase enzymes, which degrade superoxide, to impair ROS-mediated killing [172]. *S. aureus* also produces nuclease and adenosine synthase that degrade NETs, thereby evading their antimicrobial function [175, 176].

Monocytes/Macrophages

Similar to neutrophils, monocytes/macrophages are recruited to the site of a *S. aureus* infection and are important in phagocytosing *S. aureus*. Monocytes/macrophages (as well as neutrophils) express Fc and complement receptors that facilitate phagocytosis by recognizing immunoglobulin or complement components opsonized on the bacterial surface [172, 177]. The importance of phagocytosis is exemplified by the existence of several mechanisms that *S. aureus* utilizes to evade this process [177]. For example, *S. aureus* has protein A on its surface that binds the Fc portion of IgG, resulting in the binding of IgG in an incorrect orientation for detection by Fc receptors [177]. In addition, fibrinogen binding proteins and clumping factor A (ClfA) bind fibrinogen and impair macrophage phagocytosis [177]. *S. aureus* also secretes toxins that are pore-forming proteins that damage membranes of host cells such as macrophages leading to lysis and the prevention of phagocytosis [172]. There are two main families of these pore-forming toxins: (1) single-component α-hemolysin or α-toxin and (2) biocomponent leukotoxins, including γ-hemolysin or γ-toxin, Panton-Valentine leukocidin (PVL), leukocidin A/B (LukAB), LukED, LukGH, and leukocidin M/F-PV-like [172]. The toxins facilitate lysis of host cells by interacting with specific targets, some of which have been identified. Alpha-toxin targets ADAM10 [178], PVL targets C5a receptors [179], LukAB targets CD11b [180] and LukED targets CCR5, CXCR1 and CXCR2 [181, 182]. Finally, *S. aureus* possesses phenol soluble modulins, including four PSMα peptides (PSMα1-PSM α4), PSMβ1, PSMβ2, and PSMδ (δ-toxin), which have the ability to lyse human erythrocytes and leukocytes, including neutrophils [183]. In addition, CA-MRSA strains are known to produce high levels of PSMα peptides that contribute to the enhanced virulence of USA300 isolates [184].

Adaptive Immune Responses Against *S. aureus*

The innate immune system provides the first line of defense against microbial pathogens, while the cell-mediated and humoral immune responses of adaptive immunity are later recruited. The adaptive immune system can be divided into T cell- and B cell-mediated immune responses and the role of these adaptive immune responses against *S. aureus* will be discussed in this section.

T Cell Immune Response

A number of different observations have provided evidence that T cells play an important role in host defense against *S. aureus* skin infections. First, patients with HIV infection are at an increased risk for colonization and skin infection with *S. aureus* [185–188]. In addition, the low serum $CD4^+$ T cell counts of HIV patients is a risk factor for *S. aureus* infection [185].

Second, patients with the inflammatory skin disease atopic dermatitis, which is associated with a Th2 cytokine profile (i.e. IL-4, IL-13, and IL-10), have increased colonization and superinfection with *S. aureus* [189, 190]. Although the reason for the increased *S. aureus* superinfection in atopic dermatitis is likely multifactorial (including a defective epidermal barrier and impaired innate immune responses such as decreased expression of antimicrobial peptides), Th2 cytokines have also been implicated. For example, IL-4 has been shown to increase the expression of fibronectin and fibrinogen receptors on host cells, which promotes more efficient binding of *S. aureus* to the stratum corneum [191]. In addition, *S. aureus* via the *S.*

aureus-derived factors fibronectin-binding protein (fnbp) and clumping factor (Clf, also known as fibrinogen-binding protein) more efficiently bind to skin from atopic dermatitis patients [192]. Also in atopic dermatitis, *S. aureus* produces superantigens such as staphylococcal enterotoxins A and B (SEA and SEB) and toxic shock syndrome toxin-1 (TSST-1) that exacerbate the inflammatory response by nonspecifically activating T cells [190, 193–196]. *S. aureus* superantigens also can skew the cutaneous immune response towards the Th2 cytokine profile, thereby increasing *S. aureus* superinfection in atopic dermatitis [197]. Recently, *S. aureus* δ-toxin has been shown to induce allergic skin inflammation via mast cell activation in mice, providing yet another mechanism by which *S. aureus* contributes to the pathogenesis of atopic dermatitis [198]. Taken together, T cells likely play an important role in colonization and superinfection of atopic dermatitis lesions by increasing levels of Th2 cytokines, activation of T cells by superantigens and other toxin-mediated effects on promoting skin inflammation.

Third, recent evidence suggests that Th17 cells and IL-17 responses in humans may also be protective against *S. aureus* skin infections. The differentiation of naïve T cells into Th17 cells has been shown to be mediated by IL-6, IL-21, IL-23, TGFβ and/or IL-1β, which cause expression of the transcription factor RORγt in mature Th17 cells, which produce IL-17A, IL-17F, IL-21 and IL-22 [199, 200]. The strongest evidence for a protective role of Th17 cells against *S. aureus* skin infections comes from the study of the rare orphan disease hyper-IgE syndrome (HIES) (also called Job's syndrome after the biblical character Job whose faithfulness was tested by an affliction with sores and boils over his entire body [201]). HIES patients suffer from an eczema-like skin eruption and chronic and recurrent skin infections with *S. aureus* and *C. albicans* [201]. HIES patients were found to have loss-of-function mutations in the signaling molecule STAT3 [202, 203], which rendered them deficient in Th17 cells [204–206]. Additionally, patients with IL-17RA or IL-17F deficiency were found to also be susceptible to *C. albicans* and to a lesser extent *S. aureus* SSTIs [207]. These findings suggest that STAT3 and IL-17/Th17 responses, in particular, play a key role in host defense against *S. aureus* infections in skin. However, although *S. aureus*-specific Th17 cells are found in blood from healthy humans [208], it remains unknown whether Th17 cells in patients without rare genetic conditions promote protection against *S. aureus* skin infections [209, 210].

Similarly, although initial investigations in mice suggested that Th1 responses, including IFNγ responses, promoted neutrophil recruitment against a *S. aureus* skin surgical wound infection [211, 212] and intravenous *S. aureus* infections [213–215], more recent studies have revealed that IL-17 responses in mice, like in humans, are critical for cutaneous host defense against *S. aureus* skin infections. In a mouse model of *S. aureus* skin infection, IL-17A/F produced by γδ T cells (rather than Th17 cells) was essential in producing neutrophil-attracting chemokines and granulopoiesis factors to promote neutrophil recruitment and bacterial clearance (Fig. 16.5) [216]. Interestingly, IL-17A/F in the infected skin was not induced in IL-1R- or MyD88-deficient mice, indicating that the γδ T cell IL-17A/F required IL-1R/MyD88 activation [216]. Another study found that IL-17A/F-deficient mice suffer from spontaneous *S. aureus* skin infections [217]. In addition, several Th17 cell-inducing vaccines (the candidal adhesion protein rAls3p-N, *S. aureus* clumping factor A [ClfA], *S. aureus* iron regulated surface determinant B [IsdB] and a UV-irradiated *S. aureus* vaccine) protected mice against an intravenous and/or cutaneous *S. aureus* challenge [213, 218, 219], suggesting that Th17 cell-inducing vaccines could potentially be effective against *S. aureus* cutaneous infections in humans. Interestingly, IL-20 cytokines (IL-19, IL-20 and IL-24) have been shown to enhance a *S. aureus* skin infection in mice by inhibiting IL-1β and IL-17 responses and treatment with an antibody against the shared receptor IL-20RB resulted in improved outcomes [220]. Finally, two recent studies have found that Th17 cells and IL-17-producing γδ T cells were protective against a recurrent *S. aureus* skin infection model and a *S. aureus* surgical wound model in mice, respectively [221, 222]. The mechanism for how anti-*S. aureus* Th17 are generated is an active area of investigation but a recent study found that Langerhans cells in mouse epidermis produced elevated amounts of IL-6, IL-1β, and IL-23, which promoted Th17 differentiation in response to cutaneous challenge with *S. aureus* and *C. albicans* [223]. Similarly, IL-6, IL-1β, and IL-23 also contributed to Th17 cell differentiation of *S. aureus*-specific Th17 cells isolated from human blood [208].

B Cell Immune Response

The B cell-mediated immune response against *S. aureus* involves the production of antibodies directed against specific antigenic components of *S. aureus*. These antibodies play an important role in opsonizing *S. aureus* and facilitating antibody-mediated phagocytosis by neutrophils and macrophages [209, 210]. After an acute *S. aureus* infection (including skin infection), antibody levels have been shown to rise, including specific antibodies against toxins (e.g., α-toxin, PVL), virulence factors (e.g., superantigens), cell-wall proteins (e.g., ClfA) and non-protein antigens (capsular polysaccharides, LTA and PGN) [224]. One study demonstrated that the antibody repertoires differed in patients with superficial versus deep-seated *S. aureus* skin infections [225]. Studies using various animal models of *S. aureus* infection have provided further

evidence that antibodies against different *S. aureus* components can provide some level of protection against *S. aureus* infection [209, 210].

The importance of B cell responses and antibodies in host defense against *S. aureus* infections is further provided by the existence of protein A, an important virulence factor that *S. aureus* uses to counteract antibody-mediated responses [226]. Protein A of *S. aureus* binds antibody in the incorrect orientation, thus enabling *S. aureus* to evade antibody detection and subsequent antibody-mediated phagocytosis [226].

There have been attempts to develop vaccines and passive immunization strategies to promote antibody-mediated responses against *S. aureus* in humans [209, 210]. These vaccines were designed to target molecules on bacterial surfaces to enhance opsonophagocytosis such as capsular polysaccharides, ClfA and IsdB [227–229]. However, to date, all of these vaccines have failed in clinical trials [227–229]. It is unknown whether an antibody-based vaccine alone will promote durable immunity against *S. aureus* skin infections. However, more recent vaccines were designed to target toxins and virulence factors rather than mechanisms such as opsonophagocytosis and this may be a more efficacious approach [209, 210]. Recently, a study in children with and without recurrent *S. aureus* skin infections found that high natural antibodies against α-toxin correlated with protection against recurrence [230], providing rationale for these newer antibody-based vaccination strategies that target *S. aureus* toxins.

Group A *Streptococcus*

Group A *Streptococcus* (*Streptococcus pyogenes*) is a Gram-positive extracellular bacterial pathogen that causes superficial and invasive skin infections such as impetigo, erysipelas, cellulitis, scarlet fever, and necrotizing fasciitis and is the most common cause of bacterial pharyngitis (especially in children) [231, 232]. Group A *Streptococcus* infections can cause other severe infections such as streptococcal toxic shock syndrome, septic arthritis, osteomyelitis, septicemia, pneumonia and meningitis [231]. In addition, after a group A *Streptococcus* infection, immunologic-mediated diseases such as guttate psoriasis, acute rheumatic fever, and glomerulonephritis may ensue [231]. The World Health Organization estimates that there are approximately 600 million cases of noninvasive pharyngitis and 110 million skin infections caused by group A *Streptococcus* globally per year [231]. In the U.S., there is an estimated 8950–11,500 cases of invasive group A *Streptococcus* infections annually (including erysip-

Table 16.2 Soluble mediators and pattern recognition receptors that contribute to host defense against group A *Streptococcus* skin infections

	Cellular expression in skin	Group A *Streptococcus* evasion mechanisms	Mechanisms of action
Antimicrobial peptides			
α-defensins (human neutrophil peptides [HNPs])	Neutrophils	SIC, generation of dermatan sulphate, *dlt*ABCD	Antimicrobial activity, chemotaxis of T cells and immature dendritic cells
hBD2	Keratinocytes, macrophages, DCs	SIC, *dlt*ABCD operon	Antimicrobial activity, chemotaxis of immature dendritic cells and memory CD4+ T cells via CCR6
hBD3	Keratinocytes	SIC, DRS, *dlt*ABCD operon	
LL-37	Keratinocytes, macrophages neutrophils	SIC, SpeB, *dlt*ABCD operon	Antimicrobial activity, chemotaxis of neutrophils, monocytes and T cells via FPRL1
Complement	Serum	M-protein	Complement cascade
C5a	Serum	C5a peptidase (ScpA)	Chemotaxis of neutrophils
C5b-C7	Serum	SIC	MAC (membrane attack complex)
Toll-like receptors (TLRs)			
TLR9	Multiple	Sda1	Recognize group A *Streptococcus* DNA
NOD2	Multiple		Recognize cytosolic muramyl-dipeptide (a breakdown product of group A *Streptococcus* PGN)
Inflammasomes			
NLRP3/ASC inflammasome	Multiple		Activated by group A *Streptococcus* streptolysin O to induce pro-IL-1β processing

elas, cellulitis, and necrotizing fasciitis), which result in 1050–1850 deaths [233]. Thus, group A *Streptococcus* continues to be a major cause of superficial and invasive skin infections both globally and in the U.S.

Clinical Manifestations

Group A *Streptococcus* causes superficial skin infections such as impetigo and invasive skin infections such as erysipelas, an infection of the superficial layers of the skin and cutaneous lymphatics (Fig. 16.6), or cellulitis, an infection involving the deep dermis and subcutaneous tissue [234]. Group A *Streptococcus* also causes necrotizing fasciitis, which is a severe skin and soft-tissue infection that results in total destruction of the deep fat and fascia and often precedes streptococcal sepsis, shock and multi-organ failure [234]. In addition, scarlet fever, which is usually associated with a streptococcal throat infection, is characterized by a morbilliform rash, strawberry tongue and desquamation of skin [231, 232]. This constellation of clinical findings in scarlet fever is caused by streptococcal pyrogenic exotoxins (Spe), especially SpeA, B and C, which act as superantigens [231, 232].

Innate Immune Responses Against Group A *Streptococcus*

The innate immune response against group A *Streptococcus* is similar to that against *S. aureus* and includes soluble factors such as antimicrobial peptides and complement components, PRRs such as TLRs and NOD2 and innate immune system cells such as neutrophils and monocytes/macrophages. However, there are some key differences in the immune response against group A *Streptococcus*, especially with regard to the recognition and activity of M protein expressed by group A *Streptococcus*.

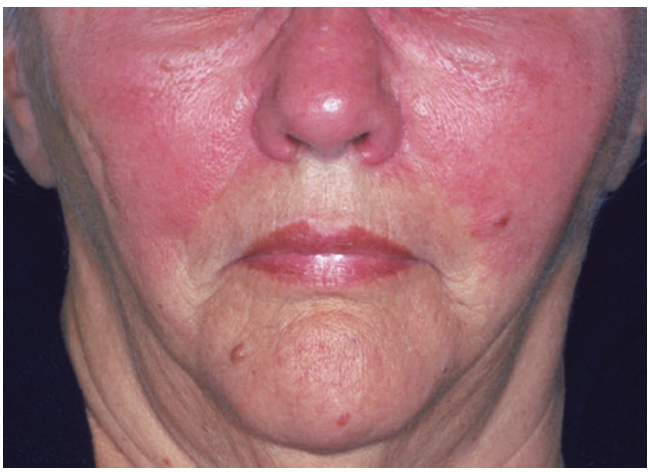

Fig. 16.6 Group A *Streptococcus* erysipelas of the face. The involved skin shows a sharply demarcated, erythematous, and edematous plaque (Photograph is courtesy of the Victor D. Newcomer collection at UCLA and Logical Images, Inc.)

Antimicrobial Peptides

Both α- and β-defensins (HBD1-3) have direct antimicrobial activity against group A *Streptococcus* [235–238]. In addition, stimulation of keratinocytes with group A *Streptococcus* increases production of HBD2 [239]. Cathelicidin also has direct antimicrobial activity against group A *Streptococcus* infections in mouse models of skin infection and in cultures of human keratinocytes or mast cells [240–244]. Cathelicidin production is upregulated in wounded human or mouse skin, which protects the healing wound from infection by group A *Streptococcus* [245]. Thus, both defensins and cathelicidin have antimicrobial activity and play a key role in the innate immune response against group A *Streptococcus*.

The importance of antimicrobial peptides in the innate immune response against group A *Streptococcus* is further illustrated by the existence of several mechanisms that group A *Streptococcus* utilizes to inhibit their function. For example, group A *Streptococcus* produces streptococcal inhibitor of complement (SIC), which inhibits human α-defensins, HBDs 1-3, LL-37, and lysozyme [236, 238] as well as DRS (distantly related to SIC), which inhibits hBD3 [238]. SpeB can cleave and inactivate LL-37 [246]. GAS can also bind plasmin on the bacterial cell surface to facilitate degradation of LL-37 [247]. In addition, extracellular proteases released by group A *Streptococcus* can generate dermatan sulphate from host proteoglycans, which subsequently binds to and inactivates α-defensins [248]. Finally, similar to *S. aureus*, group A *Streptococcus* also secretes products from the *dlt*ABCD operon to reduce the negative charge of the bacterial envelope to resist the activity of LL-37 and other antimicrobial peptides [249].

Complement Activation

The importance of complement in the immune response against group A *Streptococcus* is illustrated by the existence of multiple factors produced by group A *Streptococcus* that inhibit complement activity [231]. The M protein of group A *Streptococcus* inhibits complement activity by several different mechanisms [250, 251]. M protein directly binds to and enhances the function of factor H (FH) and FH-like protein, host proteins that inhibit complement activation and prevent C3b-mediated phagocytosis [252–258]. In addition, a

fibronectin-binding protein (Fba) was shown to have similar FH/FH-like protein binding activity as M protein to inhibit complement and enhance virulence [259–261]. Group A *Streptococcus* M protein also binds to and enhances the function of C4b-binding protein (C4BP), a host protein that downregulates complement activation by accelerating the decay and prevent formation of C3- and C5-convertases [262–267]. Furthermore, M protein binds fibrinogen, which inhibits complement-mediated phagocytosis by reducing the amount of C3 convertase on the surface of group A *Streptococcus* [268]. In addition to M protein, group A *Streptococcus* also secretes C5a peptidase (ScpA), which cleaves C5a and inhibits neutrophil recruitment [269–273]. Finally, the group A *Streptococcus*-derived protein, SIC, not only inhibits antimicrobial peptides (see above), but also binds to C5b-C7 complexes and prevents formation of the MAC [274, 275].

Pattern Recognition Receptors that Recognize Components of Group A *Streptococcus*

The recognition of group A *Streptococcus* appears to be quite different from *S. aureus*, despite both of them being Gram-positive bacteria. Prior work has found that macrophages and dendritic cells produced TNFα and IL-6 in a mechanism independent of TLR2, TLR4 and TLR9 [276–278]. Rather, a major host recognition mechanism for group A *Streptococcus* involved the induction of type I interferon (e.g., IFN-α and IFN-β), which involved DNA from group A *Streptococcus* to activate signaling molecules IRF3, STING, TBK1 and partially MyD88 in macrophages and streptococcal RNA triggering of IRF5 and MyD88 in dendritic cells (DCs) [278]. Furthermore, the type I interferon response was protective against a lethal subcutaneous group A *Streptococcus* infection model in mice [278]. Other reports found a role of TLR9 in inducing hypoxia-inducible factor-1α (HIF-1α), nitric oxide and oxidative burst in host defense and clearance of group A *Streptococcus* in cutaneous and systemic infection models in mice [279]. Interestingly, certain strains of Group A *Streptococcus* possess a bacteriophage that encodes Sda1, which is a DNase that inhibits recognition of the bacterial DNA by TLR9, thus suppressing TLR9-mediated type I interferon-mediated host defense mechanisms [280]. Other PRRs might also be involved in recognition of Group A *Streptococcus*. For example, cell wall fragments from group A *Streptococcus* induced less joint inflammation in mice deficient in NOD2 than wildtype mice and the cell wall fragments were capable of activating NOD2 expressed by human macrophages, suggesting that NOD2 is a PRR for group A *Streptococcus* [281]. Finally, group A *Streptococcus* resulted in activation of the NLRP3/ASC inflammasome to trigger caspase-1 activation and IL-1β secretion in a mechanism that was dependent upon NF-kB and the virulence factor streptolysin O but independent of exogenous ATP, P2X7R and TLR signaling [282]. Taken together, the PRRs involved in recognition of group A *Streptococcus* are not entirely clear (and are different than those of *S. aureus*), but type I interferon responses, NOD2 and NLRP3/ASC inflammasome activation have been identified to be important for host defense. It should be mentioned that the M protein of group A *Streptococcus* has been shown to interact with TLR2 on human monocytes leading to production of IL-6, IL-1β and TNF-α [283]. However, the M protein also binds to CD46 (membrane cofactor protein) on the surface of human keratinocytes and this interaction facilitates the ability of group A *Streptococcus* to invade these cells [284–287]. Thus, the M protein of group A *Streptococcus* might induce inflammatory responses via TLR2 while also promoting invasion of host cells and disease pathogenesis [284–287].

Cellular Innate Immune Responses Against Group A *Streptococcus*

Neutrophils

Neutrophil infiltrates are the characteristic of acute Group A *Streptococcus* infections, which is consistent with its scientific name, *Streptococcus pyogenes* from the Latin for 'pus-generating' [288]. The importance of neutrophils in host defense against group A *Streptococcus* is further demonstrated by the existence of numerous mechanisms that group A *Streptococcus* utilizes to counteract neutrophil recruitment and function [231, 288, 289]. Regarding neutrophil recruitment, group A *Streptococcus* not only produces the C5a peptidase (see above), but also produces another peptidase called ScpC (also called SpyCEP) that degrades CXC chemokines (including IL-8 in humans and KC and MIP2 in mice) [290]. These chemokines are critical for neutrophil recruitment to sites of infection [290].

Group A *Streptococcus* has developed mechanisms to inhibit both complement- and antibody-mediated phagocytosis. As mentioned above, group A *Streptococcus* prevents complement-mediated phagocytosis via activity of M protein and Fba. In addition, group A *Streptococcus* secretes endoglycosidase (EndoS), and streptococcal pyrogenic exotoxin B (SpeB) [291–295]. These bacterial factors inhibit antibody-mediated phagocytosis by hydrolyzing N-linked oligosaccharides on opsonized IgG molecules and by cleaving opsonized IgG molecules into Fab and Fc fragments, respectively [291–295]. SpeB has been shown to degrade most immunoglobulin subtypes (IgG, IgA, IgM, IgD and IgE) [293]. Recently, another endoglycosidase, $EndoS_2$, has similar activity as EndoS to inhibit IgG-mediated phagocytosis [296]. In a mouse

skin infection model, group A *Streptococcus* mutant strains expressing protease-inactive SpeB caused significantly less necrosis and demonstrated less efficient systemic dissemination from the initial focus of skin inoculation [297]. There are also other IgG-degrading enzymes (Ide) produced by group A *Streptococcus*, including IdeS (also known as Mac-1), which cleaves the lower Fc region of surface bound IgG [298] and is also a bacterial homolog of the α-subunit of the β2-integrin Mac-1 that binds to CD16 (FcγRIIIB) on phagocytes to inhibit Fc-mediated phagocytosis [299]. Mac-2 is a similar IgG-degrading enzyme, has weaker endopeptidase activity than IdeS but can competitively block FcγRII and FcγRIII to inhibit antibody-mediated phagocytosis [300]. In addition, the hyaluronic acid capsule of group A *Streptococcus* can act as a physical barrier to nonspecifically resist phagocytosis [301]. Group A *Streptococcus* also resists antibody-mediated phagocytosis by forming large bacterial aggregates via binding fibronectin and recruiting collagen fibers [302]. Taken together, group A *Streptococcus* produces several different factors that can inhibit both complement- and antibody-mediated phagocytosis.

There are several mechanisms that group A *Streptococcus* utilizes to inhibit neutrophil function. First, group A *Streptococcus* can directly induce neutrophil lysis or apoptosis, effectively eliminating their antimicrobial activity [303, 304]. Second, in addition to SIC, which inhibits antimicrobial peptides (see above), group A *Streptococcus* produces several enzymes that inhibit ROS-mediated microbicidal toxicity such as glutathione peroxidase, superoxide dismutase, alkylhydroperoxidase and alkylhydroperoxidase reductase [305–307]. Furthermore, the bacteriophage-encoded Sda1, which is produced by certain strains of Group A *Streptococcus*, (see above) [308, 309], as well as the nuclease SpnA [310], degrade DNA in neutrophil NETs, thus inhibiting the antimicrobial and killing mechanisms of NETs.

Adaptive Immune Responses Against Group A *Streptococcus*

Both B and T cells play a role in adaptive immune responses against group A *Streptococcus* infections. In particular, antibodies and T cells that recognize antigenic components of M protein have been shown to produce protective immune responses that prevent colonization and infection by group A *Streptococcus* [311–319]. Similar to *S. aureus*, group A *Streptococcus* also produces several superantigens, including streptococcal pyrogenic exotoxins (SPEs) (serotypes A, C and G to M) and the streptococcal mitogenic exotoxin $SMEZ_n$ [320]. These superantigens nonspecifically activate T cells and contribute to the pathogenesis of group A *Streptococcus* infections [320].

The important role of adaptive immunity in host defense against group A *Streptococcus* has led to several different vaccination strategies to produce protective antibody responses [231, 321]. A safe human vaccine against group A *Streptococcus* has been challenging given the genetic diversity among clinical isolates as well as producing a vaccine that does not increase the risk for development of autoimmune sequellae such as acute rheumatic fever and acute poststreptococcal glomerulonephritis [231, 321, 322]. Since antibodies against M protein of group A *Streptococcus* have been shown to offer protection against colonization and infection, several vaccines have targeted different antigenic epitopes of the M protein, including 26- and 30-valent N terminal domain vaccines [314, 315] as well as vaccines targeting more conserved epitopes [316–319]. In addition, other vaccine approaches have been attempted, including vaccines directed against other streptococcal antigens, including the group A carbohydrate, C5a peptidase, fibronectin-binding protein A (FbaA), fibronectin-binding protein 54 (Fbp54), streptococcal protective antigen (Spa), SpeB, SpeC, serine protease (SpyCEP), serine esterase (SSe) and several other antigens [231, 321, 322]. These strategies have had varying successes in animal studies; whether the efficacy of these vaccines will translate to humans is not yet known. As newer technologies are becoming more available, such as high-throughput proteomics approaches [323–326], defining candidate vaccine targets with enhanced efficacy and broad strain coverage will greatly help in the development of a successful human vaccine against group A *Streptococcus* infections in the future.

Conclusion

Recent discoveries involving innate and adaptive immune responses against *S. aureus* and group A *Streptococcus* have greatly improved our understanding of these common skin infections. As antimicrobial resistance is creating a serious threat to public health, novel strategies to enhance protective host immune mechanisms against bacterial skin infections represent an alternative approach to combat these infections while minimizing antibiotic resistance. The mechanisms of cutaneous host defense and bacterial pathogenesis will be important factors to consider for the development of future immunotherapies and vaccine strategies against these common bacterial skin pathogens.

Questions

1. *Staphylococcus aureus* is a common cause of all of the following skin infections, EXCEPT:
   A. Cellulitis
   B. Impetigo

C. Ecthyma gangrenosum
D. Subcutaneous abscesses
E. Folliculitis

2. Group A Streptococcus is a common cause of all of the following skin infections, EXCEPT:
   A. Gas Gangrene
   B. Erysipelas
   C. Cellulitis
   D. Impetigo
   E. Necrotizing Fasciitis

3. Which human antimicrobial peptides have bacteriostatic or bactericidal activity against *Staphylococcus aureus*?
   A. Human beta-defensin 2
   B. Human beta-defensin 3
   C. RNase7
   D. Cathelicidin
   E. All of the above

4. Which pattern recognition receptors are correctly paired with the pathogen associated molecular pattern?
   A. TLR2 – lipoteichoic acid
   B. NOD2 – muramyl dipeptide
   C. AIM2 – hypomethylated double-stranded RNA
   D. A and B
   E. A, B and C

5. Which of the following is NOT associated with inflammasome activation?
   A. Are activated by *Staphylococcus aureus* pore-forming toxins
   B. Proteolytic processing of pro-TNF-alpha to active TNF-alpha
   C. Proteolytic processing of pro-caspase-1 to caspase-1
   D. Proteolytic processing of pro-IL-1beta to active IL-1beta
   E. Proteolytic processing of pro-IL-18 to active IL-18

Answers

1. C
2. A
3. E
4. D
5. B

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Immunodermatology and Viral Skin Infection

17

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Abstract

The skin is the largest organ in the human body, acts as our fist line of defense, and has a sophisticated array of cells and signaling molecules to help protect the body from infection. Yet even with this sophisticated defense, viruses cause a range of cutaneous diseases in humans, many of which are widespread in the population and cause significant morbidity and mortality, along with psychological and financial repercussions. Each virus has unique mechanisms by which it evades the immune system, replicates, and spreads. Some viruses infect the skin directly while others gain access systemically first. Infections can be acute or subclinical and then resolve, while others are persistent or can remain latent for years. This spectrum of presentations is mirrored by an equally wide array of evasion tactics that the viruses use to manipulate and escape both the adaptive and innate immune responses. In addition to highlighting viral responses, particular attention is also paid to the local immune response generated in the skin. Five viruses will be discussed in detail: herpes simplex virus, varicella zoster virus, human immunodeficiency virus, molluscum contagiosum virus, and human papilloma virus; along with the latest information on the development and advancement of both therapeutic and prophylactic vaccines.

Keywords

Innate immunity • Adaptive immunity • Immunodermatology • Viral skin infections • Human immunodeficiency virus • Herpes simplex virus • Varicella zoster virus • Human papilloma virus • Molluscum contagiosum • HSV • HIV • HPV • VZV • Vaccines

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Key Points

- As a complex immune organ, the skin plays a major part in protecting the body against viruses.
- Innate immunity involves an early response to foreign antigens but is not pathogen specific, whereas an adaptive response is specific for antigen recognition and develops memory but takes longer to activate.
- Viruses have developed methods to evade the skin immune response in order to establish infection or release their progeny.
- Vaccines can protect against viruses by helping to produce a rapid immune response, with high levels of protective antibodies for their target viral antigens.

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The skin is the largest organ in the human body and constitutes its first line of defense. It is not only a great physical barrier, but also has an amazing cellular army ready to defend the body from microorganisms such as viruses. This immune capacity was recognized 30 years ago and has since become an important topic of research. There is a wide spectrum of immune responses in the skin, and the mechanisms by which they are triggered are not fully understood. Almost every single microorganism (including bacteria, viruses, fungi, and parasites) is capable of inducing a specific skin response that translates into a particular clinical lesion. This interplay of responses is seen even within organisms of the same family (e.g., human papillomavirus [HPV] type I produces different lesions compared with HPV type 3). Moreover, the viruses are particularly difficult to control due to their ability to change and adapt to the medium. They have developed the capacity to escape the immune system and in some cases can coexist with the host without being noticed.

Viruses such as the human immunodeficiency virus (HIV), the human herpes simplex virus (HSV), the varicella-zoster virus (VZV), and HPV have high rates of morbidity and mortality, and they have an impact not only on the physical condition of the patient but also generate an enormous psychological trauma. The majority of the populations infected with these viruses are young, sexually active people, and as a result, spread within the population is favored and the socio- economic impact within the society is significant. Therefore, the development of vaccines against these viruses is one of the priorities of any health care system. The goal of a vaccine is to decrease the rapid and progressive spread of these diseases by producing a rapid immune response with high levels of protective antibodies for the target viral antigen. To achieve this response, the virus's behavior and the immune response it elicits must be properly understood.

The Immune System and the Skin

The skin is a vast immune organ (also known as skin-associated lymphoid tissue [SALT]), and all of its cells are part of an immunologic team (key players include keratinocytes, Langerhans cells [LCs], and skin tropic T cells, among others) [1, 2]. These cells carry out specific functions and are activated upon infection with certain viruses, and the interplay between them dictates the final immunologic outcome for the infecting virus.

The immune system is divided into two components: innate and adaptive (Table 17.1). Innate immunity is characterized by an early response to foreign antigens and is dependent on the particular environment present during the initial phase of that response. It is the first line of defense against infection and has a broad spectrum of activity (not pathogen specific), including the expression of stimulatory molecules by antigen- presenting cells and the secretion of cytokines and other inflammatory cell products with a limited repertoire of antigen recognition. This initial response does not develop a memory or long-lasting protective immunity. Nonetheless, it is a rapid response and its effectiveness is crucial for the next step (which is more specific and involves antibodies and cytotoxic effector cells) [3, 4]. The innate immunity detects pathogens and clears the majority of microbial assaults. It is activated by cell injury or cell death, generating inflammation and local vascular responses. The key cellular players recruited in this response are the parenchymal cells and local phagocytic dendritic cells (DCs) [1, 3, 4]. Among the local phagocytes, macrophages are one of the most important cells in this first response. They possess special receptors capable of recognizing the pathogen- associated molecular patterns (PAMPs), known as Toll-like receptors (TLRs). The TLRs activate a variety of signaling pathways involved in antiviral, antibacterial, antitumor, and antiinflammatory activities [1, 5].

Table 17.1 Comparison of innate versus adaptive immunity

Innate	Adaptive
Early response to foreign antigens	Occurs within days of infection
Rapid response is the first line of defense	Antigen specific
Broad spectrum (not antigen specific)	Develops immune memory
No memory or long-lasting protective immunity	Aids recovery from viral infection as well as preventing re-infection
Key players include T and parenchymal cells and phagocytic dendritic cells	Key players include T and B lymphocytes

Some of these TLRs involved in the recognition of viruses have been identified. An example is TLR9, which recognizes HSV DNA on DCs and induces antiviral mechanisms that include the secretion of type I interferons (IFNs) [1, 6]. The interactions between the TLRs and the virus are necessary to guide, in this case, the anti-herpes immunity toward an adaptive (specific) cellular response (T-helper-1 [Th1] type, see below) [7]. Other examples of TLR interactions and viruses include the production of IFN-β and different chemokines activated by TLR3 and the induction of phagocytosis and inflammation by TLR4 mediated by the secretion of IFN-β [6].

The adaptive response is specific for antigen recognition, occurs within days of the infection, and is the result of the interaction of T and B lymphocytes. The immune players are multiple and capable of developing a lasting immune memory. This response not only aids recovery from the primary viral contact but also protects against re-infection. T lymphocytes recognize a processed antigen (short peptides) bound

to the major histocompatibility complex (MHC) of the antigen-presenting cells (APCs). There are two major subsets of T cells: CD4 and CD8. CD4 cells recognize antigens bound to MHC class II (exogenous antigens taken from the extra- cellular milieu and processed in the endosome of the APC), while CD8 cells recognize the antigens bound to MHC class I (usually endogenous antigens) [3]. The activation of the CD4 cell results in the secretion of a variety of cytokines. Depending on the pattern of cytokine expression, the immune response is characterized as either a Th1 or Th2 response. Th1 cells secrete IFN-γ, which activates macrophages, natural killer cells, and cytotoxic CD8+ T lymphocytes (cell-mediated immunity). On the other hand, Th2 cells secrete mainly interleukin-4 (IL-4) and IL-10, helping the primed B lymphocytes to differentiate into plasma cells and secrete antibodies (humoral response) [3]. Also, an additional type of T cell has been shown to play an important role in the immune response against viruses. These cells, designated the regulatory T (T_{reg}) cells, carry the CD4+ and CD25+ antigens. T_{reg} cells recognize self antigens and prevent autoimmunity responses, regulate the responses to exogenous antigens, and are involved in the chronic and latent phases of viral infections [8, 9].

Viruses have developed a diversity of mechanisms to evade both the innate and adaptive immune response and thus establish an infection (or persist at least until a new progeny of viruses is released). Some of the cutaneous viruses share common evasion mechanisms and others have specialized systems to survive and become latent until they have a new opportunity to flare. Several of these mechanisms are discussed in detail in the sections that follow.

Human Herpes Virus

The human herpes simplex virus (HSV) types 1 and 2 are neurotropic viruses from the α-herpesvirus family. These viruses have a large molecular weight and harbor double-stranded DNA. The genome is in an icosahedral capsid, which is protected by a proteinaceous layer (tegument). The capsid is surrounded by a lipid bilayer with glycoproteins (envelope) [10]. Herpes simplex virus is distributed worldwide, affecting developed and developing societies. Animal vectors for human HSV have not been described, and humans appear to be the only reservoir [11]. Usually the first infection is asymptomatic, but this depends on the age and immune status of the host, the amount of the infective dose, and the presence of innate defenses that may abort the infection [12, 13]. About 80 % of the adult population in the developed world becomes seropositive to HSV-1 and more than 20 % are seropositive to HSV-2. People who experience a primary infection with one or more herpes viruses carry these viruses for the rest of their lives (usually in a latent state). The virus is retained in specific neural reservoirs and may become active with periodic episodes of viral replication and shedding [7].

Herpes simplex virus infection usually initiates in the mucosa. The virus replicates in epithelial cells and then enters the nervous system through the nerve termini. Control of the acute (and persistent) HSV infection involves the activity of natural killer (NK) cells, virus-specific CD4+ and CD8+ cells, IFNs, and virus-specific antibodies [14]. After entry into the mucosa and skin, HSV establishes a lifelong persistence in the neurons of the sensory ganglia. Herpes simplex virus persistence and latency have been demonstrated in human trigeminal, facial, and vestibular ganglia, and reactivations from these locations can cause herpes labialis, vestibular neuritis, and cranial nerve disorders among others [15]. The mechanisms of viral reactivation are not fully understood but are associated with different events such as ultraviolet (UV) light, stress, fever, infections, and immunosuppression [16]. Recent studies have demonstrated that certain neurons are more prone to productive infections versus other neurons that are more likely to exhibit viral latency indefinitely. This susceptibility appears to be governed by regulatory RNAs and other regulatory proteins yet to be identified [17]. Furthermore, it has been postulated that UV light triggers reactivation by inducing apoptotic signals and uncoordinated lytic gene expression [18]. The role of the immune system during the latent phase is crucial to maintain the virus under control [19].

Immune Response

Once the virus is in contact with host cellular receptors, the processes leading to viral infection are triggered. Glycoproteins in the lipid bilayer allow the viral envelope to fuse with the epithelial plasma membrane. Viral proteins are released into the cytoplasm and viral DNA enters the nucleus. This process triggers three phases of the innate response: (1) secretion of immune proteins, such as complement and natural antibodies; (2) an early-induced response, in which the main mediators are IFNs produced by the infected epithelial cells and resident DCs; and (3) the activation of inflammatory cells, such as neutrophils, macrophages, and NK cells [20]

At the site of mucosal contact the virus usually encounters several barriers, including mucus, normal bacterial flora, the glycocalyx, complement proteins, and natural immunoglobulin (IgM) antibodies [1, 20]. Although these substances act in concert to decrease the number of infected cells, HSV usually replicates successfully and triggers the early innate immune response. Humans may express different levels of natural antibodies to HSV, which is a reflection of their own past exposure experience [7, 20]

The early innate immune response has two goals: (1) limit viral replication and spread of the virus in uninfected cells, and (2) recruit other inflammatory cells [20]. The viral interaction with the epithelial cells may stimulate cell-surface TLR2 (e.g., in the genital mucosa) [1, 20]. With the activation of this receptor, the epithelial cells activate complement, chemokines, and IFN-α and -β soon (between 8 and 12 h) after the infection. Interferon-α and -β are produced by most cells types, but the DCs are responsible for the majority of their production. Interferon-α and -β are known to be two of the most important molecules to control HSV infection at this stage. Some studies suggest that resistance or susceptibility to HSV infection is directly correlated with the amount of IFN-α and -β produced [12].

Complement, chemokines, and IFN-α and -β activate the endothelial cells that express IL-8, tumor necrosis factor-α (TNF–α), IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF), leading to neutrophil chemotaxis. Figure 17.1 illustrates the chemokine regulation of leukocyte movement [21]. The inflammatory reaction alerts the DCs and resident macrophages to the presence of the virus and induces a "state of awareness" or "antiviral status" in the uninfected cells [7]. Infected macrophages that are able to survive acute HSV infection become a significant source of inflammatory chemokines and cytokines including TNF-α, IL-1, IL-6, IL-8, IL-12, IL-18, RANTES (regulated on activation, normal T-cell expressed and secreted; a chemokine that is a chemoattractant for eosinophils, monocytes, and lymphocytes), as well

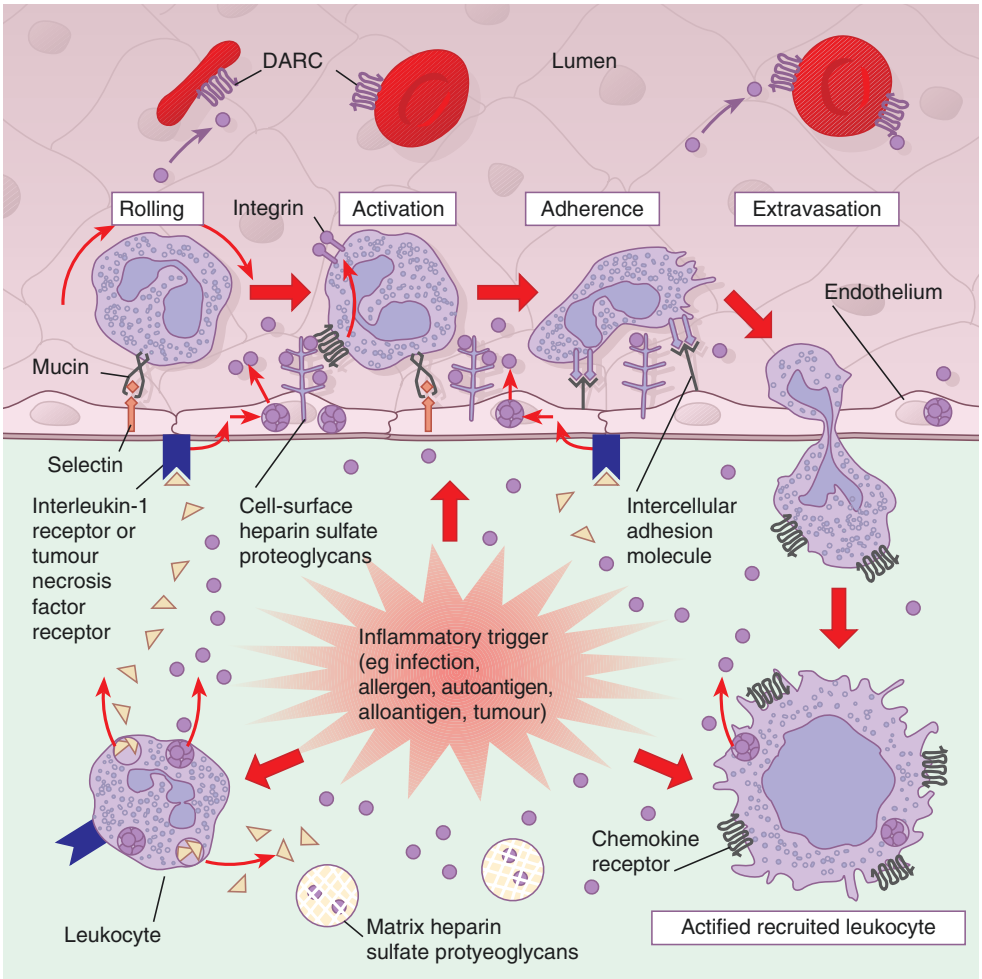


Fig. 17.1 Chemokine regulation of leukocyte movement. Chemokines are secreted at sites of inflammation and infection by resident tissue cells, resident and recruited leukocytes, and cytokine-activated endothelial cells. Chemokines are locally retained on matrix and cell-surface heparin sulfate proteoglycans, establishing a chemokine concentration gradient surrounding the inflammatory stimulus, as well as on the surface of the overlying endothelium. Leukocytes rolling on the endothelium in a selectin-mediated process are brought into contact with chemokines retained on cell-surface heparin sulfate proteoglycans. Chemokine signaling activates leukocyte integrins, leading to firm adherence and extravasation. The recruited leukocytes are activated by local proinflammatory cytokines and may become desensitized to further chemokine signaling because of high local concentrations of chemokines. The Duffy antigen receptor for chemokines (DARC), a nonsignaling erythrocyte chemokine receptor, functions as a sink, removing chemokines from the circulation and thus helping to maintain a tissue–bloodstream chemokine gradient. (From Luster [21]. Copyright © 1998 Massachusetts Medical Society. All rights reserved.)

as IFN-α and -β. Subsequently, the immature DCs capture viral antigens and transport them to the regional lymph node to alert and stimulate the adaptive immune response [7].

Once the DC exits the mucosa, the late-induced innate response begins at the infection site. Initially, an influx of neutrophils, monocytes, and NK cells is established. These cells traverse the activated capillary endothelial cells guided by the chemokines present at the infection site. The neutrophils secrete α-defensins that insert into the virion lipid envelope and trigger the degradation of phagocytosed virions [22]. They also secrete TNF-α, which acts synergistically with IFN-α and -β, as well as IFN-γ to produce lysis of the infected cells inhibiting viral replication [22]. Simultaneously, NK cells are activated by the binding of immunoglobulins to the viral antigen (through their Fc receptor [CD16] located on the cell surface) and are recruited by chemokines and cytokines (IFN-α and -β, IL-12, IL-15, IL-18) produced by the activated infected cells within 2–3 days after infection. The NKs participate in cytolysis of virus-infected cells by perforin/granzyme-mediated processes that result in phagocytosis and destruction of infected cells and viral particles [20]. The accumulation of viral antigens is followed by complement activation, facilitating the uptake of viral peptides by phagocytes [23]. These phagocytic cells produce antiviral cytokines and defensins such as nitric oxide (NO), which enhance the immune response and promote phagocytosis and destruction of virus particles and infected cells [20].

One of the key cells for the innate response against HSV is the macrophage. The molecular mechanisms of the immune response within macrophages have been extensively studied. Once HSV infects macrophages, these cells release a series of cell mediators such as TNF-α and IL-12 (which subsequently stimulates the production of IFN-α and -β). Interferon-α and -β induce the NK and T cells to secrete IFN-γ, which eventually controls the HSV replication and initiates the adaptive response [24, 25]. At this stage, the site of origin of IL-12 production is controversial. The majority of investigators suggest that DCs are the main producers of IL-12 [7, 26]. However, other authors indicate that the main source of this molecule is the recruited inflammatory cells [27].

The adaptive immune response begins when the DCs migrate to the regional lymph nodes. These cells mature and display viral peptides coupled with MHC molecules, secrete cytokines, and regulate the expression of other inflammatory molecules. The cytokines produce differentiation of the Th0 cells to either Th1 or Th2. For HSV, the Th1 response is indispensable for clearance of the infection (particularly for HSV-2) [28]. Other cells such as NK cells and macrophages also stimulate the switch from a Th0 to a Th1 response [28] (Table 17.2).

One of the most studied T cells during HSV infection is the CD8+ T cell. These cells are capable of expressing the lymphocyte-associated antigen (cutaneous leukocyte antigen [CLA]) [29, 30]. During recurrent and symptomatic infection, antigen-specific CD4+ T cells and NK cells infiltrate the dermis by day 2 of the lesion formation, whereas CD8+ dermal infiltration (which implies cytotoxic activity resulting in viral clearance) occurs a few days later [29, 30]. Therefore, CD8 cells appear to play an important role in containing HSV infection. Posaved et al. showed that the levels of CD8+ cytotoxic T lymphocytes (CTLs) correlate inversely with the severity of HSV-2 infection and temporally with the local clearance of the virus in lesions [31]. The data from the study of Koelle et al. suggest that the expression of the homing receptor (CLA) by the CD8+ T cells is programmed at the site of the original antigen encounter and promotes migration and immune responses in reactivation [29]. Additional studies indicate that this specific cell has the capacity of T-cell memory and self-renewal that is crucial to control the infection and symptomatic recurrences [31].

Table 17.2 Immune responses to herpes viruses

Complement-mediated activation of immune cells
Antibody mediated activation of immune cells
Secretion of interferons (IFNs) by the infected epithelial cells
Activation of neutrophils, macrophages, and natural killer (NK) cells
Activation of endothelial cells with secretion of tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), IFN, and granulocyte-macrophage colony-stimulating factor (GM-CSF)
Secretion of α-defensins by neutrophils
Activation of NK cells for cytolysis of virus-infected cells
Secretion of IL-12 and TNF-α by monocytes
Differentiation into Th1 and Th2 responses

Studies in vivo (mouse model) have demonstrated that CD8+ cells also play a crucial role in controlling the virus during the latent state [19, 32]. Latent herpes viral infection in humans in the trigeminal ganglia is accompanied by a chronic inflammatory process that involves elevated levels of cytokine transcripts such as IFN-γ, TNF-α (which affects viral replication), and chemokines, accompanied by persistent lymphocytic cell infiltration (CD8+ T cells, CD68+, and macrophages). It has been suggested that this phenomenon could be due to a low level of expression of viral genes during latency. The CD8+ T cells are capable of controlling the virus through cytokines, and this mechanism is likely to occur in response to the persistence of viruses that are prone to reactivate frequently, providing a survival advantage for both the host and the virus [19]. It is proposed that factors compromising CD8+ T cell function lead to virus escape from immune regulation and reactivation [33].

Immune Evasion Mechanisms

There appears to be a complex and well-balanced interaction between host cells and HSV. Success for the virus means a delicate balance between infection and latency. Therefore,

Table 17.3 Mechanisms of immune evasion in herpes viruses infection

Modulation of the activity of neutrophils, macrophages, or natural killer (NK) cells
Interference with monocyte function
Suppression of chemotaxis and phagocytosis
Modulation of the production of IL-1, TNF-α, IFN-α and -β
Reduced activity of NK cells
Inhibition of apoptosis

evasive strategies must be carefully designed to permit viral replication at certain stages that could potentially lead to transmission to other susceptible hosts in order to maintain the viral progeny. Humans and the herpes viruses have evolved together in a symbiotic relationship that has allowed the adaption of the virus to the host and the environment, maintaining a "truce" with the immune system without any serious threats to human life. Some of the identified mechanisms to evade the immune system include the ability of HSV to alter the activity of neutrophils, macrophages, or NK cells [31]. It is known that HSV interferes with monocyte function, suppresses chemotaxis and phagocytosis, and reduces the secretion of IL-1 and TNF-α [7, 20, 34]. It has also been reported that HSV reduced the activity of NK cells upon contact with infected cells, due in part to the interference with IFN-α and -β production [20, 34]. Furthermore, HSV produces many antiapoptotic factors, such as cathepsins and latency-associated transcripts (LATs), which allow for maximal viral replications. LATs have been recovered from latently infected neurons and have been shown to inhibit granzyme-B mediated apoptosis and capsase-8-dependent apoptosis [17]. It has been proposed that LATs increase the efficiency of latency and subsequent reactivation [33] (Table 17.3).

Vaccines

There are currently two different groups of vaccines targeting HSV: prophylactic and therapeutic. Prophylactic vaccines aim to protect seronegative patients against HSV infection. The goal of therapeutic vaccines is to reduce symptoms and infectivity (outbreaks and shedding) in patients who are already seropositive for HSV [35]. In the recent decades, the focus has been primarily on developing a prophylactic vaccine with varying results in trials. Two studies were carried out by GlaxoSmithKline, using a recombinant vaccine preparation containing HSV glycoproteins. The first enrolled patients who were seronegative for both HSV-1 and HSV-2. The second study allowed inclusion of HSV-1–seropositive patients [36]. The studies' primary end point was protection against genital HSV-2 clinical disease and the secondary end point was HSV-2 seroconversion. The first study did not show any difference in the primary end point between the group that received vaccine and the group that received a placebo. However, when a gender-stratified analysis was performed, the vaccine proved protective in females only (73%; 95% confidence interval [CI], 19–91%). The sex differences found in these studies might be explained by innate anatomic differences of the genital epithelial layers between men and women. The epithelial layer of male genitals consists mostly of stratum corneum, which is lacking in the genitals of women at the vaginal-cervical mucous membrane. Additionally, the secretions produced by the vagina and the genital mucous membranes contain antibodies and migratory leukocytes. Therefore, women appear to have enhanced immune responses mediated by helper T cells exhibiting a Th1 response, compared to men [37]. The Herpevac Trial for Woman was conducted to evaluate this sex difference in vaccine protection. Although the primary endpoint of HSV-1 and HSV-2 genital disease prevention was not achieved, the vaccine was found to be effective in decreasing infection caused by HSV-1 but not HSV-2 [38]. Further studies are needed to evaluate this unexpected outcome.

Recent HSV vaccine research has transitioned away from prophylactic vaccines to therapeutic vaccine candidates. There are currently three novel HSV vaccine candidates in phase I or II trials. The HerpV vaccine is composed of recombinant human heat shock protein-70, 32 different HSV-2 antigens and an adjuvant. This vaccine increased HSV-2 CD4+ and CD8+ T cell activity in HSV-2 seropositive patients in a phase I trial [39]. It is currently being evaluated for efficacy in prevention of shedding and lesions in a phase II trial [40]. The GEN-003 vaccine consists of the gD2 glycoprotein, ICP4 (a potent CD8+ T-cell immunogen) and Matrix M adjuvant. In animal models, this vaccine candidate decreased recurrence of lesions and viral shedding [41]. The GEN-003 vaccine has completed a phase I/II trial evaluating its efficacy as a therapeutic vaccine but the results of this trial have not been published [42]. Finally, the HSV529 vaccine is composed of a DNA replication defective HSV virus. This candidate vaccine has demonstrated efficacy in animal models and is in a phase I/II trial as a therapeutic and prophylactic vaccine [43]. Research on a prophylactic HSV vaccine needs to focus on inducing a stronger antibody response. In contrast, research on a therapeutic HSV vaccine needs to focus on prompting a more potent T-cell response [43].

Varicella-Zoster Virus

Varicella-zoster virus (VZV) is also an α-herpesvirus with a genome of ~125,000 base pairs (bp) with at least 70 unique open reading frames (ORFs) [44, 45]. It is characterized by a relatively short reproductive cycle, rapid cell-to-cell spread, and significant ability to establish latent infections (primarily in sensory ganglia) [45]. Humans are the only known natural

reservoir for VZV. This virus causes two different clinical syndromes: varicella (chickenpox) and herpes zoster (shingles). Varicella is usually a self-limited disease of childhood characterized by a very pruritic rash. The disease has a worldwide distribution, and transmission is more pronounced in temperate climates (where it is seen more frequently in children) than in tropical environments [11]. Approximately 20 % of those who had varicella later develop herpes zoster, which usually affects adults older than 50 years of age, although it can occur at any age [11, 46].

Immune Response

Initial clinical observations indicate that the primary site of inoculation is the respiratory tract. A rash usually develops after an incubation period of 10–12 days [11, 45, 46]. The entry of the VZV into a host cell requires fusion of the viral envelope with the host cell plasma membrane. This phenomenon is mediated by interactions of viral oligosaccharides with the heparan sulfate proteoglycans (via N-linkage) on the host cell surface. The fusion permits the entry of the viral proteins into the host cell cytosol and then to the nucleus where the nucleocapsid fuses with the outer nuclear membrane, releasing the viral DNA genome into the nucleus [47]. The spread of the virus is controlled by the innate and adaptive responses. The host cell membrane has specific glycoproteins such as gH, gL, gB, and gE that permit the fusion process between cells. These proteins are used by VZV to quickly spread to adjacent epidermal cells by inducing the fusion of the infected cells with noninfected ones [32]. Varicella-zoster virus has a special tropism for three major cell types: the peripheral blood mononuclear cells (PBMCs), skin cells, and sensory neurons [32]. This virus not only infects these cells, but also is capable of replicating inside them. This phenomenon of viral replication can be observed in intraepidermal vesicular lesions of the skin, which are loaded with free virus [11, 45, 47].

The virus infects the immature DCs of the respiratory mucosa, and these cells transport the virus to the T-cell–draining lymph nodes. From these nodes VZV is subsequently transported to the reticuloendothelial system and then is capable of gaining access to the bloodstream, causing a primary viremia. During this first viremic phase, VZV is able to reach the reticuloendothelial organs where it undergoes a phase of viral amplification. It was previously thought that VZV reached the skin during a second viremic episode that occurred in the late incubation period [47]. However, studies of viral infection in mouse models now suggest that infected T cells from the tonsil area are capable of transferring VZV to the skin immediately after entering the circulation during the primary viremia and that the prolonged interval between exposure and the skin rash reflects the time required for the virus to become recognized by potent innate immune barriers such as IFN-α [44, 47].

Varicella-zoster virus preferentially infects the active memory CD4 T cells that express skin homing markers such as CLA and the chemokine (C-C motif) receptor 4 (CCR4). These T cells are usually programmed for immune surveillance and then may facilitate the transfer of VZV into the skin [44]. It appears that VZV does not trigger any early inflammatory response that might block the appearance of virus-filled vesicles at the skin surface [44, 47]. The initial viremia is necessary to ensure that enough cutaneous lesions are formed to transmit the virus efficiently to other individuals as a viral survival mechanism. The T cells trafficking through the skin activate VZV replication at this site, and this process permits infection of more T cells that will return to the circulation [44].

The innate immune response is induced during the acute VZV infection. This response involves the release of IFN-α and IFN-γ and the secretion of cytokines such as IL-6 (by monocytes) via TLR2 [48]. This cell-mediated immune response contains VZV replication and prevents the host from a systemic disease (including severe compromise of organs such as lungs, kidneys, and spleen). The adaptive immune response begins with the presence of CD4+ T cells and the release of IL-2, IL-10, IL-12, and IFN-γ by Th1 and Th2 cells [49]. These cytokines cause the proliferation of VZV specific CD4+ and CD8+ T cells, which express MHC class I and II molecules and recognize the viral glycoproteins gE, gH, and gI, with the subsequent killing of the VZV-infected cells [50]. During the presence of rash, many mononuclear infiltrating cells are present. Most of these cells express CD4 and CD8, including CD45RO+ memory cells, skin homing CLA, and CCR4+ T cells. During this period, the skin shows expression of E-selectin (a cell adhesion molecule and CD antigen that mediates neutrophil, monocyte, and memory T-cell adhesion to cytokine-activated endothelial cells), intercellular adhesion molecule 1 (ICAM-1, a cell-surface ligand involved in leukocyte adhesion and inflammation), and vascular cell adhesion molecule-1 (VCAM-1, a cytokine- induced cell adhesion molecule present on activated endothelial cells, tissue macrophages, and DCs), allowing the migration of the inflammatory cells. The humoral immune response has a reduced role in controlling the virus. Immunoglobulin G (IgG), IgM and IgA appear to respond to some viral proteins. It seems that these antibodies neutralize cell-free virions and contribute in the lysis of infected cells [44] (Table 17.4).

During the resolution of varicella, VZV establishes latency in the trigeminal and dorsal root ganglia where it remains latent through the lifetime of its host. Approximately 20 % of infected people develop herpes zoster. Figure 17.2 illustrates the latent virus in a dorsal root ganglion and reactivated virus causing acute vesicular zoster rash [51]. In this period, the VZV multiplies and spreads centrifugally down the sensory

nerve, causing neuronal necrosis and intense neuritis. Recent studies have shown that VZV possesses multiple open reading frames (ORFs) that encode proteins present in the cytoplasm of neurons during latency and are localized in the cell nucleus during reactivation [52]. Some of these genes are involved in VZV assembly, replication (such as ORF2) and latency. ORF47 encodes a protein that is part of the VZV virion tegument and is essential for VZV growth in differentiated skin and T cells [53]. Studies of this peptide indicate that the blockage of the kinase function of this protein decreases the VZV virulence in the skin, suggesting interference with the production of complete VZV virions. This peptide has also been implicated as a latency- associated protein along with the products of the ORF4 and ORF63 [54–57]. Additionally, the mechanisms of latency appear to be controlled LATs. However, the exact mechanisms are not well understood [58].

Table 17.4 Immune responses to varicella-zoster virus (VZV)

Acute VZV infection
Release of IFN-α and IFN-γ
Secretion of cytokines such as IL-6 (by monocytes) via Toll-like receptor 2 (TLR2)
Release of IL-2, IL-10, IL-12, and IFN-γ by T-helper-1 (Th1) and Th2 cells
Proliferation of VZV specific $CD4^+$ and $CD8^+$ T cells
During skin rash
Expression of E-selectin, intercellular adhesion molecule 1(ICAM-1) and vascular cell adhesion molecule (VCAM-1)
Migration of inflammatory cells
Reduced role of humoral response in controlling the virus

Immune Evasion Mechanisms

Varicella-zoster virus utilizes several mechanisms to overcome the immune response. Major histocompatibility complex class II expression is restricted to APCs and is required to present the viral peptides to CD4 T cells. Usually, the host immune response to the virus produces the release of IFN-γ, which stimulates the expression of MHC II molecules. To evade the immune response and ensure replication, VZV produces a protein that interferes with Stat1 (a signal transducer and activator of transcription that mediates cellular responses to interferons). Stat1 interacts with P53 tumor

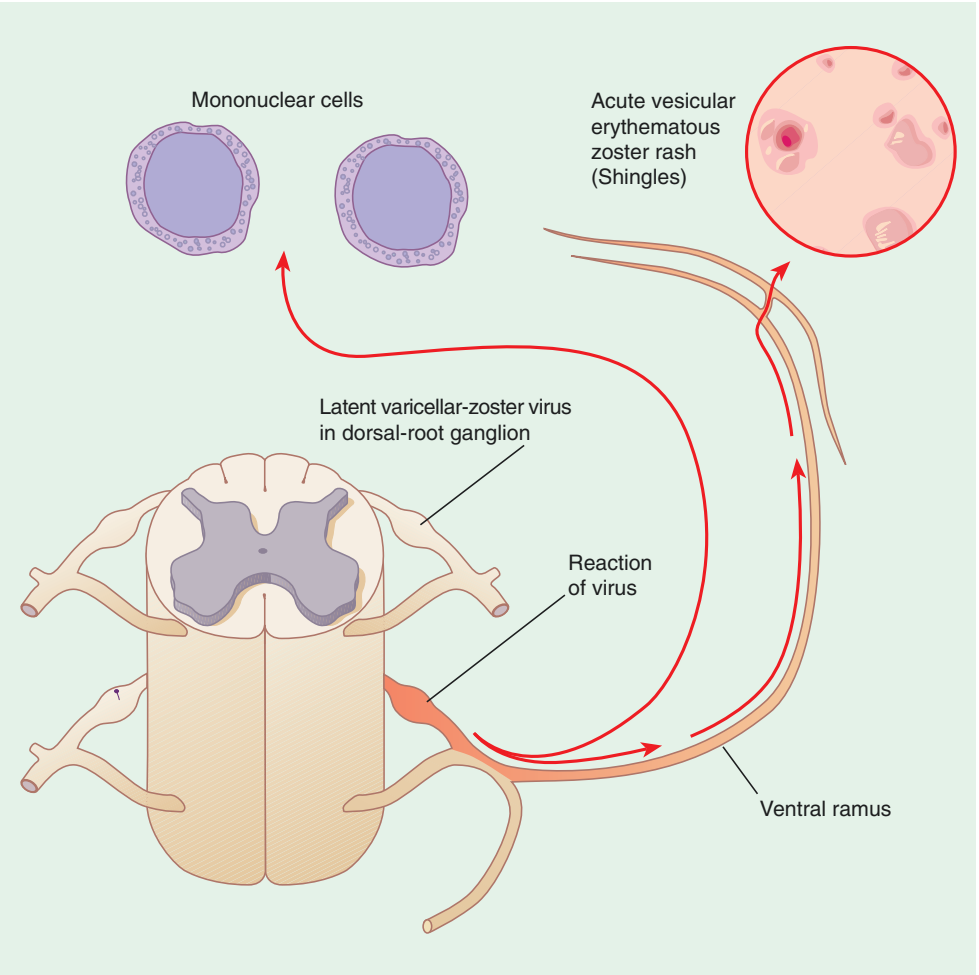


Fig. 17.2 Latent and reactivated varicella–zoster virus. Shown is a latent virus in a dorsal-root ganglion (white fusiform swelling) adjacent to the spinal cord and reactivated virus in a nearby dorsal-root ganglion (orange fusiform swelling) with transaxonal spread to the skin. The reactivated virus causes an acute, vesicular, erythematous zoster rash. (From Gilden et al [51]. Copyright © 2000 Massachusetts Medical Society. All rights reserved.)

suppressor protein and regulates expression of genes involved in growth control and apoptosis activation and thereby inhibits antiviral IFN-γ production in foci of infected skin cells [44]. Inhibition of IFN-γ results in decreased MHC II expression, which subsequently affects the efficiency of VZV specific immune responses. Varicella-zoster virus takes advantage of these circumstances, and as a consequence, the initial formation of VZV lesions in skin (varicella or during the VZV reactivation as zoster) is facilitated [44, 58, 59]. Additionally, VZV has mechanisms to delay clearance of virus-infected cells by interfering with the expression of MHC class I proteins that are necessary for CD4 and CD8 T-cell recognition [58]. This mechanism allows VZV to evade CD8 T-cell lysis during the viremic phase. The precise molecular event is related to the downregulation of MHC I by VZV via interference of its transport from the Golgi compartment to the plasma membrane [44] (Table 17.5)

Vaccines

Before the introduction in 1995 of Varivax, a live attenuated Oka strain of VZV, millions of children developed primary varicella (chickenpox). Varivax is indicated for vaccination against varicella in individuals 12 months of age and older and it is recommended as part of the childhood immunization schedule. Its effectiveness is greater than 95 % for varicella prevention. Herpes zoster is the result of the reactivation of latent VZV infection residing in sensory ganglia. In the United States, herpes zoster affects approximately 1,000,000 persons annually, with an incidence of 2.2 cases per 1000 persons of age or older [60, 61]. Results from several clinical trials have determined that a live, attenuated VZV vaccine using the Oka strain (Zostavax) is safe, elevates VZV- specific cell-mediated immunity, and significantly reduces the incidence of herpes zoster and postherpetic neuralgia in immunocompetent people over the age of 50. This vaccine is 14-fold more concentrated than VARIVAX and has been approved for use in the United States. It is expected to reduce the risk for herpes zoster by >50 % [61]. There have been concerns about the decline in Zostavax efficacy however. Although vaccine efficacy was maintained through year 5 after vaccination in the Short-Term Persistence Substudy, it steadily declined during that time period [62]. Figure 17.3 illustrates how the administration of zoster vaccine to older persons may prevent VZV-specific T cells from dropping below the threshold for zoster occurrence [63].

Table 17.5 Immune evasion mechanisms in varicella-zoster virus infection

Interference with signal transduction mechanism through Stat 1
Decreased major histocompatibility complex class II (MHC II) expression
Delay of clearance of virus infected cells through interference with MHC expression

Human Papillomavirus

Human papillomaviruses (HPVs) are small DNA tumor viruses that have a circular double- stranded DNA genome of ~8 kb in length. They belong to a large group of recognized oncogenic viruses [64]. Human papillomavirus is a member of the Papillomaviridae family that includes over 120 different genotypes (defined by differences in their nucleotide sequence) [11, 65]. The HPV genome is composed of three regions: the long control region (LCR), the early region (E), and the late region (L). The early region consists of genes E1 to E8 (which encode nonstructural proteins for transcription,

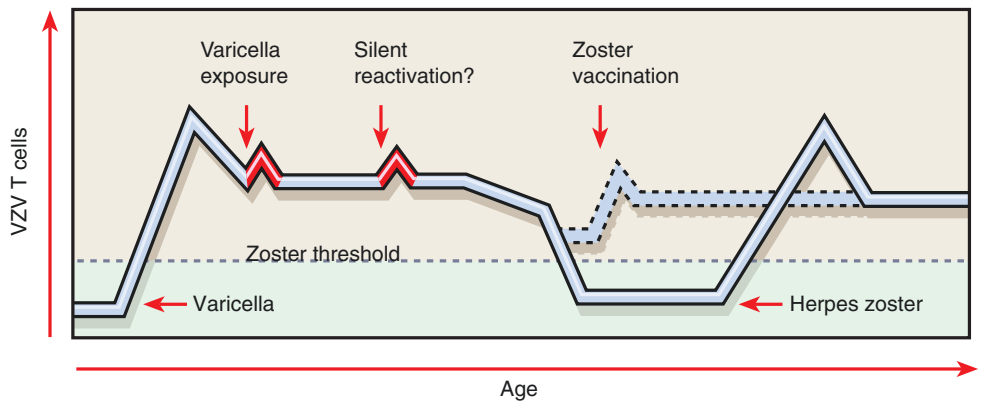


Fig. 17.3 Host factors in varicella-zoster virus (VZV) latency and reactivation. Varicella is the primary infection caused by VZV, and its resolution is associated with the induction of VZV-specific memory T cells (blue line). Memory immunity to VZV may be boosted periodically by exposure to varicella or silent reactivation from latency (red peaks). VZV-specific memory T cells decline with age. The decline below a threshold (dashed green line) correlates with an increased risk of zoster. The occurrence of zoster, in turn, is associated with an increase in VZV-specific T cells. The administration of zoster vaccine to older persons may prevent VZV-specific T cells from dropping below the threshold for zoster occurrence (dashed blue line). (From Arvin [63]. Copyright © 2005 Massachusetts Medical Society. All rights reserved.)

plasmid replication, and transformation), while the late region codes for the major (L1) and minor (L2) proteins that form the viral capsid. The current vaccines include the protein L1 as the major immunogenic antigen [66].

The HPVs are commonly categorized as having low or high oncogenic potential. Infections with the high-risk types such as HPV-16 and HPV-18 have been implicated in cervical-anogenital cancer and oral squamous cell carcinomas [11, 67, 68]. The infection of the genital tract by HPV is one of the most frequent sexually transmitted diseases [68, 69]. Approximately 75 % of sexually active individuals are infected by HPV during their lifetime. In the United States, it has been calculated that 6.2 million new cases of high-risk HPV infections occur each year, close to 20 million Americans are infected, and 1 % of sexually active adults have genital warts [65, 69]. It is estimated that >99 % of cervical, as well as >70 % of anal and vaginal cancers are related to HPV infection (cervical cancer is the second most frequent cause of death due to neoplasia among women worldwide). More specifically, about 30–40 % of penile, vulvar, and oropharynx cancers are related to the HPV-16 and HPV-18 [68, 70–72].

The majority of HPV clinical lesions are located in the genital area (70–90 %) in sexually active adolescents and young women. These lesions usually show clearance of the infection within 12–30 months [68]. Longer duration of infection is frequently related to the presence of cervical intraepithelial neoplasia (CIN) [73]. The prevalence and incidence of the HPV infection varies with age. The cause for this apparent variation is not clear. Many authors postulate that some (but not all) infected individuals develop an adaptive immune response against HPV that prevents future infections [74].

Immune Response

Human papillomavirus has a special tropism for the epithelial cells (they are epitheliotropic), infecting keratinocytes at a wide range of body sites where they cause aberrant cellular proliferation leading to benign warts or cervical cancer [3, 64, 75]. Initially, the virus infects primitive basal keratinocytes and starts DNA replication and transcription of the early genes at very low levels [76]. The peak of productive synthesis of virions is reached once the keratinocyte reaches higher strata of the epithelium. Thus, high levels of viral proteins and viral assembly occur only at the upper layers of the squamous epithelia (stratum spinosum and granulosum) [11]. At this point the cycle of replication and patterns of gene transcription are dependent on the stage of differentiation of the keratinocyte [76].

A significant amount of information regarding the immune response has been gathered from observations in animal models. Once the virus has reached the skin, it establishes itself at the basal cells of the epithelia where it starts replication. At this point the replication is minimal but becomes greater once the keratinocyte matures toward desquamation [77]. At the basal layer, there is practically no immune response against the virus (two to several months), but once it is detected by the immune system, replication increases and results in clinically detectable lesions [78, 79]. After a few months (which varies depending on the host), the infected keratinocytes express the late viral genes (encoding proteins L1 and L2) that trigger a full immune response [77–79].

The keratinocytes are able to secrete cytokines, growth factors, and chemokines upon viral stimuli. The host immune response dictates the emergence and characteristics of clinical lesions [76]. Some of the cytokines involved are transforming growth factor-β (TGF-β), TNF-α, and IFNs. TGF-β has been shown to inhibit the growth of nontumorigenic HPV-16 and HPV-18 immortalized cells, and it seems to control the expression of E6 and E7 (although some controversy exists on these facts) [76]. In normal cervical cells, TGF-β inhibits viral growth, but HPV can evade this control mechanism [76]. TNF-α, which is another product of the keratinocyte, may have an antiproliferative effect on the HPV-16–infected cells through cell cycle arrest (between the G0 and G1 phases of the cell cycle) [76, 80]. It has also been suggested that TNF-α acts as a repressor on the expression of E6 and E7 in the immortalized human keratinocytes [81]. The effects attributed to IFNs may be virus-type specific. These molecules also appear to inhibit the production of viral proteins [76].

The time between HPV infection and the development of a clinical lesion could vary from weeks to more than 9 months. This is an indication that HPV could modulate the immune system as evidenced by the following: (1) HPV infects mainly keratinocytes, which are cells programmed to die and desquamate in a specific and timely manner (apoptosis); and (2) the intracellular location of HPV in these cells allows the virus to hide and avoid immune surveillance. As a consequence, the apoptotic keratinocyte infected with HPV does not trigger a significant inflammatory reaction, eventually resulting in a persistent chronic infection [78, 82].

The adaptive immune response involves two phases: the recognition of antigen and the response to it. This adaptive immune response involves the LCs that capture the virus and its antigen for transport to local lymph nodes and presentation to naive T cells. The T cells return to the infected epithelial tissues via mechanisms that include secretion of chemokines and expression of adhesion molecules to clear the infection. In the recognition phase, the LCs are the major APCs. However, in some HPV infections, a depletion of these cells has been documented, which is associated with enhanced survival of HPV, prolonged courses of infection,

Table 17.6 Immune responses to human papilloma virus

Secretion by keratinocytes of cytokines, growth factors, and chemokines (TNF-α, transforming growth factor-β [TGF-β], and IFNs) upon viral stimuli
Langerhans cells capture of virus and its antigen
Transport to local lymph nodes and presentation to naive T cells
Large infiltration of T cells (CD4+ and CD8+) and macrophages
Th1 pattern of cytokines (IL-12, TNF-α, and IFN-γ)
Expression of the adhesion molecules for lymphocyte trafficking

and possibly malignancy [83–85]. At this stage, the infected keratinocytes produce IL-1α and TNF, which promote LC migration.

If the immune response is appropriate, the regression of warts usually follows. At this stage, a large infiltrate of T cells (CD4+ and CD8+) and macrophages is present, and the cytokines involved exhibit the Th1 pattern (IL-12, TNF-α, and IFN-γ plus the expression of the adhesion molecules for lymphocyte trafficking) (Table 17.6). A systemic T-cell response to E1 and E6 viral proteins is effective only at the peak of wart regression (although activity is present during the whole infective cycle) [78, 79].

In summary, HPV is capable of escaping the immune response for several months by modulating the inflammatory reaction from the keratinocytes after invasion. Nonetheless, in the majority of cases, the immune system finally catches up and is capable of detecting the virus, (probably when the replication turnover is highest) and thus is able to resolve the infection.

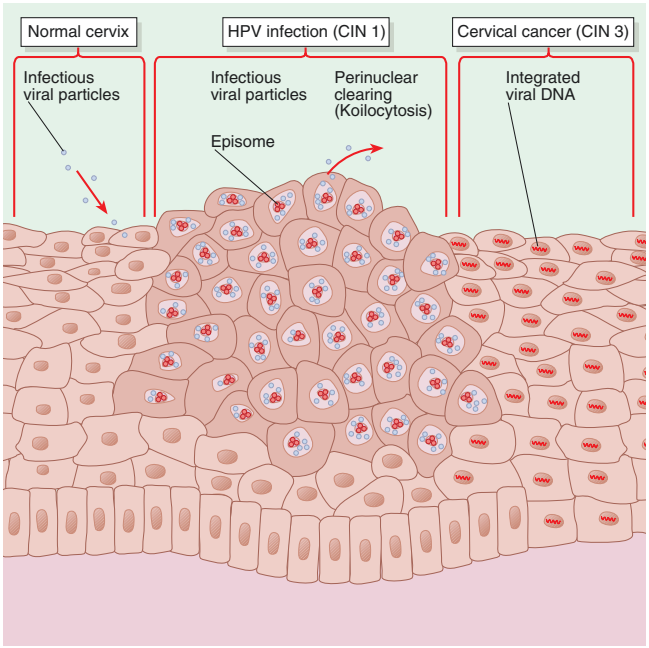


Fig. 17.4 Spectrum of changes in cervical squamous epithelium caused by human papillomavirus (HPV) infection. The left side of the figure shows normal cervical squamous epithelium. When HPV infection occurs (center), the virus exists in the cell nucleus as a circular episome. If the viral genome is intact, new infectious viral particles can be produced; their presence in the cell is indicated by perinuclear clearing, or koilocytosis. In cervical cancer (right), oncogenic portions of HPV DNA become integrated into the host's DNA, with disruption of the *E2* regulatory region and loss of other genes needed to form a complete virus. The cells are undifferentiated and do not show koilocytosis. CIN 1, cervical intraepithelial neoplasia grade 1. (From Goodman and Wilbur [93]. Copyright © 2003 Massachusetts Medical Society. All rights reserved.)

Viral Oncogenesis

It is now accepted that HPV infection is necessary (but not sufficient) to cause cervical cancer, other anogenital neoplasms, and oral squamous cell carcinomas. Several viral types (such as 16 and 18) have been clearly identified as having high oncogenic potential. Low risk types include 6 and 11 [86]. Recently, significant evidence is emerging supporting the transition zone of the cervix as the location of persistent HPV infection. Specifically, it has been demonstrated that HPV exists in the latent state in the basal epithelial stem cells even after immune clearance of papillomas [87]. A recent animal model has shown that basal epithelial cells expressing the HPV 16 genes can undergo malignant transformation [88]. For high-risk HPV types, the proteins E6 and E7 appear to play a very important role in oncogenesis since they are able to inhibit two well-known oncogenic suppressor genes encoding the pRb (product of retinoblastoma tumor suppressor gene) and p53 proteins. E6 enhances the degradation of p53 via the ubiquitin-mediated proteolysis machinery (E3 ubiquitin ligase, UBE3A) and E7 interacts with the pRb [89]. Of note, Hyland et al. and McLaughlin-Drubin et al. recently demonstrated that E6 and E7 induce epigenetic reprogramming, specifically methylation/acetylation, modification of chromatin structure and subsequent activation of homeobox genes, in infected keratinocytes [90, 91].

Many studies have shown that the absence of mature APCs, CD50, and CD86 and the downregulation of TNF-α represent an inadequate immune response to HPV that leads to persistence of the viral infection in the CIN lesions. The LCs are also decreased in numbers and lack the expression of CD11a/18, CD50, CD54, CD58, and CD86. These changes alter the antigen-presenting capacity and can induce immune tolerance to the viral infection [92]. The immortalization of epithelial cells in CIN produced by HPV-16 is related to the lack of response to the inhibitory effect of TGF-β and the inhibition in keratinocytes of TNF-α by the HPV-16 E7 protein, resulting in uncontrolled growth of the infected cells and escaping of the virus from the immune system [76]. Figure 17.4 illustrates the changes in cervical squamous epithelium caused by HPV infection [93].

Immune Evasion Mechanisms

The reasons for the failure of the immune system to recognize HPV are not well understood. It appears that HPV is able to escape detection by the APCs. To survive and have enough time to replicate without detection, HPV has developed many strategies to evade the immune system. The special tropism for keratinocytes (as discussed earlier) is one of the first mechanisms of immune evasion [78].

Keratinocytes are powerful immune cells. They constitute almost 95 % of the cervical and skin epithelium and can express MCH II and co-stimulatory signals to T cells (such as ICAM-I) during inflammation [92]. If the immune response is activated, the HPV-infected keratinocytes release TNF-α, which negatively affects the replication of HPV [70, 92]. TNF-α upregulates the expression of ICAM-1 levels, which decrease the levels of IFN-γ. In CIN, the keratinocytes express less TNF-α, decreasing the stimulus to the LCs. This alteration also affects the expression of MHC II, and as a consequence the presentation of antigens is altered and the expression of the suppressive cytokine IL-10 is increased [68, 92]. These changes in the immunologic microenvironment (with inappropriate T-cell presentation) may contribute to the persistence and progression of the viral infection and development of a CIN lesion [84, 92, 94].

Another important mechanism of immune evasion by HPV involves the downregulation of the macrophage inflammatory protein-3α (MIP-3α), which allows the virus to persist in the epithelial cells without being recognized [95]. Macrophage inflammatory protein-3α is one of the most potent attractants of LC precursors and dermal dendritic cells, as well as T cells. It has been shown that infection of keratinocytes by HPV-16 expressing the E6 and E7 proteins decreases the production of MIP-3α [96].

Similarly, it has also been noted that in most cutaneous viral infections the number of epidermal LCs is significantly reduced (the decrease in numbers of LCs is more marked in lesions of CIN) [92, 96].

Recent studies support the findings that the E5 protein, found in HPV, downregulates the MHC class I molecules, which results in impaired cell lysis by the cytotoxic T lymphocytes. The E5 oncoproteins from multiple types of HPV share similar characteristics; they are small hydrophobic peptides located in the endomembranous compartments of the infected cell that contribute to the activation of growth factor receptors and downregulation of the MHC I [11, 97]. The exact mechanisms used by E5 to downregulate MHC I are not completely clear. Two proposed hypotheses include (1) the inhibition of acidification of the Golgi apparatus (where the MHC is assembled), and (2) a direct interaction between E5 and the heavy chain of the MHC [98, 99].

It has also been shown that the proteins E6 and E7 negatively affect the immune system by inhibiting the production of immune mediators [84]. Both proteins are inversely correlated with the expression of IL-18, which induces the secretion of IFN-γ and IL-8. E6 and E7 also reduce the production of chemokines and monocyte chemoattractant proteins in the genital mucosa of women [76] (Table 17.7).

Table 17.7 Mechanisms of immune evasion in human papilloma virus infection

Modulation of the immune response in the keratinocytes
Downregulation of the production of macrophage inflammatory protein-3α (MIP-3α)
Downregulation of MHC I molecules through the HPV protein E5
Reduction of chemokine and interleukin production by the HPV proteins E6 and E7

Vaccines

The prophylactic HPV vaccines are based on L1, a major capsid protein that is self-assembled into empty capsids, called virus–like particles (VLPs). VLPs are free of viral DNA and therefore are not infectious, but can provide a source of epitopes that can stimulate a neutralizing antibody response [3, 66, 100, 101]. Figure 17.5 illustrates a proposed mechanism of the immune response to HPV vaccines [102–105].

There are currently three approved vaccines for HPV. The first one is the bivalent HPV vaccine (Cevarix marketed by GlaxoSmithKline) that provides immunity against HPV strains 16 and 18. Gardasil, which is marketed by Merck, is a quadrivalent vaccine and confers immunity against strains 6, 11, 16 and 18. The recently approved Gardasil 9 protects against these four types as well as five addition types: HPV 31, 33, 45, 52 and 58. All three vaccines have demonstrated efficacy in phase III trials [106]. An 8.4 year follow-up study for Gardasil performed in the U.S., Canada, and Brazil reported 95.1 % vaccine efficacy for incident infection [107]. Comparative trials of the vaccines have demonstrated that Cevarix evokes a higher antibody titer than Gardasil. Specifically, one trial found that the HPV 16 antibody titer was 2.4–5.8 fold higher and the HPV 18 antibody titer was 7.7–9.4 fold higher with Cevarix than with Gardasil [108]. The HPV vaccination series is recommended in females aged 11–26 years and for males aged 11 or 12 years [106].

Human Immunodeficiency Virus

The human immunodeficiency virus (HIV) pandemic is one of the tragic legacies of the 20th century. HIV is currently one of the leading causes of mortality in sub-Saharan Africa, where more than 5.5 million inhabitants are infected with the virus [109]. In 2013, 35.3 million people were estimated to

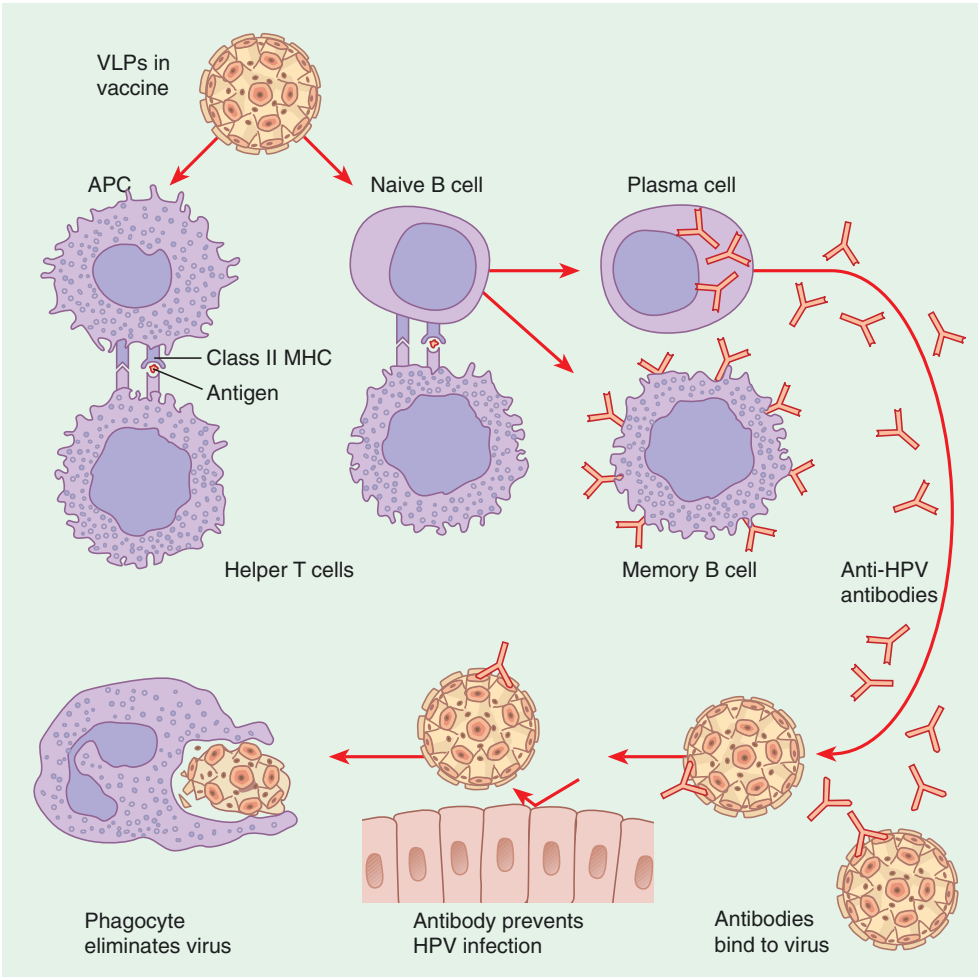


Fig. 17.5 A proposed mechanism for the immune response to HPV vaccines. It has been proposed that HPV L1 virus–like particle (VLP) stimulates an adaptive immune response in humans, resulting in the generation of type-specific neutralizing anti-HPV antibodies. The introduction of VLPs into the systemic circulation via vaccination (upper left) is followed by the ingestion, processing, and display of the VLPs complexed with major histocompatibility class II (MHC II) proteins by antigen-presenting cells (APCs). Naive T-helper cells interact with the aforementioned complex (upper left). The uptake, processing, and display of the VLPs on the B cells are shown (upper middle), which then can interact with T-helper cells that secrete cytokines necessary for B-cell differentiation. Activated B cells differentiate into either memory B cells or plasma cells (upper right). Plasma cells secrete anti-HPV antibodies with high affinity for the target antigen should the antigen enter the body again (upper right). Neutralizing anti-HPV antibodies bind to type-specific neutralizing epitopes on the surface of HPV virus antigens (lower right). The neutralizing anti-HPV antibodies can prevent HPV infection of the host epithelial cell (lower middle). The antibody-coated viruses are eventually eliminated from the body by phagocytes (bottom left) [102–105]. (From Merck & Co., Inc. Efficacy and Antibody Response to Human Papilloma Virus [HPV] Vaccines. Powerpoint Presentation. Copyright © 2006 Merck & Co., Inc. All rights reserved.)

be living with HIV or AIDS, and more than 35 million have lost their lives since the beginning of the epidemic [110].

Women are more susceptible to HIV infection than are men. It seems that the HIV male-to female transmission during sex is about twice that of female-to-male transmission [111, 112]. More than half of those living with HIV are women [110]. Among the several reasons for this variation is that women are exposed to higher concentrations of HIV present in semen (compared to vaginal fluid), and the mucosal area of exposure is greater in women than in men [113]. Furthermore, progression to AIDS is faster in females even though they have 40 % lower HIV loads and higher CD4+ T cell counts than males. Women also have increased generalized immune system activation and inflammation in response to HIV infection. These differences are thought to be due to the sex-based difference in innate immunity [114].

Immune Response

As a sexually transmitted disease, the usual portal of entry of the HIV is the anogenital mucosa. The vaginal and anal epithelium is usually moist, and secretions are continually passing through the intercellular spaces, making it more permeable

(as opposed to the tight and less easily penetrated squamous epithelium of the skin). The cells in the mucosal epithelium are connected by discontinuous patches of desmosomes (considered one of the weakest forms of intercellular junction) [11]. In the mucosal epithelium, the DCs play an important role during HIV infection. These cells are located at the sites of viral entrance, such as the rectal and vaginal mucosa, and at high viral replication sites, such as the lymph nodes [115]. The localization of DC cells makes them the key regulators of HIV transmission and subsequent viral spread [116, 117]. The DCs are APCs derived from bone marrow progenitors that home in on peripheral mucosal sites where they become immature DCs. After capturing an antigen and under the effects of the process of infection and inflammation, these cells turn into mature DCs and migrate to the lymph nodes, where the maturation process finalizes, making them capable of presenting the antigen to T cells [118].

To understand the events and mechanisms of HIV sexual transmission, the most useful animal model has been the rhesus macaque model infected with simian immunodeficiency virus (SIV), which is closely related to HIV. In macaques, the LCs are DCs located within the stratified vaginal squamous epithelia. The LC is the first infected cell after intravaginal exposure to SIV [119]. Extrapolating the animal data to HIV, upon contact with the mucosa, the HIV fuses to the immature DCs through two mechanisms: (1) CD4 cell surface receptors and the chemokine receptors (mostly CCR5), and (2) capture of the virus at the cell surface by DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN) and other C-type lectins [120]. Once inside of the DCs, HIV can replicate and produce intact virions. Figure 17.6 illustrates interactions between HIV and the cell surface [121]. Subsequently, the virus exploits the natural trafficking properties of the DCs to transfer its virion progeny to its primary cellular target, the CD4 cell, located at the draining lymph nodes [115, 122]. This strategy explains the rapid and efficient movement of HIV mediated by the LCs from mucosal tissues (the normal sites of LC activation following antigen exposure) to the lymph nodes [120]. This phenomenon also explains the fact that small amounts of infecting viruses (or relatively few infected LCs) could lead to efficient infection of large numbers of CD4+ T cells [119].

Once HIV infected cells drain to the lymph nodes, they travel to the gastrointestinal tract during the earliest days of infection. The gastrointestinal tract, and specifically the intestinal mucosa, is one of the most important organs in HIV amplification. Beneath the intestinal epithelium, the lamina propria consists of an enormous collection of memory CD4+CCR5+ T cells. This collection of T cells exceeds the total number of T cells in all other body sites combined. Thus, infection of these T cells is largely responsible for early HIV amplification and chronic viral persistence. The peak viral replication in plasma during early replication corresponds to the massive infection of gut T cells. Lastly, this rapid viral replication in the gastrointestinal tract leads to the production of many escape mutants due to selection from reactionary adaptive immune responses [123].

During immune activation, mature LCs and other types of mature DCs have the ability to cluster with the T cells. The microenvironment in these cell clusters (DCs with T cells) has been described as an explosive site for HIV replication [124–126]. While a correlation of the DCs and decreased HIV replication at this stage has been observed, the exact mechanisms responsible for this phenomenon are not known [117, 127, 128]. The clustering of DCs and T cells could decrease T-cell activation and proliferation, thus blocking the spread of HIV after sexual exposure to virus. Blocking chemotaxis of T cells toward HIV-infected DC using chemokine or chemokine receptor antagonists could be one of the strategies, while another may involve blocking full activation of T cells by HIV-infected DCs by addition of antibodies that interfere with co- stimulatory molecule function [129]. Figure 17.7 illustrates possible mechanisms of the neutralization of HIV at mucosal surfaces by antibodies [130].

For HIV replication to occur, cellular activation is critical, and some studies suggest that T cells become activated and infected through cluster formation with infected LC and not by direct contact with free viruses produced by infected LCs or T cells [129]. The study of T cells during viral infection suggests that HIV infection of DCs induces expression of chemokines in these cells that differentially attract certain T-cell subsets. Results from these studies indicate that infection of CD4+ T cells is not a random event. It seems that expression of the HIV Tat or Nef proteins increases the CXCR4 expression within monocyte-derived DCs, allowing the virus to gain access to a wider range of potential target cells [116, 131, 132]. Other observed factors in chronic generalized immune activation include translocation of intestinal microbial products that stimulate the immune system, antigen nonspecific bystander activation of T and B cells by proinflammatory cytokines and depletion or dysfunction of CD4+ regulatory T cells that normally suppress chronic immune activation. Eventually, this generalized immune activation causes effector T cells to become functionally unresponsive to antigens, leading to a state of "immune exhaustion" [133].

A hypothesis to explain the skin manifestations of HIV infection is derived from the study of LCs and their role in viral pathogenesis. Nonetheless, not all the studies are consistent. Some studies have demonstrated a lack of depletion of T cells, LCs, and DCs in the skin of infected patients (although functionality was not assessed) but an increase in CD8 T cells in the perivascular dermis [134]. On the other hand, studies in macaques have shown that during the acute SIV infection, LC density is reduced in skin, but increased in

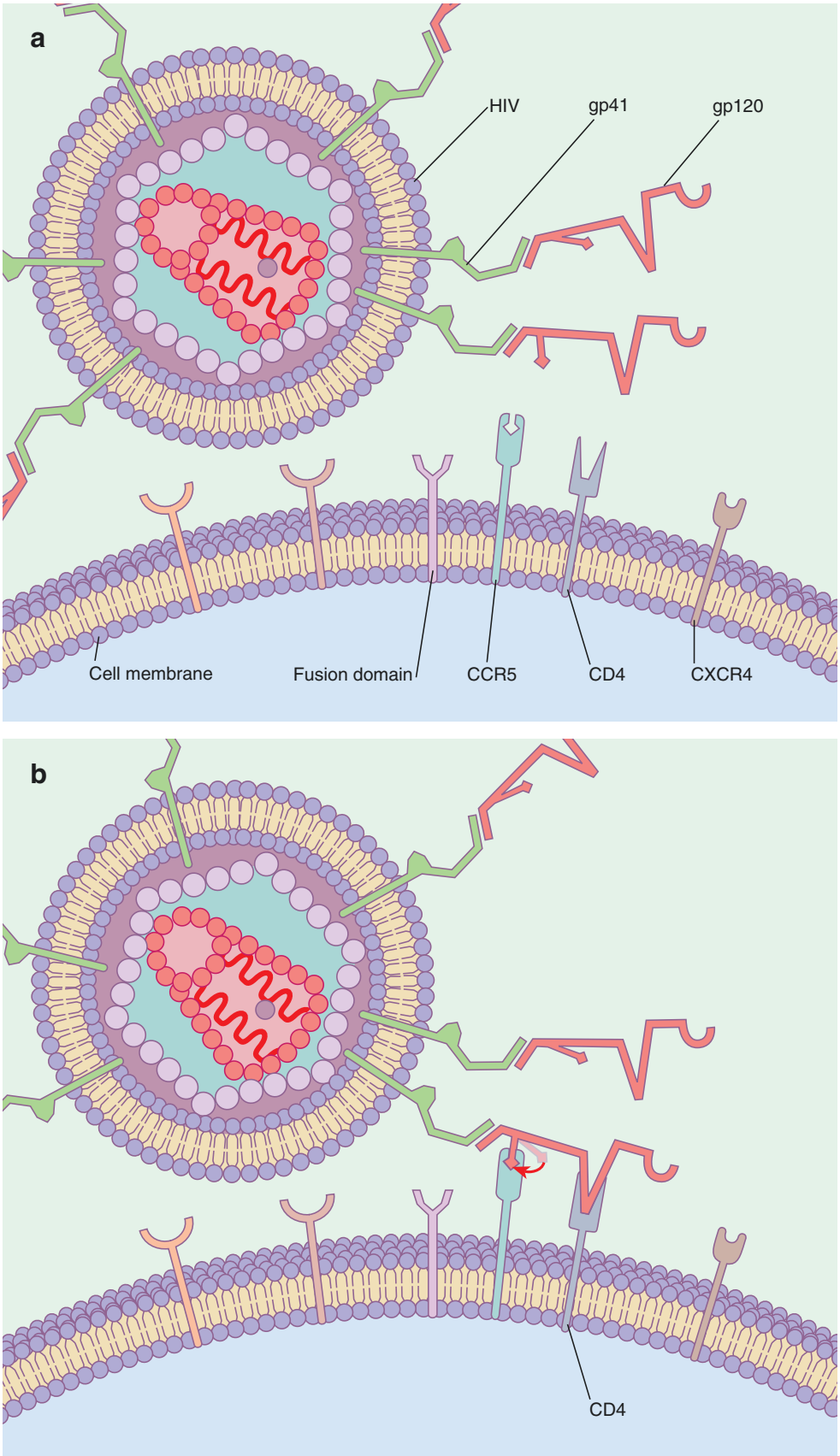


Fig. 17.6 Interactions between HIV and the cell surface. HIV interacts with a cell-surface receptor, primarily CD4, and through conformational changes becomes more closely associated with the cell through interactions with other cell-surface molecules, such as the chemokine receptors CXCR4 and CCR5 (**a**). Alternatively, some viruses, such as certain strains of HIV-2, could attach to CXCR4 directly [11]. The likely steps in HIV infection are as follows. The CD4–binding site on HIV-1 gp120 interacts with the CD4 molecule on the cell surface (**b**). Conformational changes in both the viral envelope and the CD4 receptor permit the binding of gp120 to another cell-surface receptor, such as CCR5 (**c**). This second attachment brings the viral envelope closer to the cell surface, allowing interaction between gp41 on the viral envelope and a fusion domain on the cell surface. HIV fuses with the cell (**d**). Subsequently, the viral nucleoid enters into the cell, most likely by means of other cellular events (**e**). Once this stage is achieved, the cycle of viral replication begins. (From Levy [121]. Copyright © 1996 Massachusetts Medical Society. All rights reserved.)

Fig. 17.6 (continued)

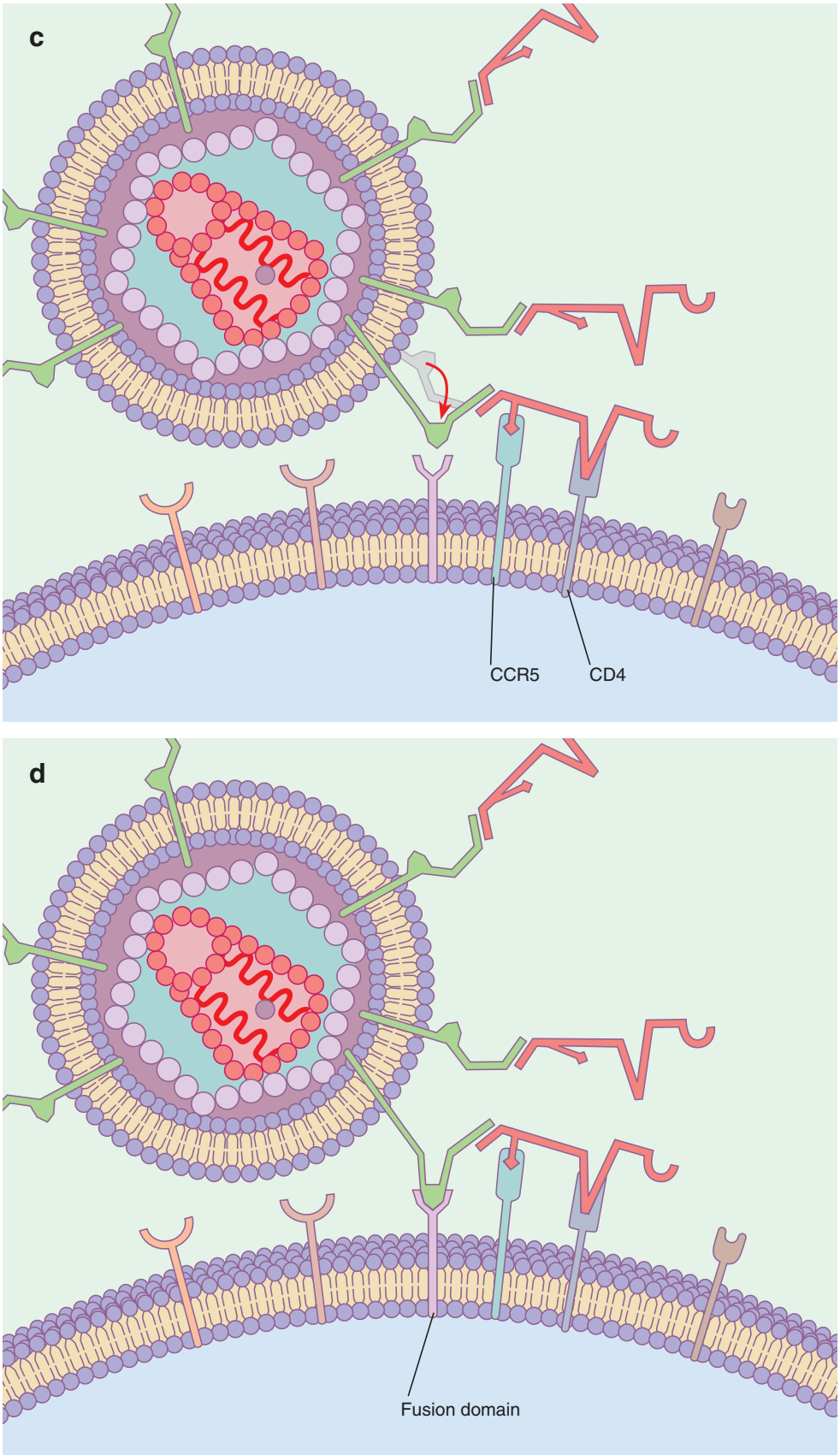
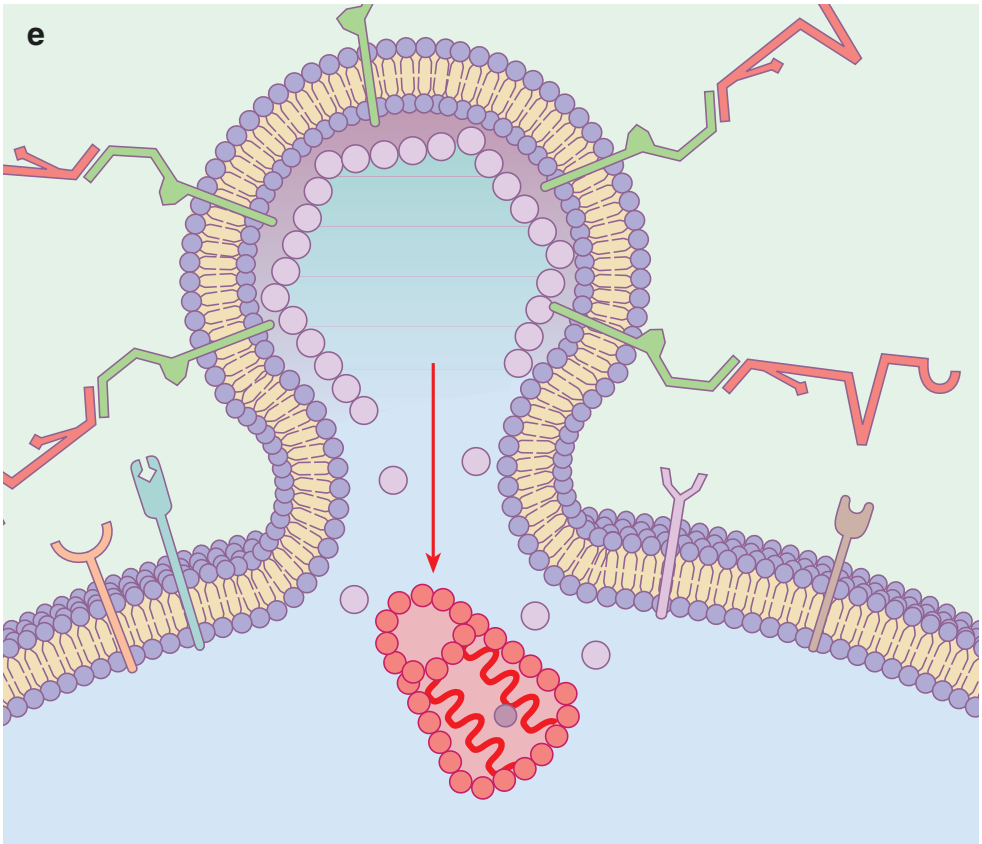


Fig. 17.6 (continued)



the lymph nodes (as activated DCs). In later stages of HIV infection (AIDS), the migration of DCs is suppressed, suggesting that changes in DC function at different stages of viral infection modulate replication, dissemination, and persistence of HIV. The depressed DC function during advanced HIV disease might increase the degree of immunosuppression [135].

Immune Evasion Mechanisms

A simple and useful way to understand viral pathogenesis has been proposed by Hilleman, who categorized viruses in two main groups: (1) those viruses that "hit and run" (e.g., cold viruses), and (2) those that "hit and stay" [136]. HIV belongs to the second group, since it infects the host and stays with it until death occurs. Nonetheless, to ensure the persistence of its progeny, it develops effective mechanisms of spread [136]. As specified before, the DCs are crucial for the generation and regulation of the adaptive immunity. HIV has developed clever strategies to exploit the DCs and carry out its replication cycle. Also, the interaction with the DCs allows HIV to disseminate and evade the antiviral immune response [116]. Since the DCs are the primary producers of IFN-α (which is a key antiviral molecule), alterations in these cells could be beneficial for viral survival and evasion [11]. Additionally, HIV is capable of downregulating the MHC class I molecules, and as a consequence has an effect on natural killer cells. HIV reduces the stimulation of NK cells by stabilizing surface expression of the nonclassic MHC class I molecule human leukocyte antigen E (HLA-E), which reduces the susceptibility to NK-cell-mediated cytotoxicity [137, 138]. Similarly, the CD8 T lymphocytes (CDL) directed against HIV recognize an important number of HIV epitopes. Mutation of these epitopes alters or abolishes CDL recognition, which results in escape of HIV from the immune system [139] (Table 17.8).

Vaccines

Despite 30 years of investigation, a vaccine for HIV still does not exist. In this time, 256 candidate vaccine trials (primarily phase I or II) trials involving more than 44,000 healthy patients have been conducted. Of these, there have been five novel HIV vaccine phase II and III trials in the past decade [140]. In 2003, the VAX 003 and 004 trials tested the immunogenicity of the recombinant protein gp120 (an HIV envelope immunogen). This vaccine stimulated antibody production but was not effective in inducing cellular immunity

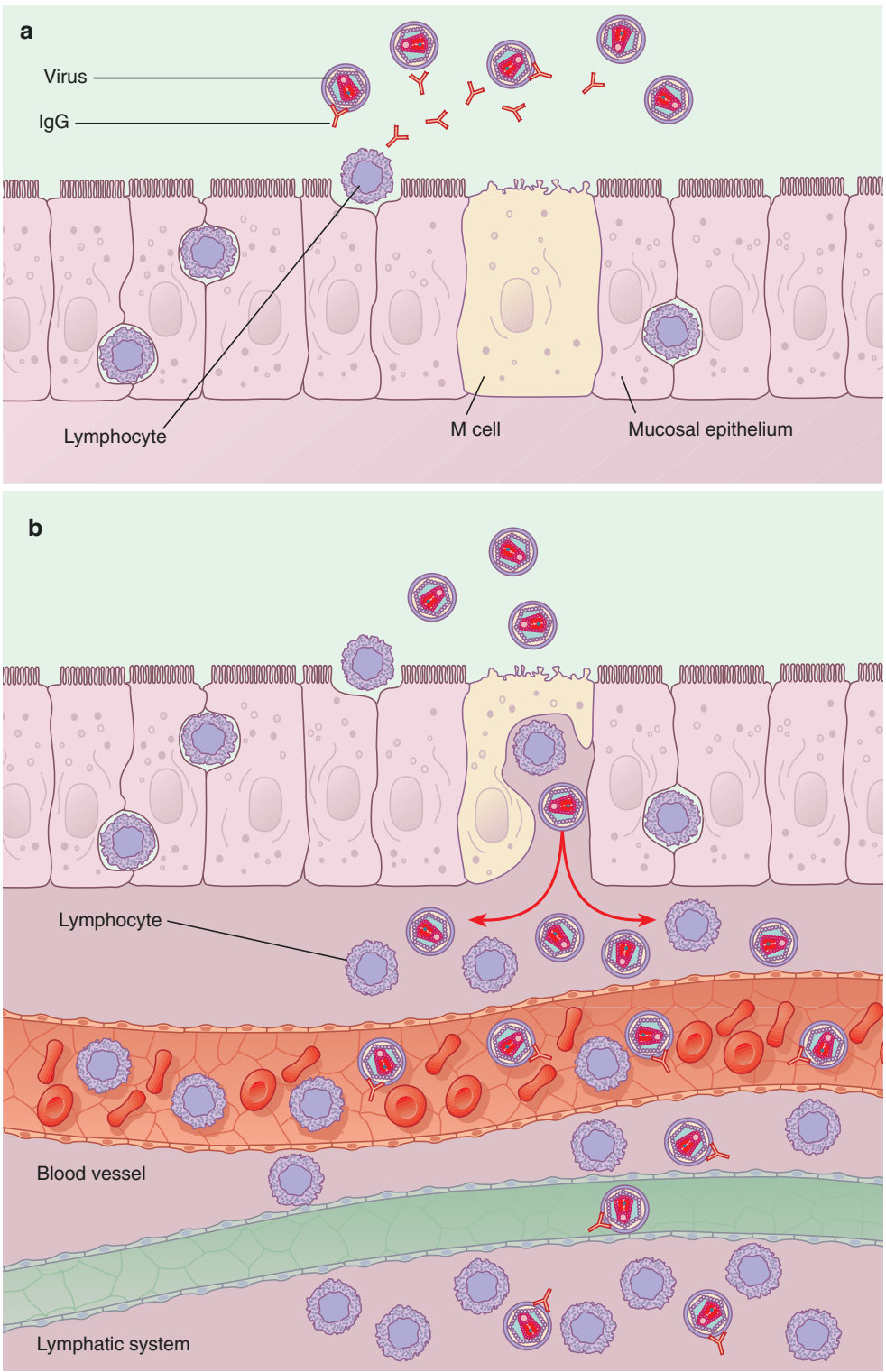


Fig. 17.7 Possible mechanisms of the neutralization of HIV at mucosal surfaces. (**a**) The transudation of passively infused immunoglobulin G (IgG) from vessels in the submucosa across the epithelium causes it to encounter virus at the mucosal surface. The virus is thus neutralized before it can encounter lymphocytes or M cells associated with the epithelium or underlying lamina propria. (**b**) IgG circulating in the blood or lymphatic system encounters virus that has been transported across mucosal surfaces by M cells. Antibodies neutralize the virus before it can spread from the site of infection. (From Nabel and Sullivan [130]. Copyright © 2000 Massachusetts Medical Society. All rights reserved.)

Table 17.8 Mechanisms of immune evasion in HIV skin and mucosal infection

Modulation of the immune activity of dendritic cells
Downregulation of MHC I molecules and reduced stimulation of NK cells
Mutations in viral epitopes recognized by CD8 T lymphocytes

[141, 142]. The 2007 STEP trial tested a vaccine composed of the three HIV core proteins (Gag, Pol and Nef) delivered in an adenovirus vehicle. This candidate vaccine was found to be clinically ineffective and might have increased HIV-1 transmission. The 2013 HVTN 505 trial which tested an adenovirus vector that delivered the Gag, Pol, Nef and Env

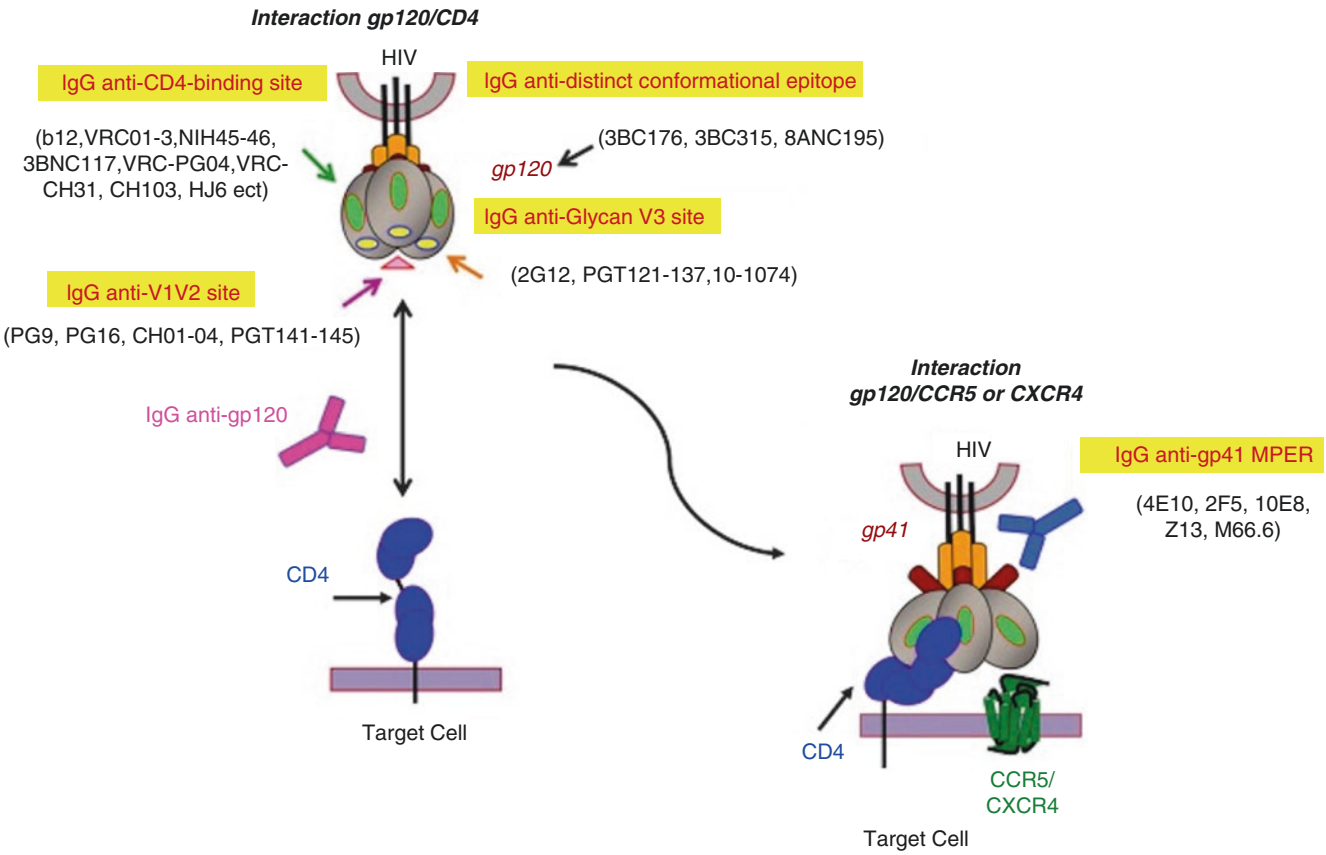


Fig. 17.8 Model representation of HIV-1 envelope glycoprotein structure and epitopes of broadly neutralizing antibodies. The surface receptor binding subunit gp120 and the fusion-mediating transmembrane subunit gp41 make up the functional HIV-1 envelope glycoproteins. The targets of broadly neutralizing antibodies (bNAbs) can be divided into several groups: (1) IgG anti-CD4-binding site, (2) IgG anti-V1V2 site, (3) IgG anti-*N*-linked glycan V3 site, and (4) IgG anti-gp41 membrane proximal external region (MPER). The IgG anti-distinct conformational epitope present on the envelope trimer, remains to be determined (Adapted from Dr. Béatrice Labrosse. Reprinted from Frontiers in Immunology, 5, Su & Moog, Which Antibody Functions are Important for an HIV Vaccine?, 289. 2014, with permission from Frontiers Media SA)

proteins after DNA priming was prematurely terminated due to inefficacy and an increase in HIV infection [143].

Finally in the 2009 RV144 trial, patients received two doses of gp120 protein from two different HIV variants and four doses of the HIV-avian pox virus [144]. The efficacy of protection of this regimen was 31 % of patients at 3 years but was deemed to be statistically insignificant [143, 144]. Further analysis showed that high anti-Env V1/V2 loop, non-neutralizing antibodies that triggered antibody dependent cellular cytotoxicity decreased the risk of HIV infection by 71 % [143]. Although the protection conferred by the RV144 trial vaccine was deemed statistically insignificant, this trial supported the idea that an ideal HIV vaccine might need to induce both humoral and cellular immune responses [145, 146]. In the last decade, generating a robust humoral response has centered on eliciting anti-HIV-1 antibodies with broadly neutralizing activity (bNAbs). These bNAbs target the V3 loop, the binding site for CD4 at the site of primary viral entry, viral glycans and the membrane proximal external region (MPER) on GP41 (Fig. 17.8) [146].

Molluscum Contagiosum

Molluscum contagiosum virus (MCV) is a double-stranded DNA poxvirus surrounded by a lipid envelope. It is the only poxvirus that commonly infects humans since the eradication of smallpox. The MCV causes benign proliferative lesions of the skin, which can last for several months in immunocompetent and immunocompromised individuals. Molluscum contagiosum virus has a worldwide distribution but is more prevalent in tropical areas [147]. It usually affects children, but it can also be transmitted sexually or through touch, such as in contact sports [148]. This virus is the largest of all animal viruses and it is easily visualized on light microscopy.

Immune Response

Molluscum contagiosum virus transmission is through direct skin contact with an infected individual. The MCV infections have a particular location on the epidermis, which

makes the virus "safe," almost beyond the reach of the immune system. Lesions in vivo are characterized by a lack of inflammatory cell infiltrates. The typical T or NK cells are usually not found at the base of molluscum lesions. Nonetheless, in the healing stage a mononuclear cell infiltrate is usually observed [11].

Regarding inflammatory mediators, studies have shown that chemokines such as growth regulated oncogene alpha (GRO-α) and IL-8 are inside the molluscum lesions and are released on clearing of the virus [150]. It is hypothesized that the immune response might be blocked by chemokine antagonists early in MCV infection, and in later stages of the infection the physical barrier (i.e., localization of the epidermis) or the formation of molluscum bodies may prevent detection by the immune system [147]. Some studies indicate that the production of antibodies does not occur in all patients with clinical MCV infection, or the antibody production is not stable during the clinical period [151].

Immune Evasion Mechanisms

Like many other viruses, MCV uses a variety of methods to escape the immune system. An MCV ORF (designated mcv148R) encodes a 104-aminoacid protein with significant homology to β-chemokines such as macrophage inflammatory protein (MIP)-1β. This homology has led investigators to believe that this virally produced chemokine may block the chemotactic activity of the host chemokines [147]. The MCV is also capable of blocking the signal necessary for successful migration of effector cells to the site of infection. Other MCV gene products such as MC53L and MC54L bind IL-18 with high affinity and prevent IFN-γ production, suggesting that these viral proteins antagonize the development of an inflammatory response to MCV infection in humans [149–151].

There is no vaccine currently available to prevent MCV infection.

Conclusion

The immune response to a viral infection is a complex phenomenon that is specific to each microorganism. Through a highly efficient process of evolution, the viruses have developed several immune evasion mechanisms that allow them to overcome the natural barriers of the skin, cause infection, and establish latency. The delicate balance between the host immune response and viral replication (and dissemination) will eventually dictate the clinical outcome. Factors influencing the host, such as age, immunocompetence, and development of immunity after exposure to the virus, all play a key role in altering this balance.

Questions

1. Herpes simplex virus produces latency associated transcripts (LATs) which have been found in high concentrations in latently infected neurons. LATs help to increase the efficiency of latency and subsequent reactivation by what mechanism?
   A. By interfering with production and secretion of IL-1 and TNF-α
   B. By destroying the homing receptor (CLA) on CD8+ T cells
   C. By inhibiting the production of proteins involved in the apoptosis pathway
   D. By altering the activity of neutrophils, macrophages, and NK cells

Answer/Explanation: answer C
The herpes simplex virus does alter the activity of neutrophils, macrophages, and NK cells. As such, it does interfere with the production and secretion of some pro-inflammatory cytokines, such as IL-1 and TNF. However, LATs are directly responsible for inhibiting granzyme-B and capsase-8 mediated apoptosis

2. Depletion of what cell line has been implicated with the enhanced survival, prolonged course of infection, and oncogenic transformation associated with some high risk HPV types?
   A. T helper cell
   B. Langerhans cell
   C. Macrophage
   D. NK cell

Answer/Explanation: answer B
Depletion of Langerhans cells has been documented in HPV infections along with a lack of expression of certain cell signaling molecules (CD54, CD86, TNF-α, etc.). Cumulatively, these features decrease the antigen-presenting capacity of Langerhans cells and can induce immune tolerance and ultimately oncogenic transformation

3. The early peak in viral replication that is integral for large scale HIV amplification and chronic viral persistence is directly attributable to infection of what cell line?
   A. CD4+CCR5+ T cells in the intestinal mucosa
   B. Dendritic cells (DC) located in draining lymph nodes
   C. CD4+ ICAM1+ T cells in keratinocytes
   D. Effector Th cells in mucosal surfaces

Answer/Explanation: answer A

HIV does initially infect DC and exploits their natural trafficking properties to transfer virus to CD4+ T cells in draining lymph nodes. However, the cell line directly responsible for the early peak in viral replication is infection of CD4+CCR5+ T cells in the lamina propria of intestinal mucosa. The collection of T cells here exceeds the total number of T cells in all other body sites combined and is responsible for the early and massive amplification of the virus

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Parasitic Infections

18

Kassahun Desalegn Bilcha and Sidney Klaus

Abstract

Parasitic diseases involving the skin represent an important segment of the globe's emerging disorders that present a threat to the health of millions of people worldwide. Host immune responses to these diseases are complex and display a wide range of variability, involving both the innate and adaptive immune systems. Not all the host's efforts at eliminating the invading parasites are successful, and in some cases the host's immune response causes more damage than the parasite itself.

This chapter examines the immune mechanisms in three widely diverse parasitic diseases that involve the skin: cutaneous leishmaniasis, onchocerciasis, and schistosomiasis.

Keywords

Parasitic Infections • Skin infections • Host • Cutaneous leishmaniasis • Onchocerciasis • Schistosomiasis • Cell receptors • TH1 • TH2 • Hypersensitivity • HIV • skin disease

Key Points

- Cell-mediated immunoregulation plays the dominant role in resolving *Leishmania* infections.
- Most promastigotes deposited in the dermis are opsonized by serum complement and killed by complement-mediated lysis.
- The remaining promastigotes are phagocytosed via complement receptors on the macrophage membrane, which binds to gp63 and lipophosphoglycan.
- Macrophages release chemokines that attract more macrophages as well as natural killer (NK) cells and dendritic cells.
- *Wolbachia* bacteria living inside *Onchocerca volvulus* are the essential target of the host's inflammatory response in onchocerciasis.
- The initial reaction in schistosomiasis is predominated by Th1 response that later shifts to Th2 response.

Leishmaniasis

Leishmaniases are a groups of zoonotic, often debilitating, diseases of humans due to infection with intracellular protozoan parasites belonging to the genus *Leishmania*. The disease is an emerging uncontrolled and neglected infection affecting more than 12 million people worldwide, with an additional 350 million at risk of infection. The global yearly incidence of all forms is approaching two million cases [1]. Leishmaniasis is endemic in more than 98 countries belonging to the tropical regions of Africa, Asia, and South and Central America. It is also found in the southwestern part of the United States; in the Middle East; and in countries surrounding the Mediterranean Sea [2].

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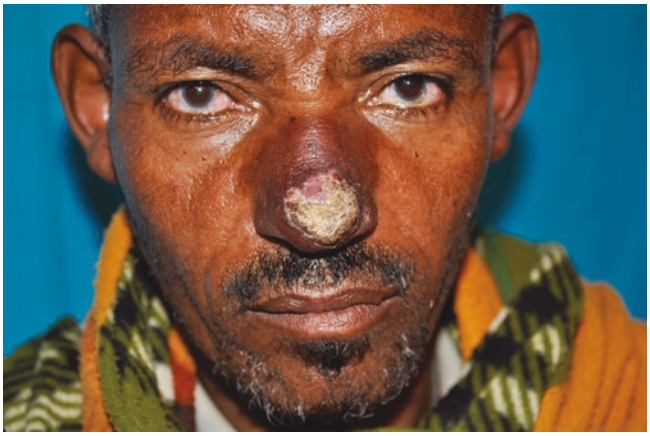

Fig. 18.1 Cutaneous leishmaniasis

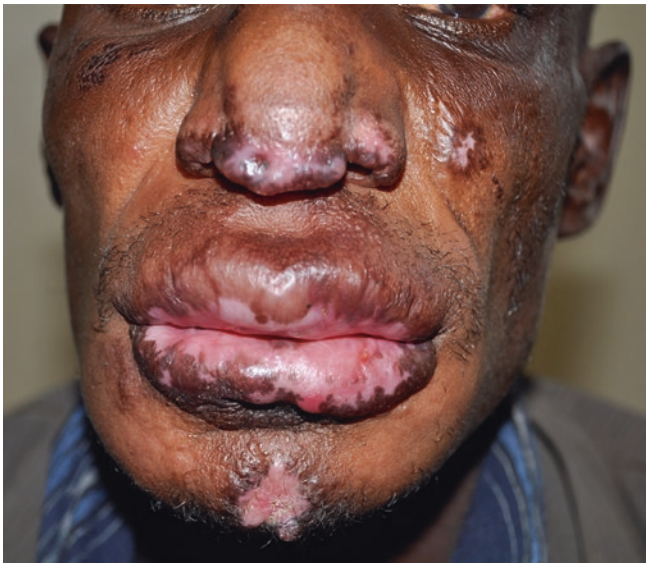

Fig. 18.2 Mucocutaneous leishmaniasis in an HIV-infected man

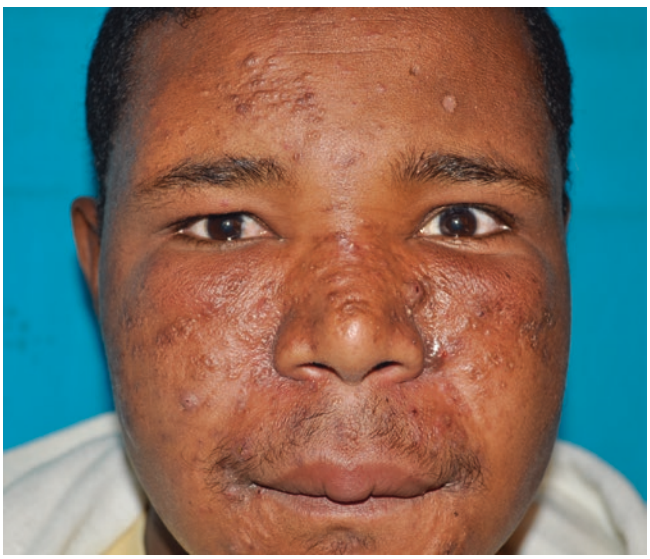

Fig. 18.3 Mucocutaneous leishmaniasis in an non-HIV-infected boy

The number of cases increases dramatically in areas of the world undergoing ecologic disruption, associated with rapid major shifts in population. More than 20 species of the genus *Leishmania* have been identified as causative agents of the disease.

The parasites exist in two morphologic forms during their life cycle: as elongated flagellated promastigotes in the gut of the sand fly vector, and as round to oval nonflagellated amastigotes in mammalian hosts.

The disease exists in three distinct clinical syndromes: The first one, cutaneous leishmaniasis (CL), is a localized lesion with single to multiple ulcers, satellite lesions, or nodular lymphangitis (Fig 18.1). Rarely, widespread involvements of the skin can occur in this form of the disease resulting in diffuse and disseminated cutaneous leishmaniases (DCL). The second, mucocutaneous leishmaniasis (MCL) occurs with marked involvement of the mucous membranes of the oral cavity, nasopharynx, and throat leading to partial or total destruction (Figs. 18.2 and 18.3). The third form, visceral leishmaniasis (VL), also called kala-azar, involves internal organs such as liver, spleen and bone marrow and is fatal if not treated. Skin involvement in the form of macular, papular or nodular widespread rash can occur in patients with VL, or, after they have been treated, and is called para-kala-azar or post-kala-azar dermal leishmaniasis (PKDL). The outcome of untreated cases depends on the species of *Leishmania* causing the infection as well as on the innate and adaptive immune responses of the host [3]. Although humoral immune responses can be demonstrated during the course of the infection, cell-mediated immunoregulation plays the dominant role in resolving the infection. Many of the details of the immune response have been derived from extensive studies of *Leishmania major* infections in two experimental mouse models: the resistant C57BL/6 strain and the susceptible BALB/c strain.

Early Events in the Immune Response

In humans, the disease is initiated by the bite of an infected female sand fly (of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World). During the bite parasites are inoculated into the dermis; in most cases less than 100 parasites are transmitted by a single bite, although the number may reach as high as 1000 or more [4].

Because the sand fly is a "pool feeder" (it severs capillaries in its hunt for a blood meal) promastigotes are deposited in a small pool of blood within the dermis. The bite of the sand fly initiates a local inflammatory response, with the recruitment of neutrophils, natural killer (NK) cells, eosinophils, and mast

cells. The cellular response is rapid; neutrophils are the first cells to arrive at the site of the infection; in mice, they have been found collecting in the skin within 1 h of a parasite injection [5].

Most of the promastigotes deposited in the dermis are opsonized by serum complement and killed by complement-mediated lysis. The remaining promastigotes are phagocytosed via complement receptors expressed on the macrophage membrane, which binds to two abundant molecules on the surface of the parasites: gp63, a 63-kd neutral metalloproteinase; and LPG, a lipophosphoglycan [6].

Macrophages that have engulfed parasites release several chemokines, including CCL2, which attracts NK cells, dendritic cells (DCs), and additional macrophages to the site of the infection [7].

Components of sand fly saliva also have been shown to affect the immune response by exacerbating lesion development in resistant strains of mice [8, 9]. When maxadilan, a vasodilatory peptide isolated from sand fly saliva, was added to human peripheral blood mononuclear cells (PBMCs) the secretion of T-helper-1 (Th1) cytokines (interferon [IFN] and interleukin-12 [IL-12]) was decreased and the secretion of Th2 cytokines enhanced [10]. In addition salivary gland lysates (SGLs) also have been shown to augment the collection of inflammatory cells at the site of the bite and when added to experimental infections caused by *L. major* and *L. braziliensis* [11].

Once the promastigotes enter the macrophages, they transform into replicative amastigotes over a period of 2–5 days and are carried within modified lysosomal compartments known as parisitophorous vacuoles (PVs) [12]. A study of the ultrastructure of the vacuoles suggests a significant difference in packaging between Old World and New World leishmania. Amastigotes of *L. major* were found to be segregated into separate vacuoles during replication; in contrast, New World species of *Leishmania* were carried in large vacuoles occupied by many amastigotes [13].

Once inside the PVs the parasites produce antioxidant enzymes and are able to resist lysosomal hydrolases (the usual mechanism for clearing ingested pathogens). Within the cells the amastigotes replicate rapidly.

Macrophages that accumulate large numbers of replicating amastigotes rupture; the released parasites are taken up by neighboring competent cells. In especially inflamed skin lesions, most parasites are found in extracellular locations. One explanation is that high levels of inflammatory mediators interfere with receptors on the macrophages, blocking re-internalization of the parasites (Klaus S, unpublished data).

Antigen Transport and Presentation

At the same time that macrophages are ingesting parasites, Langerhans cells (LCs), which normally reside in the epidermis and are a potent type of antigen-presenting cell (APC), are stimulated. In a study of *L. major* infections in mice, LCs were found to be the dominant type of APC migrating from the skin to the regional lymph nodes via lymph channels [14, 15].

The ability of LCs (and other APCs) to identify the parasites has been attributed to Toll-like receptor 4 (TLR4), a member of a family of transmembrane receptors implicated in the recognition of a variety of microbial and foreign agents [16]. A study of TLR-deficient mice showed they had larger parasite burdens and were less efficient in the resolution of cutaneous lesions [17].

The extent to which macrophages also transport parasites and parasite antigen from the skin to the nodes is unresolved, although most studies indicate that LCs are the major carriers and shoulder most responsibility for antigen presentation to $CD4^+$ naive T cells within the lymph nodes [18].

The initial stages of the uptake of antigen by the LCs are rapid; experiments in mice have demonstrated that LCs can find and engulf parasites within 4 h of exposure (although the migration to the lymph nodes may take up to 3 weeks) [19]. The movement by the LCs to the nodes is influenced by chemokine expression. Studies have indicated that during the migration the level of expression of chemokine receptor CCR7 on the LCs is enhanced, while the level of CCR2 and CCR5 is downregulated [20].

It is during this stage of the adaptive immune response that naive $CD4^+$ T-helper cells (Th0) are programmed to develop into either Th1 or Th2 cells. Interleukin-12, a cytokine released by DCs, has a critical role at this stage in the immune response [21, 22]: IL-12, along with IL-1, serves as a promotor of the differentiation of Th1 cells in the lymph nodes from naive T0 cells. It also enhances chemokine gene expression in mice during the first 3 days of infection with *L. major* [23].

It is now clear that the successful clearing of amastigotes from infected macrophages in resistant mouse strains is mediated through the predominance of Th1 cells. The mechanism is through steps that link the production of IFN-γ, a major cytokine of Th1 cells, to the release of nitric oxide (NO), a compound within macrophages harboring amastigotes leading to their destruction. Nitric oxide ordinarily is present within an inactive form in the macrophages (iNO), which needs to be catalyzed by the enzyme NO synthase to become active. Interferon along with IL-12 and tumor necrosis factor (TNF), two other cytokines released by Th1 cells, make up the major upregulators of NO synthase, and the production of NO occurs only when the Th1 cells become predominant [24].

In contrast to the events within the resistant mice, in susceptible mouse strains (BALB/c) a type 2 response is initiated following infection with *L. major,* which tends to interfere with the protective activities. Th2 cells that become stimulated produce IL-4, IL-5, and IL-10, which have the capacity through mediators to inhibit the production of IFN-γ, downregulate the

expression of iNOS (and consequently NO), and thus inhibit macrophage function.

Although a great deal is known about the process of resistance in mice, much less is known about the human immune response to *Leishmania*. While mice can mount either a Th1 or Th2 response, Rogers and Titus [25] suggested that in humans the principal response is predominantly, if not exclusively, a type 1 response. Using an *in vitro* system they cocultured PBMCs from *Leishmania*-naive donors with *L. major* parasites and found that type 1 cytokines were stimulated, (IFN-γ and IL12), and that when PBMCs were cocultured with macrophages infected with parasites, augmented intracellular killing was observed [25].

Antibody Response

Anti-*Leishmania* antibodies, as measured by enzyme-linked immunosorbent assay (ELISA), can be found in low titers in individuals recovering from CL, but their role in recovery from the acute infection and in the prevention of re-infection is debated. Polyclonal activation of human B cells leads to the production of large amounts of parasite specific antibodies, and amastigotes released into the dermis from ruptured macrophages appear to be coated with antiparasite antibodies [26].

In mice, antibody levels do not correlate in general with resistance to disease, although their effect on the course of the infection may depend on genetic factors. For example, passive transfer of antibody fractions from immune mice to BALB/c mice did not affect their susceptibility to infection [27], yet ablation of B cells in resistant mice generated a non-healing response to *L. major* [28].

Delayed-Type Hypersensitivity and the Leishmanin Skin Test

Delayed-type hypersensitivity in leishmaniasis can be measured by the leishmanin skin test (LST), in which the extent of a skin reaction is measured 48–72 h after an intradermal injection of 0.2 mL of a killed suspension of cultured leishmania promastigotes in saline. A positive reaction indicates a type 1 $CD4^+$ cell-specific immune response. Because it is usually positive in individuals who have had CL, it has been used to measure the extent of infection among individuals living in endemic regions who have no history of overt disease. A study of 470 children living in endemic foci of *L. major* infection in Tunisia found that the proportion of asymptomatic infections among this group of children was approximately 10 % [29]. The LST also has been used as a predictor of susceptibility to subsequent disease, whether or not the individual had a history of a previous skin lesion.

Persistence of Parasites

In most cases of human cutaneous leishmaniasis it had been assumed that in skin lesions that had "healed" (either spontaneously or following treatment), the parasites had been eliminated by effector mechanisms involving IFN-γ and the generation of NO within the macrophages. It is now recognized that viable *Leishmania* organisms may persist in the skin long after the resolution of the clinical lesions [30]. Persistence has been documented in several ways: in biopsy samples from normal patients who have recovered from CL, in skin lesions of patients who recovered from CL but who later contracted HIV infection, and in skin lesions of patients who recovered from CL but later developed leishmania recidivans.

Schubach et al. [31] examined skin tissue obtained by biopsy at the sites of the scars from two patients from Brazil who had been infected with *Leishmania* 8 and 11 years earlier. The tissues from both patients grew out viable *Leishmania* parasites. A more recent study analyzed skin biopsies from scars of 32 patients with who had CL but who had been treated and clinically cured. *Leishmania* specific DNA was detected by polymerase chain reaction (PCR) in 30 of the patients, and parasites were isolated by culture in three [32].

The persistence of parasites in post-recovery CL is also evident in patients who later become infected with HIV. Studies of skin biopsies from such immunocompromised patients often show large numbers of amastigotes both within macrophages and free in the dermis. Parasites can also be seen within keratinocytes surrounding sweat ducts, and within the cells of the eccrine glands themselves [33]. Skin lesions in these patients often appear as isolated papules or plaques on exposed areas of the skin and are usually indistinguishable from similar lesions seen in non-immunosuppressed individuals. Skin lesions in HIV patients may also present as diffuse scaling plaques, which on biopsy show a high concentration of parasites [34].

Leishmaniasis recidivans (LR) is a rare clinical form of CL in which skin near the site of a previously healed acute CL lesion reappears as a dusky-red granulomatous plaque with active spreading borders. The clinical features resemble lupus vulgaris. Cultures of the skin lesion for *Leishmania* are usually negative, but with perseverance sparse parasites can be detected microscopically or by PCR. Leishmaniasis recidivans patients usually demonstrate high levels of antibodies in the serum and a strongly positive LST [35].

Vaccine Development

Clinical evidence points to the conclusion that recovery from skin infection with *Leishmania* provides lifelong protection against re-infection despite ongoing exposure to sand flies,

suggesting that a vaccine would be of great value in controlling the disease.

For more than 100 years residents of endemic regions in eastern Asia and the Middle East practiced a form of vaccination known as leishmanization, which consisted of the deliberate inoculation of infective material into inconspicuous body areas (especially the buttocks) in the hope of providing protection from a subsequent infection and disfiguring scars on exposed parts of the body. A widescale trial of leishmanization was carried out among soldiers in the Iranian army in the 1990s, with more than a million individuals vaccinated, in this case using live *L. major* promastigotes obtained from cultures. Although the degree of protection initially seemed adequate (less than 3 % of a cohort of the vaccinated group developed a naturally acquired infection, compared with 14 % of unvaccinated volunteers), there was an unacceptable rate of adverse events: 2–3 % of the subjects developed large nonhealing infections at the site of the vaccinations that required treatment [36–38].

Today vaccine trials use killed or live attenuated parasites, genetically modified cells from promastigotes, and DNA encoding recombinant proteins [39–41].

Other vaccine candidates have included those using specific peptides derived from leishmania proteins such as amino acids derived from gp63, administered with certain adjuvants (such as liposomes or complete Freund's adjuvant) [42]. One novel idea was that if components of sand fly saliva were added to a standard mix of antigens, a more effective prophylactic vaccine might result [43].

Although experimental vaccines to control CL have been studied extensively over the past two decades, inoculation with live *L. major* still remains the only successful vaccine in humans [44]. Currently, vaccine development is hampered by an incomplete understanding of the immune process and by concerns about long-term safety.

Onchocerciasis

Onchocerciasis, also known as river blindness, is a neglected tropical infection caused by the filarial nematode *Onchocerca volvulus,* with significant cutaneous manifestations, including pigmentary changes (Fig. 18.4), debilitating itching and subcutaneous nodules. It also causes significant eye damage and is the second (only to trachoma) most common infectious cause of blindness globally. It affects more than 18 million people living in endemic foci mainly of sub-Saharan Africa, but also in Latin America (including Brazil, Ecuador, Guatemala, Mexico, and Venezuela), and Yemen. It is now believed that skin disease is the most important contributor to the burden of onchocerciasis, rather than eye disease [45].

The disease is spread by the bite of black flies of the genus *Simulium* that prefer to breed on fast-running (highly oxygenated) rivers and streams of tropical countries. The female blackflies that transmit the disease need to feed on human blood for ovulation. Feeding on infected individuals results in ingestion of microfilariae of the parasite which then develop into infective larvae, also called third-stage filarial larvae (L3 larvae), inside the blackfly over the course of 2 weeks. Infection of humans occurs when these blackflies deposit L3 larvae while biting for another blood meal. Bites occur during the day time and multiple bites are required for disease transmission. Humans appear to be the primary host, although the gorilla in the Congo and the spider monkey in Mexico may also be naturally infected [46].

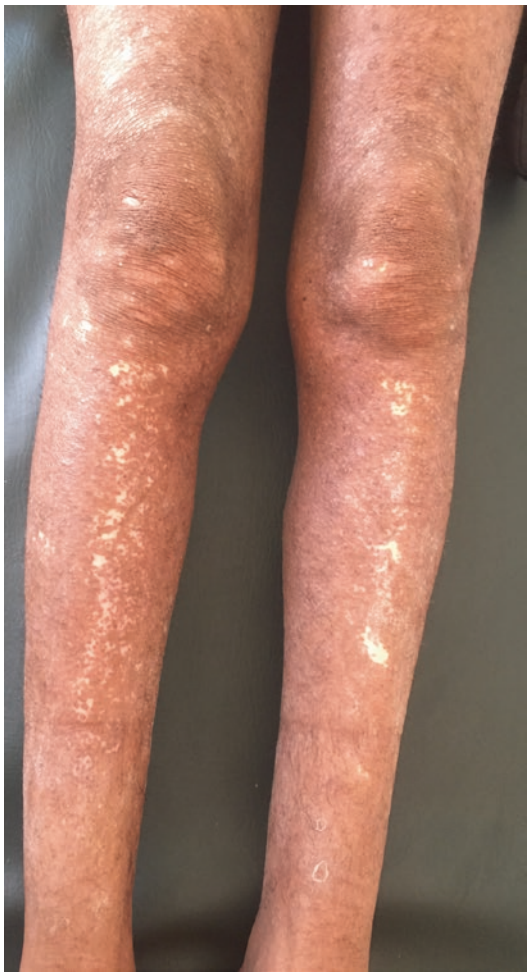

Fig. 18.4 Skin depigmentation in a patient with onchocerciasis

Once in the human host, the larvae migrate in the subcutaneous tissue forming nodules and slowly develop into adult worms over a course of about a year. Adult worms may live for 10–15 years and are usually palpable as firm, nontender nodules especially over the bony prominences of the pelvis, or on the scalp. From these sites, after a prepatent period of 3–18 months, fertilized female worms produce thousands of microfilariae daily (millions during a lifetime). The microfilariae (mfs), which can persist for 6–36 months, migrate to

the subcutaneous and ocular tissues usually without provoking symptoms. In the dermis they are accessible to re-ingestion by blackflies, restarting the cycle. In addition to the skin, mfs can also be detected in the blood, urine and sputum. Most of the skin signs and symptoms of onchocerciasis, including severe pruritus and eye damage, are related to the body's inflammatory response to dying and degenerating mfs.

The clinical features that characterize the disease include itching, commonly occurring over the lower trunk and buttocks, and an eczema-like eruption, which includes lichenification and hyperpigmentation, often called "lizard's skin". Individuals who have had the disease for many years may develop lymphedema and postinflammatory depigmentation, often on the anterior tibial surfaces (a sign called "leopard skin"). The prominent change seen on biopsy of affected skin is dermal fibrosis. In time, destruction of elastic tissue occurs, mediated by proteases from the parasites. These changes eventually lead to marked skin atrophy with redundant folds of skin in the inguinal areas, the so-called hanging groin. Two clinical patterns of reaction are evoked once the process is underway: a generalized form, characterized by widespread itching and dermatitis, and a hyperactive form, in which the skin reaction is often intense yet usually localized. The term "sowda" is reserved for severe hyperactive form that dominates one limb. A resistant form of the disease is also found among individuals living in hyperendemic areas and who remain unaffected despite being chronically exposed to the bites of infected flies. Decreased visual acuity is the most serious complication of the disease. The inflammatory reactions within the eye lead to iridocyclitis, choroiditis, and eventually optic atrophy.

The Role of *Wolbachia* Bacteria

The endosymbiotic *Wolbachia* bacteria, living inside both adult worms and mfs of *O. volvulus*, are essential for growth, development, fertility and survival of the parasite [47]. They are now considered to be an important target of the host's inflammatory response [48, 49]. Neutrophil recruitment around the encysted adult worms (onchocercomas) appears to be related to the presence of the bacteria. In patients treated with doxycycline to eliminate the symbionts, the accumulation of neutrophils adjacent to the adult worms was drastically reduced [50]. Antibiotic treatment with doxycycline has also been found to improve skin lesions in hyperergic forms of the disease, and to interrupt embryogenesis of the female adult worms [51].

Immune Responses

Although early in the course of the disease—in the prepatent phase—inflammatory cells react to protein on the surface of the adult worms, the characteristic cutaneous signs and symptoms of the disease (dermatitis and itching) do not develop until mfs are produced by the gravid female worms. The mfs represent an ongoing source of antigen; up to 3000 are released daily by the adult female worm, beginning about 6–10 months after infection [52]. It is now argued that the predominant portion of the skin reaction in onchocerciasis is a reaction not only to the death and degeneration of the mfs but to their accompanying *Wolbachia* as well.

The intensity and type of host immune response gives rise to diverse clinical manifestations with two polar forms. The first, generalized onchocerciasis, occurs in hyporesponsive individuals having palpable nodules under their skin but no strong pathology despite carrying high mf skin loads, and the second, hyperactive form, is when patients exhibit severe inflammatory response with few worms [53].

Early in the course of the infection a polyclonal B-cell activation occurs with the production of parasite-specific immunoglobulins, including IgM, IgG (both IgG1 and IgG4), and IgE. The reaction to the mfs in the dermis and subcutaneous tissue is initiated by antibodies that attach to the surface of the parasites, along with complement. The immune complexes that are formed attract a variety of inflammatory cells, including neutrophils, eosinophils, and later macrophages. Degranulation of eosinophils appears to play the major role in the death of the mfs, but it is likely that proteases secreted by the larvae also add to the tissue damage. In addition to the effect on B cells, parasite antigens also induce substantial reactions from PBMCs. The initial recognition of these antigens, both the infective larvae themselves and their *Wolbachia* cargo, is mediated by TLRs. TLR4 responds to both the larvae of *O. volvulus* as well as to surface protein of the *Wolbachia,* and initiates a Th2 immune response mediated by IL-4 and IL-5 [54]. In addition, TLR2 also responds to surface protein isolated from *Wolbachia,* which mediates the release of TNF-α, IL-12, and IL-8 from PBMCs [55]. The evolution and final expression of the type of immune reaction that develops (Th1 or Th2) has been attributed to the early presence of specific cytokines; for example, the initial presence of IL12 directs the immune response toward a Th1 reaction, while a rapid induction of IL-4 promotes the generation of a dominant Th2-type response [56].

In some individuals an activation of a subset of $CD4^+$ cells known as T regulatory cells (Tr1) occurs, which produce IL-10 and transforming growth factor (TGF), and some IFN [57].

In the usual generalized, chronic form of the disease, where the microfilarial load in the skin is high (up to 500 microfilaria per milligram of skin), the cutaneous reactions tend to be mild to moderate. In this type of onchocerciasis the cellular reactions are downregulated, with a suppression of Th1 and only a moderate Th2 response [58]. High levels

of IL-10 are produced by $CD4^+$ T-regulatory cells (Tr1), which act to inhibit activation of APCs and thus suppress proinflammatory functions. This type of immune response is thought to protect the host from acute skin damage (yet it may also be of benefit to the parasites by protecting them from some of the host's lethal immune responses) [59]. In hyperreactive forms of the disease where the concentration of mfs is low (less than 10 per mg of skin) and the inflammatory reaction of the skin is severe, a strong Th2 response is seen. It is suggested that, in this form of onchocerciasis, inflammatory cells (eosinophils, neutrophils, and macrophages) all combine to kill mfs, under the direction of T cells and APCs.

Up to now HIV infection has been reported to play only a minor role in onchocerciasis, with no significant association with HIV detected in a large case control study [60]. No differences were noted in the density of mfs [61], although antibody response to the parasite was decreased in HIV-infected individuals, and they tended to lose their reactivity to these antigens over time [62].

Mazzotti Reaction

First described in 1948, Mazzotti reaction is a set of adverse reactions following administration of diethylcarbamazine (DEC) in patients with onchocerciasis. It has also been reported with administration of ivermectin and other antihelminths [63]. It is clinically characterized by intensely pruritic rash with or without systemic symptoms. Severity of the reaction correlates with the degree of microfilarial infestation and could at times be life threatening. A complex mechanism involving eosinophil degranulation and release of other mediators following the death of mf is involved in the pathogenesis. Patch testing using DEC is sometimes used to make a diagnosis of onchocerciasis [64].

Vaccine Development

Efforts to conquer onchocerciasis have been directed largely through the control of vectors and the use of community directed mass drug administration. With increasing treat of drug-resistance *O. volvulus*, there are compelling reasons to believe that vaccines will be the main tools for disease control in the future. Protective immunity against *O. volvulus* has been demonstrated in cattle [65] and mice [66]. In one study, 15 recombinant *O. volvulus* antigens out of the 44 screened using the *O. volvulus*-mouse model were found to be protective [67]. Eight of these antigens were produced and tested under controlled conditions, and only three, namely *Ov*-103, *Ov*-RAL-2 and *Ov*-CPI-2M, were able to repeatedly induce protective immunity [68]. Immunization in mice using DNA encoding of selected parasite genes has also shown promise [69]. A new initiative called The Onchocerciasis Vaccine for Africa (TOVA) is currently pursuing development of an onchocerciasis vaccine and has proposed two potential target product profiles, preventive vaccine for children less than 5 years old and therapeutic vaccine for infected individuals, with a plan to perform proof of concept trial for efficacy (phase two trials) at least for one candidate by 2020 [70].

Schistosomiasis

Schistosomiasis, also called bilharzia, is another neglected tropical infection in which schistosomal cercaria that normally parasitizes birds and mammals, including humans, cause skin disease or establish infection in the veins of the urinary or gastrointestinal tract, and occasionally, other organs. More than 260 million people are affected worldwide mainly in the tropical and subtropical regions [71].

The disease is caused by blood flukes of the family *Schistosomatidae*. Exposure to the parasite results from fecal or urinary contamination of freshwater that contains the intermediate host snails and skin contact to the same water by the definite host. Many eggs remain in the mammalian host causing an inflammatory reaction that results in morbidity. Less than half are released through the urine or feces depending on the infecting species. Each egg then releases a free-living stage of the parasite called miracidium that infects the intermediate host snail. Inside the snail miracidia undergo asexual reproduction giving rise to cercaria which are then released back into the water. Contact with contaminated water by the definite host results in penetration of cercaria through the skin that develop to maturity inside the body. When entering non-compatible hosts, parasites do not reproduce and die at various intervals after infection causing allergic reactions only [72].

During penetration and travel into the human skin cercaria transform to schistosomula causing human cercarial dermatitis (CD) or swimmer's itch, represented by a maculopapular skin eruption associated with intense itching that occurs within few hours to days of exposure. Debilitating pruritus and constitutionals symptoms like diarrhea and fever may also occur based on the intensity of infection. Non-human schistosomes like *Trichbilharzia* are known to induce more severe skin reactions than human schistosomes, i.e. *Schistosoma* [72].

Another distinct skin manifestation of schistosmiasis is seen in Katayama syndrome (KS) or acute schistosomiasis. It is the result of immune-complex mediated response to the immature forms of the parasite and is characterized by extensive urticaria with or without angioedema, wheeze, fever, malaise, hepatosplenomegaly and other gastrointestinal symptoms that starts 2–12 weeks after exposure [73].

The pathognomonic features of skin schistosomiasis due to the adult worm is called cutaneous schistosomal granuloma (CSG) and is due to formation of a distinctive granulomatous reaction as a result of trapped eggs that took aberrant routes through the portosystemic anastomoses to the skin. Lesions are typically located in the periumbilcal region, torso, superior dorsal regions, buttock and genitalia and appear as skin colored papules that could progress to tumoral and ulcerative plaques [74].

Immune Response

Different stages of the parasite, cercaria, schistosomula, adult worms and eggs, produce hundreds of antigenic moieties many of which are capable of stimulating both the humoral and cellular wings of the human immune system.

Cercarial penetration to the skin and transformation to schistosomula is accompanied by shedding of the tail, emptying of penetration glands as well as creation of different parasite surface antigens [75]. Mice experiments with *Trichobiliharzia* cercaria showed immediate edema, thickening of the exposure site and an influx of leucocytes including neutrophils, macrophages, CD4+ lymphocytes and mast cells [76]. Mixed cytokine response (IL-1β, IL-6 and IL-12p40), including abundant IFN-γ, and significant elevations of histamine, IL-4 and IL-10 were observed within 1 h post infection. Death of immature parasites in non-competent hosts is believed to induce a severe immune response. It is generally assumed that during initial infection a Th17 -polarized immune response (elevation of IL-4 and IL-5) develops, while experiments in mice re-infected by the cercaria four times showed a Th2-polarized immune response which might characterize the maculopapular rash in human CD [72].

Acute schistosomiasis, KS, is characterized predominantly by a Th1 immune response with high levels of IFN-γ and IL-5 in the first 5 weeks followed by a Th2 shift with IL-4 and IL-5 and low IFN-γ production [77]. Many patients also have circulating immune complexes [78, 79]. The immune response in KS also has been studied with gene-deficient mice. Mice lacking IL-4 production showed severe disease with rapid cachexia and accelerated egg deposition while IL-13-lacking mice showed sufficient Th2 response and enhanced survival [80].

CSG is the result of granulomatous inflammation both in the papillary and reticular dermis elicited by deposition of schistosomal eggs. This granulomatous inflammation consists of an admixture of lymphocytes intermingled with histiocytes and eosinophils around schistosomal eggs. Once egg deposition starts around 6 weeks after infection, there is a dramatic shift towards a Th-2 type inflammatory response with up regulation of IL-13. However, it is generally believed that CSG is formed with the action of Th-1 (IFN- γ) and Th17 (IL-17) cytokines. One study has shown positive immunoreactivity of IFN-γ (Th1 cytokine) and IL-4 (Th2 cytokine) in infiltrating cells around schistosomal eggs in 90 % and 10 % of subjects, respectively [81].

HIV Co-infection

The chronic immune activation induced by helminthic infections like schistosomiasis is known to hasten HIV progression by reducing the cytotoxic effects of CD-8+ lymphocytes. Higher viral loads and accelerated decline in CD4 count has been noticed in untreated schistosomiasis patients with HIV. Untreated schistosomiasis may also be a major contributor for immunologic failure and poorer CD4 gain upon initiation of antiretroviral therapy [82]. Presence of genital schistosomiasis is also known to increase HIV acquisition and susceptibility. It is known that *S. mansoni*-infected individuals displayed higher densities of the HIV chemokine receptors CCR5 and CXCR4 on their CD4+ T cells and monocytes [83]. One study in rural Tanzania showed markedly higher prevalence of HIV among women with schistosomiasis than among women without schistosome infection [84].

Vaccine Development

The critical role that antibodies play in schistosomiasis resistance has been well established in animal models. Schistosoma candidate vaccines are being developed for control of schistosmiasis and are in the process of evaluation [85]. Among many, a recombinant *S. haematobium* 28-kD glutathione S-transferase (Sh28 GST) protein, which is produced in *Saccharomyces cerevisiae* and formulated with alum, is the only vaccine currently in a phase III clinical trial [86]. Numerous novel potential vaccine candidates having a cross-reactivity among *S. hematobium, S. japonicum and S. mansoni* have recently been identified in *S. hematobium*-infected people and monkeys who acquire drug-induced resistance after praziquantel treatment [87]. However, whether vaccination is superior to other means of disease control like mass drug administration and control of the intermediate host remains debatable.

Conclusion

The immunology of cutaneous parasitic infections can be illustrated by leishmaniasis, onchocerciasis, and schistosomiasis. Cell-mediated immunoregulation plays the dominant role in resolving *Leishmania* infections. Most promastigotes deposited in the dermis are opsonized by serum complement and killed by complement-mediated

lysis. The remaining promastigotes are phagocytosed via complement receptors on the macrophage membrane, which bind to gp63 and lipophosphoglycan. Macrophages release chemokines, which attract NK cells, dendritic cells, and more macrophages. *Wolbachia* bacteria living inside *Onchocerca volvulus* are the essential target of the host's inflammatory response in onchocerciasis. The initial reaction in cercarial dermatitis is marked Th-1 response. Understanding these components of the immune response to parasitic infections will potentially lead to better therapies and possibly to vaccines for prevention.

Questions

1. Which human cell type is most responsible for presentation of leishmania antigens to T cells within lymph nodes?
   A. Macrophages
   B. Plasmacytoid dendritic cells
   C. Neutrophils
   D. Langerhans cells

Correct Answer: (D) Langerhans cells

2. Which human cells has been shown to engulf most of the leishmania promastigotes following the bite of an infected sand fly?
   A. Keratinocytes
   B. Macrophages
   C. Mast cells
   D. Langerhans cells

Correct Answer: (B) Macrophages

3. In what disorder may viable leishmania parasites be demonstrated in patients with a history of "resolved" cutaneous leishmaniasis?
   A. Psoriasis
   B. Inflammatory bowel disease
   C. HIV infection
   D. Liver transplantation

Correct Answer: (C) HIV infection

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Fungal Infections

19

Jacqueline Guidry, Ramya Kollipara, Christopher Downing, Michael Lee, and Stephen K. Tyring

Abstract

Fungi comprise many species that are associated with a wide spectrum of diseases in humans. The clinical relevance of fungal diseases has increased markedly, mainly because of an increasing population of immunocompromised hosts, including individuals infected with HIV, transplant recipients, and patients with cancer. Fungal infections are classified according to the primary site of infection, as superficial, cutaneous, subcutaneous, and deep or systemic mycosis. Superficial mycosis is limited to the stratum corneum and elicits no or slight inflammation. Cutaneous mycosis involves the integument and its appendages, including hair and nail. Infection of the skin, which is caused by the fungal organisms or its products, may involve stratum corneum or deep layers of the epidermis. Subcutaneous mycosis involves the epidermis and subcutaneous tissues. Subcutaneous mycosis usually follows traumatic inoculation of fungal organisms. The inflammatory response that develops in the subcutaneous tissues usually involves the epidermis. Deep or systemic mycosis usually involves organs such as lung, central nervous system, bones, and abdominal viscera. The portal of entry in deep mycosis is the respiratory tract, gastrointestinal tract, and blood vessels.

Keywords

Fungi • Subcutaneous • Immune response • Dermatophytes • Mycosis

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Key Points

- The clinical relevance of fungal diseases has increased due to increased populations of immunocompromised patients
- Fungal infections are classified according to the site of the primary infection: superficial, cutaneous, subcutaneous, and deep or systemic
- Dimorphic fungi assume both yeast and hyphal states based on environmental conditions and the hosts' immune response
- Certain fungi can synthesize capsular components, which can affect host immune responses
- The innate response to fungi serves two purposes: a direct antifungal effector activity, and activation and induction of the specific adaptive immune responses
- Understanding the immune responses to fungal infections has led to better diagnostic tests and therapeutic interventions for fungal diseases.

Fungi comprise many species that are associated with a wide spectrum of diseases in humans. The clinical relevance of fungal diseases has increased markedly, mainly because of an increasing population of immunocompromised hosts, including individuals infected with HIV, transplant recipients, and patients with cancer. Fungal infections are classified according to the primary site of infection, as superficial, cutaneous, subcutaneous, and deep or systemic mycosis. Superficial mycosis is limited to the stratum corneum and elicits no or slight inflammation. Cutaneous mycosis involves the integument and its appendages, including hair and nail. Infection of the skin, which is caused by the fungal organisms or its products, may involve stratum corneum or deep layers of the epidermis. Subcutaneous mycosis involves the epidermis and subcutaneous tissues. Subcutaneous mycosis usually follows traumatic inoculation of fungal organisms. The inflammatory response that develops in the subcutaneous tissues usually involves the epidermis. Deep or systemic mycosis usually involves organs such as lung, central nervous system, bones, and abdominal viscera. The portal of entry in deep mycosis is the respiratory tract, gastrointestinal tract, and blood vessels.

Fungi that cause cutaneous, subcutaneous, or disseminated infection with skin involvement exist in a range of morphologic forms that include yeasts and molds. Yeasts (e.g., Malassezia) grow as unicellular round or oval-shaped organisms, whereas molds (e.g., dermatophytes) form long tubular structures termed hyphae that extend into a branch-like network known as a mycelium. Dimorphic fungi (e.g., *Candida albicans, Histoplasma capsulatum, Coccidioides immitis*, *Blastomyces dermatitis*, and *Sporothrix schenckii*) assume both yeast or spherules and hyphal states of growth based on environmental conditions and interactions with the mammalian immune system. Yeasts and molds are bound by a cell wall composed of polysaccharide polymers (chitin, mannans, and glucans) derived from biosynthetic pathways absent in mammalian cells [1, 1a, 2], which inhibits complement-mediated damage to the fungal cell membrane [3]. In addition, certain fungi can synthesize capsular components, melanins, and secondary metabolites that include toxins, for example, gliotoxin and aflatoxin, many of which can affect host immune responses [4–7].

This chapter discusses the general innate and acquired immune responses against fungi, particularly the cellular and molecular pathways of immune defense mechanisms that have significantly contributed to our present understanding of the host response to fungi and have provided a sound framework for development of effective strategies of immunotherapy against some fungal infections. This chapter also discusses host defenses and specific immune responses against certain fungal pathogens causing cutaneous, subcutaneous, and deep mycosis that begins with either cutaneous or subcutaneous diseases and then disseminates to become a systemic disease that involves also the skin.

Innate Immune Responses to Fungal Infection

The host defense mechanisms against fungi are numerous, and range from nonspecific, germline-encoded immunity that presents early in the evolution of microorganisms, to highly specialized and specific adaptive mechanisms that are induced during infection and disease. The relative importance of specific innate and adaptive defense mechanisms differs, depending on the organism and anatomic site of infection (skin, mucosal sites, or disseminated infection). Additionally, the morphotype of the fungal pathogen (yeast or hyphae) determines the type of host immune response. For example, yeasts and spores are often effectively phagocytosed, while the larger size of hyphae prevents effective ingestion. Pathogenic fungi have also developed mechanisms to subvert host defenses, which allow some intracellular fungi to survive within phagocytes, avoid fungal killing, and then disseminate throughout the host.

The innate response to fungi serves two main purposes: (1) a direct antifungal effector activity by mediating nonspecific elimination of pathogens through either a phagocytic process and intracellular killing of internalized pathogens or through the secretion of microbicidal compounds against undigested fungal molecules; and (2) activation and induction of the specific adaptive immune responses via the production of proinflammatory mediators, including chemokines and cytokines, providing

co-stimulatory signals to naive T cells, as well as antigen uptake and presentation to CD4 and CD8 T cells [8]. In addition to the above inducible functions of innate response, the constitutive mechanisms of innate defense that are present in the skin include the barrier function of body surfaces. Figure 19.1 illustrates the link between innate and acquired (cellular and humoral) immune responses against fungal infections of the skin.

Host innate defenses against fungi are mediated by professional phagocytes, including neutrophils, mononuclear leukocytes (monocytes and macrophages), and dendritic cells (DCs), natural killer (NK) cells, and nonhematopoietic cells, such as keratinocytes and epithelial and endothelial cells. The first step in the innate immunity involves fungal recognition and uptake by germline-encoded pattern recognition receptors (PRRs) expressed on the surfaces of several innate immune cells [8–11].

Figure 19.2 illustrates the different PRRs expressed on phagocytic cells such as macrophages and the downstream effector functions resulting from interaction of fungal antigens with these receptors.

The most important classes of PRR are the Toll-like receptors (TLRs) [10, 11], dectin-1 [12], the lectin-like receptors [13, 14], Fc receptors [15], complement receptors, the mannose receptor [16], and integrins [17].

The microbial ligands of these receptors are called pathogen-associated molecular patterns (PAMPs). Fungal structures such as β-1,3/β-1,6 glucans [17], glucuronoxylomannan, phospholipomannan, and galactomannan function as ligands for TLR2, TLR4, and TLR6 [18, 19]. The signaling pathway for mammalian TLR after ligation of PAMPs involves interaction with the adaptor molecule MYD88 (myeloid differentiation primary response gene 88) located in the cytosol [19, 20]. The activation of the MYD88 adaptor culminates in the activation and nuclear translocation of nuclear factor kB (NF-kB), which leads to activation of several cytokine and chemokine genes. Recognition of pathogens by TLR in a Myd88-dependent, and sometimes in a Myd88-independent, manner leads to release of proinflammatory cytokines, chemokines, activation of antibacterial mechanisms, and enhancing the T-cell priming ability of professional antigen presenting cells (APCs) such as dendritic cells. Compared to individual TLRs, Myd88−/− mice had higher levels of fungal growth than control animals [19, 21]. The more severe phenotype of Myd88−/− mice compared with mice deficient in individual TLRs probably reflects the broad function of Myd88 as an adaptor for multiple TLR-dependent responses.

Toll-like receptors discriminate between distinct fungal morphotypes. For example, TLR4 and CD14 expressed on human monocytes appear to recognize Aspergillus hyphae but not Candida hyphae [17, 22].

Similarly, the production of pro-inflammatory and Th1 cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) by macrophages in response to *C. albicans* phospholipomannan expressed on yeast cells depends on TLR2 and TLR4 signaling, whereas hyphal cells trigger these cytokines in a TLR2-dependent manner only [19, 22–24].

In vivo, the role of TLR2 in systemic fungal infection such as systemic candidiasis is less clear: one study reported that TLR2−/− mice are more sensitive to primary infection than control mice [25], while another study reported no difference between TLR2−/− and TLR2+/+ mice, regardless of whether the animals were inoculated with yeast or hyphal forms [26, 27]. TLR2− mediated recognition of Candida has also been shown to expedite differentiation of hematopoietic stem and progenitor cells into macrophages, which likely helps boost the body's innate immune response [28]. In addition to their function in fungal recognition, uptake, and production of pro-inflammatory cytokines, signaling through individual TLR signaling can determine the type of acquired immune responses against fungi. TLR2 ligation by fungal zymosan, and possibly β-glucan, leads to the prevalent production of anti-inflammatory cytokines such as interleukin-10 (IL-10), which can suppress macrophage microbicidal functions and also drive the induction of Th2 response [26, 27, 29, 30].

Lectin receptors are other PRRs on phagocytes that not only play a role in fungal recognition but also mediate distinct downstream intracellular events related to clearance of fungi. Recognition of fungal PAMPs by lectin receptors induces rapid and broad host defense responses such as opsonization, activation of complement, activation of coagulation cascades, phagocytosis, inflammation, and direct microbial killing [31, 32].

Among several lectin-like families, galectin-3 binds to β-1,2-linked oligomannan, an uncommon PAMP present on the surface of *C. albicans* but absent on *Saccharomyces cerevisiae* [33, 34]. The binding of galectin-3 to yeast cell walls of *C. albicans* is inhibited by C. albicans mannans but not by *S. cerevisiae* mannans. More importantly, binding of galectin-3 results in opsonization of Candida expressing different combinations of β-1,2-linked oligomannosides and death of yeast cells [34] and as such gal3−/− mice are more susceptible to infection, have larger fungal burden and die faster than wild type controls [35]. In addition to its direct effects on innate immunity, galectin-3 also plays an important role modulating the Th1 versus Th2 response in infected tissues [36].

Other fungal receptors such as complement receptors (CRs), mannose receptors (MRs), and dectin-1 receptors mediate fungal internalization following binding of various fungal ligands such as complement-associated products, mannosylefucosyl glycoconjugate ligands, and β-glucans, respectively. Internalization through MRs does not lead to effective clearance of fungi in the absence of opsonins. However, MRs expressed by DCs activate specific programs that are relevant to the development of antifungal acquired specific immune

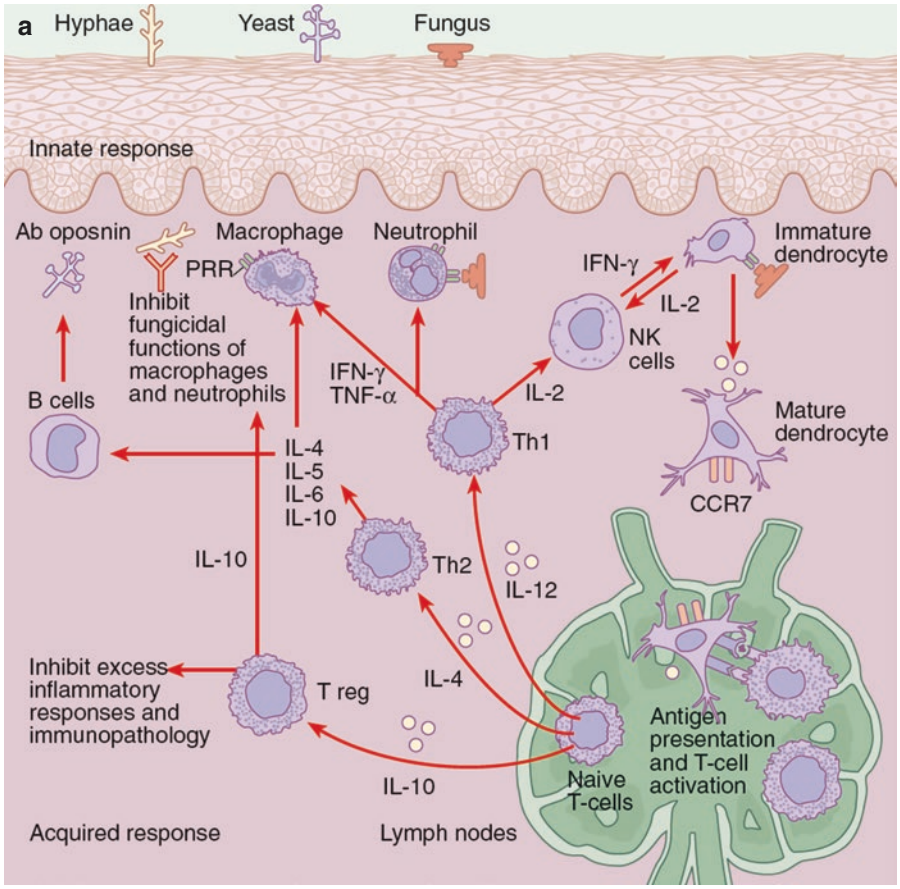


Fig. 19.1 Possible pathways for immunologic responses stimulated by fungi. (**a**) Most fungi are detected and destroyed within hours by innate nonspecific defense mechanisms mediated by phagocytes such as macrophages, neutrophils, immature dendritic cells, and opsonins (antibodies, Ab) through the involvement of distinct pattern-recognition receptors (PRRs). Fungal organisms on the skin surface also release antigens (Ag) that penetrate the skin and are captured by an antigen-presenting cell (APC) such as dendritic cells (DCs). Cross-linking of PRR on the surface of immature DCs by fungal antigen lead to their maturation. In addition, production of inflammatory cytokines such as IFN-γ and TNF-α by other innate cells such as NK cells further enhance activation of microbicidal functions of phagocytic cells as well as maturation of DCs. The DCs sampling fungal antigens from the skin and migrating to secondary lymphoid organs process and present antigens through class I or class II major histocompatibility complex (MHC) molecules to antigen-specific naive T cells endowed with the capacity to recognize the peptide epitopes through specific T-cell receptors (TCRs). This process lead to activation of different antigen-specific T helper (Th) effector cells, regulatory T (Treg) cells and B cells that specifically target the pathogen and induce memory cells. Differentiation of naive CD4+ Th cells in the peripheral lymphoid organs into Th1, Th2, or Treg depend on several factors, among which is the cytokine environment stimulated by different fungal morphotypes. Thus, the production of interleukin-12 (IL-12) by DCs leads to the outgrowth of T-helper-1 (Th1) cells that produce IFN-γ, TNF-α, or both. IFN-γ and TNF-α are required for further activation of fungistatic and fungicidal activities by phagocytes that results in clearance of infection with most, if not all, of these fungal pathogens. The induction of IL-4 (and failure to produce IL-12) by DCs leads to a Th2 response, which blunts the generation of protective immunity. (**b**) Progressive disease in immunodeficient or susceptible hosts is associated with a shift in the balance between Th1 and Th2, toward the Th2 response. The latter is characterized by upregulation in IL-4, IL-5 and IL-10, an increase in tissue eosinophils, antibody isotype switch and production of antigen-specific antibodies including IgG and IgE. The IgE antibodies bind to mast cells (MCs) and upon subsequent encounter with allergens, trigger degranulation leading to inflammation and clinical features of type I hypersensitivity reactions. IL-10 production by mast cells suppresses cell-mediated immune responses in certain cutaneous fungal diseases. Neutralization of IL-4, IL-5, and IL-10 in vivo can sometimes restore protective immunity. Thus, the activation of the appropriate Th-cell subset is critical in the generation of a successful immune response to fungi. Although IL-4 and IL-10 cytokines block the expression of a protective response against fungi, the elaboration of at least some Th2 cytokines also helps to balance the immune response. Finally, induction of T-regulatory (Treg) cells mediated by IL-10 might serve to dampen the excessive inflammatory reactions through cell contact or secretion of immune suppressive cytokines such as IL-10 (**b**)

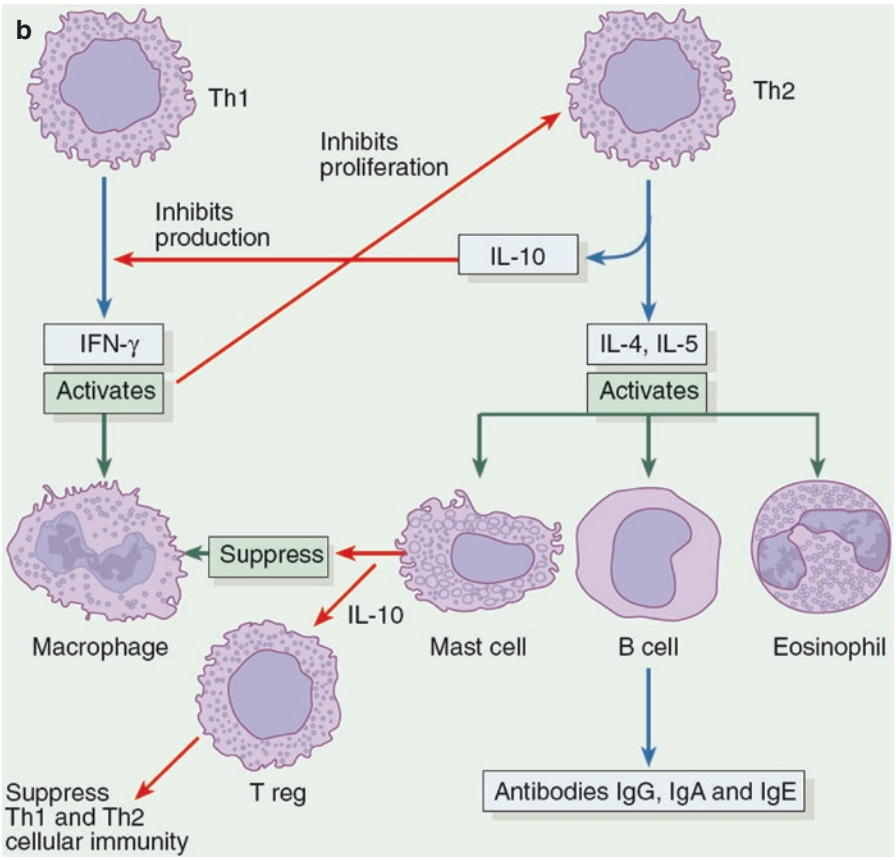
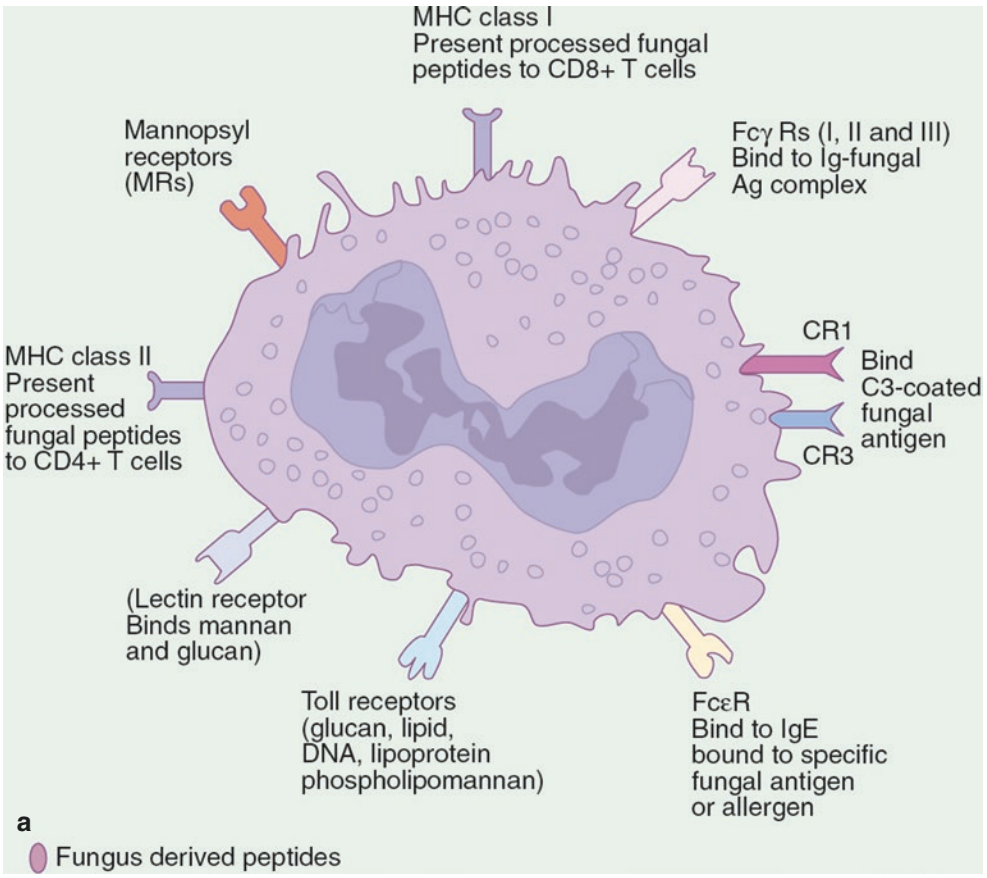


Fig. 28.1 (continued)

responses as will be discussed later. Ligation of CR3 (also known as CD11b/CD18) is one of the most efficient means of engulfing opsonized fungi, but it also has broad recognition capacity for diverse fungal ligands. Interestingly, yeasts such as *Histoplasma capsulatum* establish intracellular fungal parasitism within macrophages when they enter the cells through the CR3. In contrast, a concomitant ligation of both CR3 and FcγIIIR on macrophages triggers an effective phagocytosis and respiratory burst that interferes with fungal infectivity and mediates elimination of fungal pathogens [37, 38].

Neutrophils, macrophages, and monocytes constitute the major cellular effectors of innate immunity against fungal pathogens [39–42]. Following phagocytosis, fungi are killed by intracellular microbicidal effector molecules produced by macrophages and neutrophils, including oxygen-dependent (i.e., nitric oxide, reactive oxygen intermediates, reactive nitrogen intermediates, and peroxynitrite) and oxygen-independent (i.e., release of cationic proteins, lysozyme, and antimicrobial peptides such as defensins, arachidonic acid, myeloperoxidase, and iron sequestration) [42–45]. Enzymes such as the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and inducible nitric oxide synthase initiate the oxygen-dependent pathways known as respiratory burst that produces toxic reactive oxygen intermediates (ROIs) [43–45]. In retribution, fungi have evolved strategies to selectively inhibit the respiratory burst through the production of specific scavengers of oxidative killing by phagocytes, such as catalase, mannitol and melanin.

Patients with inherited X-linked chronic granulomatous disease, resulting from a deficiency in oxidant formation due to mutations in any of the four genes that encode the subunits of NADPH oxidase, have increased susceptibility to fungal infection, mainly aspergillosis [45]. These patients could be treated effectively with IFN-γ, which increases the non-oxidative as well as the oxidative intracellular microbicidal mechanisms mediated by phagocytic cells such as macrophages and neutrophils. The involvement of neutrophils or macrophages in host defense against fungi depends on the morphotype of the fungi causing infection. For example, neutrophils play a predominant role in phagocytosis of filamentous fungi [40–42, 45], while macrophages play a predominant role in host defense against fungal yeast [39, 46]. In addition to the ability of macrophages to ingest organisms that have been opsonized with antibody, or complement, they are also able to phagocytose unopsonized fungal elements through recognition receptors such as the integrins. Although the main contribution of neutrophils and macrophages resides in their phagocytic and microbicidal functions, they can produce cytokines and chemokines that can modulate the protective immune response. Furthermore, macrophages function also as APCs that activate CD4+ and CD8+ T cells through presentation of fungus-derived peptides in the


MHC class I
Present processed fungal peptides to CD8+ T cells
Mannopsyl receptors (MRs)
Fcγ Rs (I, II and III) Bind to Ig-fungal Ag complex
CR1
Bind C3-coated fungal antigen
CR3
MHC class II Present processed fungal peptides to CD4+ T cells
(Lectin receptor Binds mannan and glucan)
Toll receptors (glucan, lipid, DNA, lipoprotein phospholipomannan)
FcεR Bind to IgE bound to specific fungal antigen or allergen
a
Fungus derived peptides

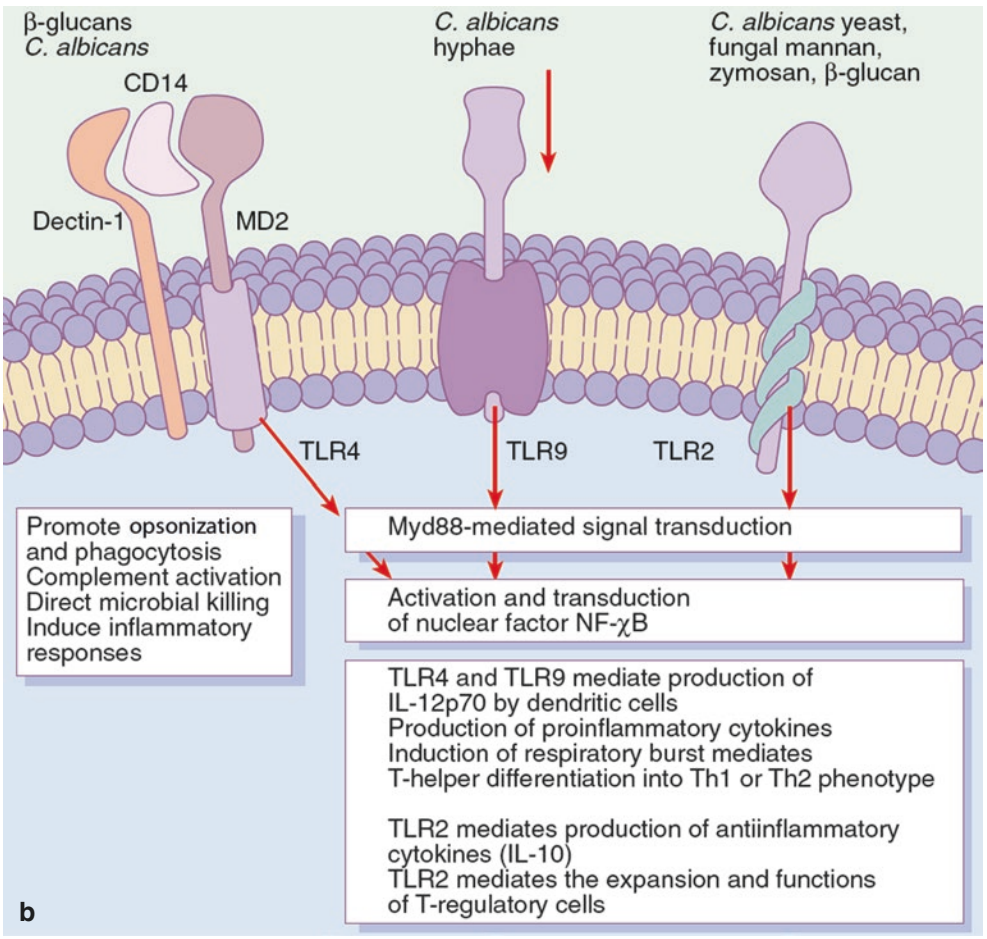

β-glucans C. albicans
C. albicans hyphae
C. albicans yeast, fungal mannan, zymosan, β-glucan
CD14
Dectin-1
MD2
TLR4
TLR9
TLR2
Promote opsonization and phagocytosis
Complement activation
Direct microbial killing
Induce inflammatory responses
Myd88-mediated signal transduction
Activation and transduction of nuclear factor NF-χB
TLR4 and TLR9 mediate production of IL-12p70 by dendritic cells
Production of proinflammatory cytokines
Induction of respiratory burst mediates
T-helper differentiation into Th1 or Th2 phenotype
TLR2 mediates production of antiinflammatory cytokines (IL-10)
TLR2 mediates the expansion and functions of T-regulatory cells
b

Fig. 19.2 Recognition of fungal ligands by different pattern recognition receptors (PRRs) expressed on the surface of macrophages: the role of Toll-like receptors (TLRs) and other receptors as activators of innate and adaptive immunity to fungi. (**a**) Innate cells such as macrophages, monocytes, and dendritic cells express several pattern recognition receptors (PRRs) that recognize various fungal ligands, promote fungal internalization, activate intracellular fungicidal effector mechanisms, and play a role in the induction of the acquired immune response against fungi. Concomitant interaction of antibody and complement-coated fungal cells with Fc receptors (FcRs) and complement receptors (CRs) on host phagocytic cell membranes results in prompt ingestion of the fungal cell, which can lead to the death of the ingested fungal cell. Furthermore, phagocytic cells express several TLRs that bind to specific fungal ligands referred to as pathogen-associated molecular patterns (PAMPs). The signaling pathway for mammalian TLRs after ligation of PAMPs involves interaction with the adaptor molecule MyD88 (myeloid differentiation primary response gene 88) located in the cytosol. The activation of MyD88 results in activation and translocation of nuclear transcription factor $\chi B\,(NF{-}\chi B)\,.\,NF{-}\chi B$ controls the activation of several downstream cytokines and chemokine genes; therefore, its activation is usually linked to production of proinflammatory and antiinflammatory cytokines and chemokines. Although all TLRs signal through MyD88, ligation of certain TLR can result in unique effector functions. For example, TLR2 stimulation leads to production of IL-10, which promotes the expansion and function of immunoregulatory T cells. On the other hand, stimulation of TLR4 or TLR9 leads to the activation of antifungal effector functions in phagocytes, such as respiratory burst and degranulation, and production of interleukin-12p70 (IL-12p70) by dendritic cells. This leads to inflammatory and protective antifungal T-helper-1 (Th1)-cell responses. However, the differential TLR responses could also function by unidentified MyD88–independent pathways (**b**)

context of major histocompatibility complex (MHC) class II and I, respectively as well as providing co-stimulatory signals as illustrated in Fig. 19.3. Nevertheless, for some intracellular fungal pathogens, such as *H. capsulatum*, their intracellular location protects them from host defenses, and these organisms thrive within macrophages [37, 38].

To overcome the fungal immune evasion mechanism within phagocytic cells, other innate immune cells such as NK [46–48], NKT, and γδ T cells [46, 49, 50] play a pivotal role in host defense against fungi. These cells mediate their antifungal response through different mechanisms that include the following: (1) early production of cytokines such as IFN-γ and TNF-α that are important for full activation of macrophage phagocytic and antimicrobial effector functions; (2) direct cytotoxic killing of pathogens or growth inhibition; (3) activation of dendritic cells through either cytokines or cell–cell contact, which in turn mediate activation and differentiation of specific CD4+ and CD8+ T cells as described later. Evidence that supports the protective role of NK, NKT, and γδ T cells in immunity against fungi stems from studies conducted in knockout mice that lack a particular cell subset. These mice are susceptible to various fungal infections, mainly *C. albicans* [48, 49].

Langerhans cells (LCs) and immature dermal DCs are some of the first cells to encounter fungi and play pivotal roles in induction of acquired responses against fungi as well as restriction of fungal growth [51–53]. Immature DCs constantly monitor the epidermal microenvironment by taking up antigen and processing it into fragments that can be recognized by cells of the adaptive immune response. However, receptors on the surface of DCs may be selective for only certain species, or for certain forms within that species, and may explain in part the ability of fungi to survive within the host. Compared to yeasts, hyphae cause DCs to produce cytokines leading to local induction of T regulatory cells (Treg), which serve to limit the immune response [54].

Because of their unique migratory ability, DCs can transport fungal antigen from the epidermis or dermis to regional lymph nodes, where they initiate specific immune responses. Fungal infection provides danger signals, leading to a local production of proinflammatory cytokines that induce local DC maturation [55, 56]. Maturation of DCs is associated with a selective change in chemokine receptor

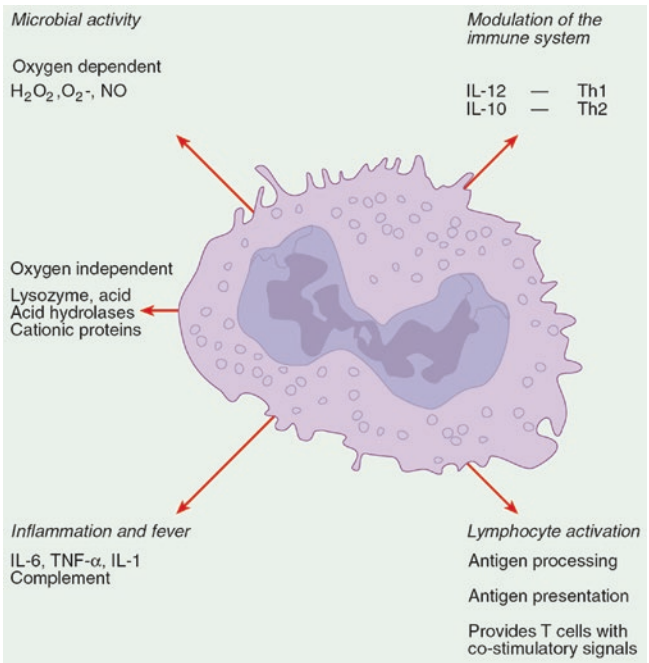


Fig. 19.3 Different functions of macrophages in innate and acquired immune responses against fungal infection. Macrophages play a pivotal role in innate and acquired immune responses against fungal pathogens. Local activation of macrophages at early stages of infection by fungal antigens and later by IFN-γ and TNF-α produced by NK cells or effector CD4+ Th1 cells results in (1) increased oxygen–dependent and independent fungicidal activities; (2) production of proinflammatory cytokines and chemokines that enhance migration of effector immune cells to the skin as well as play a role in activation of T cells. In addition, macrophages can also function as professional antigen presenting cells (APC) that process fungal antigens and present fungus-derived peptides in the context of MHC class II and I to CD4+ and CD8+ T cells, respectively

profile [57–65]. For example, immature DCs express a number of chemokine receptors for inducible chemokines, such as IL-8 (CXCL8), RANTES (regulated on activation, normal T-cell expressed and secreted; CCL5), macrophage inflammatory protein 1α (MIP-1α; CCL3), or monocyte chemoattractant protein 3 (MCP-3; CCL7), by which immature DCs are attracted to the site of inflammation (i.e., skin) [61–63].

Following maturation, DCs downregulate these receptors, which may allow them to leave the inflammatory site (i.e., the site with the highest chemokine concentration). At the same time, maturing DCs upregulate receptors for constitutively expressed chemokines such as the CXC chemokine receptor 4 and CCR7 [62, 63]. Interestingly, the CCR7 ligand secondary lymphoid tissue chemokine (SLC; CCL21) is constitutively expressed by stromal cells in T-cell zones of lymph nodes and by lymphatic endothelial cells in the dermis [61–63]. Thus constitutive expression of SLC by lymphatic endothelium seems to provide the first chemotactic gradient for activated CCR7 positive DCs, leading to a selective recruitment of DCs from the epidermis to the afferent lymphatics [63–65]. Once they enter the lymphatics, they are likely to be transported passively with the lymph to the subcapsular region, where they then encounter an additional chemotactic gradient of SLC that directs their migration into the paracortical T-cell zone of the lymph node where they activate T cells specific for the invading fungal pathogen. In the draining lymph nodes, these DC-capturing antigens initiate T-cell immune responses by virtue of their abilities to present fungal antigens to T cells, provide lymphocytes co-stimulatory molecules, and secrete cytokines. The DCs use distinct receptors to recognize each form of a particular fungus, thereby activating different signaling pathways with distinct functional consequences [55, 65–67].

Finally, the noncellular effectors of innate immunity comprise complement and natural antibodies [68–71]. As described before, these molecules mediate opsonization and therefore promote the ingestion of fungi by phagocytes. However, the fate of opsonized fungi can differ from that of unopsonized organisms, because in phagocytes, opsonized fungi may traffic through a different pathway than do the unopsonized organisms [37, 38].

Adaptive Immunity

For many fungal pathogens, the effective immune response to invasion is a cell-mediated immune response. The role of CD4+ T lymphocytes in protection against fungal infections is underscored by the susceptibility of patients with acquired immunodeficiency syndrome (AIDS) caused by HIV to fungal infections caused by *C. albicans, H. capsulatum, Cryptococcus neoformans*, and *Aspergillus fumigatus*. For all of the pathogenic fungi, a T-helper-1 (Th1) response characterized by production of IL-2 and IFN-γ is the dominant adaptive protective response. The production of IL-12 by DCs leads to expansion and increased number of Th1 cells that produce IFN-γ or TNFα [39, 56, 57, 72, 73]. Interestingly, IL-12 secretion seems to be dependent on the morphologic form of the fungi where the yeast form of *C. albicans* stimulates IL-12 production, while the hyphal form inhibits such production [67, 73]. Interferon-γ production by Th1 cells is essential for optimal activation of phagocytes (e.g., macrophages, neutrophils, and immature DCs) at sites of infection and enhances their fungicidal effector functions [39, 66]. Therefore, deficiency of this cytokine or Th1 response might predispose patients to overwhelming fungal infections, and also favor fungal persistence. On the other hand, the Th2 response characterized by production of Th2 cytokines (IL-4, IL-13, IL-5, and IL-10) is often associated with a subversion of the host response to fungi. Increases in the Th2 cytokines are commonly observed in progressive disease, and neutralizing their activity restores protective immunity [73–77].

The role of Th1/Th2 paradigm in outcome of fungal disease is exemplified in skin infection with *Paracoccidioides brasiliensis* (PCM), which stimulates the formation of granulomatous lesions in the skin [77, 78]. The presence of well-formed granulomas and local Th1 responses in the skin of PCM patients is associated with mild disease, while the presence of poorly formed granulomas and local Th2 responses is associated with progressive and severe disease. These observations suggest that well-organized granulomas and cutaneous Th1 response reflect a better cellular immune response, while the presence of Th2 cells expressing Th2 cytokine such as IL-4 and IL-5 indicate an ineffective response in PCM skin lesions.

More recently, Th17, a distinct CD4+ T cell outside the family of Th1 and Th2 cells, has been discovered and like Th1 cells can confer a protective effect in fungal infections [79]. In the same manner that different fungal pathogens can induce a Th1 versus Th2 immune response, certain fungal PAMPs predominantly activate a Th17 immune response. Thus far, the understanding of Th17 activation pathways relies on the same signaling pathways (e.g. TLR, dectin-1), though further research may elucidate receptors unique to Th17 development [80]. If not regulated, Th17 cells can yield a deleterious effect [54, 80].

Compared to CD4+ T cells, the role of CD8+ T cells during fungal infection has not been defined as clearly [81]. CD8+ T cells mediate protection against fungal infection mainly via the production of IFN-γ. However; the role of CD8+ T lymphocytes in mediating cytotoxic lysis of fungus-infected target cells is not well delineated [82].

Although CD8+ T cell activity against filamentous fungi such as *A. fumigatus* has not been demonstrated in mice, the expansion of cytotoxic, class I–restricted, *A. fumigatus*–specific CD8+ T cell clones from human peripheral blood suggests that CD8+ T cells might contribute to cell-mediated defense [82]. Interestingly, unlike infection with bacteria and viruses, the priming of fungus-specific CD8+ T cells does not appear to require CD4+ T-cell help; in fact, CD8 T-cell responses are enhanced in CD4-knockout mice. In the absence of CD4+ T cells, CD8+ T cells can protect mice from *H. capsulatum* infections by secretion of IFN-γ [83–85].

Although Th1-biased responses to fungal infections are protective, Th2-biased responses are deleterious; an excess or unregulated Th1 response may also generate unnecessary tissue damage. The Th1 response can be downregulated by simultaneous elaboration of Th2 cytokines or suppression of Th1 and Th2 responses that usually are associated with either chronic fungal infection or overwhelming infection, respectively. Thus, the induction of regulatory mechanisms in immunity to fungi is pivotal as they ensure that under physiologic conditions, an effective protective antifungal immunity is generated while avoiding immune pathology. One important immunoregulatory cytokine is IL-10, which is a potent immunosuppressive cytokine, produced in a non–antigen-specific manner by innate cells such as macrophages and DCs or in an antigen (Ag)-specific manner by regulatory CD4+ T cells [86, 87].

Interleukin-10 acts by impairing (1) the antifungal effector functions of phagocytes; (2) the secretion of proinflammatory cytokines such as TNF-α, IL-1, and IL-6; and (3) the production of Th1-promoting cytokines such as IL-12, IFN-γ, and IL-23 [86–89].

The IL-10-mediated suppressive functions would result in defective protective antifungal cell-mediated immunity. Production of IL-10 at early stages of infection suppresses immune responses and enhances susceptibility to fungal infection.

Histopathologic and double immunohistochemical examination of skin lesions from patients with severe PCM infection revealed an increased number of mast cells expressing IL-10 [90, 91]. Early production of IL-10 by mast cells, as part of the innate system, could contribute to an ineffective response against fungal antigens. It is of interest that IL-10 expressing mast cells were detected only in skin lesions characterized by loose granulomas and local Th2 response, but not in lesions that are characterized by compact granulomas and Th1 response. Other studies have shown that patients treated with anti–TNF-α antibodies, which resulted in increases in IL-10 production are susceptible to fungal pneumonia. On the other hand, production of IL-10 at later stages of infection by CD4 T regulatory cells is beneficial by contributing to resolution of excessive inflammatory responses, thus avoiding immune-mediated tissue damage [88–92].

Role of Antibodies in Protective Immunity Against Fungi

Nearly all fungi elicit an antibody response; however, the role of these antibodies in pathogenesis or protective immunity is not completely clear. Initially, the presence of specific antibodies in patients with progressive fungal infections and the lack of increased susceptibility to fungal infections in patients with antibody deficiencies argued against a protective role. However, studies involving opportunistic fungal diseases, mainly candidiasis and aspergillosis, provided evidence that supports a role for antibodies in protective immunity against fungal diseases [96–99]. More recent evidence suggests that the role of fungal antibodies is dependent on the specificity and isotype, which can yield three distinct host responses: protective, non-protective or indifferent, and disease-enhancing [3]. Possible targets for protective antibodies included fungal cell wall polysaccharides of Candida and Aspergillus species, heat shock protein [94], histone-like proteins, and mannoprotein in *C. albicans* [96–100].

Recently, another fungal cell-wall polysaccharide, β-glucan, has been identified as a possible target for the induction of protective antibodies [101]. The conserved structure suggested that β-1,3-glucan could be part of a universal antifungal vaccine. In the light of known inverse relationship between Th1 and Th2 cytokines and immunoregulation of the Th1 and Th2 responses, a role for antibodies in defense against fungal disease might seem to be in conflict with the underlying Th1-directed response that is the most widely accepted explanation for host-acquired specific immunity against fungi. However, some studies show that Th2-derived antibodies have a protective effect by augmenting cell-mediated immunity.

Antibodies can function as opsonins, promoting fungal ingestion and even killing by phagocytes, and several antibodies are directly fungicidal [102–104]. These kinds of activities are complementary, rather than exclusive, to cell-mediated mechanisms. The central importance of granulocytes and macrophages in innate defense against opportunistic fungal pathogens, and of activated neutrophils and macrophages against fungi in general, is consistent with an expectation that opsonic antibodies facilitate host defense. Antifungal antibodies can contribute to the activation of the classic complement system and antibody mediated cytotoxicity by NK cells [105, 106] and can change the gene expression making the fungus more vulnerable to antifungal therapy [3].

Th1-derived antibodies are usually of immunoglobulin G2 (IgG2) isotype, which are excellent opsonins that promote fungal ingestion and even killing by phagocytes. In contrast, the association of antibodies with nonprotective responses or progressive fungal diseases could be due to the possibility that these antibodies are of Th2 isotype (usually IgG1), which are produced by antigen-specific B cells interacting with antigen specific CD4+ Th2 cells producing Th2 cytokines such as IL-4 or IL-10. In that case, Th2-derived antibodies are associated with a Th2 response that causes suppression of protective Th1 and cell-mediated immunity [103, 107].

Cutaneous Fungal Diseases

Cutaneous Candidiasis

Clinical Manifestations and Pathogenesis of Cutaneous Candidiasis

Cutaneous candidiasis is an infection of the skin that is caused by the yeast *C. albicans* and can be either acute or chronic in nature. Cases of cutaneous candidiasis caused by other Candida species such as *C. parapsilosis* or *C. tropicalis* are sometimes seen, but these are rare. *Candida albicans* is part of the normal flora of the gastrointestinal tract rather than of the skin, although it can be found on the skin [108–110]. This organism can grow as either yeast cells or filamentous forms, with mixtures of the two phases generally seen in tissue infections. Acute cutaneous candidiasis may present as lesions with intense erythema, edema, creamy exudate, and satellite pustules within folds of the skin. Other infections may be more chronic, as in the feet, where there can be a thick, white layer of infected stratum corneum overlying the epidermis of the interdigital spaces. Candida paronychia is marked by infections of the periungual skin and the nail itself, resulting in the typical swelling and redness of this type of candida infection. The virulence of *C. albicans* has been attributed to various causes, including its ability to grow at particular temperatures, its ability to produce filamentous forms, its adherence capabilities, and the activity of different enzymes. In some cases superficial *C. albicans* infections may be particularly severe and refractory to treatment, producing the uncommon disorder known as chronic mucocutaneous candidiasis (CMC) [111–113], which consists of persistent and recurrent infections of the mucous membranes, skin, and nails. Oral thrush and Candida vaginitis are fairly common in patients with CMC. There is often infection of the esophagus, although further extension into the viscera is unusual. The typical skin lesions are generally red, raised, and hyperkeratotic but usually are not painful. Epidermal neutrophilic microabscesses, which are common in acute cutaneous candidiasis, are rare in the lesions of CMC. Nail involvement can be severe in this condition, producing marked thickening, distortion, and fragmentation of the nails, with chronic swelling of the distal phalanx.

Immune Responses and Host Defenses Against Candida

The initial events in stimulation of both innate and acquired immune responses against cutaneous Candida infection include recognition of fungal ligands and phagocytosis. The immunoreactive components of *C. albicans* are thought to be the carbohydrate derivatives found on the yeast surface, including glucans, mannans, and chitin. The activation of TLRs by these fungal products presumably contributes to the inflammatory tissue injury seen in mucocutaneous candidiasis by signaling the release of proinflammatory cytokines and by directing the recruitment and activation of other inflammatory cells [114–119]. TLR2, TLR4, TLR6, and TLR9 play a role in host recognition of C. albicans. C-type lectin-like receptors (dectin-1 and 2) also recognize candida infections by binding to β-glucan or mannose, and patients with dectin-1 deficiency appear more susceptible to mucosal candida infections. When both dectin-1 and TLR2 or TLR4 are activated, there appears to be a synergistic effect on the host's proinflammatory response [79].

Recognition of mannan by the mannose receptor on macrophages results in the internalization of Candida. Mannan drives TNF-α production by murine alveolar macrophages in vitro and increases the serum TNF-α level upon intravenous administration in vivo. Earlier studies of the immune response against yeasts have relied heavily on the use of zymosan, an insoluble fungal cell wall preparation, which contains large amounts of β-glucan and mannan [17, 21–27, 29]. It was shown that TLR6 and TLR2 formed heterodimers to coordinate the macrophage activation by zymosan [29, 120]. These findings also suggested that immunity against C. albicans requires the collaboration between receptors responsible for phagocytosis (e.g., mannose or complement receptors) and TLRs designed to induce proinflammatory cytokines. Recent studies highlighted the role of Myd88 [12–19, 121, 122], a signaling molecule for TLR in the phagocytosis of *C. albicans* [19–21]. Macrophages harvested from Myd88–deficient mice and challenged with live *C. albicans* showed a diminished capacity to engulf and kill the yeast, and a diminished capacity to produce TNF-α when compared to wild-type cells [21].

In contrast to immune mechanisms involved in protection against widespread, systemic candidiasis, where cells of the innate immune system, particularly neutrophils, are crucial [17, 38], it has long been recognized, based on both clinical and experimental data, that in the mucocutaneous form of candidiasis, cell-mediated immunity is essential for protection. Chronic mucocutaneous candidiasis

represents a group of syndromes with a variety of predisposing or secondary abnormalities in host defense function. The most common deficiency appears to be one of cell-mediated immune responses against candida antigens, although abnormalities in chemotaxis or phagocytic cell function have also been reported [111–117]. Other host defense mechanisms, such as humoral immunity and the complement system, have generally been found to be normal in these patients [118]. Although treatment of this condition with amphotericin B could be successful in prompt clearance of the cutaneous lesions, relapses usually occurred, presumably because of the underlying immunodeficiency state. HIV infection or severe combined immune deficiencies affecting cell-mediated immunity predispose to mucocutaneous candidiasis. Evidence of impaired cell-mediated immunity to Candida spp. in these patients is represented by negative delayed-type hypersensitivity skin tests, absent or low T-cell proliferation in vitro, and impaired production of leukocyte/macrophage inhibitory factor [123].

Several human and murine studies suggest that impaired clearance of Candida spp. in patients with CMC is due to imbalance between Th1 and Th2 responses with bias toward the Th2 phenotype. Appropriate and timely induction of Th1 responses characterized by production of Th1 cytokines such as IL-2, IFN-γ, TNF-α [124, 125], and IL-12 [39, 66, 126], which activate and recruit effector cells such as macrophages or CD8+ cytotoxic T cells, are of major importance for protection and clearance of the yeast [123, 124]. In contrast, induction of Th2 responses marked by production of Th2 cytokines such as IL-4 and IL-10, which are necessary for mounting an antibody response but also downregulate type 1 cytokine production, had opposite detrimental effects [125]. Neutrophils are thought to be the primary effector cells and their production of either IL-10 or IL-12 likely directs the immune system toward either a Th1 or Th2 response [126]. Recruitment of neutrophils to the site of candida infection is dependent upon recognition of mannans by TLR4 [127].

Peripheral blood mononuclear cells from patients with CMC produce high levels of IL-10, but normal levels of IL-4 and IL-5, upon in vitro stimulation with carbohydrate and mannan fractions of C. albicans. Although Th1 cytokines such as IL-12 and IFN are also decreased in CMC patients, receptors for IL-12 and IFN are shown to be intact [125]. Two possibilities can account for the bias toward Th2 response in CMC patients. The first possibility is that altered cytokine environment caused by interaction of the yeast or hyphal forms of Candida with innate immune cells could inversely influence the induction of CD4+ Th1 cells producing IFN-γ and IL-2. The second possible mechanism could lie more upstream, involving the co-stimulatory functions of APCs such as DCs or macrophages [30, 128].

In support of the first possibility, recent evidence in mice indicates that innate immune cells such as neutrophils and macrophages discriminate between the hyphal and yeast forms of the fungus through binding to different PRRs such as TLR and lectin, being able to produce IL-12 in response to C. albicans yeasts, and IL-10 in response to C. albicans hyphae [30, 66]. The production of IL-12 and IL-10 can thus bias the CD4+ Th response to Th1 or Th2 phenotype, respectively. Th17 cells have also been recently implicated in maintaining a balanced immune response to CMC, which are thought to control fungal growth in the epithelium. Th17 cells produce IL-12, IL-21, and IL-22, cytokines which play a role in neutrophil recruitment, and therefore appropriate recognition, to the area of candida infection [127].

Interestingly, in vitro culture of DCs with the two forms showed that hyphae escaped the phagosome and were lying free in the cytoplasm of the cells, while the yeast form remained within the phagosome. The differences in fungal localization within DCs, therefore, may influence the access of fungal antigens to MHC class I (cytoplasmic) or II (phagosomal) pathways of antigen presentation to CD8+ and CD4+ T cells, respectively, which influences the ability of each fungal form to stimulate CD4+ or CD8+ T cells. Therefore, DCs can influence the type of specific immune response by discriminating between yeast and hyphal forms of the fungus. This can be accomplished through different TLRs, the downstream effect of differential TLR ligation on the cytokine environment, and differential fungal localization within DC.

Nevertheless, it remains to be identified whether the differential immune responses to different phenotypes of Candida is due to altered responses of immune cells or to expression of different virulent genes that allow evasion of host immune response and persistence. In support of the latter possibility, several studies suggested that phenotypic switching represents a pathogenic strategy for the combinatorial expression of batteries of genes leading to a variety of pathologic states. This conclusion was based on the findings that the process of phenotype switching between yeast and hyphal forms, which is characterized by different colony morphology, regulates the expression of a number of phase-specific genes, including PEP1 (SAP1), SAP3, OP4, CDR3, CDR4, NIK1, WH11, and EFG1 [23, 129, 130]. High-frequency phenotypic switching has also been demonstrated to regulate a number of phenotypic characteristics that have been implicated in pathogenesis, including antigenicity, sensitivity to neutrophils and oxidants, adhesion, and susceptibility to common antifungal agents [130, 131]. Whether similar host-microbial interaction involving putative inherent defects of dendritic cells in terms of different dendritic cell subsets or receptors on the cells or differential gene expression upon phenotype switch exists in CMC patients is not yet known.

Dermatophytosis

Clinical Manifestations and Pathogenesis of Dermatophytosis

The dermatophytes include a group of fungi (ringworm) that under most conditions have the ability to infect and survive only on dead keratin, that is, the top layer of the skin (stratum corneum or keratin layer), the hair, and the nails. Dermatophytes cannot survive on mucosal surfaces such as the mouth or vagina where the keratin layer does not form. Very rarely, dermatophytes undergo deep local invasion and multivisceral dissemination in the immunosuppressed host. Dermatophytes are responsible for the vast majority of skin, nail, and hair fungal infections. These types of infections, termed "dermatophytoses," are widespread and increasing in prevalence on a global scale [132, 133]. Reasons for this increase are not clear, although it may be due, in part, to an aging process. This observation is consistent with the view that changes in the immune response that occur with advancing age lead to disease susceptibility. In keeping with this, fungal nail infections are more frequent in immunocompromised patients such as those who are HIV positive and those who have diabetes [134, 135]. Alternatively, age-related changes in peripheral vasculature may be important in predisposing to infection. In addition to the effects of aging, genetic susceptibility has been proposed to contribute to infection. Although *Trichophyton rubrum* infection has been reported to show a familial pattern of autosomal dominant inheritance, more recent epidemiologic findings challenge this view [135]. Other factors that have been implicated include those associated with a modern lifestyle, including the use of footwear made from synthetic materials and exposure to dermatophytes in communal areas with damp environments that favor fungal growth, such as swimming pools and school gymnasiums [135–137]. Despite the identification of multiple predisposing factors, there is no consensus of opinion regarding a single mechanism to explain the increased incidence of foot disease that has occurred in recent years.

Dermatophytes are classified in several ways. The "ringworm" fungi belong to three genera: Microsporum, Trichophyton, and Epidermophyton. There are several species of Microsporum and Trichophyton, and one species of Epidermophyton. The inflammatory response to dermatophytes varies [133–138]. In general, zoophilic and geophilic dermatophytes elicit a brisk inflammatory response on skin and in hair follicles. The inflammatory response to anthropophilic fungi is usually mild. One very characteristic pattern of inflammation is the active border of infection. The highest numbers of hyphae are located in the active border, and this is the best area to obtain a sample for a potassium hydroxide examination. Typically the active border is scaly, red, and slightly elevated. Vesicles appear at the active border when inflammation is intense. This pattern is present in all locations except the palms and soles. Infections of the feet are particularly troublesome and affect both the skin (athlete's foot) and nails (tinea unguium). Despite the availability of new systemic antifungal therapies, nail infections are difficult to eradicate, with recurrence reported in up to 25 % to 40 % of cases. Since lesions vary in presentation and closely resemble other diseases, laboratory confirmation is often required.

Innate Immune Responses to Dermatophytes

Interaction of dermatophytes with many dermal and epidermal immune cell types including keratinocytes results in a cascade of chemokines and proinflammatory and anti-inflammatory cytokine responses [139–142]. Activation of the alternative complement pathway by dermatophytes such as the case with Trichophyton mentagrophytes also results in production of chemotactic factors. These chemotactic events and inflammatory responses are thus responsible for the observed macroscopic changes associated with dermatophyte infections including scaling, vesicles, pustules, annular dermatitis, and severe inflammatory reactions (kerions). Microscopically, the lesions are characterized by an accumulation of neutrophils in the infected skin of acute infections or a mononuclear cell infiltrate in the dermis of the more chronic ones [139–142]. The acute inflammatory responses may be manifested as epidermal microabscesses, while chronic dermatophyte infection is usually manifested as epidermal hyperkeratosis and parakeratosis [135–139].

Similar to other cutaneous mycosis, neutrophils mediate elimination of dermatophyte infections by a variety of microbicidal processes, including (1) microbicidal oxidants such as superoxide, hydrogen peroxide, hypochlorous acid, and monochloramine; and (2) nonoxidative microbicidal granules such as cathepsins, defensins, lactoferrin, lysozyme, elastase, azurocidin, and a number of other proteins [140]. Furthermore, neutrophils inhibit the growth of dermatophytes through release of large amounts of calcium and zinc-binding protein, called calprotectin that has potent microbiologic static activity against these fungi. This protein is released into inflammatory exudates as neutrophils degranulate at sites of infection [140, 141].

Acquired Immune Responses Against Dermatophytes: Immediate and Delayed-Type Hypersensitivity Reactions

Several of dermatophyte antigens are cross-reactive with airborne molds. These antigens stimulate IgE production, which mediates immediate hypersensitivity (IH) reaction in infected patients, mainly those with chronic dermatophytosis. The IH reaction in these patients is mediated by IgE antibody, and is characterized by a local wheal and flare occurring 5–20 min after injection of antigen into the skin. In this

process, binding of antigen to IgE antibody (Ab) on the surface of mast cells results in cross-linking of IgE Ab, which, in turn, triggers the degranulation of mast cells and release of histamine and other proinflammatory mediators [141, 142].

The IH skin tests to Trichophyton are associated with the presence of serum IgE and IgG Ab to Trichophyton antigens. IgG4 Ab is a major component of the IgG Ab response [136]. Thus, IH reactions to dermatophytes bear the hallmarks of a Th2 response. In this type of response, IL-4 produced by CD4+ Th2 cells induces antibody isotype switching to IgG4 and IgE [142, 143]. A role for fungal antigens (Ags) in allergic disease is strongly supported by the association between chronic dermatophytosis and allergic respiratory symptoms, and the improvement of late-onset asthma after antifungal therapy in patients with IH to Trichophyton.

By contrast, Trichophyton antigens are also able to induce delayed-type hypersensitivity (DTH) responses in some patients [133, 143]. Delayed-type hypersensitivity is a form of cell-mediated immunity in which the ultimate effector cell is the activated macrophage. In the classic DTH reaction, activation of macrophages is mediated by IFN-γ–producing Th1 CD4+ T lymphocytes [142–144]. However more recent studies have shown that both CD4+ and CD8+ T cells can have direct cytotoxicity to *T. mentagrophytes* and *T. rubrum*, although to a lesser extent [145].

These T cells recognize and respond to foreign antigen presented in the form of peptide complexed with MHC class II molecules expressed on the surface of APCs. This cell-mediated response is characterized by induration at the injection site, which is maximal at 48 h. There is considerable evidence that DTH is pivotal in the eradication of dermatophyte infection. This conclusion is based on data showing (1) the development of DTH in association with inflammatory responses in primary infections, (2) the association between acute highly inflamed lesions and DTH, and (3) the failure to develop infection after experimental inoculation when DTH is present. Interestingly, DTH to Trichophyton extract is associated with lower titers of IgG Ab to Trichophyton antigens and no IgE or IgG4 [142, 144, 146]. These observations suggest that humoral response to Trichophyton is less protective. A subset of patients also mounts "dual" skin test responses in which a DTH response follows the IH reaction. Whether this represents a transitional stage in DTH-to-IH conversion (or vice versa) is not known.

Several hypotheses have been proposed to explain the mechanism of induction or suppression of protective DTH responses in different individuals. One study suggested that development of IH suppresses DTH responses in patients with chronic dermatophytosis since most IH responses occur in the absence of DTH [146, 147]. Treatment of chronic dermatophytosis associated with IH responses with the systemic antifungal terbinafine can restore DTH responsiveness to intradermal trichophyton antigen. Another study proposed that prolonged antigen exposure can induce immunologic unresponsiveness, or anergy, by activating suppressor T cells, which then downregulate cell mediated responses. However, several studies have failed to demonstrate diminished T-cell proliferative responses to dermatophyte antigens in patients with IH [143, 146, 148, 149].

An alternative hypothesis was that the properties of the fungus itself may prevent the development of cell-mediated responses. For example, mannans derived from *T. rubrum* have been proposed to inhibit DTH by interfering with antigen-processing pathways required for T-cell activation. However, despite all these data, it is not yet clear whether chronic infection results from a lack of DTH or from the presence of an IH response. Thus, a major question is whether IH is a prerequisite for the development of chronic infection and, if so, whether it reflects a more broad-based immune dysregulation. The answer to this question is complicated by the fact that host factors, such as integrity of the skin barrier as well as immune status of the host, play a central role in determining the outcome of dermatophyte infection. Indeed, it is well recognized that patients with HIV infection or those receiving immunosuppressive therapy are predisposed to develop chronic dermatophytosis and sometimes an invasive disease. This observation is consistent with impairment of cell-mediated immunity associated with these conditions.

Changes in the balance between Th1 and Th2 responses with bias toward Th2 response have been implicated in the progression of several diseases such as HIV infection associated with chronic dermatophytosis [146]. Indeed, this would be consistent with the dogma that Th2 polarization contributes to disease progression in HIV-infected subjects. Similarly, chronic *T. rubrum* infection associated with IH and a markedly elevated total IgE level in the serum was proposed to contribute to the development of severe measles infection by favoring the development of Th2 responses [143, 144, 146, 147]. However, this does not explain the paradoxical association between chronic dermatophytosis and Th1-mediated diseases such as diabetes. Nevertheless, the association between diabetes and dermatophytosis is also complex, as diabetic complications such as peripheral vascular disease could contribute to persistent dermatophyte infection.

Although immune mechanisms involved in the natural resolution of infection have yet to be resolved, recent findings suggest that T cells with a defined specificity for Trichophyton antigens or epitopes are critical for development of DTH or IH responses in different individuals. This conclusion was based on comparison of the T-cell repertoire in subjects with distinct immune responses to a single Ag such as the 29-kd Tri-r-2 derived from *T. rubrum*. Interestingly, the differences in the T-cell repertoire between patients with IH and those with DTH were independent of

human leukocyte antigen (HLA) haplotype [143, 144, 150–152]. The clinical relevance of different T-cell repertoires remains to be determined. However, it is possible that certain antigenic peptides derived from Trichophyton antigens contain an epitope that specifically promotes the development of a DTH response, making progression to chronic dermatophytosis unlikely [150, 151, 153].

Malassezia Infection

Clinical Manifestation and Pathogenesis of Malassezia Infection

Yeasts of the genus Malassezia undergo asexual reproduction by monopolar budding. The yeast cell is actually a phialide that has a small collarette at its apex, which gives it an overall bottle-shaped appearance. Some of the species are able to undergo a phase transition from yeasts to hyphae, although the factors that control this transition are not clearly understood. The genus Malassezia is known to include at least seven species of yeast (M. furfur, M. pachydermatis, M. sympodialis, M. globosa, M. obtusa, M. restricta, and M. slooffiae). Except for M. pachydermatis, the species require an exogenous source of lipid owing to their inability to synthesise C14–C16 saturated fatty acids because of a block in the de novo synthesis of myristic acid [152]. Although Malassezia can be found on normal human skin, it has been implicated in a range of both cutaneous and systemic diseases such as seborrheic dermatitis, dandruff, folliculitis, atopic dermatitis (recently renamed atopic eczema/dermatitis syndrome [AEDS]), psoriasis, confluent reticulate papillomatosis, and seborrheic blepharitis [154–161].

Malassezia is most frequently associated with pityriasis (tinea) versicolor (PV), which is one of the most common disorders of pigmentation seen in dermatologic clinics worldwide [154, 155]. PV is a chronic superficial fungal infection of the skin. The Malassezia species that have been isolated from patients with PV include M. furfur, M. globosa, M. restricta, M. slooffiae, and M. sympodialis, with more than one species of Malassezia being present. PV most often occurs on the trunk, neck, and proximal extremities. It is characterized by scaly hypopigmented or hyperpigmented macules and patches with minimal pruritic reaction [152, 154, 155].

Malassezia folliculitis is characterized by follicular papules and pustules localized to the trunk, upper arms, neck, and, less often, the face [159–161]. These lesions are generally pruritic. Diagnosis is based on clinical signs, cytology, and culture in combination with histopathology. Although it has been suggested that follicular occlusion was the primary cause of Malassezia folliculitis with a secondary overgrowth of Malassezia organisms, colonization of normal pilosebaceous units by these yeasts can also be extensive. The exact role of Malassezia in Malassezia folliculitis, therefore, awaits further elucidation. Therapy for Malassezia folliculitis is similar to that described for PV. As with PV, recurrence tends to be a common problem.

Seborrheic dermatitis and dandruff are other diseases caused by Malassezia. Seborrheic dermatitis is characterized by inflammation and desquamation in areas that are rich in sebaceous glands such as the scalp, face, and upper trunk, whereas dandruff is a noninflammatory scaling condition of the scalp. Dandruff is now generally considered the mildest form or a variant of seborrheic dermatitis [160, 161]. The decreased severity of these diseases upon fungal therapy, the increase in the number of Malassezia organisms, and relapse upon discontinuation of therapy support a role of Malassezia as the etiologic agents in these two conditions. The species that have been isolated from patients with seborrheic dermatitis are similar to those causing PV, with M. furfur and M. globosa being most common. Atopic dermatitis is another chronic, multifactorial, inflammatory allergic skin disease associated with abnormal immunologic regulation and is associated with Malassezia infection [161–163].

Similar to dermatophyte antigens, allergens from Malassezia organisms have been implicated in its pathogenesis [162–164]. For atopic patients with a hypersensitivity response to Malassezia spp., antifungal therapy should be included in the treatment regime. In addition to the diseases described above, Malassezia spp. have also been shown to cause more deep-seated infections, including mastitis, sinusitis, septic arthritis, malignant otitis externa, fungemia, pulmonary vasculitis, peritonitis, and meningitis [159–162].

Immunology of Malassezia-Associated Diseases

There have been large numbers of studies examining the innate and acquired (cellular and humoral) immune responses to Malassezia in patients with many Malassezia-associated diseases [165]. For many of the diseases, the responses reported have varied widely. The innate response against Malassezia involves several components such as complement, phagocytosis, and NK-mediated lysis. Several groups have reported the ability of Malassezia to activate the complement system, via either the alternative pathway or the classic pathway [166–168]. The extent of activation of the alternative pathway was cell concentration and time dependent, reaching a plateau after 30 min. Although the molecule responsible for triggering the

alternative pathway is not well defined, ß-glucan in the cell wall may be involved [168]. The ability to activate complement has been suggested as a mechanism responsible for the inflammation associated with seborrheic dermatitis. Phagocytosis and intracellular killing of the yeast, mainly by neutrophils, is another important innate mechanism by which nonspecific effector cells play a role in host defense against Malassezia [169, 170].

The importance of phagocytosis in protection against fungal infections is highlighted by the increased susceptibility of neutropenic patients to many mycoses. In vitro, neutrophils take up Malassezia in a complement-dependent process. On the other hand, the receptors involved in phagocyte–yeast cell binding have been characterized in a human monocytic cell line as the mannose receptor, ß-glucan receptor, and complement receptor type 3 [171]. Recent studies have shown that when a monocytic cell line, THP1, was stimulated with either live or heat-killed Malassezia, the production of IL-8 was increased, while stimulation of a granulocytic cell line, HL-60, resulted in increased levels of both IL-8 and IL-1β [172]. The effects of IL-1β in host defense against cutaneous fungal organisms include the activation of lymphocytes, chemotaxis, and neutrophils and the induction of inflammation [172–174]. Interleukin-8 also induces chemotaxis and activation of neutrophils and T cells. Therefore, the interaction of Malassezia with phagocytic cells may serve to amplify the inflammatory response and encourage further recruitment of phagocytic cells.

Opsonized live Malassezia yeast cells are more stimulatory than were nonopsonized or heat-killed Malassezia yeast cells. However, the ability of neutrophils to kill Malassezia seems limited compared to efficient killing of *C. albicans* yeast cells and other fungal genera. The mechanism by which Malassezia may resist or prevent phagocytic killing is not completely clear. In addition to escaping phagolysosomal killing, in vitro studies suggested an immunosuppressive effect of Malassezia on activation of different innate effector cells [174, 175]. *M. furfur* was able to invade human keratinocytes and resist phagolysosomal fusion, which allows their survival inside host cells. In addition, culture of yeast cells of *M. furfur* with normal human keratinocytes did not stimulate cytokines or chemokines production.

Low levels of monocyte chemotactic protein 1 (MCP-1), TNF-α, and IL-1β (IL-1β) were found, which were associated with overproduction of immunosuppressive cytokines IL-10 and tumor growth factor-β (TGF-β) [175]. It was further postulated that the suppression of proinflammatory cytokines might allow Malassezia to survive within host cells without causing an inflammatory response. Suppression of IL-1β, IL-6, and TNF-α also has been reported when Malassezia was co-cultured with peripheral blood mononuclear cells (PBMCs), and that the suppression was IL-10 dependent [175]. This correlates with the situation seen in normal healthy skin and also with the limited inflammation seen in PV, despite the large fungal burden seen in the lesions [176, 177]. It is known that the immunosuppressive effects of Malassezia on PBMCs can be reversed by removal of the lipid-rich capsular-like layer around the organism, and it will be interesting to determine if this is also the case with keratinocytes [178].

The role of DC and NK cells in innate and acquired immune responses against Malassezia in atopic and nonatopic individuals has been extensively studied. Uptake of whole M. furfur yeast cells and various allergenic components from the yeast, including M. furfur extracts, recombinant M. furfur allergen 5 (Mal-f-5), and M. furfur mannan by immature monocyte-derived dendritic cells (MDDCs) has been demonstrated in vitro [179, 180].

The internalization of Malassezia was shown to occur via binding to the mannose receptor (other receptors may also be involved) or pinocytosis and is not influenced by IgE. The presence of M. furfur was also shown to induce maturation of immature MDDCs by upregulation of CD83 expression, and also resulted in increased expression of the co-stimulatory molecules CD80 and CD86 [179–181].

The uptake of the yeast by the DCs also induced a significant production of TNF-α, IL-1β, and IL-18, but not IL-10 or IL-12, after 46 h of co-culture. Although immature DCs are highly phagocytic, mature DCs are excellent at presenting antigen-derived peptides on MHC molecules to T cells [182]. Thus, the DCs that had been exposed to Malassezia induced proliferation of autologous T lymphocytes in a dose-dependent way [182–184].

The interaction of Malassezia-infected mature DCs with NK cells in atopic patients also has been examined by comparing the numbers of NK cells in the normal skin of healthy controls with those in the atopic skin of atopic dermatitis or AEDS patients [179, 185]. These studies showed that there were only scanty NK cells in normal skin, but that they were numerous in the atopic skin, and they were in close apposition with DCs. Dendritic cells that had been preincubated with Malassezia for 46 h were less susceptible to NK-mediated lysis, and this resistance to NK lysis was mediated by soluble factors [185–189].

This protection of DCs against NK-mediated lysis, if it were to occur in vivo, would allow the mature dendritic cells to remain in the epidermis, presenting Malassezia antigens to T cells and hence contributing to the maintenance of the inflammatory response in AEDS lesions [188, 189].

The effect of different forms of Malassezia on the responses of DCs has been examined. Similar to that described in immunity against *C. albicans*, the yeast phase

elicits production of IL-12 and priming of Th1 cells, while the hyphal phase inhibits IL-12 and Th1 priming, and induces production of IL-4, a Th2-type cytokine [190–192]. Similar to other fungi, Th1 T-cell–mediated immunity is important in the prevention and recovery from infections. A deficiency in cell-mediated Th1 responses could therefore predispose the host to overgrowth of Malassezia. Atopic patients with specific IgE antibodies against M. furfur were shown to have increased synthesis of the Th2-related cytokines IL-4, IL-5, and IL-10 by Malassezia-stimulated PBMCs [190–192].

In regard to humoral immune responses against Malassezia, it is clear that IgG responses to Malassezia yeasts are common in both healthy individuals and patients with Malassezia-associated diseases. This probably reflects exposure of the immune system to antigens produced by commensal organisms [193]. However, enhanced IgG responses can be seen in humans with atopic dermatitis. The role of this IgG response in the pathogenesis of skin disease is currently unclear. IgG antibodies are known to be able to act as opsonins coating microorganisms and to activate phagocytes, which in turn ingest and destroy extracellular pathogens. This could in theory provide protection for the host. However, as overgrowth with Malassezia does not appear to be a self-resolving condition, it seems likely that these antibodies are not protective [194, 195].

Alternatively, IgG antibodies could activate the complement system, as has been demonstrated with *Pityrosporum ovale* and *P. orbiculare*, and exacerbate the inflammatory response [196]. A final possibility is that IgG responses to the yeast are merely an association and neither contributes to nor inhibits the ongoing disease process [195]. Further studies are therefore required to determine the precise role played by these antibodies in Malassezia-induced skin disease. Using in vitro serologic tests such as enzyme-linked immunosorbent assay (ELISA), the radioallergosorbent test (RAST), and Western immunoblotting, Malassezia-specific IgE has been detected in human atopic patients for over a decade [194, 195].

Stimulation with Malassezia extracts and IL-4 led to a dose-dependent increase in IgE synthesis from PBMCs only in RAST(+) atopic patients, indicating a Th2-type skewed response towards Malassezia in these patients. The Malassezia-specific IgE antibodies in human atopic patients could play a key role in enhancement of immune responses [196–198]. The allergen-specific IgE antibodies could bind to Langerhans cells in the skin, thus enhancing their allergen capturing and presentation capacity upon a second encounter with the allergen. In addition, IgE could mediate mast cell-mediated hypersensitivity responses to Malassezia allergens, and that may be involved in the pathogenesis, and contribute to the clinical signs, in some cases of human atopic dermatitis.

Subcutaneous Mycosis

Chromoblastomycosis

Clinical Manifestations and Pathogenesis of Chromoblastomycosis

Chromoblastomycosis is a term that designates a group of chronic cutaneous and subcutaneous mycoses caused by several species of dematiaceous (darkly pigmented) fungi. As a member of the heterogeneous group of subcutaneous mycoses, chromoblastomycosis commonly presents the following typical features: lesions beginning at the site of a transcutaneous trauma, chronic evolution associated with survival of the fungal agent, and fibrotic reaction [199–201] as seen in Fig. 19.4. In tissues, all agents form thick-walled, dark multiseptate structures—the muriform (sclerotic) cells. It is common worldwide but occurs mostly in tropical and subtropical areas of Africa, Asia, and South America. Chromoblastomycosis is considered an occupational disease in many tropical and temperate countries (Madagascar, northern Venezuela, and the Amazon region of Brazil) [202–204].

The disease is caused by a large number of fungi that exist in the soil, plants, flowers, and wood. The most common agents are *Fonsecaea pedrosoi* and *Cladophialophora carrionii*. The latter is considered to be the most important etiologic agent in deserts in South Africa and Australia [202–204]. Less frequently, the disease is caused by *Phialophora verrucosa, Rhinocladiella aquaspersa*, as well as *Wangiella* or *Exophlaia* dermatitidis. Other etiologic agents that were discovered recently are *Exophiala jeanselmei* and *Exophiala spinifera. Fonsecaea compacta* has also been observed in lesions of chromoblastomycosis. The etiologic agents enter the host through cutaneous puncture wounds, usually on a thorn or a splinter. Other kinds of trauma such as animal-associated trauma (insect bite or sting) are identified as the portal of entry of the fungus.

A primary lesion is represented by a papule that slowly enlarges over time and can ulcerate. The primary lesion can progress to polymorphic skin lesions, including nodular and verrucous lesions. Sometimes the lesions can heal as sclerotic plaques, scars, or keloid formations [199–204]. The most frequent clinical presentation is a cauliflower-like lesion that develops at the site of inoculation, and satellite lesions gradually arise from scratching, autoinoculation and spread via the lymphatic system. Secondary lymphedema and pruritus are common findings, but the disease remains confined to the subcutaneous tissue and does not invade underlying muscles and bone, except in immunosuppressed patients such as those on high-dose corticosteroids. The lower limb is the most common site followed by the upper limb, ear pinna, and nose [199–204]. The diagnosis can be

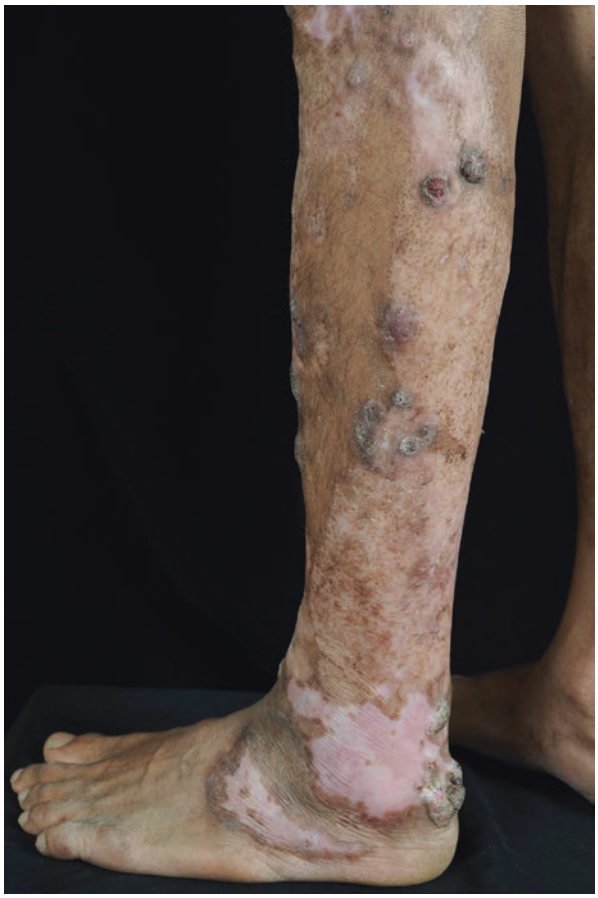


Fig. 19.4 Leg of a patient with chromoblastomycosis

made by detection of multiform cells, referred to as sclerotic bodies, from tissue biopsies or from fungal culture. Hydrogen peroxide and hematoxylin and eosin (H&E) are recommended stains for elucidation of the sclerotic bodies in tissues [204, 205]. The hyphae and budding cells are usually seen in the surface of the lesion, while the muriform cells are often seen in the deep part of the lesion. The treatment of chromoblastomycosis is difficult due to its limited response to oral antifungal therapy [206, 207]. Medical treatment usually includes combined use of antifungal drugs such as itraconazole with terbinafine. Other treatment strategies include a combination of cryotherapy, 5-fluorocytosine, and amphotericin B. Surgical treatment is the ultimate effective therapy, which involves surgical removal of lesions, electrodesiccation, and cryosurgery.

Immunology of Chromoblastomycosis

The host defense mechanism in chromoblastomycosis has not been extensively investigated. The first line of defense utilizes HLA-DR and costimulatory molecules, like CD86, TNF-α, IL-10, and IL-12, to activate dendritic cells. Although melanin produced by *F. pedrosoi* has been suggested to play a role in immune evasion, other research suggests that melanin stimulates the immune response by helping to activate TLR4 leading to the production of IL-8, increasing fungal internalization and amplifying oxidative burst. Mannose in the cell wall may also contribute to this immune reaction [208].

Some studies have focused on fungus–host interactions, showing a predominantly cell-mediated immune response, with activated macrophages involved in fungus phagocytosis [209–211]. The highly organized inflammatory reaction and chronic nature of chromoblastomycosis is characterized by the presence of a granulomatous reaction in association with neutrophil-rich, purulent abscesses. This granulomatous reaction shows extensive phagocytosis of brown thick-wall fungal cells, which is considered the main factor explaining the chronic and inflammatory nature of the disease [204, 212]. However, the degree of phagocytosis and cytotoxicity depends on the fungal species causing infection. *P. verrucosa* and *R. aquaspersa* rely on complement-mediated phagocytosis and macrophages are only cytotoxic on *R. aquaspersa* [208].

Macrophages (referred to as epithelioid or giant cells) are highly activated as marked by higher expression of TNF-α and their enhanced in vitro antifungal activities marked by H2O2 and NO production [213, 214]. Inhibition of macrophage synthesis of NO by fungus-derived melanin has been proposed as an immune evasion mechanism that prevents the host from clearing *F. pedrosoi*, leading to a chronic disease [215]. CD4+ Th1 cells and type 1 response are important for host defense against causative agents of chromoblastomycosis [216]. Patients with a mild form of the disease have predominant production of IFN-γ, low levels of IL-10, and efficient T-cell proliferation, consistent with a type 1 response, while patients with the severe form of the disease have predominant production of IL-10, low levels of IFN-γ, and inefficient T-cell proliferation, consistent with an immunosuppression rather than Th2 response [216, 217] Histopathologic and immunohistochemical staining of skin tissues from patients with the verrucous form of the disease has identified CD4+ T lymphocytes at the periphery of the granulomas, with immunostaining for IL-10. These findings suggest that either a Th2 phenotype or immunosuppression is linked to severe or verrucous forms.

Furthermore, patients with chromoblastomycosis produce specific IgM, IgG, and IgA antibodies [209] and levels may correlate with disease chronicity [208]. Several cell wall and secreted immunoreactive antigens of *F. pedrosoi* have been identified. Antifungal antibodies specific to secreted melanin inhibit fungal development, support fungal internalization, and enhance macrophage functions as marked by greater degrees of oxidative burst [52]. Sera from infected human patients also reacted with secreted melanins, suggesting that

F. pedrosoi synthesizes melanin in vivo [52]. Antibodies against melanin purified from patients' sera also reacted with sclerotic cells from patients' lesions as well as with sclerotic bodies cultivated in vitro—conidia, mycelium, and digested residues [218].

Taken together, these results indicate that melanin from *F. pedrosoi* is an immunologically active fungal structure that activates humoral and cellular responses that could help the control of chromoblastomycosis. Glucosylceramide (GlcCer) is another immunoreactive conserved lipid component in the cell wall of many fungi including dark fungi. Antifungal antibodies specific to GalCer directly inhibit fungal development, which supported the use of monoclonal antibodies to GlcCer as potential tools in antifungal immunotherapy [219, 220]. However, unlike the clear correlation between mild disease and cell- mediated type 1 response, there is inconclusive evidence to support a protective role of the humoral immune response in host defense against chromoblastomycosis.

Mycetoma

Clinical Manifestation and Pathogenesis of Mycetoma Infection

Mycetoma is a chronic granulomatous infection caused by fungi (eumycetoma) or actinomycetes (actinomycetoma). The first description of a case of mycetoma is usually attributed to Dr. John Gill, who reported "Madura foot" in a report of the Madras Medical Service of the British Army in India in 1842 [221–224]. The mycetoma lesion is characterized by a subcutaneous mass and multiple sinuses draining pus, blood, and fungal grains as seen in Fig. 19.5. The morphologic characteristics and color of the grains provide clues about the species of the agents. The mycetoma infection has a prolonged and indolent course. The mycetoma lesion might ultimately extend to deeper tissues and bones, leading to deformity of the affected site and subsequent disability for the patient. This disease has been reported in many countries including Sudan, Somalia, Senegal, Mauritania, Kenya, Niger, Nigeria, Ethiopia, Chad, Cameroon, Djibouti, India, Yemen, Mexico, Venezuela, Columbia, and Argentina [221–225].

Mycetoma infection is not self-limited and, if untreated, leads to massive lesions, which in the end necessitate surgical amputation. Mycetoma initially presents as a slowly progressive and painless subcutaneous swelling, sometimes in combination with a history of preceding trauma [225–227]. However, the incubation time before classic signs develop is not well defined because in many cases the patients usually present at later stages of disease when most early clinical symptoms have disappeared.

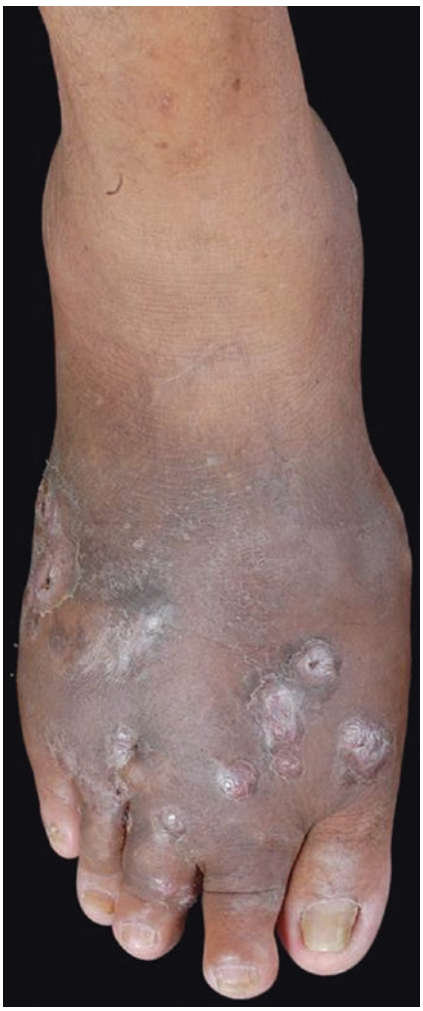


Fig. 19.5 Clinical example of Madura foot from mycetoma infection

The duration of the disease, the type of causative organism, the site of the infection, and the immune response of the host can all affect the clinical presentation of mycetoma. However, patients with a short disease history might present with massive lesions and severe destruction of deep tissues and even of bones. The subcutaneous swelling is usually firm and rounded but it can also be soft and lobular. It is rarely cystic and is often movable. The subcutaneous nodule increases in size, and secondary nodules might evolve as well. The nodules might suppurate and drain through multiple sinus tracts, which can close transiently after discharge during the active phase of the disease. The disease process involves opening of fresh adjacent sinuses while the old ones heal completely. The nodules are connected to the skin surface and to each other through deep sterile abscesses.

Mycetoma can affect any part of the body. Most cases are usually seen in the feet, followed by the hands, legs, and knee joints. Rarely, the chest and abdominal walls, facial bones, paranasal sinuses, eyelid, orbit, scrotum, and old surgical incisions might also be affected [224–228]. Mycetoma

spreads locally or through the lymphatic system, and, rarely, through the bloodstream. Compared to actinomycetoma where secondary nodules that represent lymphatic metastasis, are common, eumycetoma is rarely associated with secondary nodules [229].

Mycetoma is usually painless, and the mycetoma lesion has been suggested to produce substances that have an anesthetic effect. At a late stage of the disease, the absence of pain might be due to nerve damage by the tense fibrous tissue reaction or endarteritis obliterans, or alternatively, poor vascularization of the nerves. In the final stages of the disease, pain might be due to invasion of the bone or to secondary bacterial infection. As the mycetoma granuloma increases in size, the skin may become smooth and shiny, and areas of hypopigmentation or hyperpigmentation can develop [226–230].

Diagnosis of mycetoma is based on the presence of subcutaneous masses, sinuses, and granular discharge in patients from an endemic area. Madurella species, the causative agents for eumycetoma, in general are slow-growing fungi that produce dark colonies composed of a dense, melanized, and mostly sterile mycelium. Madurella species are well-known agents of black-grain mycetoma. Two species are recognized, *M. mycetomatis* and *M. grisea.*

In different culture media, *M. mycetomatis* strains show various colonies and moderate growth rate. The colonies, which are white and woolly at first, becoming olivaceous, yellow, or brown, and produce a brownish, diffusing pigment. Colonies are mostly sterile, composed of dense melanized mycelium. No efficient sporulation has ever been seen [231, 232].

However, phialides with minute conidia in balls and collarettes may be seen. Species-differentiation of *M. mycetomatis* and *M. grisea* can be made by differences in sugar assimilation and optimal growth temperature. *M. mycetomatis* assimilates lactose but not sucrose, whereas *M. grisea* assimilates sucrose but not lactose. *M. mycetomatis* grows well at 37 °C, while *M. grisea* does not grow at 37 °C (growth is seen at 30 °C). This finding might also explain the observed difference in virulence.

Molecular tests have recently been developed and have significantly improved the diagnosis. The polymerase chain reaction (PCR) test is useful for detection and identification of *M. mycetomatis* in patients [233]. The test showed that a wide range of agents can actually cause eumycetoma. When different isolates of *M. mycetomatis* derived from patients originating from endemic areas were genetically compared, they did not show major differences, despite differences in phenotypes seen when cultured [231–233]. The diverse clinical presentations thus seem to be due to differences in host susceptibility rather than gross genetic differences among the fungal strains involved. In addition to culture, biochemical, and molecular identification of the fungal etiologic agents of mycetoma, other diagnostic tests are important in developing an appropriate plan of treatment [234–236]. Magnetic resonance imaging has been shown to be valuable in the detection and even identification of fungal grains and for the assessment of therapeutic success [236]. Furthermore, radiology helps in clinical diagnostics, especially in the follow-up of disease progression, development of a surgical strategy, and assessing the clinical cure. At the level of the fungus, direct examination of grains might be useful in determining the type of mycetoma [234, 235]. The large numbers of causative organisms and the poor in-vitro differentiation of fungi that cause mycetoma complicate the identification process. Histopathologic examination is generally not useful for differentiation of fungi, although some of the pigmented fungi may be categorized to a certain extent [237]. Fine-needle aspiration cytologic methods for mycetoma have also been described and are considered to be useful. Finally, serodiagnosis also can be helpful in identification of the etiologic agent [238–242]. The common serologic tests are immunodiffusion and ELISA; however, due to possible cross-reactivity between some species, the specificity of these assays is compromised [229, 238–242].

In tissues, *M. mycetomatis* forms numerous black sclerotia (grains). Grains are vegetative aggregates of the fungal mycelium embedded in a hard brown matrix, which consists of extracellular cement that seems to be 1,8-dihydroxynaphthalene melanin in combination with host tissue debris. This rigid matrix might act as a barrier protecting the fungus from the natural immunity of the host and antifungal agents. Melanins are thought to be protective in cases of host-induced oxidative stress. Two types of grains have been identified: filamentous and, less commonly, vesicular. Triple-layered tissue reaction zones have been described around the grains. An inner neutrophil zone immediately around the grain, an intermediate zone containing mainly macrophages, and an outer zone consisting of lymphocytes and plasma cells mainly can be seen under the microscope [234, 235, 237]. The medical treatment of eumycetoma is difficult, as the in vivo activity of azoles is often poor especially in late, advanced cases, but lesions of patients under ketoconazole treatment remain localized and well encapsulated. Thus, long-term treatment with itraconazole seems to be the best therapeutic regimen at present. Treatment of advanced cases usually implies amputation of the infected limb.

Immune Responses in Mycetoma

In 1964, Mahgoub [238] was able to demonstrate that eumycetoma patients developed Abs against *M. mycetomatis*. Counterimmunoelectrophoresis, immunodiffusion, and ELISA were developed to detect Abs raised against different mycetoma causative agents, using crude culture extracts as

Ag [239–241]. However patients without mycetoma infection who live in endemic areas also have elevated Ab levels, yet do not develop disease. In mouse models, mycetoma only developed when introduced with an adjuvant, which led to a Th2-dominant response [243].

It was not until the second half of the 1980s, that it was demonstrated that the cytoplasm, organelles, and, predominantly, the cell wall of *M. mycetomatis* were antigenic. About the same time it was also determined that IgM and IgG were the dominant immunoglobulins resulting from mycetoma. In 1991, the first attempts were made to characterize the nature of the epitopes present in the crude extracts used for the initial experiments. Cytoplasmic proteins were extracted from several eumycetoma agents, and, although the different *M. mycetomatis* isolates had very heterogeneous protein profiles by sodium dodecyl sulfate–polyacrylamide gel (SDS-PAGE), the antigenic makeup was quite similar within the species [242]. However, information about the individual immunoreactive Ags was not examined in this study [239–242].

A recent study identified the first immunogenic Ag, a protein homologous to the translational controlled tumor protein (TCTP), a well-conserved histamine release factor in a range of eukaryotes [244]. The gene for this Ag was demonstrated to be present in two variants in *M. mycetomatis*, with 13 % amino acid difference between the two proteins encoded. In vitro, TCTP was secreted into the culture medium. In vivo, this protein was found to be expressed on hyphae present in developing stages of the eumycetoma-characteristic black grain. Significant IgG and IgM immune responses, against the whole protein and selected *M. mycetomatis*–specific peptides, were detected. The Ab levels correlated with lesion size and disease duration. Overall, the patients with the largest lesions had the highest Ab level, which lowered with decreasing size of the lesion. Similarly, prolonged duration of the disease was associated with the highest Ab levels. Whether these TCTP-specific antibodies are markers of progressive and chronic disease or whether they play a protective role in host defenses against *M. mycetomatis* infection is not yet known. Nevertheless, this TCTP is considered to be the first well-characterized immunogenic Ag that can be used as a monomolecular vaccine candidate against *M. mycetomatis*.

Dimorphic Fungi with Cutaneous Involvement

Sporotrichosis

Clinical Manifestations and Pathogenesis of Sporotrichosis

Sporotrichosis is caused by a thermo-dependent dimorphic fungus, *Sporothrix schenckii*. The hyphal form that is present in the normal environment consists of both conidia and hyphae, while the yeast form develops at 37 °C [245]. The conidia or hyphae enter the body through either traumatic implantation or inhalation. However, *S. schenckii* is observed only as the yeast form in biopsies or excised specimens. Such conversion from hyphal to yeast form seems to occur in both the implantation and inhalation sites. Clinical manifestations of sporotrichosis are variable [245–249].

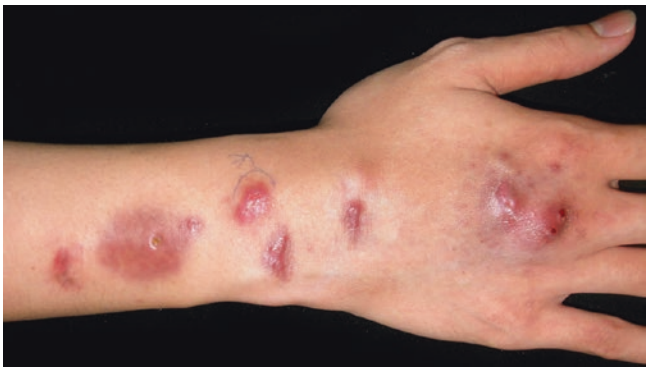


Fig. 19.6 Sporotrichoid lesions on the upper extremity

The major clinical manifestations occur in the skin and present primarily as either fixed cutaneous or lymphocutaneous forms. The lymphocutaneous form is depicted in Fig. 19.6. However, cases of disseminated cutaneous or visceral forms in immunosuppressed patients have reportedly increased.

Immune Responses in Cutaneous and Visceral Sporotrichosis

Both virulence factors of the individual *S. schenckii* strains and the immunologic status of the host determine the clinical manifestations of sporotrichosis [250, 251]. From the host perspective, cell-mediated immunity to *S. schenckii* antigen is a key immunologic defense mechanism that controls infection with *S. schenckii* [250, 251]. Both CD4+ Th1 cells producing IFN-γ and macrophages are required for the development of granuloma formation, which is a critical and essential component of normal host defense against the pathogens. Interferon-γ messenger RNA (mRNA) is detected in the granulomatous skin lesions of sporotrichosis [252], and immunohistochemical analysis demonstrated the existence of IFN-γ–producing CD4+ T cells in the periphery of such lesions [253]. The Th1 response activates macrophages to kill intracellular *S. schenckii*. Other studies demonstrated a CD83+ DC subpopulation in the granulation tissue of sporotrichosis, which indicates that activated DCs that express co-stimulatory molecules such as CD83 may play important roles in the Th1 immune response against *S. schenckii* [254].

From the fungal pathogen perspective, infection with different strains of *S. schenckii* possessing special virulence factors is a critical factor that contributes to different clinical manifestations of individual *S. schenckii* strains (cutaneous

versus visceral strains) [255, 256]. *S. schenckii* of cutaneous origin and yeast forms are more potent at activating DCs to induce subsequently stronger Th1-prone immune responses than those of visceral origin as evidenced by (1) higher expressions of HLA-DR and costimulatory molecules such as OX40L on DC and (2) higher induction of Th1 cytokines (IFN-γ and TNF-α). In contrast, *S. schenckii* of visceral origin positively induced a Th2 cytokine environment as evidenced by significantly higher IL-4 production and the inability to induce strong Th1 immune responses. Thus, similar to immune responses generated against other fungi described in this chapter, the Th1/Th2 balance may explain the differential clinical manifestations observed in cutaneous versus systemic or visceral sporotrichosis [55, 255–257].

Although the exact mechanism that accounts for differential Th1/Th2 responses following cutaneous and visceral sporotrichosis is not known, two possibilities are postulated: (1) different efficacies for the internalization of individual *S. schenckii* strains may affect the immunostimulatory response of DCs; and (2) differential expressions of surface molecules on *S. schenckii* could contribute to differential abilities of these strains to stimulate DCs via TLR or other PRRs such as lectin [258–260]. Examination of fungal internalization reveals no difference between the cutaneous and visceral strains. Therefore, the second possibility involving differential interaction of different strains with different PRRs on various APCs is the most likely mechanism that accounts for different clinical manifestations between the two strains of Sporothrix [121, 261–263]. The PRRs that recognize *S. schenckii* have not yet been identified in humans; however, TLR2 and TLR4 appear to be plausible because many fungi such as *C. albicans*, *Aspergillus* spp., and *S. cerevisiae* are recognized through TLR2 and TLR4. However, recent murine models of sporotrichosis implicate TLR4 in the induction of the oxidative burst [264].

Recognition of fungi via TLR2 and TLR4 activates several signaling molecules including JNK, ERK, p38 MAPK, and NF-kB pathways. While TLR induces release of proinflammatory cytokines such as IL-6 and TNF-α, TLR2 signals mediate anti-inflammatory effect by release of IL-10 that shifts the immune responses toward the Th2 phenotype. Since IL-10 was not detected in studies involving interaction of *S. schenckii* with DCs, these studies suggested that TLR4, but not TLR2, might be the receptor to recognize *S. schenckii* of cutaneous, but not visceral, origin, which induces a strong Th1 immune response. In conclusion, *S. schenckii* of cutaneous, but not visceral, origin may be localized to the skin due to stimulation of protective Th1 responses [121, 261–263, 265]. Nevertheless, the immune status of the host might be another contributing factor that accounts for the limited versus disseminated disease in cutaneous and visceral sporotrichosis, respectively.

Coccidioidomycosis

Clinical Manifestations and Pathogenesis of Cutaneous and Systemic Disease

Coccidioidomycosis (San Joaquin Valley fever) is a mycotic disease caused by *Coccidioides immitis* and the newly proposed phylogenetic species *C. posadasii*. The fungus propagates in soil in the regions of the southwestern United States, Mexico, and Central and South America, in a region corresponding to the Lower Sonoran Life Zone [266]. The saprobic phase is characterized by mycelia that give rise to infectious arthroconidia, which become aerosolized when the soil is disturbed. Humans acquire the infection by inhalation of the arthroconidia, which differentiate into large, endosporulating spherules once they are in the host. The disease presents a diverse clinical spectrum that includes inapparent infection, primary respiratory disease (usually with uncomplicated resolution), stabilized or progressive chronic pulmonary disease, and extrapulmonary dissemination involving skin, which can be acute, chronic, or progressive. The degree of severity varies considerably within each category and depends, in part, on the dose of inhaled arthroconidia, the genetic predisposition of the host, and the host's immunologic status [266–270]. Cutaneous infection with Coccidioides can also be acquired via a percutaneous route. Most percutaneous infections occur in laboratory workers as a result of a hypodermic injection of Coccidioides. Primary cutaneous coccidioidomycosis is characterized by a painful suppurative lesion at the site of inoculation, often with regional lymphadenopathy. Most of the cutaneous coccidioidomycosis acquired via this route is self-limited, and most cases have remained localized [266–270].

Immune Response Against Cutaneous and Systemic Coccidioides

Recognition of coccidioides spherules is dependent upon macrophages, TLR2, and dectin. TLR4 is not involved in recognition of infection, but is necessary in preventing dissemination of disease [271]. Polymorphonuclear leukocytes (PMNLs) comprise the earliest cellular influx to be arthroconidia. Ingestion of the arthroconidia is followed by a respiratory burst. However, fewer than 20 % of the arthroconidia are killed by this mechanism, and some studies suggest that PMNL may promote the maturation of arthroconidia into endosporulating spherules. Transformation of arthroconidia into spherules renders the latter impervious to phagocytosis and killing by PMNLs [272, 273]. Rupture of the spherules and release of the endospores triggers an influx of PMNLs. Ingestion of the endospores triggers an oxidative burst, and the level of intracellular killing is less effective compared to that observed in the killing of arthroconidia by PMNLs [272]. Both arthroconidia and endospores are phagocytosed by

monocytes/macrophages, but less than 1 % of the phagocytosed cells are killed. One mechanism that Coccidioides might use to survive intracellularly is the inhibition of phagosome-lysosome fusion, a strategy used by many intracellular pathogens to evade the antimicrobial effects of phagocytes. Co-incubation of monocytes/macrophages with immune T lymphocytes or IFN-γ significantly enhanced their anticoccidioidal activity [272–274].

Natural killer (NK) cells are a major component of innate immunity against Coccidioides. On activation, NK cells secrete cytokines, notably IFN-γ, and chemokines that induce inflammatory responses and control the activation of monocytes and granulocytes. Before adaptive immunity has fully developed, NK cells are thought to the main source of IFN-γ, in response to macrophage-derived IL-12 and IL-18 [275]. In addition, some studies suggest a direct cytotoxicity of NK cells to Coccidioides. Dendritic cells also play a pivotal role in innate immunity and adaptive immunity against Coccidioides by producing IL-12 [271]. As described before, DCs are sentinel cells that trigger T-cell responses against several fungi. A study has shown the potential immunotherapeutic use of DCs when the anergy demonstrated by peripheral blood lymphocytes from patients with disseminated coccidioidomycosis could be reversed by the addition of DCs pulsed with coccidioidal antigen [276]. Although the study was conducted in vitro, additional studies of the restoration of immunity by DC immunotherapy in animal models could reveal a new avenue for adjunctive therapy in severe coccidioidomycosis [277, 278].

Pro-inflammatory and Th1 cytokines play a dominant protective role in antifungal host defense. It has been reported that heat-killed spherules and arthroconidia of Coccidioides induced the production of TNF-α by adherent mononuclear cells from healthy human donors. TNF-α has several biologic functions, including its ability to activate neutrophils, enhance the cytolytic activity of macrophages, augment NK-cell activity, and promote T and B-cell proliferation. TNF-α has also been implicated as a major component in host-mediated destruction of host tissue. Similarly, IFN-γ production is associated with protection against Coccidioides infection, and significantly lower levels of IFN-γ are detected more frequently in patients with disseminated disease than in healthy, skin test–positive persons. Incubation of the monocytes with recombinant human IFN-γ or recombinant TNF-α augmented the fungicidal capabilities of the monocytes via increases in phagosome-lysosome fusion [274–276, 279, 280]. The mechanism by which IFN-γ or TNF-α activates human monocytes to kill Coccidioides is not known, but in studies with other intracellular pathogens such as those examining responses of human alveolar macrophages from tuberculosis patients, IFN-γ and TNF-α activate the macrophages to generate nitric oxide and related reactive nitrogen intermediates via nitric oxide synthase, using l-arginine as the substrate.

Recently, Th2 and Th17 immune responses have also been found to play a protective role against coccidioidal infection. Eosinophilia is a common finding in patients with coccidioidomycosis and cytokines IL-5 and eotaxin (CCL11), which are chemoattractants for eosinophils may also serve as stimulators of the Th2 response. In recent vaccine studies, it has been suggested that the Th17 response may be the most vital reaction to coccidiodial infection and lack of this response, as demonstrated by a mouse model with no IL-17 receptor on lymphocytes, may lead to increased mortality [281].

Different profiles of adaptive immune responses in persons with various clinical forms of coccidioidomycosis have been characterized. Persons with primary, asymptomatic, or benign disease characteristically have strong skin test reactivity to coccidioidin (the classic antigen preparation that was used in the early skin test and serologic studies), and low or nondemonstrable levels of anti-Coccidioides complement fixation (CF) antibody [279, 280, 282]. The converse pattern develops in patients who develop severe, chronic, or progressive pulmonary or disseminated disease. Typically, these persons, in particular those with disease involving two or more organ systems, are hyporesponsive or show anergy to coccidioidal skin testing but have high levels of anti-Coccidioides IgG to the CF antigen. Recovery from active disease, either spontaneous or in response to antifungal therapy, is in many patients associated with a reacquisition of T-cell reactivity to Coccidioides antigens and decreased CF antibody titers [279, 280, 282].

Chronic or progressive coccidioidomycosis is associated with a polyclonal B-lymphocyte activation, as evidenced by elevated levels of IgG, IgA, and IgE in serum. Antibodies reactive with coccidioidal antigens have been demonstrated within each of these Ig classes [2285-286,291]. Two separate antibody classes are involved, coccidioidomycosis-tube precipitins (TP) and complement fixing (CF) antibodies. The latter tends to be associated with IgG, while the former is usually associated with IgM [271]. Serum IgG levels directly correlate with disease involvement, being highest in patients with multifocal involvement. High CF titers often indicate significant disease severity and therefore suggests that a robust humoral response may be detrimental in patients with coccidioidomycosis. However, more likely, this indicates that antibodies to certain antigens are protective while antibodies to others are not [271]. The serum IgA level is elevated in approximately 20 % of patients, being manifested most often in patients with chronic pulmonary disease. Hyperproduction of IgE would be consistent with a Th2 response and has been demonstrated in approximately 23 % of patients with active disease, with the highest incidence occurring in patients with disseminated disease and, within

this group, in patients who have disease involving two or more organ systems [280, 282, 283].

Longitudinal studies of coccidioidomycosis patients with excessive IgE levels revealed that, in most patients, IgE production diminished to normal or near-normal levels after clinical remission, suggesting that IgE hyperproduction is a consequence of the disease [282]. This interpretation is countered, however, by the report that atopic persons are at greater risk of developing symptomatic coccidioidomycosis than are persons who are nonatopic [284]. Binding of circulating immune complex complement products has been detected in sera from coccidioidomycosis patients and has been shown to correlate with disease severity. Whereas 33 % of sera from patients with disease involving a single organ system had elevated immune complex levels, 67 % of sera from patients with disseminated multifocal disease showed circulating immune complexes. The role, if any, of immune complexes in the immunopathogenesis of coccidioidomycosis is not known.

Investigators reported suppression of lymphocyte proliferation responses when lymphocytes from healthy coccidioidin skin test–positive persons were assayed in the presence of patient sera and, conversely, augmentation of the responses of patient lymphocytes when assayed in sera from healthy subjects (versus autologous serum). However, addition of immune complexes formed in vitro (by the addition of coccidioidin to a serum sample with high levels of anti-Coccidioides IgG) to cultured mononuclear cells from healthy, coccidioidin skin test–positive persons did not suppress their proliferation response to coccidioidin. These results, taken together, argue against suppression by immune complexes and raise the question of whether the suppression observed with patient sera was merely attributable to the neutralization of coccidioidin in such a manner that it was not available to stimulate lymphocytes [277, 278, 284].

Cutaneous Histoplasmosis

Clinical Manifestations and Pathogenesis of Cutaneous and Systemic Histoplasmosis

Histoplasmosis, caused by the dimorphic fungus *Histoplasma capsulatum*, is a deep mycosis endemic to regions in the Western Hemisphere, including southern Mexico and some areas of the southeastern United States. The infection is acquired by the inhalation of spores from soil contaminated by bird and bat excreta. *Histoplasma capsulatum* is an opportunistic pathogen residing in the macrophage phagolysosome. Histoplasma infection commonly results in mild or inapparent clinical symptoms in immune-competent individuals [285–287]. However, in immune-compromised individuals deficient in CD4+ T-cell function, failure of adequate granuloma formation allows the fungus to disseminate systemically and can lead to a life-threatening disease. Thus, in endemic areas, histoplasmosis affects a susceptible population of patients with secondary immune defects due to HIV infection, immune suppression after transplantation, or anti–TNF-α immunotherapy

Three forms of histoplasmosis in susceptible individuals, including acute pulmonary, chronic cavitary, and disseminated histoplasmosis, have been described. In the pre-AIDS era, disseminated histoplasmosis was rare and the cutaneous manifestations were reported infrequently, mainly in patients at the extremes of age and in those with decreased cell immunity secondary to inborn errors of immunity, malignancies, and cytotoxic or steroid therapy. Whether HIV-associated disseminated histoplasmosis is a manifestation of progressive primary infection or whether it represents reactivation of old infection, is currently unknown. The cutaneous manifestations of histoplasmosis are reported to occur in 10–25 % of AIDS patients with disseminated histoplasmosis [288, 289]. Clinical manifestation of cutaneous histoplasmosis varies depending on the stage of the disease and immune status of the host. The cutaneous lesions usually have not only different morphologic appearances in different patients but also varying morphology in the same patient. The cutaneous histopathologic spectrum comprises necrotizing tuberculoid and nonnecrotizing granulomatous inflammation with focal organisms, diffuse dermal histiocytosis (DDH), and diffuse dermal karyorrhexis (DDK) characterized by the presence of sheets of heavily parasitized histiocytes [288–291]. Clumps of H. capsulatum var. capsulatum, released from disintegrating macrophages, are identified predominantly extracellularly in an interstitial location in DDK, while a dense infiltrate of heavily parasitized histiocytes is identified in biopsies demonstrating DDH. Exfoliative dermatitis and cutaneous vasculitis have not been documented in AIDS patients with disseminated histoplasmosis. The lesions are usually painless unless they ulcerate. The skin lesions are widely disseminated, but they are common on the trunk, face, and upper limbs. In the majority of patients, cutaneous histoplasmosis is accompanied by disseminated histoplasmosis involving lung, bone marrow, esophagus, duodenum, and liver [288–291].

Since the clinical lesions of histoplasmosis are nondiagnostic and the morphologic spectrum is shared by a range of infective and noninfective diseases that are common in AIDS patients, the gold standard for differentiating disseminated cutaneous histoplasmosis (DCH) from other processes is the identification and isolation of the infective agent in tissue sections and in fungal cultures, respectively [292–296]. Cutaneous histoplasmosis entails disease caused by two morphologically different forms of the fungus, *H. capsulatum var. capsulatum* and *H. capsulatum var. duboisii*. *H. capsulatum var. duboisii* infection causes a relatively indolent form of histoplasmosis with skin, bone, and lymph node involvement,

and rarely fatal disseminated disease, while *H. capsulatum var. capsulatum* causes widespread visceral (including pulmonary) involvement that is commonly fatal. However, the cutaneous clinical spectrum of *H. capsulatum var. capsulatum* is similar to that caused by *H. capsulatum var. duboisii* infection in some features including isolated or disseminated papules, nodules, plaques, or large subcutaneous nodules. The most important distinguishing feature is the larger size of *H. capsulatum var. duboisii* yeasts (i.e., 8–15 μm) and their predominant location within foreign body giant cells. *H. capsulatum var. capsulatum*, 2–4 μm in diameter, is located predominantly within histiocytes and less commonly in giant cells. Under special circumstances, *H. capsulatum var. capsulatum* is known to transform to a larger size that mimics *H. capsulatum var. duboisii.* Both organisms may be identified extracellularly. Round and oval configuration of the yeasts is common to both variants [292–296].

The yeast forms of *Blastomyces dermatitidis* may resemble *H. capsulatum var. capsulatum*; however, the former forms are multinucleate and demonstrate broad-based budding, while *H. capsulatum var. capsulatum* is uninucleate with narrow-necked budding. Other fungal pathogens with similar morphology are encapsulated and nonencapsulated *Crytococcus neoformans* that stains black or dark brown with the Fontana–Masson stain, while *H. capsulatum var. capsulatum* is characteristically negative. Intracellular small yeast forms have also been documented in sporotrichosis. *S. schenckii*, however, grows easily in cultures as dark brown colonies at 25 °C within 5 days [292–296]. Furthermore, identification of *Sporothrix schenckii* within microabscesses is facilitated when a Splendore–Hoeppli phenomenon is present. The presence of pseudohyphae, and intercellular location of the yeast cells in tissues allow the differentiation of Candida species that have a comparable size range to *H. capsulatum var. capsulatum*. Finally, *H. capsulatum var. capsulatum* must be distinguished from Leishmania species, which may be difficult on low-power examination, as both organisms share a 2–4-μm size range, round and oval morphology, and intracellular location. High power examination, however, demonstrates the kinetoplast of *Leishmania amastigotes*, which on Giemsa-stained sections appears red and is not seen in *H. capsulatum var. capsulatum* [296].

Immune Response Against Histoplasma Capsulatum

Histopathologic examination of skin biopsies from HIV patients with disseminated cutaneous histoplasmosis demonstrated granulomatous inflammation with a histiocytic palisade bordering central necrosis. Although intracellular organisms were identified focally within histiocytes and giant cells, they were not numerous as in the biopsies that lacked a granulomatous component [73, 297]. The granuloma is a form of delayed-type hypersensitivity. Localized inflammatory lesions composed of infected macrophages and fused giant cells can subsequently form granulomata with the help of CD4+ T lymphocytes. CD4+ T cells are very important for initiating and regulating granuloma function, but macrophages are the dominant cell type. A Th1 immune response, in particular, is necessary for control of the infection and is stimulated by IL-12 production as depicted in Fig. 19.7. Similar to other fungal infections, Th17 cells have also been shown to play a proinflammatory role in histoplasmosis infection. However, it seems that Th17 activity is driven more by IL-21 than IL-6 in *H. capsulatum* [298].

The benefit of granuloma formation for the host is that it isolates the inflammation, protects the surrounding healthy tissue, controls the growth of pathogens, and prevents systemic dissemination. At the same time, the microorganism may also benefit from localization to the granuloma. As an isolated microenvironment, granulomas presents a special environment for the pathogen in the host [73, 297, 299]. The chronic granulomatous lesion may be the reservoir from which surviving pathogens emerge to reactivate the infection after a long term latency is broken by a compromised immune system. Thus, granulomata formed in response to macrophages infected by Histoplasma are most likely a dominant component of the highly effective antifungal immune response. Conversely, the progressive, disseminated histoplasmosis observed in immune-compromised persons arises in large part from failures of established granulomas and failure to form new inflammatory lesions in response to recently infected macrophages. While there is growing knowledge about the nature of systemic immunity during the course of histoplasmosis, there is little known about local immune responses within granulomas despite these lesions representing the main interaction between the fungi and the host. A recent study in mice isolated *H. capsulatum*–induced granulomas in order to determine the cellular composition and cytokine milieu of granuloma-infiltrating cells during the course of disseminated infection that involves multiple organs [289].

The average granuloma size reaches a maximum at day 10 of the infection and subsequently declines. Furthermore, this study shows that IL-10 and TGF-β were elevated in the Histoplasma granuloma, mainly at early stages of granuloma formation. The main source of TGF-β was macrophages. In addition to production of IL-10, the decreased granuloma size in this animal model of histoplasmosis was postulated to be due to fungal clearance leading to decreased antigenic stimuli, inflammatory agents, chemoattractants, and attenuated cellular recruitment. Although the new granuloma formation was detected after clearance of the yeast, it is not completely clear whether granulomatous protection is completely sterilizing. However, it is possible that very low

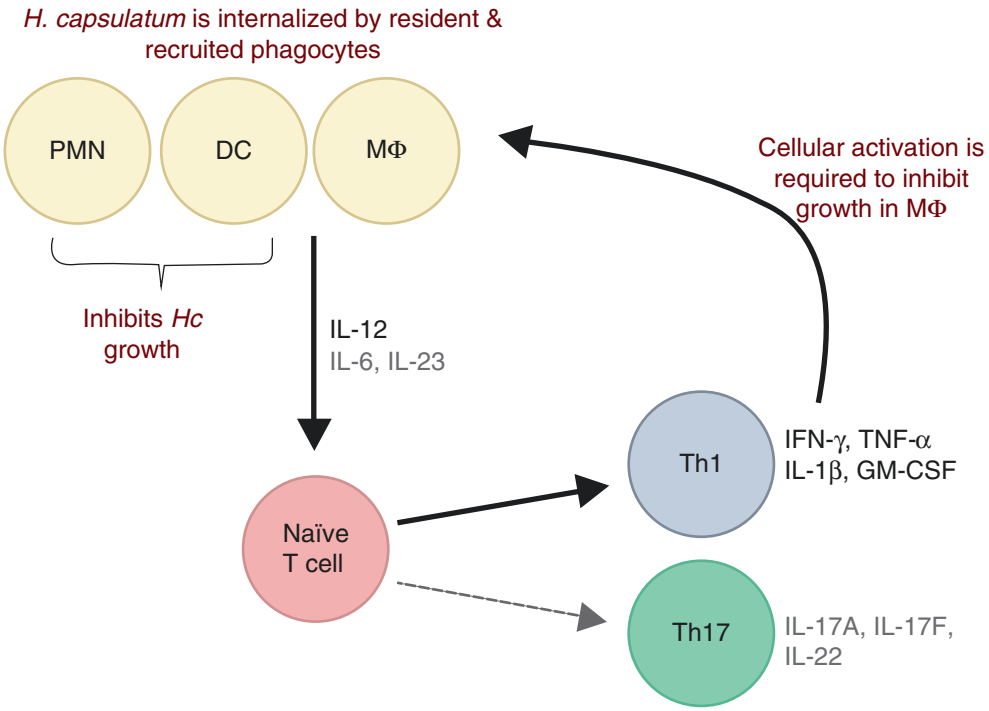


Fig. 19.7 *H. capsulatum* induces a proinflammatory response. Upon inhalation, the H. capsulatum is internalized by host phagocytes. Whereas polymorphonuclear (PMN) and DCs inhibit fungal growth, the organism replicates within macrophages (MΦ) prior to cellular activation. Cytokines such as IL-12, IL-6, and IL-23 are produced during the innate response and are involved in the polarization of naïve T cells. Th1 cells, characterized by IFN- γ, are the predominant T cell population during infection. A smaller population of Th17 population emerges, but only plays a minor role in resolving infection. In addition to IFN- γ, other Th1 cytokines including TNF-a, IL-1b, and GM-CSF activate macrophages to prevent intracellular growth (Reprinted from Cytokine, 58, Kroetz and Deepe [298], with permission from Elsevier)

numbers of yeast survive within granulomas similar to *Mycobacterium tuberculosis* and serve as a source for reactivation during immune deficiency.

Identification of the type of cells infiltrating the granuloma indicates that macrophages are the dominant cell type in the lesion reaching up to 70% of the granuloma. The local expressions of IFN-γ and TNF-α in the lesion indicate that most of infiltrating macrophages are activated. The main cellular source of IFN-γ in the granulomas is CD4+ and CD8+ T cells. Interferon-γ plays a central role in the immunity against H. capsulatum as marked by findings that IFN-γ–deficient animals are killed by H. capsulatum infection [300]. Local IFN-γ activates macrophages to produce reactive radicals crucial to control of the yeast and directly prevents the yeast from replicating by limiting the amount of available minerals (iron, zinc) [298]. The primary cellular source of TNF-α is the macrophage, and T cells contribute little if any to local TNF-α levels. Therapeutic anti-TNF-α treatments induced reactivation of latent *H. capsulatum* infection in some patients, emphasizing the role of this mediator in control of the yeast and preventing dissemination [301]. The H&E-stained sections suggest that most lesions have few, if any, neutrophils, and flow cytometry analysis indicates that only a low percentage of granuloma-infiltrating cells express a dendritic cell phenotype (CD11c+DEC205+) [289]. The latter could be due to the fact that DCs are professional APCs that sample antigens from peripheral sites of infection, and migrate to lymph nodes where they stimulate T cells.

The presence of DCs may provide a local reactivation for the recruited effector T cells and raises the possibility that they sample granuloma Ags and may carry them to draining lymph nodes. The idea that Ags in granulomas might prime systemic T cells needs further investigation. Both effector CD25 low CD4+ and CD8+ T cells are recruited to the lesions. At early stages, there was more CD4+ T cells present, but later the ratio was close to 1:1. This temporal change in the CD4/CD8 T-cell ratio likely reflects the somewhat earlier systemic activation of CD4+ T cells relative to activation of CD8+ T cells. Interestingly, in HIV patients with disseminated cutaneous histoplasmosis, a predominant infiltration of CD8+ T cells was detected in biopsy samples from skin lesions [289]. Both T cells contribute to the cytokine milieu of the granulomas.

In contrast to high infiltration with CD4+ CD25 low T cells, CD25 high CD4+ T cells, thought to be regulatory T cells, are present at much lower levels in the granuloma relative to both infected spleen and naive spleen, suggesting an exclusion of those cells from the local inflammatory site. Thus, the role of regulatory T cells in Histoplasma-induced granuloma formation warrants more investigation since these cells are reported to regulate Leishmania and Schistosoma-induced lesions.

However, due to the limitation of regulatory T-cell classification based on CD25 expression alone, additional phenotypic and functional characterization will be needed in further studies of regulatory T cells in Histoplasma infection. The level of γδ T cells was also lower in granulomas compared with systemic sites; γδ T cells have been reported in granulomas affecting granuloma size during the chronic stage of infection. A low level of B cells was also present having a conventional B cell phenotype (CD5−, Mac-1−) characteristic of peripheral blood [289]. The local Ab production in the granuloma could be potentially important since there are reports that Abs can protect against intracellular yeasts. Taken altogether, local granuloma responses in histoplasmosis at infection site(s) are similar to systemic responses represented by the spleen where responses against Histoplasma infection are dominated by CD4+ Th1 cell responses and require IFN-γ and TNF-α for activation of macrophages.

Conclusion

Due to the increased populations of immunocompromised patients, the clinical relevance of fungal infections has risen in the last three decades. Fungal diseases are classed according to the site of the primary infection: superficial, cutaneous, subcutaneous, and deep or systemic. Dimorphic fungi assume both yeast and hyphal states based on environmental conditions and the hosts' immune response. Certain fungi can synthesize capsular components, which can affect host immune responses. The innate response to fungi serves both a direct antifungal effector activity and an activation and induction of the specific adaptive immune responses. Understanding the immune response to fungal infections has led to better diagnostic tests and therapeutic interventions for fungal diseases. In the future, it is hoped that this knowledge will lead to vaccines for their prevention.

Q&A

1. Which of the following is not a fungal structure that acts as a ligand for toll-like receptors?
   A. β-1,3/β-1,6 glucans
   B. glucuronoxylomannan
   C. cholesterol*
   D. phospholipomannan
   E. galactomannan

2. Which of the following is true regarding the immunoregulatory functions of interleukin 10?
   A. It is produced in a non-antigen specific manner by macrophages*
   B. It is produced in an antigen specific manner by dendritic cells
   C. It is produced in a non-antigen specific manner by regulatory CD4+ cells
   D. It activates the antifungal effector function of phagocytes
   E. It enhances the secretion of proinflammatory cytokines

3. Which toll-like receptor (TLR) is not known to play a role in host recognition of *Candida albicans*?
   A. TLR2
   B. TLR4
   C. TLR6
   D. TLR8*
   E. TLR9

4. Which of the following are thought to be the primary effector cells against Candida spp. that direct the immune system toward either a Th1 or Th2 response via production of either IL10 or IL-12?
   A. CD4+ cells
   B. CD8+ cells
   C. neutrophils*
   D. CD17+ cells
   E. monocytes

5. Which component of the immune response to coccidioides spherules is not involved in recognition of infection, but is necessary in preventing dissemination of disease?
   A. Macrophages
   B. Polymorphonuclear leukocytes
   C. Dectin
   D. TLR2
   E. TLR4*

Answers

1. C
2. A
3. D
4. C
5. E

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HIV/Opportunistic Infections

20

Kemunto Mokaya and Toby Maurer

Abstract

HIV adversely affects the immune system via destruction of T cells, macrophages and related effector cells. Clinical manifestations include a spectrum of opportunistic infections, neoplasms and inflammatory conditions. Control of HIV replication involves combination of drugs from two or more classes that significantly lower the viral load and allow increases in immune effector cells. They extend the length and quality of life of HIV patients. These drugs, however, are very expensive and can be associated with resistance and to a number of adverse events. Although effective use of these drugs can reduce transmission of HIV, they do not produce a cure. The best hope for control of the pandemic of HIV is the development of a safe and effective preventative vaccine.

Keywords

HIV • AIDS • cART • IRIS • Opportunistic infections • Viral load

Key Points

- The two major glycoprotein subunits on the HIV envelope that interact with target cell surface receptors are gp120 and gp41.
- CD4, as well as co-receptors, on the target cell surface interacts with gp120 to initiate infection. The co-receptor used by HIV on T cell targets is primarily CXCR4 while the co-receptor used by HIV on macrophage targets is primarily CCR5.
- Mucocutaneous manifestations of HIV affect up to 90% of infected individuals and are due to opportunistic infections, neoplasms and inflammatory dermatoses. Cutaneous findings in individuals often correlate with their immune status.
- Antiretroviral therapy (ART) includes inhibitors of viral reverse transcriptase, protease, and integrase, as well as blockers of fusion and co-receptors (e.g., CCR5).
- ART has prolonged the life of HIV positive individuals, but complications of treatment include various drug reactions and toxicities, and the immune reconstitution inflammatory syndrome (IRIS).

Introduction

Human immunodeficiency virus (HIV) infection continues to be a major global pandemic. Since AIDS was first described in 1981, about 78 million people have contracted HIV, and over half of these have died from AIDS-related causes. Over 35 million people are currently living with HIV [1]. In 2013, an estimated 2.1 million people became newly infected with

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the virus, while about 1.5 million people died from AIDS. Despite these sobering statistics, some progress has been made globally to reduce HIV infection, with a 33% decline in new infections since 2001, and a decline in the number of annual AIDS-related deaths since the late 1990s [2]. This progress can be attributed to preventive interventions (such as prevention of mother-to-child transmission efforts, male circumcision, global educational campaigns) and to increased access to healthcare and the use of combined antiretroviral therapy (cART). Since AZT was approved as the first drug to treat AIDS in 1987 and combination anti-retroviral treatments were shown to be highly effective against HIV in 1996, there has been a robust pipeline of new effective antiretroviral therapies. To date, 26 individual HIV drugs have been approved by the United States Food and Drug Administration [3]. These drugs effectively suppress viral load below detectable levels, however, they are unable to eliminate HIV from the body. The successes of cART are tempered by the need for lifelong treatment to suppress the virus – which is costly, drug toxicities, drug resistance, and chronic immune activation, which predisposes HIV-infected individuals to other non-AIDS disorders. There is also a treatment access gap, and it is estimated that just about 13 million individuals (37% of the total HIV-infected population) have access to antiretroviral therapy [4]. To combat the HIV/AIDS pandemic, more efforts are needed to reduce the rates of new HIV infection and to increase access to life-saving cART. The ultimate hope in the war against the virus lies in finding a cure for it – whether this comes in the form of a vaccine, gene therapy or a drug. Therefore, understanding basic virologic and immunologic mechanisms in the pathogenesis of HIV disease continues to be of critical importance.

The HIV Life Cycle

Human immunodeficiency virus (HIV) is a retrovirus. This means that the basic genetic material encoded by HIV is RNA, and that the virus contains an enzyme, reverse transcriptase, which is able to convert viral RNA to viral DNA. Double-stranded viral RNA is contained within the core of the virus, and is immediately surrounded by the viral capsid, which is surrounded by the viral envelope. The viral envelope gene encodes a precursor protein, gp160 which is cleaved into the two major glycoprotein subunits of the viral envelope: gp120 and gp41. These glycoproteins are trimeric, consisting of three gp120 molecules (surface unit), and three gp41 molecules (transmembrane region). The gp 120/41 trimers on the viral envelope interact with cell-surface receptors on target cells to initiate infection [5, 6]. The number of gp120/41 trimers on each virion ranges from 10 to 100 depending on the isolate [7].

The life cycle of HIV begins with viral entry into a new target cell and ends with release of new virions from the infected cell. The first event involves binding of gp120, the major envelope protein of HIV, with the molecule CD4 on the surface of target cells. CD4 extends from the cell membrane, and it is believed that CD4–gp120 interactions induce conformational changes that bring virus close to the cell membrane, and subsequently allow for another region of gp120 to interact with and bind to a HIV co-receptor embedded in the cell membrane [8]. HIV co-receptors are seven-transmembrane–domain molecules, members of the G-protein-coupled receptor family, and normally function as chemokine receptors, helping to mediate chemotaxis during inflammatory processes. The two major HIV co- receptors for HIV are CCR5 [9–11], and CXCR4, [12] although several other minor HIV co-receptors with similar structure and function have also been described [13]. Viruses that are predominantly macrophage-tropic use CCR5 for entry (labeled R5 viruses), whereas viruses that are predominantly T-cell-tropic use CXCR4 for entry (labeled X4 viruses). Viruses that use both receptors are termed R5X4. Determination for co-receptor usage is dictated by the amino acid sequence in the co-receptor binding region of gp120. Recent studies reveal that different R5 viruses prevail during the chronic phase of infection, while X4 viruses are associated with pathologic disease progression [14].

Binding of gp120 to an HIV co-receptor then leads to activation of gp41, the other major envelope glycoprotein, causing conformational changes that lead to fusion of the viral envelope with the cell membrane [8, 15]. Following fusion, viral RNA is released into the cytoplasm of the cell. Detailed knowledge of these events has been greatly facilitated by the discoveries of HIV co-receptors and by identification of the crystal structures of gp41 and gp120 [16, 17]. Importantly, these advances in basic research allow for better understanding of HIV disease pathogenesis and have led to design of new therapeutic strategies aimed at blocking viral entry.

Viral RNA is reverse transcribed to DNA in the cytoplasm by the viral enzyme reverse transcriptase. Much of the genetic variability and the development of drug-resistant HIV strains can be attributed to errors introduced into the viral genome during reverse transcription. Proviral DNA is then transported to the nucleus, where it becomes incorporated into the host genome with the aid of the viral enzyme integrase. Host cellular machinery, in combination with the viral proteins Tat and Rev and transcription factors such as P-TEFb (human positive transcription elongation factor), drive viral transcription [18]. In cytoplasm, viral messenger RNA (mRNA) is then translated into viral proteins, and viral capsids form around paired strands of viral RNA. Capsids obtain outer envelopes upon budding from cell membranes, which are studded with the viral glycoproteins gp120 and gp41. The viral protease enzyme orchestrates release of virions from infected cell membranes. Currently available antiretroviral drugs were designed to specifically inhibit these various steps in the HIV life cycle (see Fig. 20.1).

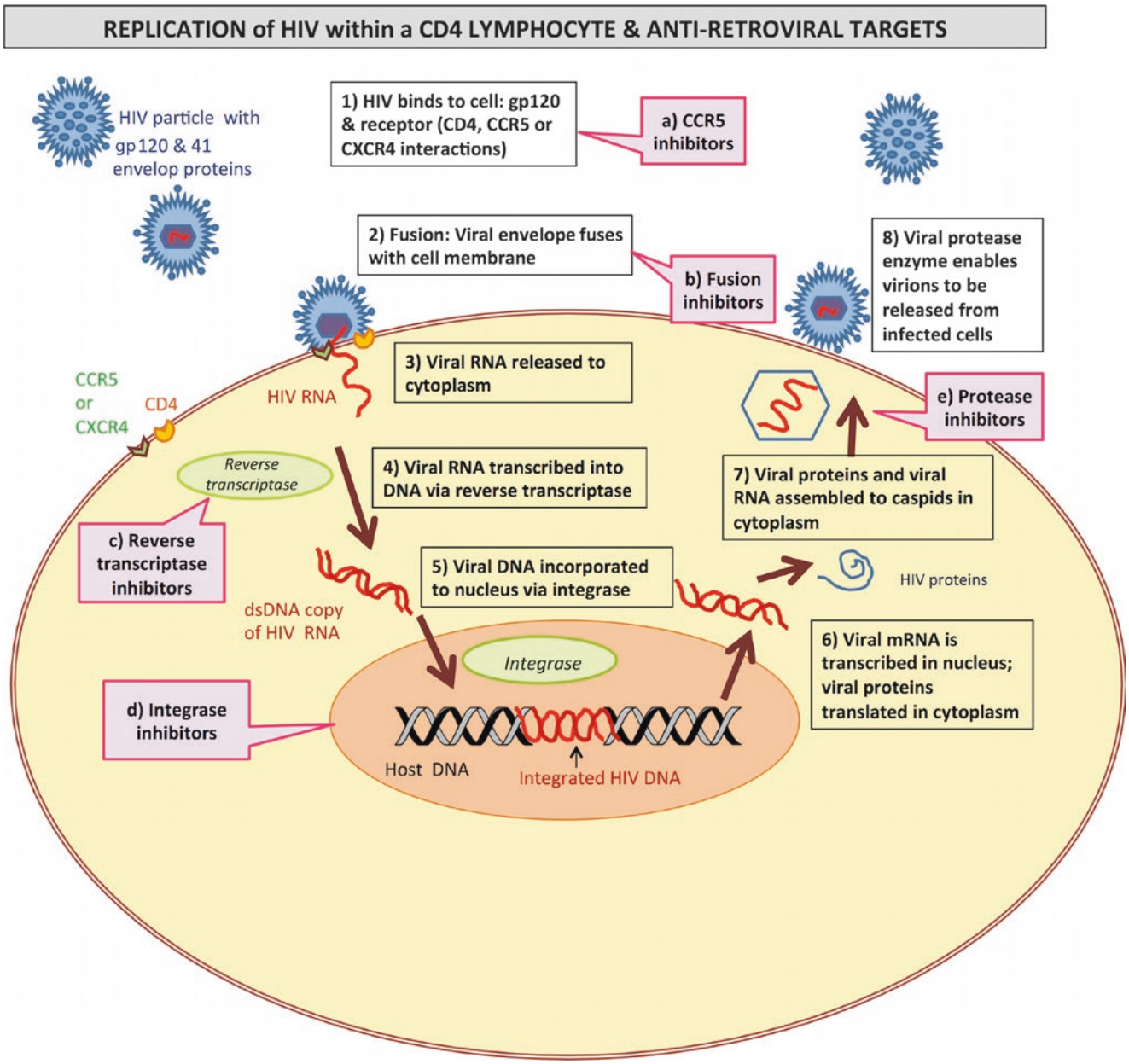

Fig. 20.1 Represents the major steps in the infection of a CD4 lympocyte with HIV. Anti-retroviral drugs have been developed to target various steps in the replication of HIV

Virologic, Immunologic, and Clinical Features of HIV Disease

Early-Stage HIV Disease

Langerhans Cells as Initial Targets for HIV Following Sexual Exposure to Virus

Most HIV infections are transmitted through the mucosa. Most adults acquire the virus via vaginal, penile and rectal transmission, while most pediatric HIV infections occur through the oral ingestion of maternal fluids [19].

Langerhans cells are antigen-presenting cells found within skin and in oral, vaginal, cervical, and anal epithelial layers, and normally function as outposts of the immune system within these tissues [20–23]. Here, they capture surface antigens and emigrate from the epithelium to draining lymph nodes via afferent lymphatics. Langerhans cells then present processed antigenic peptides to T cells, thereby leading to antigen-specific T-cell activation. Intraepithelial Langerhans' cells have been shown to be the first cells infected following vaginal exposure to simian immunodeficiency virus (SIV) in a rhesus macaque model of primary HIV infection [24].

Following infection, Langerhans' cells are believed to migrate from mucosal surfaces to draining lymph nodes via afferent lymphatics, where they transmit HIV to paracortical activated CD4+ T cells, thus establishing infection within the lymph node compartment [25, 26].

In situ, immature Langerhans cells express CD4 and are CCR5+CXCR4− [27–29]. Epithelial cells are CD4− and HIV co-receptor negative and are thus not predicted to be easily infected by HIV. However, they express HLA-DR, CD1a and some mannose dependent C-type lectin receptors (MCLRs) that can function as viral attachment factors [30]. The differential surface expression of CCR5 and CXCR4 may explain why macrophage-tropic, or R5 type, viruses are the dominant type of HIV to be sexually transmitted (90–95 % of cases) [31, 32]. In vitro, human immature Langerhans cells are much more easily infected by R5 viruses when compared to X4 viruses, and cells can be completely protected from infection by blocking CCR5 [33–36]. The importance of CCR5 in initiating primary HIV infection is underscored by the fact that individuals who have homozygous deletion of their CCR5 gene are relatively protected from becoming infected by HIV despite numerous exposures [37–40].

Development of Topical Microbicides to Prevent Sexual Transmission of HIV

In the absence of a prophylactic vaccine against HIV, health measures designed to limit the numbers of new cases of sexually transmitted HIV infection have included abstinence and condom use. Unfortunately, these methods can be impractical in certain countries and social situations where women are not empowered to influence decisions regarding sexual intercourse [41]. Thus, additional means to block sexual transmission of HIV are urgently needed. The use of topical microbicides, drugs or compounds that can be applied to genital tissue prior to sexual intercourse and potentially block sexual transmission of HIV, is being actively investigated [42]. Microbicide acceptability studies in populations of at-risk women have suggested that an effective topical microbicide would likely be used by women [43, 44], potentially making a major impact on improving world health.

In the past decade, several potential microbicides have been developed, many of which have reached Phase III human clinical trials. These include Nonoxynol-9 and cellulose sulfate, which failed due to inducing damage in vaginal epithelia of users thereby increasing HIV infection; and Carraguard, which failed due to lack of efficacy [45–47].

A promising microbicide currently undergoing Phase III clinical trials is tenofovir 1 % gel. In the CAPRISA 004 Phase IIb clinical trial, this gel demonstrated prophylactic efficacy against HIV acquisition [48]. Should it demonstrate efficacy, tenofovir 1 % gel will be the first, successful gel microbicide. Another highly promising potential microbicide that is currently undergoing phase III clinical trials is the dapivirine vaginal ring. Its advantages include its long-acting nature (for a month or longer), its convenient use and discrete nature, its durability and high acceptability among potential users [49]. Other new microbicides in the pipeline include vaginal tablets of tenofovir and emtricitabine [50]. Many other compounds are in the development pipeline, and the future of effective microbicides in multiple formulations to empower at-risk women to prevent themselves from acquiring HIV is promising.

Immunologic Features and Cutaneous Manifestations of Acute Primary HIV Infection

The clinical syndrome in acute HIV infection often develops within 2–4 weeks following exposure and can range from asymptomatic to a severe illness, lasting from a few days to several weeks. 50–90 % of acutely infected patients are symptomatic, often with fevers, lymphadenopathy and the nonspecific flu-like signs and symptoms outlined in Table 20.1 [51, 52]. Rash occurs in a relatively high percentage of patients (50–75 %). Lesions are described as non-pruritic erythematous macules and papules, with a predilection for the upper trunk, head, and neck (Fig. 20.2). The cutaneous eruption is probably caused by infiltration of anti–HIV-specific CD8+ cytotoxic lymphocytes, as has been shown in the skin of rhesus macaques during acute infection with SIV [53]. Painful

Table 20.1 Signs and symptoms of primary HIV infection

Common	Uncommon
Fever (95 %)[a]	Diarrhea (30 %)
Lymphadenopathy (75 %)	Headache (30 %)
Pharyngitis (70 %)	Nausea/vomiting (25 %)
Rash/oral ulcers (70 %)	Hepatosplenomegaly (15 %)
Myalgia/arthralgia (55 %)	Thrush (10 %)
Neurologic symptoms (10 %)	

[a]Approximate incidence

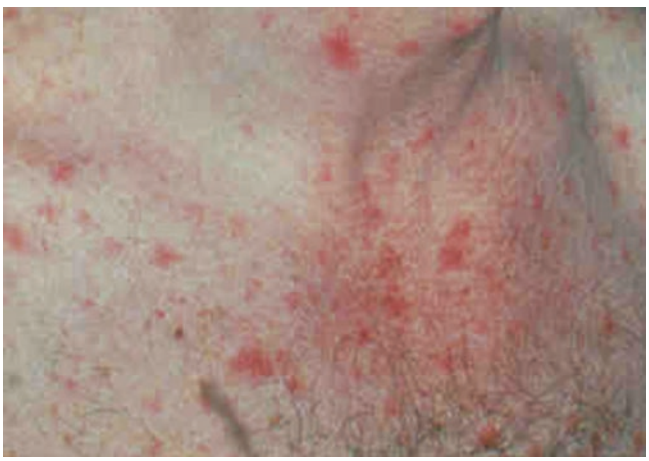

Fig. 20.2 Rash of primary HIV infection. Lesions are characteristically erythematous macules involving the head, neck, and upper trunk. Oral ulcers are also common

oral ulcers are also common in primary HIV infection. Because of the nonspecific nature of these signs and symptoms, clinical diagnosis of acute HIV infection requires a high index of suspicion. Accordingly, it is now suggested that all individuals with known or suspected risk factors for acquiring HIV, who present with rash and fever, should be questioned in detail about possible HIV exposures and have laboratory tests to investigate this possibility [54].

Characteristically, HIV plasma viremia is high during this acute syndrome, with plasma usually containing >100,000 copies of HIV RNA/mL as measured by standard viral load assays, whereas routine HIV antibody tests are negative (Fig. 20.3). The combination of a high viral load and no HIV-specific antibodies confirms the diagnosis of primary HIV infection. Signs and symptoms resolve and plasma viremia gradually drops as cellular and humoral immune responses are initiated to control initial infection. HIV antibody tests usually become positive within 3 months following infection, although this interval may be prolonged in unusual cases.

Most clinicians and HIV researchers promote the widespread use of antiretroviral therapy at the earliest possible time point following diagnosis of acute HIV infection [55, 56]. There are several pieces of data that support this recommendation. First, early use of cART (defined as the use of three or more HIV medications from at least two different drug classes) most likely decreases the "viral set point"—the level of plasma viremia following resolution of acute HIV infection. This level of plasma viremia is linked with the ultimate prognosis for a given HIV-infected individual [57]. Second, cART has been shown to preserve both number and function of anti–HIV-specific CD4+ and CD8+ T cells, believed to be critical in the partial, albeit incomplete, control of HIV replication. Third, early cART likely blunts loss of antigen-specific memory T cells, which are preferentially lost in early HIV disease [58]. Preservation of cellular immunity to common antigens would be predicted to prevent opportunistic infections. Fourth, early initiation of cART reduces plasma HIV-1 concentrations, and has been shown to decrease the risk of viral transmission to others [59, 60]. The drawbacks of treatment with cART in early HIV disease include the possible emergence of drug-resistant strains of HIV, the high cost of medications, drug-related side effects and chronic immune activation resulting in increased risk of several non-AIDS disorders [61, 62].

Middle Stage HIV Disease

Virologic and Immunologic Features That Determine Progression to AIDS

Following resolution of acute infection, HIV-infected individuals often go into a prolonged period without clinical symptoms of HIV disease. This is the clinical latency period. Although plasma viremia is usually low or undetectable during this asymptomatic stage, viral replication continually occurs unabated within lymph nodes and there is gradual damage to the architecture of lymph nodes and other lymphoid tissues [63]. During middle stage HIV disease, CD4+ T cell counts range between 200 and 500/μL, with a slow gradual decline often observed (Fig. 20.3).

As mentioned earlier, three strains of HIV can be classified based on their cellular tropism: (1) macrophage-tropic (R5) strains that preferentially infect peripheral mononuclear cells, monocytes and macrophages via the co-receptor CCR5; (2) T-cell line tropic (X4) strains that preferentially infect T cell lines via the CXCR4 co-receptor; and (3) Dual-tropic (R5X4) strains that infect macrophage and T cell lines using both receptors. R5 strains are present in all stages of HIV. During the middle stage of HIV, there is a gradual increase of X4 strains, which are more cytopathic to CD4+ T cells leading to their gradual decline in numbers.

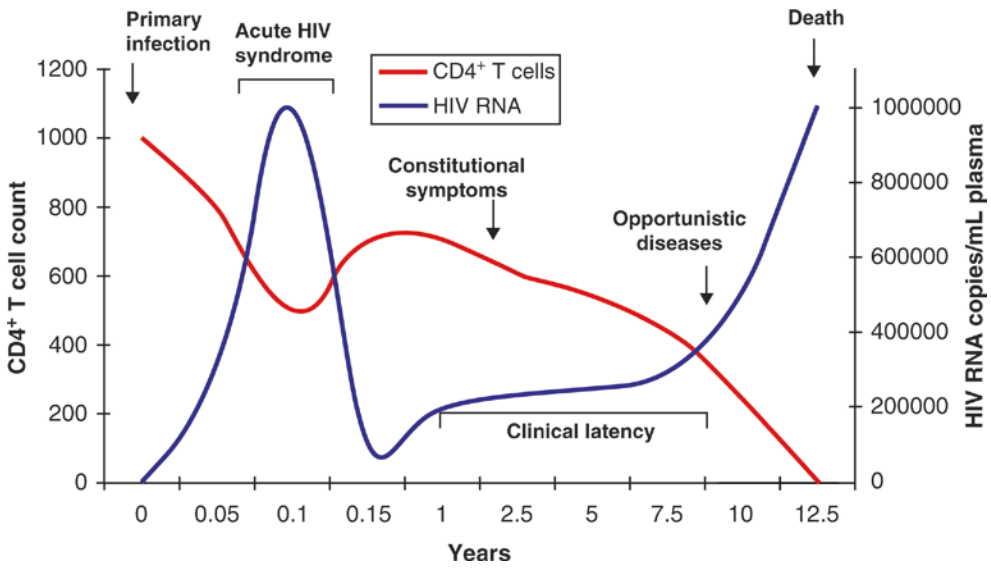

Fig. 20.3 Natural course of HIV disease. Potent highly active antiretroviral therapy (HAART) has a strong influence on this course by prolonging life for HIV-infected individuals

The V3 loop of the gp120 viral protein determines the viral phenotype (R5, X4 or R5X4). It is thought that mutations in gp120 lead to more X4 viruses, which in turn deplete CD4+ T causing the progression to AIDS [64]. However, it should be noted that even individuals with predominant R5 or R5X4 strains still develop AIDS; only at a much slower rate, since the R5 strains are still able to deplete T cells [65].

Deletions or polymorphisms in genes encoding HIV co-receptors (i.e., chemokine receptors) and their ligands (i.e., chemokines) have been associated with delayed HIV disease progression. For example, individuals with a particular polymorphism in their SDF-1 gene, the gene that encodes for the natural chemokine ligand of CXCR4, progress to AIDS less quickly than individuals without this particular polymorphism [66]. Functional studies on the two forms of SDF-1 protein suggest that the variant form is degraded less rapidly than the wild-type form, and would therefore be predicted to have more prolonged binding periods with CXCR4. In addition, heterozygous mutations of the CCR5 gene or its promoters as well as gene duplications in CCR5 ligands prolong the onset of AIDS, presumably by limiting infection and spread of macrophage-tropic viruses early in HIV disease [67–69].

Cutaneous Manifestations of Middle-Stage HIV Disease

Human immunodeficiency virus–infected individuals with CD4+ T-cell counts in the 200 to 500/μL range are mostly asymptomatic, although this certainly is not always true. Signs and symptoms that belie underlying HIV infection often manifest as cutaneous diseases during this phase [70]. In particular, herpes zoster and treatment-resistant seborrheic dermatitis in young persons should alert clinicians to the possibility of coexisting HIV infection and lead to questioning about known HIV risk factors. Zoster involving multiple dermatomes, zoster involving the head and neck, and prolonged healing of lesions are distinct clues to possible underlying immunosuppression, and thus are more common in HIV-infected individuals [71]. Subtle loss of antigen-specific cell-mediated immunity is believed to be the mechanism by which these diseases occur. Although not limited to patients with particular CD4+ T-cell counts, diagnosis of any sexually transmitted disease (syphilis, condyloma, herpes simplex, etc.) should also prompt questioning about possible concomitant HIV infection. Additionally, there is an increase in Staphylococcus aureus colonization and infections in HIV infected people, especially in the middle and late stages of the disease [72].

Acute illnesses, such as herpes simplex reactivation and herpes zoster, lead to tissue inflammation, immune activation, and increases in HIV plasma viremia. The basis for increased plasma viremia is most likely multifactorial. Inflammatory cytokines released during acute inflammation or infection can directly lead to enhanced viral replication within HIV-infected cells. As well, immune activation and cytokine production can stimulate HIV infection of previously uninfected CD4+ T cells. Thus, there is a strong basis for both preventing and aggressively treating all acute infections and illnesses in HIV-infected individuals. Of note, immunizations, which lead to transient activation of the immune system, also trigger transient increases in HIV plasma viremia [73]. The benefits, however, of protecting against future illnesses accorded by immunizations outweigh any potential harm caused by them.

Late Stage HIV Disease, or AIDS

Virologic and Immunologic Features of AIDS

Viruses of all types (T-cell-tropic, macrophage-tropic, dual-tropic) can be isolated from most AIDS patients [64]. Plasma viremia is also usually high in untreated patients (Fig. 20.3). In addition, destruction of lymph node architecture also contributes to high viral loads in blood. This occurs because many infectious HIV virions previously trapped by follicular dendritic cells are released into blood following breakdown of lymphoid tissue [63]. Viral loads can drop dramatically in AIDS patients who are placed on cART for the first time, although it can be difficult for AIDS patients to get to the point where plasma viremia is undetectable [74].

Immune defects in advanced-stage HIV disease are profound. Due to chronic immune activation from persistent viral antigens, both the innate and adaptive immune systems develop immune exhaustion: a state of relative unresponsiveness to the persistent pathogen [75]. T cell exhaustion is weak or absent virus-specific T cell reactivity characterized by impaired cytokine production (such as the loss of interleukin-2 production, a key cytokine involved in normal T-cell function), decreased T cell proliferation, poor effector cytotoxic activity, and sustained expression of inhibitory receptors – such as PD-1, LAG-3, CTLA4, and Tim-3 [76, 77]. Eventually, this results in apoptosis of T cells – preferentially the memory subset of T cells – leads to AIDS. By definition, patients with AIDS have CD4+ T-cell counts less than 200/μL.

B cell exhaustion is also seen in AIDS, characterized by hypergammaglobulinemia, decreased sub-populations of memory B cells, over-representation of exhausted B cells that have decreased capacity to proliferate in response to de novo stimuli, and increased numbers of aberrant naïve B cell subsets – many of which express increased inhibitory receptors [78, 79]. Macrophages also demonstrate numerous defects in AIDS and may also display immune exhaustion, although the mechanism of this is less well understood. Surprisingly, most studies show that dendritic cell function, including epidermal Langerhans cell function, remains relatively intact, even in late-stage AIDS patients [33, 80, 81].

In conclusion, due to immune exhaustion, HIV-infected individuals develop loss of antigen-specific cell-mediated immunity and are susceptible to opportunistic infections. Early initiation of cART is important in reducing viral loads and preventing T-cell exhaustion. Use of cART often leads to increases in CD4+ T-cell counts for AIDS patients [82]. Additionally, new approaches to combating immune exhaustion are being

explored such as the development of molecules that block inhibitory ligand-receptor interactions, leading to the rescue of exhausted T cells. Immunotherapy targeting the inhibition of inhibitory receptors (PD-1, CTLA-4, Tim-3, LAG-3) is not only being studied in chronic viral infections such as Hepatitis C and HIV, but also in fields like oncology in which PD-1 blockage has been used to target tumors in humans [83].

Cutaneous Manifestations of AIDS

In patients with AIDS, especially those that are not on anti-retroviral therapy, the most cutaneous manifestations of their disease are opportunistic infections, which can be viral, bacterial, fungal, parasitic and ectoparasitic (infestations). Additionally, they develop neoplasms such as Kaposi's Sarcoma, lymphomas, non-melanoma skin cancers, and other cutaneous neoplasms. They also develop forms of non-infectious dermatoses such as papular pruritic eruption of AIDS and severe ichthyosis. They are susceptible to many severe drug-reactions such as toxic epidermal necrolysis. Due to AIDS and the initiation of anti-retroviral drugs, they develop metabolic changes such as HIV/ART-associated lipodystrophy. Finally, patients with AIDS who then receive anti-retroviral therapy may develop the immune reconstitution inflammatory syndrome (IRIS), which is the paradoxical worsening of pre-existing infections after initiation of cART due to inflammatory sequelae that result when the immune response to those pathogens is enhanced [84]. These cutaneous manifestations of AIDS are discussed in more detail below.

Kaposi's Sarcoma

Kaposi's sarcoma (KS) is the most common neoplasm in HIV-infected individuals [85]. However, due to more widespread use of anti-retroviral therapy, its incidence is declining, except in resource-poor settings where cART is not readily available. Most investigators now believe KS is not a true malignancy, but rather a multicentric proliferative process driven by inflammation and immune dysregulation. In a landmark study from 1994, the KS-associated herpesvirus (KSHV) – now more commonly referred to as human herpesvirus 8 (HHV-8) – was discovered within lesions of AIDS-associated KS [86]. HHV-8 is transmitted sexually and also through saliva and blood, and it infects endothelial and spindle cells [87]. Factors that play a role in the pathogenesis of KS include loss of HHV-8-specific cell-mediated immune immunity and inflammatory cytokines [88]. Only a small number of cells (<2%) within KS tumors are productively infected with HHV-8 and produce virions. The remaining cells are latently infected with virus [89]. It is thought that the viral genes expressed in latently infected cells contribute to the abnormal spindle cell growth observed in KS lesions [90, 91].

Clinically, KS patients present with violaceous lesions ranging from small papules to large plaques and ulcerated nodules. It typically affects the upper body, usually along the skin-lines. It has a predilection for the face, especially around the nose and oral mucosa. KS can also affect internal organs such as the lymph nodes, gastrointestinal tract and lungs. Multiple treatments exist for KS depending on the stage of HIV, extent of KS and other patient comorbidities. Most patients on cART note regression of their disease: most likely due to improved HHV-8-specific immune function and decreases in HIV-associated inflammatory cytokines that directly stimulate KS spindle cell growth [92]. However, patients on cART may develop KS flares due to immune reconstitution inflammatory syndrome (IRIS). Other options include local destruction (for example cryotherapy), laser therapy, topical alitretinoin, radiotherapy, and intralesional chemotherapy (for example vinblastine). Systemic therapies for disseminated KS include IV liposomally encapsulated doxorubicin, duanorubicin and paclitaxel [93].

Other Viral Infections

Loss of cell-mediated immunity predisposes to viral infections. Infection with herpes simplex virus types 1 and 2 in AIDS patients often results in slow-healing, painful cutaneous ulcerations in the perianal region, genitalia and tongue. These lesions can get large and verrucous, and often take longer to respond to treatment. Varicella zoster virus (VZV) in AIDS patients is usually multidermatomal, and lesions can be chronic, verrucous, ulcerative and widely disseminated with systemic involvement [70]. Treatment and prophylaxis with acyclovir or one of its derivatives is indicated, although clinicians should be wary of the development of acyclovir-resistant strains of both HSV and VZV in the setting of AIDS.

Lytic replication of Epstein-Barr virus within lingual epithelial cells produces oral hairy leukoplakia. This manifests as white corrugated adherent plaques on the lateral aspects of the tongue. CMV viremia can rarely lead to cutaneous diseases, such as ulcers in the anogenital area [94]. Human papillomavirus and molluscum contagiosum virus infections can be particularly aggressive and treatment-resistant in individuals with AIDS. Giant mollusca are seen, and the lesions of HPV can also be quite extensive. Of note, anogenital cancer, like cervical cancer, has been linked to human papilloma virus infection and occurs more commonly in HIV- infected persons compared to the general population [95].

Cutaneous viral infections, like all other cutaneous manifestations of HIV disease, are best treated by first ensuring that patients are on proper cART, which completely suppresses HIV plasma viremia. Second, specific treatment as dictated by the clinical disease, biopsy findings, and culture results should be instituted. As learned in the era prior to cART, specific treatment of AIDS- associated dermatoses is unlikely to be optimally effective in face of uncontrolled HIV plasma viremia and continued destruction of the immune system.

Fungal Infections

Loss of cell-mediated immunity also predisposes to fungal infections. Candidiasis is the most common mucocutaneous manifestation of AIDS, often presenting as friable non-adherent white plaques within oral and vaginal mucosa. Esophageal candidiasis is a particularly painful complication and can lead to impaired swallowing. Treatment and prophylaxis with systemic antifungals is often indicated. Dermatophytosis can also be particularly widespread and difficult to treat.

Systemic fungal infections are also seen in AIDS, with the most common being cryptococcosis and histoplasmosis, where up to 10–15% of patients have cutaneous involvement. Cutaneous cryptococcosis in AIDS usually presents as papules and nodules with central umbilication (i.e., molluscum-like) or necrosis. Histoplasmosis can present with acneiform papules and pustules, which often involve the face. Other systemic fungal diseases that can affect the skin when they are disseminated include coccidioidomycosis, blastomycosis, paracoccidioidomycosis, sporotrichosis, penicilliosis and aspergillosis [96]. Fungal infections in AIDS are best managed by optimizing cART, making accurate diagnoses, and instituting specific antifungal therapy.

Bacterial Infections

Bacillary angiomatosis is caused by gram-negative *Bartonella* bacteria (specifically *B. henselae* and *B. quintana* species), and is thought to be a reactive, vasoproliferative condition. Lesions affect any body site and present as red-purple vascular papules, nodules and ulcers. Staphylococcal infections in AIDS present as impetigo, folliculitis, furunculosis, botryomycosis and cellulitis and tend to be more refractory to therapy. Mycobacterial infections such as cutaneous tuberculosis are seen in AIDS. Other mycobacteria that produce cutaneous lesions include: *Mycobacterium avium* complex, *M. kansasii*, *M. haemophilum* and *M. fortuitum*.

Parasitic and Ectoparasitic Infestations

Scabies, caused by infestation with the *Sarcoptes scabiei* var. *hominis* mite is the most common ectoparasitic skin infestation patients with AIDS. Lesions range from the usual burrows, vesicles and papules – to a widespread dermatitis of thickened, dry, scaly hyperkeratotic eruption called crusted scabies in which thousands to millions of mites may be present on an individual. Demodicosis is also frequent in AIDS. Parasites such as leishmaniasis, acanthamebiasis and strongyloidiasis are also seen in AIDS. In patients with disseminated strongyloidiasis, thumb-print purpura on the lower trunk may be seen.

Neoplastic Cutaneous Disorders in AIDS

In addition to Kaposi's Sarcoma (which is considered both infectious and neoplastic), other neoplastic disorders that may develop in AIDS include lymphomas such as non-Hodgkin B-cell and T cell lymphomas. The lymphomas usually present as violaceous papules and nodules that sometimes ulcerate and resemble panniculitis. Roughly half of non-Hodgkin lymphomas in HIV are associated with EBV infection. Cutaneous T cell lymphomas, squamous cell carcinomas and basal cell carcinomas are also seen in not only AIDS, but also patients with higher CD4 counts. HPV-induced genital SCCs – such as vaginal, cervical, penile and anal SCC – occur at increased frequency and with more rapid progression in HIV-infected individuals.

Other Cutaneous Diseases

Eosinophilic folliculitis is an extremely common cutaneous manifestation of AIDS. Patients present with intensely pruritic urticarial papules surmounted by tiny central vesicles usually on the face and upper trunk [97]. This morphology may not be preserved at the time of presentation due to scratching of lesions, in which case the lesions appear as excoriated papules or small round scars (Fig. 20.4). Lesions are distributed on the face,

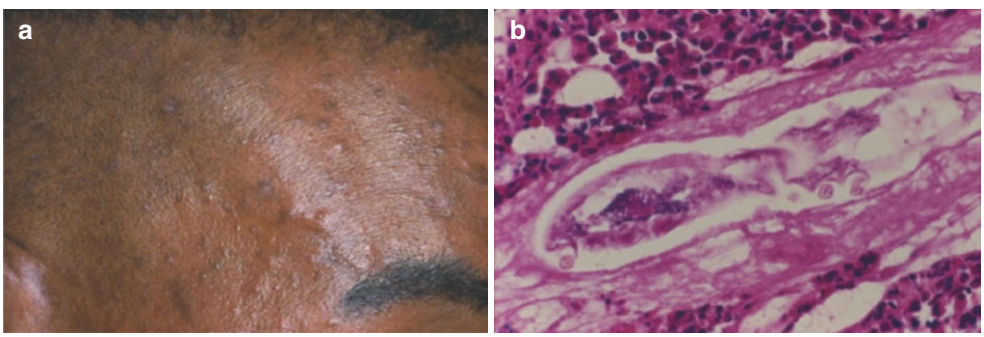

Fig. 20.4 (**a**) Typical urticarial papules of eosinophilic folliculitis. (**b**) High-power histologic view of an early lesion of eosinophilic folliculitis. Demodex mites are characteristically seen within centers of affected hair follicles surrounded by sheets of eosinophils. Excoriated or older lesions show a mixed infiltrate and will not demonstrate mites

neck, and upper chest and back. In early nonexcoriated lesions, Demodex mites are observed within hair follicles at the center of heavy eosinophilic infiltrates [81]. Eosinophilic folliculitis is thought to be an aberrant immune response directed against Demodex mites or *Malassezia* yeast. Treatment options for eosinophilic folliculitis – which is often refractory to therapy – include topical permethrin, topical corticosteroids, topical tacrolimus, UVB phototherapy, systemic antibiotics, itraconazole, dapsone and oral retinoids [98]. Papular pruritic eruption (PPE) of AIDS is believed to be in the same spectrum of pruritic disorders as eosinophilic folliculitis. It primarily affects the extremities more than the trunk and face [99].

Other non-infectious cutaneous disorders seen in AIDS include an acquired ichthyosis with generalized dryness and scaling of skin, refractory seborrheic dermatitis, severe psoriasis, development of refractory oral aphthae, and photosensitive dermatoses such as chronic actinic dermatitis and generalized UV light sensitivity (often with a photolichenoid reaction) [100].

Drug Reactions in AIDS

Drug reactions are common in advanced- stage HIV disease, particularly severe reactions, like Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrosis (TEN), which occur with increased frequency in these patients [101]. The most common drug eruptions in AIDS are morbilliform eruptions. Others include urticarial eruptions, vasculitis, exfoliative erythrodermas, photodermatitis and SJS/TEN. Common triggers of AIDS-associated drug reactions include medications like trimethoprim-sulfamethoxazole and the antiretroviral drug nevirapine. Up to 8 % of patients treated with nevirapine develop Steven Johnson's syndrome [102]. Additionally, patients taking protease inhibitors may develop a syndrome with clinical features resembling Cushing's disease. These individuals have central fat deposition ("buffalo humps," protuberant abdomens, gynecomastia), wasting of facial fat and peripheral fat of the arms and legs, hypertriglyceridemia, glucose intolerance, and increased risk for myocardial infarction (Fig. 22.6) [103]. Unlike in Cushing's disease, the pituitary axis is unaffected. Facial wasting may be treated by switching drug regimens that have less lipodystrophic effects and by injection of filler substances [104, 105]. Other cART-associated cutaneous drug reactions have also been reported, including abacavir hypersensitivity, zidovudine-associated hyperpigmentation of the nails, and retinoid-like effects due to protease inhibitors (Table 20.2) [106]. The pathogenic basis for all of these drug reactions occurring in HIV-infected individuals is unclear (Figs. 20.5 and 20.6).

Table 20.2 Major adverse cutaneous manifestations of cART

Hyperpigmentation due to zidovudine
Hypersensitivity due to abacavir
Stevens-Johnson syndrome due to nevirapine
Lipodystrophy due to indinavir, ritonavir, stavudine, or zidovudine
Retinoid-like effects due to indinavir
Injection site reactions to enfuvirtide
Morbilliform eruptions to most drugs

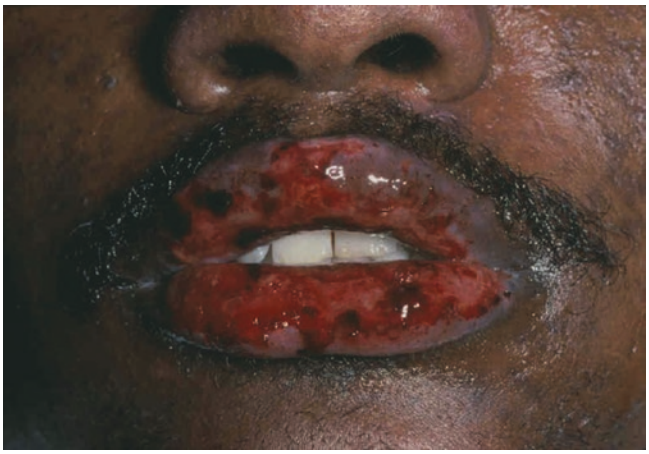

Fig. 20.5 Nevirapine-induced Stevens-Johnson syndrome. This side effect occurs in approximately 8 % of individuals treated with this drug

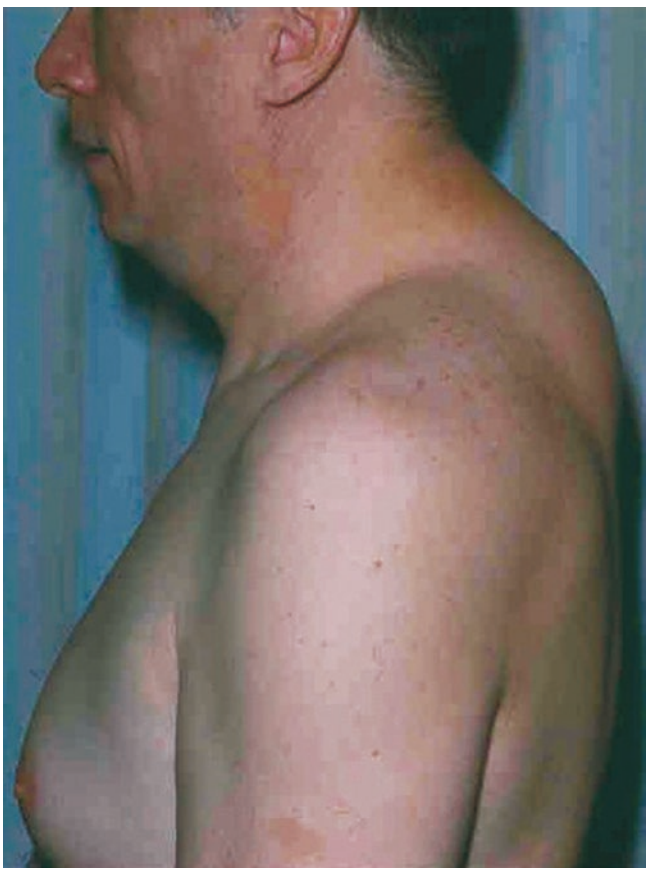

Fig. 20.6 Protease inhibitor-induced lipodystrophy. "Buffalo hump" along with other fat accumulations centrally (i.e., in breast and abdomen) occurs in combination with loss of fat on the face and extremities

Immune Reconstitution Inflammatory Syndrome (IRIS)

The immune reconstitution inflammatory syndrome (IRIS) is the paradoxical worsening or unmasking of pre-existing infections after initiation of cART due to inflammatory sequelae that result when the immune response to those pathogens is enhanced. This clinical deterioration of patients on cART typically presents within the first weeks to months of cART, especially when CD4+ cell counts increase by at least two-fold from low pre-treatment levels. An estimated 10–25 % of patients on cART develop IRIS [107]. The mechanism of IRIS is not fully understood; proposed mechanisms include the uncoupling of the innate and acquired immune responses, restoration of pathogen-specific immune responses and defective regulatory responses [108]. Examples of cutaneous diseases that can flare due to IRIS include: mycobacterial infections (*m. tuberculosis*, *m. leprum*, *m. avium* complex), cryptococcus, VZV and HSV, cytomegalovirus, Kaposi's Sarcoma and many others. Strategies to prevent IRIS include initiation of cART before the CD4+ T cell count drops below 100 cells/mm^3, delaying cART initiation until an opportunist infection resolves, starting cART with prophylaxis against suspected infections, and the use of corticosteroids and nonsteroidal anti-inflammatory drugs [109].

Conclusion

Basic science advances in immunology and virology have led to key advances in the care and management of HIV-infected individuals. For instance, knowledge of the structure and function of the viral proteins and life cycle of HIV has led to the development of novel antiretroviral medications. As well, increased understanding of the initial and later stages of HIV disease through epidemiologic, clinical, immunologic, and genetic studies has provided a solid basis for using these medications in a proper manner. As has been consistently demonstrated in the past, therapeutic advances to combat HIV disease in the future are likely to be based on discoveries in basic virology, biology, and immunology. As evidenced by numerous cutaneous manifestations that can occur throughout the course of HIV disease, the dermatologist remains an important member of the team caring for infected individuals.

Questions

1. **What are the steps required in the life cycle of the HIV virus: from infection of a target cell to the production of virions?**
   Answer: See Fig. 20.1. Binding of HIV to target (gp120 binds CD4 and co-receptors (CCR5 or CXCR4) → Fusion of HIV to target cell → Release of viral RNA to cytoplasm → Transcription of viral RNA to viral DNA (via reverse transcriptase) → Integration of viral DNA to human genome → Transcription of viral mRNA → Translation of viral mRNA to viral proteins in cytoplasm → Assembly of viral proteins into capsids in cytoplasm → Release of virions from infected cells

2. **What are the 5 major classes of currently available anti-retroviral drugs? Describe how they target the HIV life cycle**
   Answer: See Figure 1:
   A. CCR5 inhibitors that try to prevent viral interactions with co-receptor
   B. Fusion inhibitors that prevent HIV from fusing to target cell
   C. Reverse transcriptase inhibitors that block the virus reverse transcriptase. Of 2 types: nucleoside reverse-transcriptase inhibitors (NRTIs) and non-nucleoside reverse-transcriptase inhibitors (NNRTIs)
   D. Integrase inhibitors that inhibit integration of viral DNA to human genome
   E. Protease inhibitors

3. **A HIV positive patient who you normally see for psoriasis has been increasing the frequency of his visits because his scalp lesions are increasingly difficult to control. He presented last month with thrush, and today, comes in with worsened psoriasis and a painful vesicular eruption affecting 4 dermatomes on his left chest and back. Based on his clinical presentation, what do you estimate his CD4+ T cell count is? What other findings would you expect in someone with a similar CD4+ T cell count?**
   Answer: CD4 count is below 500 cells/mm^3. See Table 20.2 for other findings

4. **What is the immune reconstitution inflammatory syndrome (IRIS)? List some conditions where IRIS has been noted**
   Answer: IRIS is the paradoxical worsening or unmasking of pre-existing infections after initiation of cART due to inflammatory sequelae that result when the immune response to those pathogens is enhanced. It has been seen in Kaposi Sarcoma, mycobacterial infections, Cryptococcus, etc

5. **What are some common side effects of anti-retroviral medications?**
   Answer: Lipodystrophy → Morbilliform reactions to most drugs, retinoid-like effects, lipodystropyhy, SJS/TEN, hyperpigmentation, abacavir hypersensitivity

6. **What is immune exhaustion in T cells and B cells, and what are some strategies to prevent/ delay it?**

Answer: Immune exhaustion is a state of relative unresponsiveness of the innate/ adaptive immune system due to chronic immune activation from persistent viral antigens. Leads to impaired cytokine production by T cells, decreased T cell proliferation, poor effector cytotoxic activity, and sustained expression of inhibitory receptors in T cells. In B cells, it is characterized by hypergammaglobulinemia, decreased sub-populations of memory B cells, over-representation of exhausted B cells that have decreased capacity to proliferate in response to de novo stimuli

– Strategies to prevent it include early initiation of antiretroviral therapy; and new immunotherapy agents in development that target inhibitory receptors on B/T cells

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Immunopathogenesis of Psoriasis

21

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Abstract

Psoriasis is a common, complex, inflammatory skin disease resulting from the interplay of genetic, environmental and immunological factors. In the last four decades, advances in understanding psoriasis etiopathogenesis have resulted from pivotal genetics studies, as well as, the integration of clinical and experimental models of disease. This has resulted in the identification of several psoriasis susceptibility genes as well as cellular and molecular mediators, with some of these findings already translated in novel targeted therapies. Here we review the psoriasis literature, describing the elucidation of key pathogenic mechanisms and their translation into effective drugs. Moreover, we describe how the stratified medicine approaches being developed in psoriasis and the quest for psoriasis biomarkers are poised to predict individual susceptibility to disease, detect the onset of disease at the very earliest stages pre-empting its progression, and to develop and prescribe safe and effective medicines to each patient.

Keywords

Skin • Genetics • T cells • Keratinocytes • Targeted therapies • Biomarker • Stratified medicine

Introduction

Psoriasis is a complex disease, resulting from the interaction of genetic, environmental, and immunological factors [1, 2]. The complexity of its etiopathogenesis is mirrored by a spectrum of clinical phenotypes, which often associates with comorbidities that are also multi-factorial and possibly feed back onto the primary disease. Being one of the most common skin conditions, psoriasis has received a great deal of attention from clinicians and basic scientists alike in the past four decades, becoming a model to study chronic skin inflammation. This joint effort has resulted in the elucidation of many underlying pathogenic mechanisms and, more importantly, has been translated in novel therapeutic strategies. Here, we review recent advances in understanding the complex genetic, environmental and immunological basis of psoriasis, identify research gaps which need to be filled in, describe how some of the more recent findings have resulted in novel targeted therapies already available in the clinic or in advanced stages of clinical trials, and highlight how stratified medicine approaches and the quest for psoriasis biomarkers has begun in earnest to further improve patients' life.

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Epidemiology, Clinical Subtypes and Histological Features

Psoriasis is a common disease, affecting 2–4 % of the population in western countries, with prevalence rates influenced by age, geographic location and genetic background [3]. Prevalence is higher in adults (from 0.91 to 8.5 %) as

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compared to children (from 0 to 2.1 %) with a dual peak of incidence: an early onset, before the age of 40, which is often associated with familiar disease history and showing high association with the human leukocyte antigen (HLA)-Cw0602 allele (type I psoriasis), and a late onset, after the age of 40 (type II psoriasis). Geographical patterns of prevalence suggest lower prevalence in countries closer to the equator, in keeping with the beneficial effects of UV radiation exposure [4]. Prevalence is higher in individuals of European descent (from 0.73 to 2.9 %) as compared to those of African and Asiatic background (from 0 to <0.5 %).

Psoriasis has traditionally been considered to affect both genders equally; however recent data about age stratification within gender shows a higher incidence in females <18 years old, and conversely a higher incidence in males ≥18 years old [5, 6].

The term psoriasis (from the Greek word *psora*, to itch) encompasses a number of distinct clinical phenotypes [7]. This heterogeneity often represents a dynamic, anatomical or qualitative spectrum of the same disease (e.g. large and small plaque-psoriasis), while in other cases most likely pins down the existence of quite different disease entities (e.g. generalized pustular psoriasis, GPP). According to the International Psoriasis Council, there are four main forms of psoriasis: plaque-type, guttate, GPP, erythroderma, plus several further sub-phenotypes defined based on different parameters; i.e. the distribution, anatomical localization, size and thickness of plaques, onset and disease activity [8].

Plaque-type psoriasis, occurring in 85–90 % of affected patients is the most common form of psoriasis, the most studied and also the one usually targeted in randomized controlled clinical trials. Plaque-type psoriasis is characterized by oval- or irregularly-shaped, red, sharply demarcated, raised plaques, covered by silvery scales [1, 7] (Fig. 21.1). Plaques occur mainly on the extensor surface of elbows and knees, on the scalp and in the lower back, but can affect every area of the body, often with a symmetrical distribution. Key histological features of psoriasis are epidermal thickening (acanthosis), incomplete keratinocyte terminal differentiation with retention of the nucleus by corneocytes (parakeratosis) and thickening of the *statum corneum* (hyperkeratosis), elongation of the rete ridges extending downward between dermal papillae (papillomatosis), together with blood vessel dilation and immune cell infiltration into the skin. In the epidermis, neutrophils accumulate into the parakeratotic scales in the *stratum corneum* forming Munro microabscesses while lymphocytes, mainly CD8 + T cells, are interspersed between keratinocytes. The dermis is heavily infiltrated by T cells (mainly CD4+) and dendritic cells (DC).

Psoriasis is a dynamic disease: newly formed lesions evolve into an advanced plaque that can slowly enlarge (active lesion) or remain static (stable lesion) [8]. Resolving lesions after therapy can be encased by a distinctive rim of blanching (Woronoff's ring), predictive of clearing, and are histologically characterized by orthokeratosis, that is thickening of the *stratum corneum* without parakeratosis and restoration of the *stratum granulosum*.

Guttate psoriasis, from the Latin "*gutta*" for tear drop, is characterized by multiple small scaly plaques, commonly occurring around the trunk and upper arms and thighs. The rash has often abrupt onset, usually within 2–4 weeks after streptococcal pharyngitis in children and young adults and is therefore associated with type I psoriasis [8]. Guttate psoriasis can either completely clear spontaneously or following topical treatment, become chronic, or worsen into the plaque-type.

Generalized pustular psoriasis (*GPP*), also known as von Zumbush psoriasis, is a rare but potentially life-threatening disease characterized by episodic, widespread skin and systemic inflammation. A typical histological feature of GPP is the presence of conspicuous aggregates of neutrophils infiltrating the *stratum spinosum* (spongiform pustules of Kogoj) and giving rise to sterile cutaneous pustules [7]. Acute attacks can occur during pregnancy and may be triggered by infection, exposure to or withdrawal of drugs. GPP is frequently associated with plaque type psoriasis and/or palmoplantar pustular psoriasis (PPP). Although still classified as a variant of psoriasis, the salient clinical and histological feature of GPP have long suggested that is a disease of distinct aetiology.

Recent genetic data lend further support to this hypothesis suggesting that, at least in certain cases, GPP is inherited as an autosomal recessive trait, due to mutations in the *IL36RN* gene encoding the anti-inflammatory IL-36-receptor antagonist, IL-36Ra [9, 10]. IL-36Ra blocks the pro-inflammatory cytokines IL-36α/β/γ: when *IL36RN* is mutated, IL-36 signaling is unrestrained, with enhanced production of further pro-inflammatory cytokines [10]. However, IL36RN mutations only occur in a minority of patients [11], thus more genetic determinants are likely to be involved. Interestingly, a *de novo* mutation in the epidermal NF-kB activator *CARD14* [12] has been described to underlay a sporadic case of severe GPP, suggesting that keratinocytes dysfunction is likely to play a predominant role in this disease phenotype.

Erythrodermic psoriasis is characterized by diffuse erythema, with or without scaling, involving more than 75 % of the skin surface. It represents the most severe, albeit rare, psoriasis phenotype. Systemic manifestations such as hypothermia and limb oedema might occur, due to the generalized vasodilation underlying the erythema, as well as myalgia, fatigue and fever. Both administration and abrupt withdrawals of systemic corticosteroids or methotrexate, sunburn and emotional stress have been suggested as possible triggering factors [13].

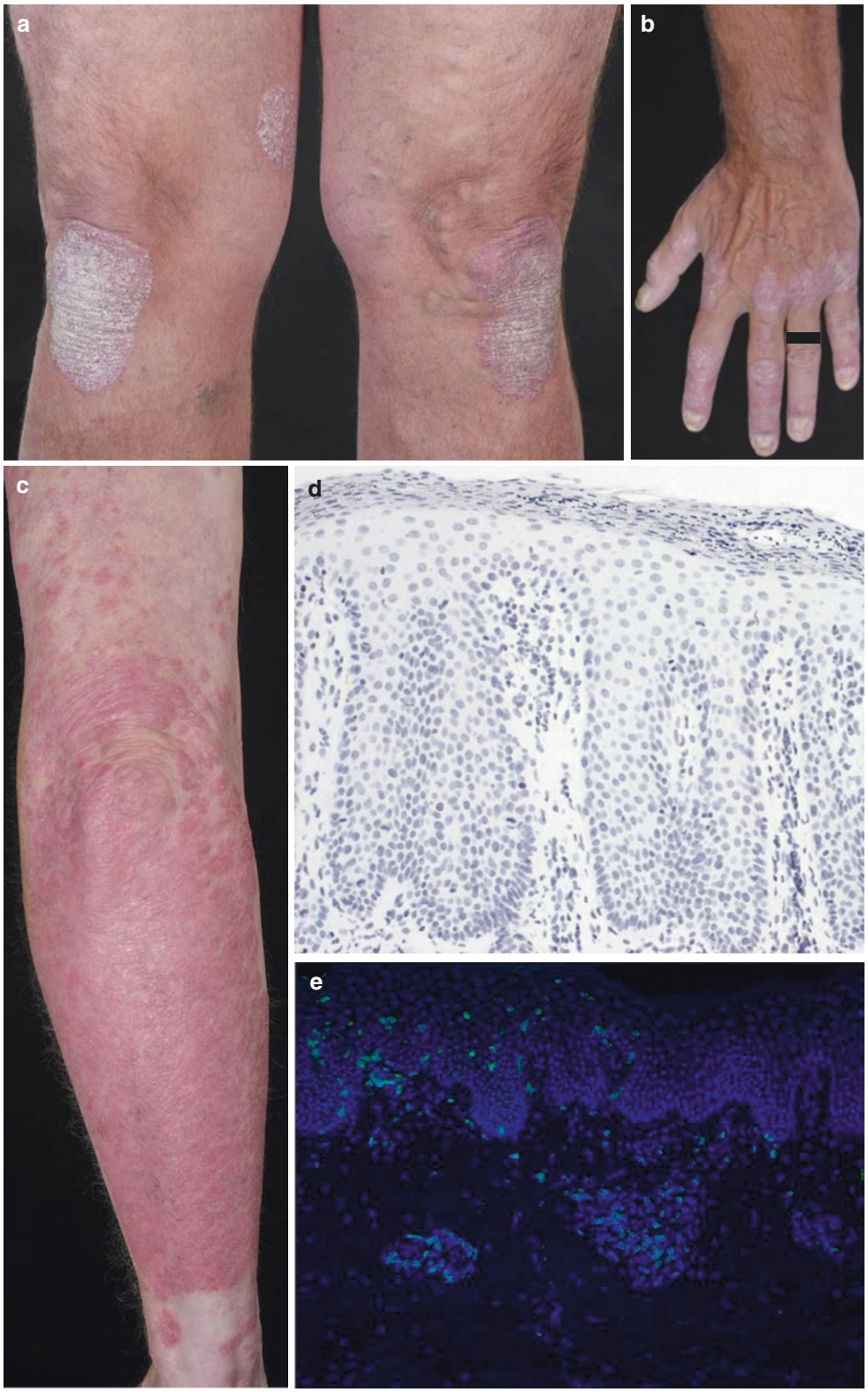

Fig. 21.1 Clinical and histoimmunological features of psoriasis- (**a**–**c**) clinical photographs of chronic plaque-psoriasis, note nail involvement in (**b**). (**d**) Hematoxylin-stained skin section from chronic plaque-psoriasis showing acanthosis, papillomatosis, parakeratosis, as well as Munro abscess. (**e**) Immunofluorescence staining of chronic plaque-psoriasis showing skin-infiltrating CD3+ T cells in green (Reproduced with permission from: Di Meglio et al. [242])

Added together, these last three forms of psoriasis represent 10–15 % of psoriasis cases. Both their relative infrequency as compared to the plaque-type form, and their peculiar epidemiological and clinical features (e.g. high prevalence in children and young adults for the guttate form or the extremely severe systemic manifestation for GPP and erythrodermic psoriasis) have hampered so far a thorough investigation of the genetic determinants and molecular and cellular mechanisms underlying their pathogenesis, which might diverge from that of plaque-psoriasis. Moreover, very few or no randomized controlled clinical studies have been specifically conducted with these forms of psoriasis thus far, leaving open only the option for off label use of approved therapy for plaque-type psoriasis which might or might not be effective. Thus, a concerted effort by the wide international scientific community is requested to fill both these gaps in the near future.

Co-morbidities

Psoriatic Arthritis

About 20–30 % of psoriasis patients develop psoriatic arthritis (PsA), a seronegative, chronic inflammatory muscoskeletal disorder, occurring in most cases about a decade after the appearance of psoriasis [14].

PsA has a complex aetiology mirrored in a wide spectrum of clinical disease presentation, expression and clinical course [15]. PsA can affect different tissues (synovium, cartilage, bone, entheses, tendons); it presents common involvement of distal joints, asymmetric articular distribution, erythema over affected joints, spinal involvement and enthesitis [14] and eventually leads to erosion and loss of function of the affected areas.

Since about 80 % of the patients develop PsA following psoriasis [16], PsA is sometimes considered as a disease within a disease with the skin manifestation being the parent disease [17]. PsA has a stronger genetic component than psoriasis (sibling recurrence risk (λ_s) of 27–47 for PsA v [18] *vs.* 4–11 for Ps [19]), however it is not as well defined as that of psoriasis due to phenotypic heterogeneity, disease overlap and smaller number of patients analyzed. Several PsA susceptibility genes, such as *HLA-C*, *IL12B*, *IL23R*, *TNIP1*, overlap with psoriasis [16, 20, 21]. On the other hand, differences in the genetic background of the two conditions do exist and unique genetic determinants have been identified, although not at a genome-wide significant level [21]. Nevertheless, PsA shares several key cellular and molecular mediators with psoriasis, such as lymphocytes infiltrating the inflamed skin or joint [22] and the pro-inflammatory cytokines TNF, IL-23, and IL17. TNF is a critical disease player and about 70 % patients successfully respond to anti-TNF therapy in terms of signs and symptoms improvement, and, in some cases also by radiographic progression [15]. Moreover, clinical trials showed efficacy for the anti IL-12/IL23p40 antibody ustekinumab [23–25] or anti-IL17 secukinumab [26] in the treatment of PsA.

Other Co-morbidities

The association of psoriasis with physical and psycho-social co-morbidities, such as cardiovascular disease (CVD, i.e. myocardial infarction (MI) and stroke), metabolic disorders (obesity, non-alcoholic fatty liver disease, dyslipidaemia and diabetes), depression and Crohn's disease (CD), is increasingly being appreciated and results in a more modern definition of the disease as a systemic inflammatory disorder in which the chronic nature of the skin inflammation is likely to contribute to the development of associated co-morbidities [27]. In analogy with that occurring in atopic dermatitis, the concept of "psoriatic march" has been proposed to describe the synergistic interplay of psoriasis and its co-morbidities in the establishment of systemic inflammation [28].

Moreover, in analogy to hypertension being called the "silent killer", psoriasis has been recently dubbed the "visible killer" [29] as patients with severe psoriasis have a 6-year reduction in life expectancy, mainly due to excess risk of CV death [30, 31]. The association between psoriasis and CVD has attracted considerable interest since a significant increased risk of death from CVD for psoriasis patients requiring hospital admission was reported in 2004 [32]. Nevertheless, it has been long known that psoriasis patients have a higher prevalence of traditional cardiovascular risk factors such as diabetes, hypertension, dyslipidaemia, obesity, and metabolic syndrome compared to the general population [33–35] that might account for some or all of the increased CVD risk. Therefore, a number of population-based epidemiological studies have been performed in the last decade to address this issue. Unfortunately, these studies are often heterogeneous in their data collection methods, outcomes, sample size and thus in statistical power, and display variable degrees of control for confounding factors [29, 36, 37]. This heterogeneity is likely to account for some conflicting results [38–40].

Nevertheless, a growing body of epidemiologic literature suggests that severe psoriasis (defined in most of the studies as affecting >10 % body surface area or requiring systemic treatment or phototherapy) confers a clinically significantly increased risk of CVD and cardiovascular mortality that is independent of conventional risk factors [39, 41]. A recent meta-analysis, including 14 previous epidemiological studies of which ten were population-based, showed increased risk of CVD in patients with severe disease, with OR relative

to the general population of 1.37 for CVD mortality, 3.04 for myocardial infarction (MI) and 1.59 for stroke [42]. Thus, psoriasis has been included as an independent risk factor for CVD in recent guidelines for CVD prevention (Fifth Joint Task Force of the European Society of Cardiology, 2012).

Moreover, severe psoriasis has been shown to be an independent risk factor for atherosclerotic CV disease, as defined by outcomes such as imaging of coronary and carotid arteries, and measurements of endothelial function and arterial stiffness [43–45].

As mentioned earlier, high prevalence of metabolic syndrome has been reported among psoriasis patients, with more than two-fold increased odds ratio (OR) in psoriasis patients, compared to matched healthy controls [46]. In particular, the association of type 2 diabetes with psoriasis is stronger in patients with severe disease (OR = 1.97) as compared to those with mild disease (OR = 1.53).

Clinical data and a better understanding of psoriasis immunopathogenesis also support these epidemiological observations. Elevated levels of nonspecific inflammation markers (e.g. as C-reactive protein), pro-inflammatory cytokines (such as TNF and IFN-γ) and immune cells (such as T helper type 1 (Th1) and Th17) in the circulation of psoriasis patients [47, 48], and the fact that most of these inflammatory markers are also increased in the skin lesions [49], strongly support that psoriasis is not only *skin deep*. Indeed, psoriasis patients have high levels of lipids and lipid peroxidation, altered adipokine function [50, 51] as well as abnormal coagulation profile [52, 53].

Despite a definitive biological link between co-morbidities and psoriasis has yet to be identified, it is reasonable to infer that the systemic inflammation and dyslipidaemia present in patients may predispose to impaired glucose tolerance and cardiovascular damage [27]. It has been suggested that the pro-inflammatory molecules produced by the skin could be released into the systemic circulation; in fact several genes differentially regulated in psoriasis are linked to functional pathways associated with metabolic diseases/diabetes and cardiovascular risk [49]. For instance renin, an enzyme involved in the renin–angiotensin pathway ultimately regulating blood pressure, is over-expressed in psoriatic skin, suggesting a functional link between expression profile at the skin level and peripheral functions. The presence of IL-17A/IL-17 F and CD4+ cells expressing IL-17 and IFN-γ in atherosclerotic lesions [54, 55], and risk alleles shared between psoriasis and its metabolic and cardiovascular co-morbidities [56], also lend support to the association between psoriasis and these diseases.

Epidemiological [57–59] and genetic [60, 61] studies support a close relationship between psoriasis and inflammatory bowel diseases (IBDs). Increased occurrence of psoriasis has been reported in patients with CD (8.9 %) yielding an aggregate relative risk of 7.1 ($\chi^2 = 139$, $P < 1 \times 10^{-9}$) [62]. Conversely, Li et al recently reported that women with psoriasis have a significantly increased risk of CD (relative risk (RR), 3.86, 95 % CI 2.23–6.67), but not ulcerative colitis (UC) (RR, 1.17, 95 % CI 0.41–3.36) [58].

Remarkably, psoriasis and IBDs share a number of common genetic determinants such as *IL23R* [60, 61].

Finally, psoriasis carries a severe psychosocial burden with anxiety, depression and perceived stress appearing at higher rate in psoriasis patients [63]. The impact of psoriasis on health-related quality of life is similar to that of other major medical diseases, including cancer, arthritis, hypertension, heart disease, diabetes, and depression [64]. Psoriasis patients find it hard to adapt to the chronic yet variable and unpredictable nature of the disease. Another major component of psychological distress is the anticipated negative reactions of others which can be of shame or stigmatization. Coping mechanisms include avoidance and seclusion which in turn affect their quality of life.

Changes in cognitive processing of facial expressions of disgust have been identified in psoriasis patients, who display smaller signal responses to disgusted faces to protect them from stressful emotional responses [65].

Depression, which is one of the stronger predictors of suicidal ideation, has been observed in more than 60 % of patients [66], and higher prevalence of suicidal ideation has been detected in psoriasis patients as compared to healthy controls and patients affected by other skin disease [67, 68].

Taken together, clinicians are presented with the challenging task of managing a multifaceted and lifelong disease which, although apparently not lethal, can severely affect patients' quality of life and, in some cases, life expectancy.

Thus, the current focus is not to only treat but to manage psoriasis patients [69]. A multidisciplinary approach is needed, particularly in cases of severe psoriasis with multiple comorbidities or cardiovascular disease with dermatologists alerted to detect and react to early indications of cardiovascular risk factors and comorbidities in psoriasis patients. While all patients should be encouraged to correct their modifiable cardiovascular risk factors, particularly obesity and smoking, and to adopt healthy lifestyle behaviours, patients with moderate to severe psoriasis are to be recognized and managed as being at intermediate risk of CVD with appropriate counselling and treatment [70].

Moreover, a patient-centred therapeutic approach, undertaken early in the psoriasis treatment pathway ("early intervention") aimed at complete clearance, has been advocated to improve control of cutaneous symptoms and modify disease course and burden [71].

A number of outstanding questions arise from the study of psoriasis and its comorbidities, including what is the effect of systemic therapy for psoriasis on CVD and diabetes [42, 72] and whether an association exists between specific subtypes of psoriasis and co-morbidities.

Etiopathogenesis

Psoriasis is a complex disease occurring in genetically predisposed individuals, in which a dysregulated immune response takes place following exposure to certain environmental triggers.

Genetics

Genetic predisposition to psoriasis is supported by population and family studies, as well as, higher concordance rates in monozygotic twins, compared with dizygotic twins (up to 73 vs. 20 %, depending on the population studied) [73–76]. Lack of complete concordance between monozygotic twins and familial occurrence of disease not following a clear inheritance pattern, support the definition of psoriasis as a complex genetic trait, resulting from gene–gene and gene–environment interactions [77, 78]. Large efforts to understand the genetic architecture of psoriasis have resulted in the identification of a number of psoriasis genetic determinants. The psoriasis genetic landscape emerging from recent genome-wide association studies (GWAS) and their meta-analysis [20, 60, 79–86], as of mid-2014, includes 36 independent psoriasis-associated genetic regions in individuals of European ancestry (Table 21.1), plus five more uniquely associated in the Chinese population [87]. Psoriasis- susceptibility genes encompass skin and immune-related genes, with the latter belonging to either the innate or the adaptive immunity, as well as bridging the two arms of the immune system. SNPs and Copy Number Variation (CNV) [85, 86] in genes of the late cornified envelope (LCE) family within the epidermal cell differentiation complex, a cluster of genes involved in skin barrier formation, support a critical role for skin-specific genes in psoriasis susceptibility. Among immune genes, the over-representation of four pivotal immunological processes and pathways strongly points towards their critical contribution to disease susceptibility: antigen presentation (*HLA-C* and *ERAP1*), NF-kb signalling (e.g. *TNFAIP3*, *TNIP1*, *TRAF3IP2*, *CARD14*), type I IFN pathway (e.g. *IL28RA* and *RNF114*), and IL-23/IL-17 axis (e.g. *IL23A*, *IL12B*, and *IL23R*) [77]. Psoriasis susceptibility region 1 (PSORS1) within the major histocompatibility complex is the strongest susceptibility locus [88, 89] and the HLA- Cw*0602 allele of the MHC class I molecule HLA-C is considered to be the primary associated allele, as confirmed by early sequence and haplotype analysis [90], and more recently by GWASs [81, 82, 86], and analysis of high density SNP data [91]. The HLA-C association has not only the greatest statistical significance observed in GWAS studies [86] but also accounts for about 6 % of the total genetic variance [92], which is at least ten fold more than that explained by any other known psoriasis susceptibility locus [86]. The expression pattern of MHC class I molecule on all nucleated cells makes HLA-C capable of regulating both innate and adaptive responses [93]. Nevertheless, despite the strong genetic evidences and the obvious immunological function of HLA-C, functional studies addressing the precise mechanism by which -Cw*0602 alleles predispose to psoriasis are still missing and no -Cw*0602 specific antigen or interacting protein has been identified to date. Among genes of the innate immunity, more than half belong to the NF-kB pathway, which has a pivotal role in amplifying and sustaining chronic inflammation. Two recent studies have identified and evaluated the functional consequences of rare and common gene variants [86, 94] and missense mutations [12] in CARD14, an activator of NF-kB primarily expressed in skin epidermis. Genes belonging to the type I IFN pathway support clinical and experimental findings indicating an important role for antiviral responses in psoriasis [95, 96]. Finally, several susceptibility genes belong to the IL-23/IL17 pathway, whose critical involvement in disease pathogenesis has been extensively documented by a wealth of studies showing a pivotal role for IL-23-induced and IL-17-mediated responses in psoriasis [97]. Moreover, the genetic association with *IL23R* is one of the very few supported by functional evidence with reduced IL-17 responses in carriers of the protective Arg381Gln *IL23R* allele [98, 99]. Interestingly, some genes from the above pathways influence multiple phenotypic traits, in particular other immune-mediated conditions such as Crohn's disease (CD), celiac disease and ankylosing spondylitis [86], thus confirming the presence of a shared genetic basis among immune-mediated inflammatory diseases.

The signals identified in the European population collectively account for approximately 20 % of estimated psoriasis heritability, and the gene variants identified have only modest- effect size [86]. It has been hypothesized that rare variants with bigger effects may explain this "missing heritability" [100]. However, two recent re-sequencing studies in individuals of European or Chinese descent, have shown that rare variants at known immune-related loci have a negligible role in psoriasis genetic susceptibility [101, 102], suggesting that the estimated missing heritability either results from the co-existence of many common variants of weak effect, or has been overestimated, owing to the existence of gene-gene and gene-environment interactions [103].

Environmental Factors

In contrast to the fast-growing list of psoriasis susceptibility genes, the environmental component concurring in initiating the disease is still ill-defined. Among known environmental triggers of psoriasis there are drugs, infections, physical trauma, smoking, alcohol and stress.

Table 21.1 Psoriasis susceptibility genes

Chr	Gene(s)	Class	Protein function	Pathway	Reference
1	LCE3B/3C/3D	Skin-related	Keratinocyte structural protein	Skin barrier formation	[80, 84, 85]
1	IL28RA	Immunity	IL-29 receptor subunit	IFN signaling	[82]
1	IL23R	Immunity	Unique subunit of IL-23 receptor complex	IL-23/IL17 axis	[79–82, 86]
1	RUNX3	Immunity	Transcription factor	Tbet pathway	[86]
1	TNFRSF9*	Immunity	Adaptor molecules involved in T cell biology	T cell differentiation	[16, 86]
2	REL	Immunity	NF-kB subunit	NF-kb signaling	[82]
2	IFIH1	Immunity	Innate antiviral receptor	IFN signaling	[82]
2	B3GNT2	Other	Enzyme	Carbohydrate metabolism	[86]
5	TNIP1	Immunity	Inhibitor of TNF-induced NF-kB activation	NF-kb signaling	[81, 82, 87]
5	IL12B	Immunity	Shared subunit of IL-12/IL-23	IL-23/IL17 axis	[79–81, 84, 86]
5	ERAP1	Immunity	Enzyme processing MHC class I ligands	Antigen presentation	[82, 83]
5	IL4/IL13	Immunity	IL-4 and IL-13 cytokines	IL-4/IL-13signaling	[81]
6	TNFAIP3	Immunity	Inhibitor of TNF-induced NF-kB activation	NF-kb signaling	[81, 82]
6	TRAF3IP3	Immunity	Adaptor molecule mediating IL-17-induced NF-kb activation	IL-23/IL17 axis, NF-kB signaling	[20, 80, 82]
6	IRF4*	Immunity	Transcription factor	IL 17 signaling	[86]
6	HLA-C	Immunity	MHC class I antigen	Antigen presentation	[79–82, 84, 86]
6	TAGAP	Immunity	RhoGTPase-activating protein	T cell activation	[86]
7	ELMO1	Immunity	Involved in TLR-mediated IFN-a signaling	IFN signaling	[86]
9	KLF4	Skin-related/ immune	Transcription factor	Skin barrier formation, IL 17 signaling	[86]
9	DDX58	Immunity	Innate antiviral receptor	IFN signaling	[86]
10	ZMIZ1	Immunity	Protein inhibitor of activated STAT(PIAS) family of proteins	TGF-β signaling	[16, 86]
11	PRDX5	Other	Antioxidant enzyme	Intracellular redox signaling	[16, 86]
11	ETS1	Immunity	Transcription factor	Unknown	[86]
11	ZC3H12C	Other	Zinc finger protein with putative RNase function	Unknown	[86]
12	IL23A	Immunity	Unique subunit of IL-23	IL-23/IL17 axis	[81, 82]
14	NFKBIA	Immunity	Inhibitor of NF-kB activation	NF-kb signaling	[82, 83]
16	FBXL19	Immunity	Putative inhibitor of NF-kB activation	NF-kb signaling	[83]
16	SOCS1	Immunity	Suppressor of cytokine signaling	Type II IFN signaling	[86]
17	CARD14	Immunity	Activator of NF-kB pathway	NF-kb signaling	[12, 86, 94]
17	NOS2	Immunity	Induced nitric oxide synthase	Inflammation	[83]
17	STAT3*		Transcription factor	IL-23/IL17 axis	[86]
18	MBD2*	Other	Transcriptional repressor	Unknown	[86]
19	CARM1*	Immunity	Transcriptional co-activator of NF-kB	NF-kb signaling	[86]
19	TYK2	Immunity	Tyrosine kinase associated with cytokines receptors	IL-23/IL17 axis, IFN signaling	[82]
20	RNF114	Immunity	E3 ubiquitin ligase	IFN signaling	[79, 81–83]
22	UBE2L3*	Immunity	Ubiquitin conjugating enzyme	NFkb signaling	[83, 86]

* denotes more then one gene in the locus; the most plausible one is reported

Drugs, such as the anti-viral and anti-proliferative agent imiquimod, anti-depressants (lithium), anti-hypertensives (beta-blockers), cytokines (IFN-α) and anti-cytokine therapies (anti-TNF) have all been clinically associated with initiation, exacerbation and worsening of psoriasis [104]. Imiquimod, a Toll Like Receptor (TLR) 7/8 agonist used to treat genital warts and non-melanoma skin tumours, represents one of the best investigated examples of psoriasis triggers so far. The initial clinical observation of a case of psoriasis exacerbated by topical treatment with imiquimod [105] has prompted research into plasmacytoid DC (pDC) and type I IFN pathway, which is downstream of TLR7/8 signaling [95]. Moreover, it has also been translated into the imiquimod-induced psoriasiform skin inflammation mouse model, which faithfully reproduces most of the features of the human disease and has quickly become one of the most widely used experimental models to study psoriasis [106].

An association between preceding streptococcal throat infection and psoriasis [107] has been reported, mainly with guttate psoriasis [108] and homologous T cell clones have been found in both the tonsils and skin lesions of plaque-type psoriasis patients [109].

Tattoos and surgical incisions give rise to the Koebner phenomenon with psoriasis plaques appearing at the site of the trauma [110].

The association of modifiable behavioural risk factors, such as smoking and alcohol consumption, as well as comorbidites such as stress, are traditionally more difficult to investigate. Although a number of studies have offered evidence linking stress and smoking with psoriasis [33, 111, 112] there is no consensus on whether these factors do actually cause or aggravate psoriasis [113].

While more often included among risk factors, environmental cues can also have a protective role against disease. We have recently uncovered an unexpected protective role in psoriasis for the ligand-activated transcription factor Aryl hydrocarbon receptor (AhR) [114], an environmental sensor which responds to a wide range of stimuli including environmental pollutants (e.g. dioxin), but also more physiological ones (e.g. tryptophan metabolites of dietary or light-exposure origin), by inducing detoxifying enzymes belonging to the cytochrome P450 family. In particular, physiological ligands are believed to mediate the beneficial effect exerted by AhR, via short-term activation of the receptor in contrast to the prolonged and deleterious activation sustained by dioxin and tobacco smoke [115]. By combining data from the imiquimod model of psoriasis-like skin inflammation in mice lacking AHR, with the analysis of human psoriasis skin biopsies treated *ex vivo* with AhR ligands we found that absence of AhR signalling results in an exacerbation of psoriasis [114]. In particular AhR exerts its beneficial effect in keratinocytes as in its absence they become over-reactive to pro-inflammatory stimuli and release a greater amount of cytokines and chemokines, thus instigating an excessive inflammatory reaction. Thus, physiological AhR activation acts as an immunological brake that prevents dysregulation of the inflammatory response. Based on this data it is tempting to speculate that psoriasis patients might have impaired activation of the AhR pathway, possibly due to genetic variants in genes of the AhR pathway or perhaps shortage of physiological ligands, and ongoing studies are addressing these hypotheses.

Immunopathogenesis

The contribution of the immune system to psoriasis is not less complex than the overall disease pathogenesis, with a variety of innate and adaptive immune cells and pro- inflammatory mediators involved, possibly at different stages of the disease.

The recent integration of findings drawn from studies of clinical samples, xenotransplant models in which human skin is transplanted onto immuno-compromised hosts, and experimental mouse model of psoriasis-like skin inflammation, has contributed and accelerated a better understanding of disease pathogenesis. It is worth highlighting that, although no mouse model can fully recapitulate the development and features of a disease only occurring in humans [116], lessons from a number of animal models, exhibiting most of the crucial clinical traits and molecular signatures of psoriasis [117], should not be discarded as they can provide valuable insights to dissect pathogenic mechanisms.

The question of whether psoriasis is primarily an epithelial- or immune-mediated disease has recurred for several decades in the scientific community, with researchers torn between the prominent changes in the skin and the increasingly recognized importance of immunological pathways. Not surprisingly, given the macroscopic alterations occurring in psoriatic skin, the focus has initially been on keratinocytes and the accelerated and aberrant terminal differentiation program they undertake in psoriasis. Following the unexpected efficacy of serendipitously administered immunosuppressive agents, immune cells have attracted attention and have been almost the exclusive focus of clinical and experimental studies for two decades. These efforts have elucidated many of the pathogenic immune mechanisms and highlighted the critical contribution of tissue resident T cells and TNF, leading to effective anti-T cell and anti-cytokine targeted therapies. Nevertheless, KC, equipped with innate immune receptors and actively taking part in inflammatory skin responses, are nowadays considered non-hematopoietic

immune cells and gained the status of skin sentinel cells [118, 119], thus prompting a re-evaluation of their role in psoriasis.

The current view of psoriasis pathogenesis implies that the aberrant immune and epidermal response seen in psoriasis is sustained by a pathogenic cross-talk between epithelial and immune cells [120, 121]. This interplay is primarily driven by the critical pro-inflammatory molecules, TNF, IL-23 and IL-17, whose direct therapeutic targeting has proven to be clinically effective, with other mediators, such as IFN-α, IFN-γ and IL-22 possibly contributing to the initiation, amplification and maintenance of the disease.

One of the best characterized initiation mechanisms, leading to dysregulated skin immune responses, involves damaged skin KC releasing the cationic antimicrobial peptide (AMP) LL-37, following physical trauma or infection. LL37 binds to self-DNA/RNA fragments forming LL-37/self-DNA/RNA complexes [122–124] that activate TLR7/9-bearing pDC. pDC are a subset of circulatory DC normally absent in human healthy skin but highly infiltrating developing psoriasis lesions [95], and once activated, specialize in releasing type I IFN which is thought to play important roles in the early phases of psoriasis development. In the AGR129 xenotransplantation model, where human non-lesional psoriatic skin is transplanted onto AGR129 mice lacking T/B cells and having severely impaired NK activity, Type I IFN triggers activation and expansion of autoimmune T cells present in the transplant, leading to full-fledged psoriasis plaque formation [95]. Moreover, self-RNA-LL-37 complexes, and pro-inflammatory cytokines IL 1β, IL 6, TNF and IFNα activate myeloid DC. Psoriatic skin lesions have a 30-fold increase in myeloid dermal DC (DDC) [125] and harbour both "classical" CD11c + CD1c + DDC, also found in healthy skin, and a distinct population of "inflammatory" CD1c- DC [126] producing TNF, iNOS, IL-20 and IL-23 [127–129]. DDC are thought to migrate to skin-draining lymph nodes to present an as-yet elusive antigen (either of self or of microbial origin) to naïve T cells. T cells, particularly those residing in the skin as tissue-resident memory T (T_{RM}) cells, are critical players in the initiation phase of disease. T_{RM} are memory cells which do not circulate but are strategically positioned as the first-line defence in the tissue [130, 131] and have been implicated in long term peripheral immunity [132, 133]. One of the very first evidences of their existence came from the AGR xeno- transplantion model of human non-lesional psoriatic skin onto immunodeficient mice. In this model development of full-fledged psoriatic lesions occurs in the absence of T-cell recruitment from blood [134] and depends on the ability of locally activated skin T_{RM} cells, present in the initial graft, to migrate into the epidermis [135].

Activation of DDC and their interaction with T cells is central to plaque progression as it creates an IL-23/IL-17 inflammatory environment in which DC and macrophages-derived IL-23 promotes an IL-17-rich pro-inflammatory environment sustained by Th17 cells, [48, 121], Tc17 [136–138], γδ-T cells [139, 140], NCR+ group 3 Innate lymphocytes (ILC3) [141–143] and possibly neutrophils, mast cells [122] and regulatory T cells [144]. The initial definition of psoriasis as Th1 and IFN-γ-driven disease, based on a strong type II IFN transcriptomic signature and the high frequency of Th1 and Tc1 cells in both psoriasis plaques and peripheral blood [145–147], has been challenged by the discovery of IL-23 and Th17 cells and a wealth of genetic, clinical and experimental findings indicating a key role for the IL-23/IL-17 axis in psoriasis [97].

IL-17A and IL-17 F, sharing high structural and functional homology, activate keratinocytes to produce an array of molecules with chemoattractant properties, including neutrophil- (CXCL1, CXCL2, CXCL5, CXCL8) and T cell- (CCL20) recruiting chemokines and AMP (LL37, S100A7/8/9/15) [148, 149]. Moreover, another IL-17 family member, IL-17C induces an autocrine pro-inflammatory loop in KC [150, 151] and IL-22, produced by Th, Tc and NCR+ ILC3, which mediates most of the epidermal hyperplasia by impairing KC differentiation [152, 153]. Finally, a recent study has identified IL-9-producing Th cells FOXP3+ in psoriatic lesions although their pathogenic relevance has not been established to date [154].

Taken together, a pathogenic cross-talk between KC, DCs and T cells, sustained by TNF, IL-23 and IL-17 and supported by other immune cell players and further pro-inflammatory molecules, underlie the dysregulated immune response observed in psoriasis (Fig. 21.2).

Targeted Therapy

No definitive cure is available for psoriasis and current treatments are aimed at decreasing disease activity and improving symptoms. Therapies are administered according to disease severity, assessed by the Psoriasis Area and Severity Index (PASI, ranging from 0 to 72), which takes in account appearance and extension of the lesions. Most psoriasis patients present with a mild form of disease which is usually treated with topical agents, with local anti-inflammatory and/or anti-proliferative action. However, moderate and severe disease cases require systemic treatment. Systemic treatment usually comes after unsuccessful topical strategies, in a two-tiered approach where systemic therapy is used as a second-line treatment of moderate to severe psoriasis. Traditional systemic therapies aim at general immunosuppression and include the use of cyclosporine and/or methotrexate (MTX). In the past decade, a

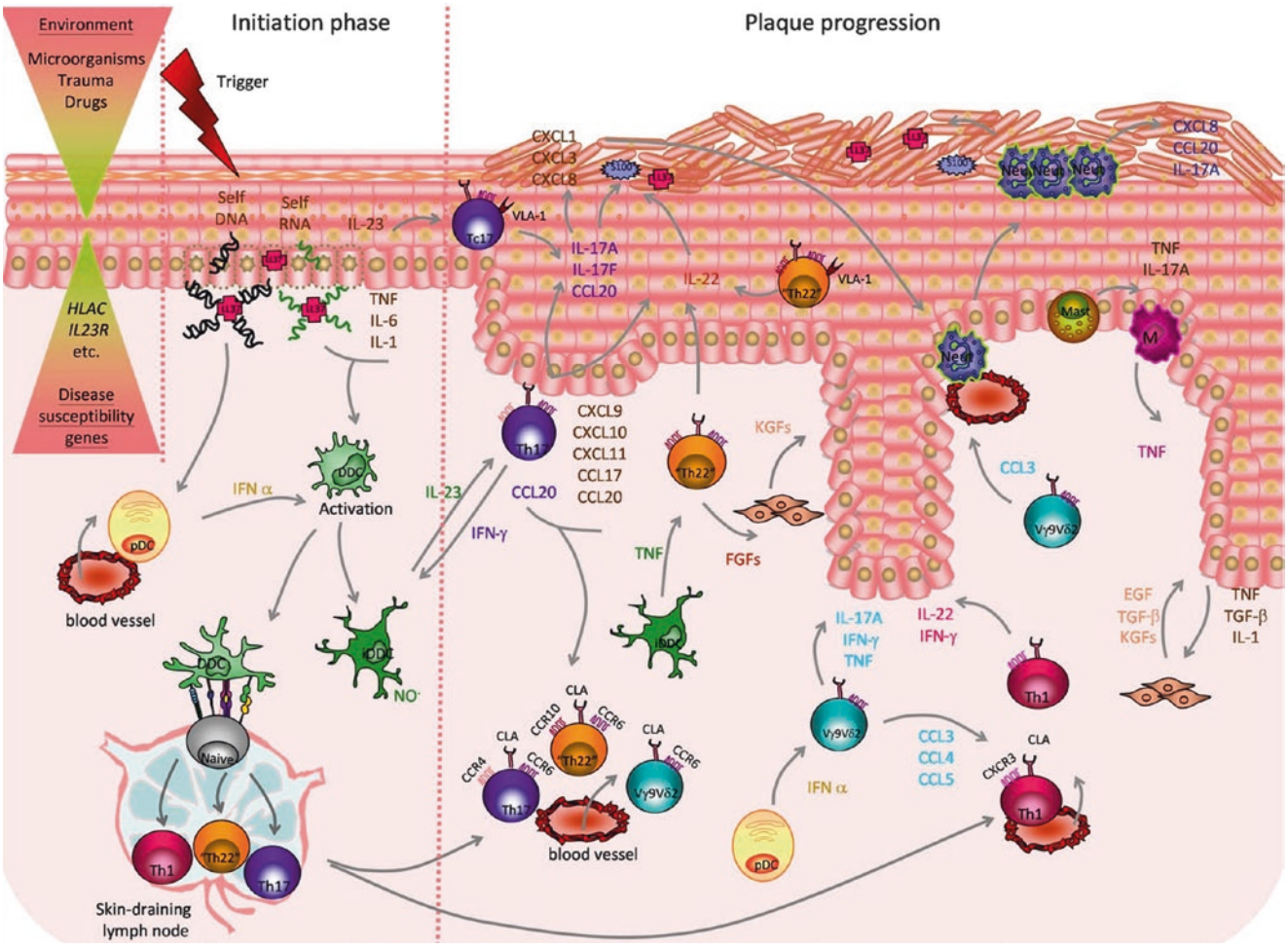

Fig. 21.2 Psoriasis etiopathogenesis- The combination of environmental factors with psoriasis susceptibility genes triggers a cascade of pathogenic events leading to disease initiation and plaque formation. In the initiation phase, pro-inflammatory cross-talk between damaged keratinocytes (KCs), releasing self-nucleic acids and LL-37, recruited plasmacytoid dendritic cells (pDCs), activated dermal DC (DDCs) and inflammatory DDC (iDDCs), producing IL-23, TNF and nitric oxide radicals (NO.), promote the activation of skin resident and newly recruited T cells that lead to plaque formation. IL-23 stimulates T helper 17 (Th17) and T cytotoxic 17 (Tc17) cells, expressing cutaneous leukocyte antigen (CLA), CCR6 and CCR4, plus very late antigen (VLA)-1 in the epidermis, to release IL-17A, IL-17 F, IL-22 and IFN-γ. IL-17A and IL-17 F act on KCs promoting production of T cells and neutrophils-attracting chemokines (CXCL1,3,8-11;CCL17-20) and antimicrobial peptides (AMPs): S100 proteins and LL-37. IL-22, produced by Th17 cells, as well as Th1 cells, expressing CXCR3 and skin-homing marker CLA, and "Th22"/"Tc22" cells, expressing CCR6, CCR10 and CLA, induces epidermal hyperplasia by impairing KC terminal differentiation. Recruited unconventional V 9v 2 T cells, expressing CLA and CCR6, are activated by pDCs-derived IFN-and release further pro-inflammatory cytokines and chemokines (CCL3-5) attracting neutrophils (Neut) and Th-1. Infiltrating Neut, mast cells and macrophages (M) contribute to the pro-inflammatory environment producing cytokines (IL-17A, TNF), AMPs (S100 proteins, LL-37) and chemokines. Cross-talk between keratinocytes producing IL-1, TNF and transforming growth factor beta (TGFβ), and fibroblasts, which in turn release keratinocyte growth factor (KGF), epidermal growth factor (EGF) and TGFβ, and possibly Th22 cells releasing fibroblast growth factor (FGFs), contribute to tissue reorganization (Reproduced with permission from Di Meglio et al. [120])

better understanding of disease immunopathogenesis has been successfully translated into new drugs, known as *biologics*, targeting key inflammatory mediators and currently representing an effective third-line therapy in moderate-to-severe psoriasis patients, unresponsive to non-biologic systemic agents. As of mid-2014, there are five biologics approved for the treatment of psoriasis, targeting either T cells or cytokines such as TNF or IL-12/IL-23 (see also Chap. 43) [155] (Table 21.2).

Anti T-Cell and Anti-cytokines Biologic Drugs

The first biologics to be approved for psoriasis treatment were anti-T cell therapies targeting T cell adhesion or activation.

The first biologic to be approved was alefacept in 2003, a LFA-3/IgG1 fusion protein binding CD2 on T cells and thus, selectively inducing apoptosis of CD2(+) human memory-effector T cells [156]. Clinical efficacy was shown in phase

Table 21.2 Targeted therapy for Psoriasis in 2014

Mechanism of action	Name	Molecular target	Phase	Formulation	Administration route	Company	Reference
Anti-Tcells	Alefacept	CD2	Approved 2003 (US)	Human LFA-3/ IgG1 fusion protein	IM or IV	Biogen	[157, 158]
Anti-cytokine	Etanercept	TNF	Approved 2004 (US and EU)	Human TNF-R(p75)-lgG1 fusion protein	SC	Amgen	[169–171]
	Infliximab	TNF	Approved 2006 (US and EU)	Mouse-human IgG1 chimeric monoclonal antibody	IV	Janssen Biotech	[172–174]
	Adalimumab	TNF	Approved 2007 (EU) 2008 (US)	Human IgG1 monoclonal antibody	SC	Abbott	[175, 176]
	Ustekinumab	IL12p40 (IL-12,IL-23)	Approved 2009 (US and EU)	Human IgG1 monoclonal antibody	SC	Janssen Biotech	[179, 180]

III studies with 40 % of patients achieving a PASI75 response (75 % reduction of the PASI) [157] and more that 50 % achieving PASI 50 (50 % reduction of the PASI) [158]. A further antibody, anti-T cell strategy was approved in 2003 and consisted in a humanized antibody (efalizumab) binding and blocking CD11a, a key molecule for T-cell activation and migration through the circulation into the skin [159]. Despite a good efficacy profile, [160–166], efalizumab has been withdrawn from the market in 2009, due to three cases of progressive multifocal leukoencephalopathy [167], highlighting the importance of carefully monitoring the long-term safety of immunomodulatory therapies.

An alternative strategy to anti-T cell targeting aims at interfering with the psoriasis cytokine network using anti-cytokine biologic drugs. TNF blockade was serendipitously found to ameliorate psoriasis in a patient with IBD treated with the anti-TNF drug infliximab and had concomitant psoriasis [168]. Currently there are three anti-TNF biologics approved for psoriasis: etanercept, a human p75 TNF receptor fusion protein (approved in 2004), infliximab, a humanized chimeric anti-TNF monoclonal antibody (approved in 2006), and adalimumab, a fully human monoclonal antibody (approved in 2008). The efficacy of TNF inhibitors has been tested in phase III clinical trials, showing up to 80 % of treated patients achieving PASI75 within 10–12 weeks of treatment [169–176]. TNF neutralization causes early down- modulation of myeloid cell-related genes, with decrease of Th17 cell products and downstream molecules in just 2 weeks after commencing therapy [125, 177, 178]. The latest biologic to be approved for psoriasis in 2009, ustekinumab, is a monoclonal antibody simultaneously blocking the heterodimeric proteins IL-12 and IL- 23 via its binding to the shared subunit p40. Its efficacy is quite high, with 67 % of patients achieving PASI75 at 12 weeks of treatment [179, 180].

Some serious adverse events, such as opportunistic infections and reactivation of latent tuberculosis, have been reported for these monoclonal antibodies [181]. Long term safety data (up to 4 and 5 year treatment) are now available for etanercept and ustekinumab [169–171, 182, 183], suggesting a safe use of these drugs.

Emerging New Drugs: Biologics and Small Molecules in Clinical Trials

A number of other biologic drugs is currently being investigated in clinical trials, as of mid-2014 (see also Chap. 43) (Table 21.3). In line with the prominent role of IL-23/IL-17 axis in psoriasis, there are three antibodies (BI655066, tildrakizumab, and guselkumab) specifically targeting the IL-23p19 subunit which are currently being tested in phase II [184], III [185] or have recently completed phase II [186] clinical trials, respectively. Data from a small phase 1 study showed between 50 (10 mg) and 100 % (300 mg) of guselkumab-treated patients achieving PASI75, with improvements generally maintained through week 24 and rate of adverse event similar to placebo-treated patients [187]. Preliminary phase IIb data presented at the American Academy of Dermatology (AAD) meeting 2014 showed up to 81 % of patients receiving the highest dose achieving PASI75 response [188]. Phase 3 data for tildrakizumab presented at AAD 2013 showed PASI75 response rates of 64–74 % [189].

Monoclonal antibodies blocking either IL-17A (ixekizumab and secukinumab) [190–192] or IL-17R brodalumab [193], have shown remarkable efficacy in phase 2 clinical trials with more than 70 % of patients achieving PASI 75, and more than half achieving a striking PASI 90. While phase 3 clinical trials are currently ongoing, initial molecular data

Table 21.3 Emerging drugs in clinical trials as of 2014

Type	Mechanism of action	Name	Molecular target	Phase	Formulation	Administration route	Company	Reference
Biologics	Anti-cytokine	Tildrakizumab (MK-3222)	IL-23p19	Phase III	Humanized IgG1 monoclonal antibody	SC	Merck	[185, 189]
		Guselkumab (CNTO 1959)	IL-23p19	Phase II	Human IgG1 monoclonal antibody	SC	Janssen Biotech	[186–188]
		BI655066	IL-23p19	Phase II	Humanized IgG1 monoclonal antibody	SC	Boehringer Ingelheim	[184]
		Brodalumab (AMG 827)	IL-17R	Phase III	Human IgG2 monoclonal antibody	SC	Amgen	[193]
		Ixekizumab (LY2439821)	IL-17	Phase III	Humanized IgG4 monoclonal antibody	SC	Eli Lilly	[190]
		Secukinumab (AIN457)	IL-17	Phase III	Human IgG1 monoclonal antibody	SC or IV	Novartis	[191, 192]
	PDE4 inhibitor	Apremilast (CC-10004)	PDE4	Phase III	N/A	Oral	Celgene	[201, 202]
Small molecule	JAK inhibitor	Tofacitinib (CP-690,550)	JAK1 and JAK3	Phase II	N/A	Oral	Pfizer	[199, 200]
		Tofacitinib (CP-690,550)	JAK1 and JAK3	Phase II	N/A	Topical	Pfizer	[198]

showed that the effect of IL-17 blockade on expression of genes synergistically regulated by IL-17 and TNF is greater than in previous studies with anti-TNF therapy [194].

Notwithstanding the efficacy of the biologic drugs currently available in the clinic, at least one third of patients do not respond to biologic therapy [195] or lose initial responsiveness, due to the development of anti-drug antibodies (ADA), which causes decreased drug efficacy and/or induction of adverse events [196, 197]. Moreover, biologic drugs pose a considerable economic burden due to their cost of about £10 k per patient/per year. Finally, a sizable number of patients with mild-to-moderate psoriasis still rely only on traditional topical treatments to manage their disease. Thus, other therapeutic options are being explored, such as small molecules, i.e. low molecular weight organic compounds targeting key molecules involved in cellular signalling. Based on the importance of cytokine- driven inflammatory pathways in psoriasis, the most promising small molecules currently under testing are targeting key cellular components in cytokine signalling, such as Janus kinases (JAK), as well as enzymes involved in cytokine production. Among JAK inhibitors, tofacitinib, which specifically inhibits JAK1 and 3 and is approved for RA treatment, has showed good efficacy in phase II trials for both its oral and topical formulation [198, 199] with 66.7 % of patients treated with the highest dose of oral tofacitinib reaching PASI 75 at 12 weeks in a phase 2b study [200]. Apremilast, a phosphodiesterase four inhibitor which inhibits an enzyme involved in the breakdown of cAMP, thus suppressing the production of pro-inflammatory cytokines is also been tested in Phase IIb clinical trials [201, 202] showing 41 % of patients achieving PASI75 at week 16 with the highest dose. A cheaper manufacturing process, a route of administration (oral or topical vs injectable) which ensures high patient compliance and an overall good safety profile, makes the use of these small molecules in mild-to-moderate psoriasis foreseeable

Novel Integrative Approaches for Stratified Medicine in Psoriasis

Stratified medicine, that is tailoring medicine to the individual characteristics of each patient, aims at predicting individual susceptibility to disease, detecting the onset of disease at the very earliest stages, predicting and pre-empting disease progression, developing novel targeted therapies, and prescribing safe and effective medicines to each patient.

At the heart of stratified medicine is patient stratification, or the classification of individuals into sub-populations that differ in their susceptibility to a particular disease, or the

natural history of their disease or their response to a specific treatment. To this end, stratified medicine encompasses the entire spectrum of molecular medicine (including the genome, proteome, metabolome and epigenome) and relies on recently developed technologies allowing deep analysis of biological samples available in limited quantity, in a high-throughput manner.

The psoriasis research community has embraced these innovative tools with enthusiasm, not only to better understand disease mechanisms but, ultimately, to overcome patients' heterogeneity and improve disease diagnosis, prognosis and therapy, by implementing stratified medicine approaches.

For instance, high-throughput genotyping has enabled the identification of 36 psoriasis susceptibility genes by means of GWAS in which common genetic variation such as SNPs are examined in patients and control individuals to identify association with the disease [86]. Moreover, next-generation sequencing (NGS) that parallelize the sequencing process, producing millions of sequences concurrently and at a fraction of the initial costs, have enabled the refining of the psoriasis transcriptome via RNA-sequencing [203, 204], building upon initial array-based analysis [205–207]. Array-based technologies have also been used for DNA methylation profiling resulting in the detection of different methylation profiles between lesional and non lesional psoriatic skin [208]. Other powerful techniques allowing multiparameter analysis of samples at the cellular level are multiparameter flow cytometry with the use of lyoplates, (pre-formatted plates containing lyophilized cocktails of antibodies) to increase reproducibility, standardization and medium throughput processing of the samples [141, 209].

Not only all the aforementioned technologies each generate a vast amount of data, but they are often used in combination to analyse the same biological sample, thus resulting in an escalating amount of data which require powerful bioinformatics tools not only to analyse but also to integrate, handle, manage, and store these "omics" data [210]. Computational analysis approaches applied to the analysis of large data in psoriasis has already proven successful in a number of studies, especially taking advantage of the large amount of publicly available gene expression data from psoriasis skin. Integrative approaches have been used to combine the results of five microarray datasets obtaining the Meta-Analysis Derived (MAD) psoriasis transcriptome [205]. The over-representation of atherosclerosis signaling and fatty acid metabolism pathways in lesional skin, supports the close relationship between psoriasis and systemic manifestations [205]. A set of 20 "classifier" genes clearly separating lesional from non lesional psoriasis skin has also been identified, to contain many genes that were part of the residual disease genomic profile, or "molecular scar', still present in psoriasis skin after successful treatment [211]. Moreover, by integrating differentially expressed genes with altered expression in psoriasis lesions, as well as candidate genes near susceptibility loci from psoriasis GWAS studies and candidate cell types for genes near susceptibility loci, a recent study has prioritized SNPs at which susceptibility variants are predicted to influence transcription factor binding [212]. This has resulted in the identification of potentially causal non-coding SNPs for which susceptibility variants influence binding of key pro-inflammatory transcription factors, such as AP-1, NF-kB, IRF1, STAT3 and STAT4. From skin gene expression data, ensembles of decision tree predictors were used to cluster psoriatic samples and the analysis revealed distinct molecular sub-groups within the clinical phenotype of plaque psoriasis [213]. In another study, cytokines and cell-type specific signatures were identified according to differentially expressed genes in the lesions, uncovering a range of inflammatory- and cytokine- associated gene expression patterns able to differentiate between etanercept responders and non-responders [207]. Data from human samples can also be integrated with data from in vivo models to overcome the translational gap in the development of new targeted therapies. For example, the role of the cytokine IL-22 in psoriasis has been recently evaluated by comparing the publicly available psoriasis transcriptome with the transcriptome derived from humanized mouse models of disease (i.e. IL-22 injection into xenografts of normal human skin) for inhibition of disease (i.e. antibody-mediated blockade of IL-22 into xenografts of psoriasis human skin). Mapping the in vivo experimental data over the psoriasis transcriptome, resulted in the identification of PIM1, a serine/threonine kinase, subsequently validated as a critical checkpoint for human skin inflammation and potential future therapeutic target in psoriasis [214]. Finally, a systems biology approach has been used to model and quantify immune cell interactions contributing to skin inflammation via cytokine signalling [215].

The Quest for Psoriasis Biomarkers

Critical to the implementation of stratified and personalized medicine approaches are biomarkers, or biological characteristics that are measured and evaluated objectively as indicators of normal biological processes, pathogenic responses or to pharmacological responses to therapeutic intervention [216]. Biomarkers can be classified according to their function in diagnostic biomarkers, indicating the existence of disease; prognostic biomarkers, able to forecast disease progression, with or without treatment, and predictive or theranostic biomarkers, able to predict the probable response to a particular treatment. A further

alternative classification, according to the NIH Biomarkers Definitions Working Group, distinguish biomarkers in three categories: type 0 biomarkers, correlating longitudinally with the severity of disease; type 1 biomarkers, reflecting the effect of an intervention according to the mechanism of action of therapy itself (or drug endotype), and type 2 biomarkers which are surrogate endpoints for a therapy [217].

Examples of stratified medicine approaches are already in clinical practice in oncology, where a number of reliable biomarkers aiding in patients stratification have been identified and implemented (e.g. human epidermal growth factor receptor (Her2) in breast cancer tissue [218]. Following in these steps, large efforts aimed at the identification of psoriasis biomarkers are ongoing [216].

Psoriasis is in most of the cases diagnosed by clinical assessment by a dermatologist, with rare cases of histological confirmation of the disease. However, in patients difficult to diagnose, e.g. those with other skin diseases, such as atopic dermatitis, a molecular signature may be useful. Using patients simultaneously affected by both psoriasis and non-atopic or atopic dermatitis, a recent study has developed a disease classifier consisting of NOS2 and CCL27 which was able to identify initially misdiagnosed or clinically undifferentiated patients [219].

Prognostic biomarkers indicating disease severity progression or the onset of comorbidities would be extremely useful for better patient management. Moreover, biomarkers for early diagnosis are an unmet need in psoriatic arthritis, where the large disease heterogeneity often hampers proper diagnosis and the progressive course of the disease also calls for prognostic biomarkers. Finally, despite the growing number of therapeutic alternatives to treat psoriasis, not all patients respond to the same treatment and a recent survey has indicated that more than 50 % of patients polled are dissatisfied by the management of their disease [220]. The current therapeutic approach to treat psoriasis, especially in its moderate-to-severe forms, contemplates the use of different treatments, in an empirical attempt to find the most effective one. Patients are therefore likely to experience one or more ineffective therapies with relative associated side-effects. Moreover, this approach also results in increased public health costs which are especially relevant in the case of expensive biologic drugs. Thus, biomarkers for better disease management are both a clinical and a public health need which would benefit both patients and the healthcare system (Fig. 21.3).

Despite large efforts in the quest for psoriasis biomarkers, none of those identified so far have entered into clinical use for disease diagnosis, prognosis or for predicting therapy response [216]. Here, we describe them according the type 0, 1 and 2 classification and discuss their potential use in stratified medicine approaches in psoriasis.

Type 0 Psoriasis Biomarkers: Markers of Disease Severity

Type 0 biomarkers, correlating with the severity of psoriasis, include genetic, as well as, tissue and systemic biomarkers. It is known that HLA-Cw*0602- positive patients have more severe disease and early onset (Type I Psoriasis) compared to HLA-Cw*0602- negative patients [221]; while HLA-Cw*0602- positive PsA patients have a less severe clinical course [222].

The aberrant skin architecture of psoriatic plaques is reflected by the altered expression of tissue-specific molecules, such as keratins, as well as pro-inflammatory molecules in the tissue. Keratins associated with cell proliferation such as K6 and K16 are upregulated in psoriatic skin, while K1 and K10, indicating keratinocyte terminal differentiation, are decreased [223].

Moreover, being psoriasis is “more than skin deep”, it is not surprising that alteration of inflammatory mediators, both at the tissue and peripheral level, correlate with disease status. Pro-inflammatory cytokines such as TNF, IFN, IL-6 and IL-8 and IL-12 [47, 224] are increased in psoriatic skin and in the circulation of psoriatic patients, as compared to healthy individuals, and their expression rapidly reduces after successful anti-TNF treatment [177]. Inflammatory cytokines increased in psoriatic serum are also increased in the skin, suggesting that peripheral blood can mirror the skin [49]. The study of circulating markers has been extensively investigated due to the easy access to patients’ peripheral blood samples. IL-22 serum levels are increased in psoriasis patients and positively correlate with PASI [225, 226]. Th17 cells and cytokines are associated with psoriasis severity. IL23, IL23R and Th17 cytokines are increased in lesional psoriatic skin [227] and IL23R is overexpressed in circulating T cells [228]. However, it is not clear yet whether IL-17A serum levels are altered in psoriatic disease as inconsistent results have been found in different studies, possibly due to low levels and sensitivity issues of the detection assays used [47, 49, 225, 226, 229]. Nevertheless, high IL17-A serum levels, together with high IL1RA, correlate with the eruptive inflammatory form of the disease and not with chronic and stable psoriasis [230]. Increased frequency of T cells subsets such as Th1, Th17 and Th22 cells is detected in both the circulation of psoriasis patients as well as in the tissue [48, 231, 232]. Interestingly, also innate cells involved in the production of IL-17 and IL-22 such as ILC3 are increased in the skin and blood of psoriasis patients, and decrease in the circulation after successful anti- TNF treatment [141–143]. Finally, psoriasis patients have high levels of generic inflammation markers (e.g. CRP, haptoglobin and platelet P-selectin), shared with other inflammatory conditions [233], as well as lipids and oxidative status alterations [234, 235], shared with metabolic diseases.

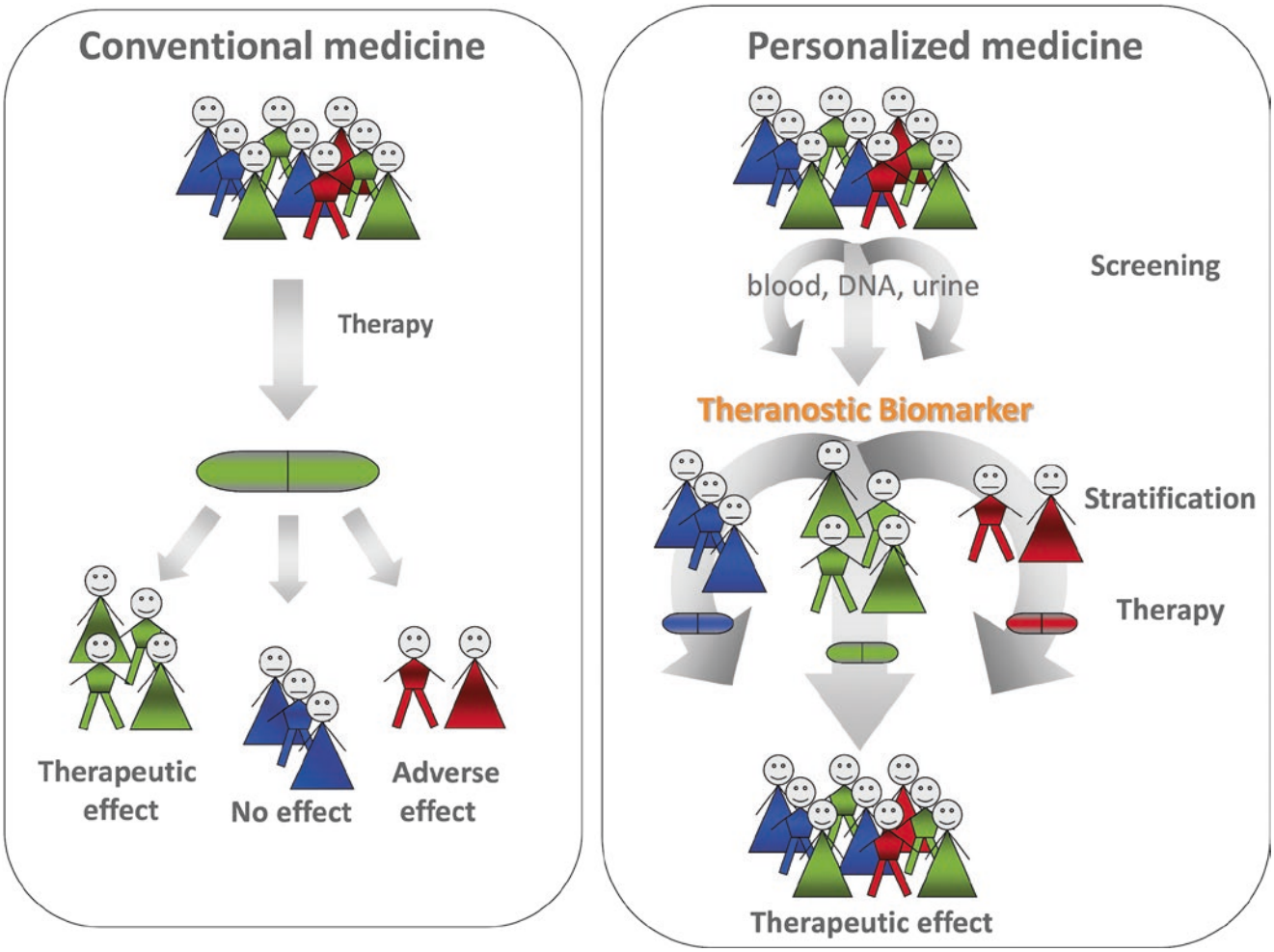

Fig. 21.3 Conventional versus stratified medicine- In conventional medicine approaches (*left*) patients receive the same drug which will have a therapeutic effect on the majority of them but will be ineffective in some and cause adverse events in others. In personalized medicine approaches, patients undergo screening using biological materials (DNA, urine, blood) to identify theranostic biomarkers allowing their stratification to receive the most appropriate and effective drug for each individual (Reproduced modified with permission from: Targeted therapies and biomarkers for personalized treatment of psoriasis (Villanova et al. 2014, submitted) in: Personalized Treatment Options in Dermatology -Springer)

Type 1 Psoriasis Biomarkers: Drug Endotype

Type 1 biomarkers, reflecting the effect of an intervention according to the mechanism of action of therapy itself, are being identified as results of targeted therapies aimed at restoring specific immune circuits which are dysregulated in psoriasis. In particular, anti-IL12/23 and anti-IL17 biologics act on the central IL23/IL17 axis in psoriasis. The molecules directly targeted by these drugs can potentially be used as prognostic or theranostic biomarkers to monitor the actual suppression of the targeted pathway and the associated clinical improvements.

Blockade of IL-17 with ixekizumab results in reduced IL-17, IFN-γ, and IL-22 in the tissue, as well as in the downregulation of IL-17-regulated molecules (LL37, beta defensin 2, S100A7, S100A8) in epidermal keratinocytes, within 2 weeks of treatment. This in turn leads to decreased infiltration of lymphocytes and DC, as well as, normalization of keratinocyte structural and activation markers within 6 weeks of treatment. These cellular and molecular changes correlate with the rapid clinical improvements suggesting that IL-17 is a key marker whose regulation is sufficient to normalize many other deregulated circuits in psoriatic skin [194]. Downregulation of IL17 and its immediate regulated genes is also necessary for positive response to the anti-TNF biologic etanercept [125, 236], suggesting that the efficacy of TNF blockade is ultimately down to the inhibition of IL17 signaling.

Type 2 Biomarkers: Predictive or Theranostic Biomarkers

Type 2 biomarkers, which are surrogate endpoints for therapy, have predictive value and theranostic use.

The identification, validation and implementation of this type of biomarker is the most urgently needed in psoriasis. Soluble, circulatory or genetic markers associated with therapeutic response would be ideal theranostic biomarkers in psoriasis, being not or minimally invasive.

In a small study, a disease response classifier including 23 genes using gene expression of PBMCs has been obtained to accurately predict response to alefacept [237]. The relative frequency of specific immune cell populations within the first few weeks of treatment has been shown to correlate with more long-term therapy response. In particular, an increase in T regulatory cells within 8 weeks of anti-TNF therapy predicts good therapeutic response [238], while the expression of cutaneous lymphocyte-associate antigen (CLA) on lymphocytes negatively correlates with PASI at 6 weeks [239]. Among genetics biomarkers, psoriasis susceptibility genes identified by GWASs represent a potential gold mine for the identification of theranostic biomarkers. SNPs in TNAFAIP3, encoding for a zinc finger protein (A20) that is a negative regulator of TNF-induced pathways, are associated with improved response to anti-TNF agents [240] Moreover, a small cohort of HLA-Cw06+ patients have been shown to respond better and faster to ustekinumab [241]. An obvious potential candidate to probe is the *IL23R* R381Q SNP in IL-23R, given its functional role in down-regulating IL-23 responses in psoriasis patients [99].

Conclusions and Future Perspectives

The complex pathogenesis of psoriasis has been untangled in the last 40 years, resulting in an increase of effective therapeutic options available to patients. Future directions call for a refinement of the current knowledge using novel integrative approaches. Psoriasis susceptibility genes identified so far clearly point towards critical pathogenic pathways warranting further studies. More genetic studies are required to identify the so called "missing heritability" while functional investigation of genetic determinants is needed to better exploit them in a clinical setting, e.g as theranostic biomarkers. The integration of different types of large datasets, obtained through high-throughput platforms and powerful analytical tools, will aid in the discovery of disease biomarkers with multiple applications in patient stratification, treatment of co-morbidites and new drug discovery. Taken together, these efforts hold the promise to further benefit psoriasis patients.

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Questions

1. Which is the **correct** definition for psoriasis:
   A. Psoriasis is a chonic skin disease due solely to an alteration of the immune system
   B. Psoriasis is a infectous skin disease
   C. Psoriasis is a chonic skin disease due to the interplay of genetic and environmental factors resulting in altered immune system and aberrant skin differentiation
   D. Psoriasis is a monogenic skin disease

2. What **are** the clinical features of plaque-type psoriasis:
   A. Infected, white pustules
   B. Inflamed, sharply demarcated, raised plaques, covered by silvery scales
   C. Diffuse erythema, with or without scaling, involving more than 75 % of the skin surface
   D. Violaceous or pinkish papules

3. Which signaling pathway below is **not** involved in the pathogenesis of psoriasis
   A. Type I Interferon
   B. NF-kB
   C. IL-23/IL-17
   D. Sonic/Hedgehog

4. Which statement below about T helper 17 (Th17) cells **is correct**:
   A. Th17 cells are innate immune cells and protect against infections
   B. Th17 cells are key players in the pathogenesis of psoriasis and an important therapeutic target
   C. Th17 cells have a minor role in psoriasis, which is insted a Th1-mediated disease
   D. Th17 cells produce esclusively IL-17, hence their name

5. Which statement below about therapy of psoriasis is **not correct**:
   A. Biologics drugs used in psoriasis target key inflammatory mediators such as IFN-γ
   B. Systemic therapy is used as a second-line treatment of moderate to severe psoriasis

C. The first wave of biologics to be approved for psoriasis treatment were anti-T cell therapies targeting T cell adhesion or activation
D. Apremilast, which inhibits an enzyme involved in the breakdown of cyclic AMP, is in clinical trials for psoriasis

Answers

1. C
2. B
3. D
4. B
5. A

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Atopic Dermatitis

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Abstract

Atopic dermatitis (AD) is a common skin disease that has received much scientific attention over the recent years. Although disease mechanisms had been difficult to dissect, with multiple factors that seem to affect disease activity, skin barrier, aberrant immunity and the microbiota appear to be the important components in pathogenesis. In this chapter, we will discuss the current concept of AD, its related genetic disorders, and the above pathological components to try to provide cutting-edge understanding on pathogenesis of this disease.

Keywords

Atopic dermatitis • Pathogenesis • AD • Inflammatory skin disorder • Skin leisons • T cells • Dendritic cells • Altered skin microbiota • Dry skin • Immunodeficiency • Genetic skin disorders

Key Points

- Atopic dermatitis is a common chronic inflammatory skin disorder. Patients suffer from eczematous lesions with distinct distribution and dry skin, complicated with progressive development of other allergic diseases, called the atopic march.
- *FLG* gene mutations are a major genetic predisposing factor, suggesting that barrier disruption is prerequisite for eczematous inflammation.
- Several rare genetic disorders manifest eczematous skin phenotype that resembles atopic dermatitis. Netherton syndrome and hyper-IgE syndrome are such prototypes with mutations in well-defined genes.
- *Staphylococcus aureus* colonization is universal in skin of patients with not only atopic dermatitis but also other genetic disorders manifesting eczema.
- Impaired epidermal barrier, immunological abnormalities and altered skin microbiota are three major factors that play a role in atopic dermatitis.
- A variety of helper T cells, such as Th1, Th2, Th17 and Th22, are involved in the pathogenesis of atopic dermatitis.
- Restoring barrier, modulating immune responses and normalizing dysbiosis are three important strategies for the treatment of atopic dermatitis.

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Atopy

Atopic dermatitis (AD) is a chronic inflammatory skin disorder and one of the most common skin diseases seen by dermatologists [1, 2]. Defining AD in simple words is rather difficult, but atopy in terminology has been recognized as a personal or familial tendency to become sensitized and produce specific IgE antibodies to foreign

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antigens and lead to the development of asthma, rhinitis or eczema [3]. Progressive development of asthma, allergic rhinitis and food allergy in patients with AD is referred to as 'atopic march' [4]. The main clinical feature of AD is eczematous dermatitis with pruritus that has distinctive distributions and that arises upon dry skin [5]. As will be discussed below, it is becoming clearer that barrier disruption, manifesting as dry skin, is attributed to mutations in genes that are necessary for normal epidermal differentiation and function. The role of IgE is not yet clear in eczema formation, and a minor subset of patients exhibit symptoms indistinguishable with AD.

Epidemiology

AD is a prevalent disease that affects 15–30 % of children and 2–10 % of adults. In the past, AD had been more prevalent in highly developed countries. Recently, there has been an increase in the prevalence of AD in relatively under developed countries. A number of population-based prevalence surveys have been conducted to find epidemiological factors for the development of AD and have revealed significant differences in the prevalence of AD between countries. It was suggested that not only genetic factors, but also environmental factors might be pathological factors that contribute to the development of AD. A relatively recently reported environmental factor that had not received much attention is the climate. Based on ecological analysis of AD prevalence, the results, which were adjusted for national per capita income, suggested that AD symptoms correlate positively with latitude, which is attributed to less exposure time to UV light, and negatively with annual outdoor temperature [6].

Consistent with the above finding, a recently performed ecological analysis has also suggested the negative correlation of UV index with decreased prevalence of eczema [7]. AD patients have decreased levels of serum vitamin D, suggesting that low UV exposure time and resulting low serum vitamin D are risk factors for AD [8].

Dry skin is a major clinical manifestation of AD patients and low humidity is a potential exacerbating factor. Consistent with this, AD prevalence was significantly lower with the higher relative humidity [7].

Another prevailing concept is the so-called 'hygiene hypothesis'. It holds that the lack of early childhood exposure to microbes, parasites or other infectious agents increases the risk of allergic diseases due to defects in immune tolerance [9]. Inverse relationship between the risk of AD and endotoxin exposure has been found [10]. Early life exposure to antibiotics can lead to an increased risk of developing eczema [11], which, at a superficial level, is consistent with the report that AD patients harbor low intestinal microbial diversity [12]. The surroundings of homes of atopic individuals are reported to be lower in environmental biodiversity and that such AD patients exhibit significantly lower genetic diversity of gammaproteobacteria on their skin [13], suggesting that natural environment could influence the composition of the human microbiota, and that the reduced contact with such environment could lead to less opportunity to acquire microbes that have the ability to regulate immune functions.

Genetics

Family history of AD or other allergic diseases has been known to be a major risk factor of AD in children suggesting genetic background as a strong risk factor for the development of AD [14]. Recent advances of molecular technologies in genetic analysis have accelerated studies on genetics of patients with AD. Candidate gene approach or genome-wide linkage analysis has identified a number of possible AD-related genes [14]. Since the first genome-wide association study (GWAS) of AD patients reported in 2009, several susceptibility loci for AD have been identified based on GWAS [15, 16].

Although the genome-wide analysis provides a comprehensive and unbiased data set on AD associated-genes, a genetic risk factor stronger than filaggrin gene (*FLG*) has yet to be discovered. The gene *FLG* encodes a large insoluble polyprotein, profilaggrin, which undergoes enzymatic cleavage to generate multiple filaggrin peptides. Such filaggrin peptides function as structural proteins that bind actin filaments to maintain structural integrity, and as so-called natural moisturizing factors, a group of molecules believed to hold moisture within the stratum corneum.

In 2006, two common loss-of-function mutations within the *FLG* gene were found to cause ichthyosis vulgaris [17]. Since ichthyosis vulgaris had been well recognized to associate with AD, investigations of *FLG* mutations were further conducted in the context of AD. As a result, significant associations between AD and two *FLG* null alleles were found in 15 families with ichthyosis vulgaris, and additional AD cohorts from Ireland, Scotland and Denmark demonstrated a strong and highly significant association between *FLG* null mutations and AD [18]. About 40 truncating mutations have been found so far, with distinct mutations in the European and Asian patient populations [19]. Recent meta analyses of multiple studies assessing *FLG* mutations as well as GWAS also confirmed the association of *FLG* mutations with AD [16, 20]. Thus, although some conflicting data exist, most reported studies are congruent with the original finding that *FLG* mutations represent a major predisposing factor for AD.

AD-Like Genetic Disorders with Aberrant Barrier Formation

It is not only *FLG* mutation that results in eczema formation, but several rare genetic disorders also manifest symptoms that resemble AD (Fig. 22.1 and Table. 22.1). Because of their specific and well-characterized genetic defects, these diseases could represent useful prototypes that might allow us to gain deeper insight into the mechanisms of eczematous dermatitis at the molecular level. These diseases could be considered as a part of the extended spectrum of atopic diseases.

Netherton syndrome is an autosomal recessive skin disorder characterized by congenital ichthyosis and severe atopic manifestations including eczematous lesions and staphylococcal skin colonization, food allergy and rhinitis with high serum IgE. It is caused by mutations in *SPINK5*, encoding the serine protease inhibitor LEKTI [21]. LEKTI inhibits several members of kallikreins (KLK) including KLK5, KLK7 and KLK14, the balance of which is crucial to regulate desquamation. Loss of LEKTI leads to aberrant enhancement of multiple proteolytic events in epidermis, and Stimulates proinflammatory signals via protease activated receptor 2 (PAR2). *Spink5*-deficient mice and KLK5-overexpressed mice manifest Netherton syndrome-like, as well as AD-like phenotypes, such as eczematous inflammation, and high serum IgE and TSLP [22–24], which was attributed to PAR2-mediated TSLP up-regulation [23]. A significant association of a *SPINK5* polymorphism with AD has been found, indicating an overlap of disease pathogenesis between NS and AD [25].

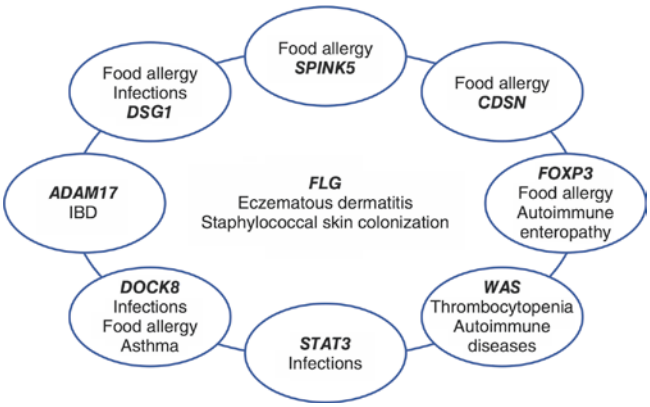

Fig. 22.1 Spectrum of atopic/eczematous dermatitis. List of genes in which mutations can cause eczematous dermatitis associated with staphylococcal colonization bacterial and fungal infections, and other clinical manifestations such as food allergy and asthma

A generalized, inflammatory type of peeling skin syndrome (type B) also manifests as eczematous dermatitis and food allergies. This disease is associated with mutations in *CDSN*, which leads to a complete loss of corneodesmosin [26]. Corneodesmosin is an adhesion glycoprotein located in corneodesmosomes, and deletion of *Cdsn* resulted in hair and skin abnormalities also in mice [27].

Recently, loss-of-function mutations of another important transmembrane structural protein have been reported in patients with eczematous dermatitis. Homozygous mutations in *DSG1*, which encodes desmoglein1, a major constituent of desmosomes, cause a syndrome featuring severe dermatitis, multiple allergies and metabolic wasting (SAM syndrome) [28].

Interestingly, like *FLG*, gene defects in the above diseases and those listed in Table 22.1 directly impair normal differentiation and formation of the upper epidermis to the stratum corneum. Furthermore, it is interesting to note that disease affecting lower parts of the epidermis, such as epidermolysis bullosa, does not result in eczematous dermatitis or asthma and food allergies despite the formation of ulcers or erosions that potentially allow antigen penetration. Existence of upper epidermis ~ stratum corneum with dysfunctional barrier appears crucial for the development of AD and subsequent atopic march.

AD-Like Genetic Disorders with Immunodeficiency

Patients with primary immunodeficiencies, which are caused by mutations in genes mainly associated with the development and regulation of immune cells, can also exhibit

Table 22.1 AD-like genetic disorders

Disease	Causative gene	Staphylococcal infections
Netherton syndrome	*SPINK5*	Skin
Peeling skin disease	*CDSN*	Skin
SAM syndrome	*DSG1*	N.D.
Autosomal dominant hyper-IgE syndrome	*STAT3*	Skin and respiratory tract
Autosomal recessive hyper-IgE syndrome	*DOCK8*	Skin and respiratory tract
Wiskott-Aldrich syndrome	*WAS*	Skin and respiratory tract
ADAM17 deficiency	*ADAM17*	Skin
IPEX syndrome	*FOXP3*	Skin and respiratory tract

SAM severe dermatitis, multiple allergies and metabolic wasting, *N.D.* not determined, *IPEX* immunodysregulation polyendocrinopathy enteropathy X-linked

eczematous dermatitis that closely resembles AD. An autosomal dominant form of hyper-IgE syndrome (HIES) is caused by mutations in *STAT3*, and manifests as eczematous dermatitis with recurrent staphylococcal infections [29, 30]. Staphlycococcal infections not only occur in skin and soft tissue, but also in the respiratory tract. Patients with an autosomal recessive type of HIES caused by *DOCK8* mutations also have eczema and recurrent staphylococcal skin infections [31]. Another rare primary immunodeficiency, Wiscot-Aldrich syndrome, is characterized by eczematous dermatitis with recurrent skin infections, in addition to thrombocytopenia [32]. It is caused by mutations in *WAS*, a protein that is important for proper actin cytoskeleton function during cell motility, and is expressed in most hematopoietic cells.

The hallmark of the above-mentioned diseases is immune dysregulation that leads to increased susceptibility for recurrent infections in skin as well as other organs. Gene mutations responsible for these diseases have not been implicated to be involved in epidermal differentiation. Although it is clear that barrier dysfunction can lead to AD, a pathway that arises from immune dysfunction to barrier dysfunction is also possible. AD-like genetic diseases that result from barrier dysfunction and immunodeficiency might represent opposite ends of the AD spectrum.

A Gene Mutation That Affects Both Immunity and Barrier Formation

A new genetic disorder has been reported that could be fitted within the atopic spectrum. Patients with loss-of-function mutations in *ADAM17* manifest eczematous dermatitis and pustules with *Staphylococcus aureus* skin infections [33]. The function of ADAM17 in skin has been investigated in murine studies, which demonstrated that deficiency of ADAM17 down-stream signaling pathways such as EGFR or Notch are responsible for impaired barrier integrity, immune dysregulation and staphylococcal dysbiosis [34–36]. Note that many of these diseases in the atopic disease spectrum have staphyloccoccal skin colonization in common [37], which may be responsible for the induction of eczematous dermatitis.

Pathomechanisms

Pathophysiology of AD is complex and, as suggested in the above section, multiple pathways can lead to one common clinical phenotype. There are three major drivers that contribute to the pathogenesis of AD: (1) Impaired epidermal barrier (2) immunological abnormalities and (3) altered skin microbiota. Immunological disturbance as the primary cause of AD has been widely investigated because the mainstream of AD has been considered as Th2 type allergic responses against foreign antigens. High serum IgE concentration and eosinophilia are main laboratory features that support the Th2 concept. Barrier function in AD has been a point of focus over the past several years, which gained huge momentum after the discovery of *FLG* mutations. Meanwhile, increased colonization of *S. aureus* in skin of patients with AD has long been known, but was difficult to explain in the context of allergic inflammation. Recent advances in microbiome research are beginning to provide better understanding of interactions between the microbiome and host immunity and highlight the importance of *S. aureus* colonization in AD pathogenesis (Fig. 22.2).

Impaired Epidermal Barrier

Dry skin is caused by barrier alteration followed by increased trans epidermal water loss, and is considered an important underlying condition in AD. Since loss-of-function mutations in *FLG* have been reported in patients with AD, the manner in which filaggrin defects contribute to barrier dysfunction has been intensively studied. Given the fact that filaggrin is a major structural protein in the stratum corneum, filaggrin insufficiency would be expected to result in a number of structural, biophysical and functional changes, and in aggregate result in impaired barrier formation. Filaggrin breakdown products form natural moisturizing factors (NMF), which are thought to be essential for stratum corneum hydration. Although it is widely accepted that *FLG* mutations result in outside-in barrier dysfunction, the mechanism of this defect is yet to be fully elucidated.

Better understanding of filaggrin biology has been gained from murine models of filaggrin deficiency. The spontaneous mutant flaky tail/matted mice exhibit eczematous inflammation and enhanced immune response to cutaneous allergen exposure. A 1-bp deletion mutation of *Flg* was found [38], enforcing the previous finding in humans. Further studies investigating flaky tail (*Flgft*) mice showed clinical AD-like manifestations with barrier abnormalities and Th17-dominated skin inflammation [39–41]. Those studies suggested a crucial role of filaggrin in the maintenance of physical epidermal barrier. Unexpectedly, however, genomic deletion of filaggrin in mice led to altered stratum corneum barrier and enhanced percutaneous sensitization, but did not lead to the onset of spontaneous dermatitis under specific pathogen free conditions [42], demonstrating that filaggrin deficiency alone is not sufficient to induce eczematous dermatitis. In fact, recent studies revealed that the matted (*ma*) mutation, but not *Flg* mutation, is responsible for the dermatitis phenotype in *Flgft* mice [43, 44], which raises the question of whether eczematous inflammation is truly a result of enhanced antigen penetration subsequent to barrier dysfunction.

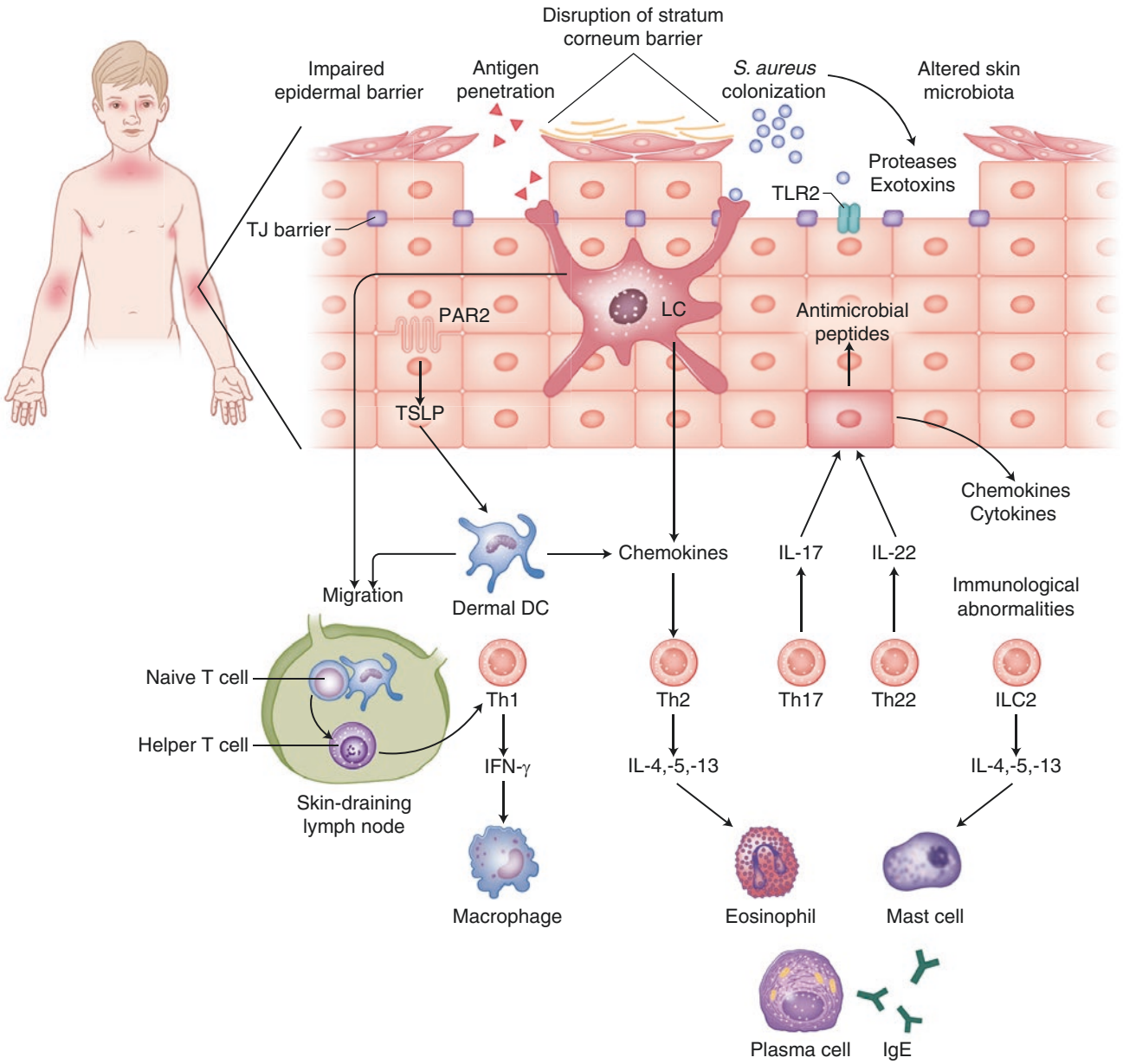

Fig. 22.2 Three major driving forces for the pathogenesis of atopic dermatitis: Impaired epidermal barrier, immunological abnormalities and altered skin microbiota. Loss of barrier integrity is believed to lead to antigen penetration and initiates inflammatory responses driven by a mixed type of cytokine producing T cells. Although the contribution of altered skin microbiota is not yet conclusive, staphylococcus aureus colonization is universal in AD as well as other genetic dermatosis and immunodeficiencies that exhibit eczematous dermatitis

Nevertheless, filaggrin is also reported to play an important role in maintaining pH balance of SC. Acidic conditions caused by filaggrin breakdown products, urocanic acid and pyrrolidone carboxylic acid, have been reported to inhibit the growth of *S.aureus* [45]. Moreover, reduced SC NMF components and consequent increased SC pH result in enhanced cleavage of proinflammatory IL-1 cytokines, which were increased in AD patients with *FLG* mutations as well as filaggrin deficient *Flgft/ft* mice [46]. In light of the matted mutant mice and *Flg* deficient mice, it remains to be clarified whether *Flg* mutation alone is responsible for the above findings.

Immunological Abnormalities

Th2 and Th1

Based on the clinical observation that patients with AD often manifest blood eosinophilia and elevated serum IgE levels, AD was long considered to be a Th2 type allergic disease. Th2 cells produce IL-4, IL-5 and IL-13 and activate eosinophils, basophils and mast cells as well as IgE-producing B cells, which are all involved in allergic reactions. In the 1990s, two studies detected mRNA expression of cytokines via *in situ* hybridization and reported increased

expression of Th2 cytokines in skin of AD patients [47, 48]. In addition, the serum level of the Th2-attracting chemokine, CCL17/TARC, correlates with AD activity [49], indicating contribution of Th2 type response in the disease flare. In contrast, elevation of Th1 cytokines, such as IFN-γ and IL-2, has also been detected in the late phase in patch test reactions of AD patients [50]. Those studies supported the evidence of Th2 cytokine bias as well as involvement of Th1 cytokines in AD.

Findings on TSLP have provided new insights into the mechanism of Th2 biased-pathogenesis in AD. TSLP is a potent activator of dendritic cells and plays an important role in the induction of CD4+ T cell-mediated allergic inflammation. TSLP promotes DCs to induce Th2-chemokines and consequently enhances the production of Th2 cytokines from T cells. TSLP was highly expressed in epidermis of AD patients [51]. Murine models of epidermis-specific overexpression of TSLP (K5-TSLP transgenic mouse) developed T cell-mediated dermatitis [52]. Other murine studies showed that TSLP promotes basophil responses and Th2 cytokine-mediated inflammation [53], and that TSLP induction through PAR2 activation induces dermatitis and basophil accumulation in flaky tail mice [54].

Although a number of studies have been conducted to prove Th2 involvement in the pathogenesis of AD, therapeutic targeting of Th2-mediating factors has not proven effective. Neutralization of Th2 cytokines or IgE and inactivation of B cells has not yielded promising outcomes. True clinical relevance of Th2 has yet to be demonstrated.

Interestingly, epithelial cells directly regulate cutaneous sensory neurons via TSLP activation to promote pruritus, which is dependent on PAR2-triggering Ca influx and NFAT translocation [55], suggesting the contribution of a non-immunological mechanism mediated by TSLP in AD.

Th17 and Th22

Emerging reports suggest the contribution of Th17 and Th22 in atopic skin [56]. IL-17 and IL-22 function to accelerate innate immune responses against extracellular pathogens and are linked to many immune/autoimmune related diseases. IL-17 and IL-22 are overexpressed in skin of AD [57, 58], and infiltrations of Th17 and Th22 cells into lesional skin have been reported [59, 60]. Correlation of serum IL-22 with CCL17 [61] and increase of IL-13/IL-22 producing cells in AD [62] suggest a link between Th22 and Th2 cytokines.

Th17-associated skin inflammation can be observed in several mouse models with AD-like inflammation, such as the flaky tail mice [40], transgenic kallikrein 5 mice [24] and Adam17 conditional knockout mice [36]. Importantly, IL-17 and IL-22 synergistically promote the induction of antimicrobial peptides from keratinocytes [63], and *S. aureus*-derived enterotoxin induces IL-17 production of T cells isolated from AD patients [64], suggesting that production of IL-17 and IL-22 is associated with colonization of *S. aureus* in AD skin (Table 22.2).

Other Cytokines

Overexpression of IL-18, an IL-1 superfamily, in murine epidermis resulted in dermatitis with elevated serum IgE and Th2 cytokines [65]. Epidermal IL-18 levels of patients with AD was correlated with SCORAD and prevalence of *S. aureus* colonization [66]. Transgenic mice overexpressing IL-31, an IL-6 family cytokine that is produced by Th2 cells, developed pruritic dermatitis [67]. Serum IL-31 is also increased in atopic individuals and the production of IL-31 in human PBMCs can be induced by staphylococcal enterotoxin B [68].

Innate Lymphoid Cells

After their recent discovery, innate lymphoid cells (ILCs) have emerged as essential effectors of innate immunity and potent players in the pathogenesis of many inflammatory diseases. ILCs resemble lymphocytes in morphology and function as effector cells through the production of several cytokines, but lack a T cell receptors. ILCs are divided into three groups based on their producing cytokines. Group 2 ILCs produce type 2 cytokines (IL-4, IL-5, IL-9, IL-13) and are implicated in allergic immune responses. The first report in the context of AD revealed that ILC2 are enriched in human AD skin lesions and in mouse skin with Vitamin D3-induced inflammation, and that ILC2 responses were dependent on TSLP [69]. Another report also found accumulation of ILC2 in lesional skin of atopic patients as well as in skin of house dust mite-exposed murine skin with dependency on IL-25 and IL-33 [70]. IL-25 and IL-33 are inducers of ILC2 expansion, and in fact, transgenic mice with epidermis-specific IL-33 overexpression developed AD-like skin lesions with increase of ILC2 [71]. Consistently, the expression of IL-33 receptor is increased in human AD skin [72].

Regulatory T Cells

Regulatory T cells (Treg) are CD25+, Foxp3+ T cell subsets, which play important roles in suppressing T cell responses. Although it is generally accepted that Treg modulate allergic inflammation by suppressing the Th2 response, the exact role of Treg in AD pathogenesis still remains unclear [73]. Treg was absent in AD lesional skin in one study [74], while it existed in other studies [75, 76]. Mutations of the Treg master regulator gene, *FOXP3*, result in the absent or dysfunctional Tregs and cause IPEX syndrome. Importantly, some of IPEX syndrome patients manifest allergic disorders including asthma, food allergy and eczema [77, 78], suggesting a strong link between the loss of Tregs and the onset of allergic diseases.

Table 22.2 Mouse models of AD

Mouse strain	Characteristics	References
Spontaneously developing models		
Nc/Nga	Scratch, increased TEWL, dermatitis, high IgE and increased expression of Th2 cytokines in skin	[126, 127]
Flaky tail (*ma/ma, Flgft/ft*)	Scratch, increased TEWL, dermatitis, high IgE and enhanced percutaneous sensitization to OVA	[38, 43, 44]
Genetically engineered models		
IL-4 transgenic	Scratch, dermatitis, *S. aureus* colonization and high IgE	[128]
IL-13 transgenic	Scratch, dermatitis, high IgE and increased expression of Th2 cytokines in skin	[129]
IL-31 transgenic	Scratch and dermatitis	[67]
TSLP transgenic	Dermatitis, high IgE and increased production of Th2 cytokines	[52]
CASP1 transgenic	Scratch, dermatitis and high IgE	[65]
Foxp3 knockout	Dermatitis and high IgE	[130]
ADAM17 conditional knockout	Scratch, high IgE, increased TEWL, *S. aureus* colonization and increased production of Th2 and Th17 cytokines	[34, 35, 36]
EGFR conditional knockout	Scratch, high IgE, increased TEWL, *S. aureus* colonization and increased production of Th2 and Th17 cytokines	[34, 36]
Notch1 and 2 conditional knockout	Dermatitis, high IgE and increased expression of Th2 cytokines in skin	[131]
Epicutaneous sensitization models		
OVA sensitized	Scratch, dermatitis, high IgE, increased expression of Th2 cytokines and chemokines in skin	[132, 133]
Hapten induced	Th2-associated dermatitis and high IgE	[134]

Dendritic Cells

Specialized antigen-presenting cells (APCs), or dendritic cells (DCs), play pivotal roles in the initiation of immune reactions through activation and modulation of T cells. Although the contribution of Langerhans' cells (LC) in initiating humoral responses against protein antigens that were taken up through the epidermal tight junctions have been established in mice [79–81], the role(s) of DCs in AD pathogenesis still remains enigmatic. It has been reported that human epidermal LCs express the high affinity receptor for IgE (FceRI) [82]. In addition, inflammatory DCs, that expressed CD206, CD209 and FceRI, but did not contain Birbeck granules, were found in lesional epidermis of AD patients, and were termed 'inflammatory dendritic epidermal cells' (IDEC) [83]. IDECs can be found to infiltrate not only epidermis, but also dermis of AD lesions [84].

FceRI on DCs mediate IgE-facilitated allergen presentation [85]. It is therefore possible that FceRI expressed on surfaces of LCs and IDECs are involved in IgE-mediated allergic reactions also in AD skin. TSLP receptor deficient mice reconstituted with normal bone marrow (therefore keratinocytes and LCs are the only relevant cells that lack TSLP receptors) resulted in decrease of clinical manifestations and OVA-specific IgE upon epicutaneous OVA sensitization in mice [86]. Moreover, vitamin D3 analogue-induced eczematous inflammation was decreased in the absence of LCs in mice [87]. Those experimental settings provide evidence that LCs contribute to Th2 as well as eczematous responses in mice, and further suggest that they may contribute to AD pathogenesis.

Altered Skin Microbiota

Skin harbors a diverse community of microorganisms, referred to as the microbiota. Composition of microbiota is greatly influenced by the condition of skin, and possibly, vice versa. Skin resident microbes are implicated in cutaneous immune system development and function and pathology of skin diseases. It has been long known that skin of patients with AD is heavily colonized with *S. aureus* [88]. Density of *S. aureus* colonization correlated with SCORAD [89, 90], suggesting the contribution of *S. aureus* to the mechanism of the disease flare.

Recent technological advances on next generation DNA sequencing have allowed the utilization of culture independent metagenomic approaches to investigate the collective genomes of resident bacteria, the microbiome, and have uncovered the details of bacterial community structure in skin [91]. Bacterial 16S rRNA sequencing analysis

in children with AD revealed temporal shifts in skin microbiome during flares, in which bacterial diversity decreases, and *S. aureus* predominates [92]. A microbiome study in patients with primary immunodeficiencies featuring AD-like eczema revealed that microbial compositions were altered in patients compared with controls, and prevalence of *Staphylococcus* and *Corynebacteirum* were positively correlated with disease severity [37]. Consistent with previously conducted studies with culture dependent detection techniques, these recent studies confirmed the emergence of *S. aureus* in eczematous lesions and provided new insight into bidirectional influences between microbes, particularly *S. aureus*, and atopic skin. However, the cause-and-effect relationship between staphylococcal colonization and eczema formation is still inconclusive, and the clinical studies provide evidence for anti-bacterial therapies only for secondary infections of eczematous lesions, but not for the treatment of eczema itself.

S. aureus is commonly found in healthy human skin, but could cause staphylococcal skin infections such as impetigo, folliculitis and abscesses under certain conditions. *S. aureus* produces a wide array of virulence factors, and it is not clear yet whether such factors contribute to eczema formation. Nevertheless, it is easily imaginable that virulence factors could have direct or indirect contributions, via host immune responses, to eczema formation or aggravation. A major group of virulence factors of *S. aureus*, such as proteases, hyaluronidase, lipase and nuclease, comprise extracellular enzymes. Another group is a family of exotoxins including cytolytic membrane-damaging toxins (mainly α, β, γ, and δ-toxins and Panton-Valentine leukocidin) and superantigens such as staphylococcal enterotoxin (SEA and SEB), toxic shock syndrome toxin-1 and exfoliative toxin A and B. An important characteristic of *S. aureus* is its ability to secrete exotoxins that disrupt host cell membrane. The correlation between the colonization of exotoxin-producing *S. aureus* strains and disease severity of AD has been reported [93].

The mechanism by which superantigens contribute to disease flares in AD has not been completely elucidated. Several reports have suggested effects of staphylococcal exotoxins on immune cells, by activating T cells by specific interaction between TCR and the toxin [94, 95], and by promoting allergic reactions through IgE generated against staphylococcal exotoxins [96–98]. A recent report showed that mast cell degranulation is induced by δ-toxin, suggesting pathological role(s) of secreted toxins in allergic skin disease [99].

Why *S. aureus* is capable of abundantly colonizing skin of AD patients is still another matter of debate, and might represent important mechanisms that could affect future therapeutic strategies. Skin is equipped with innate mechanisms that prevent overgrowth of *S. aureus*. Such mechanisms include protection against adherence of *S. aureus* to corneocytes. Acidic conditioning of skin inhibits the growth of *S. aureus*, and the production of antimicrobial peptide (AMP) by keratinocytes, sebocytes and eccrine glands likely targets *S. aureus*. Neutrophil responses are also promoted by IL-1β produced by keratinocytes [100]. It is possible that one or several of these factors are impaired in skin of AD patients.

One of the initial immune responses against bacterial pathogens is innate immunity via the recognition of pathogen-associated molecular patterns of bacteria through pathogen related receptors, and the most studied family of such receptors are Toll-like receptors (TLRs). Genetic variants of TLR2 have been studied in the context of AD, because it recognizes cell-wall products of *S. aureus*, and its polymorphism has been reported in AD patients with a history of *S.aureus* infections and increased serum IgE [101, 102].

AMPs are evolutionarily ancient innate immune effectors and are thought to contribute in immune defense of epithelial surface. Expression levels of AMPs have been reported in several studies, but the results of those are controversial. Reduced expression of cathelicidins (LL-37) and human β-defensin 2 (HBD-2) in AD skin was first reported in comparison to psoriatic skin [103], and was attributed to Th2 cytokine milieu in AD [104]. However, follow-up studies revealed the expressions of AMPs in lesional skin of AD are not actually decreased, but rather increased, when compared with healthy control skin [105] [90]. Meanwhile, mobilization of HBD-3 appears to be inhibited in keratinocytes derived from AD patients [106].

Increasing number of studies in the recent years has shown that imbalances in the composition of the intestinal microbiota are associated with both intestinal and extra-intestinal diseases. The association of altered gut microbiota with an increased risk of developing AD has been reported in several studies [12, 107–109]. Efforts have been made to "normalize" the imbalance of microbial composition in the intestine via the supplementation of probiotics. A double-blind, randomized placebo-controlled clinical trial demonstrated that taking Lactobacillus GG has a preventative effect in children at high risk of AD [110]. In the era of microbiome, such studies should be corroborated with microbiome analysis via next generation sequencing.

Treatment

Management of AD requires multiple approaches, targeting various aspects of pathogenesis. The cores of such aspects that were discussed in this chapter are barrier disruption, inflammation and, for future perspectives, dysbiosis. While strategic development of novel therapeutics will heavily depend on basic science, treatment strategies at the patient level should be determined based on clinical conditions of individual patients as well as evidence that is provided by

randomized controlled trials (RCTs), optimally supported by data from basic and clinical research.

Dry skin is a fundamental clinical element in AD, and the importance of SC barrier has been highlighted after the discovery of *FLG* mutations in patients with AD. Skin care aimed to maintain barrier integrity, thereby preventing epicutaneous sensitization against environmental allergens, would be a fundamental therapeutic strategy that accommodates most, if not all, disease phases in AD. There is strong evidence that supporting skin hydration by topical application of moisturizers reduces disease severity and prevents flares [111]. Pharmacologic interventions that directly target filaggrin expression that enforce barrier function may be a potential therapeutic approach in the future.

Control of inflammation by the use of anti-inflammatory or immunomodulating agents is another mainstream therapeutic strategy. Topical corticosteroids and calcineurin inhibitors are recommended based on evidence provided by a number of RCTs [111–113]. Systemic application of cyclosporin A is effective but only indicated for patients with AD refractory to conventional topical treatment [114, 115].

Although a large amount of scientific data on immunological pathophysiology of AD has been accumulated, specific therapies for inhibiting specific types of immune cells or cytokines are being studied in clinical trials. The results of clinical trials with new biologics such as monoclonal anti-IgE antibody (Omalizumab) and a chimeric monoclonal antibody against B-cell surface antigen CD20 (Rituximab) had been rather disappointing, although effective in a fraction of patients [112, 116]. Some early clinical trials have shown beneficial effects of recombinant IFN-γ in patients with severe AD [117, 118], but the level of evidence is still of limited-quality. With the variety of helper T cell subsets that have become known, it might be worth revisiting whether modulation and deviation of certain helper T cell subset(s) is a relevant strategy in the treatment of AD.

Inducing immunologic tolerance against specific allergens is an ultimate goal for the treatment of IgE-mediated allergic disorders in which major antigens have been established. There is enough evidence to support the effectiveness of allergen immunotherapy for the treatment of allergic rhinitis, allergic conjunctivitis and allergic asthma, but evidence for AD is still limited [119].

Consistent with epidemiological data that prevalence of AD negatively correlates with exposure time to UV, efficacy of phototherapy has been noted [120]. UV therapy is well established to exert immunosuppressive effects *in vivo*, for example by increasing IL-10 production [121], and can be utilized to attenuate skin inflammation. Meanwhile, specific recommendation of UV for a definitive AD condition has not been made, and its contraindication in patients treated with tacrolimus renders it to be considered a second line treatment.

In addition to initial culture-based studies, microbiome studies in humans have established that *S. aureus* dominates the microbiota during AD flares [92]. Consistently, it has been demonstrated in epidermal Adam17-deficient mice that *S. aureus* is capable of driving eczematous dermatitis, and that intense antibiotic cocktail targeting *S. aureus* nearly eradicates eczematous inflammation [36]. The concept that eczematous dermatitis is driven by *S. aureus* offers novel and attractive therapeutic strategies. However, despite the evidence of *S. aureus* colonization, and despite the longstanding interest in targeting this bacteria to try to improve AD symptoms, the efficacy of anti-bacterial treatment for AD patients remains unclear.

A Cochrane review of RCTs found a lack of quality trials to support the use of anti-bacterial therapies, for both topical and systemic antibiotics [122]. If antibiotics are to be utilized, well-designed randomized studies need to be conducted, considering the presence of Staphylococcal dysbiosis. With the current available evidence, the recommendation for the use of systemic antibiotics for AD patients is limited to those manifesting clear clinical signs of bacterial infections [120], and not for mere colonization. Intensified antibody cocktails might be necessary to target colonizing *S. aureus*, as have been done experimentally in mice. On the contrary, sustained systemic exposure to antibiotics will have adverse effects at distant sites such as the gut [123, 124], thus the approach utilizing systemic antibiotics might not be of practical use. In this context, the use of bleach baths is recommended for moderate to severe AD [125]. It is possible that bleach baths might be targeting Staphylococcal dysbiosis, and such skin microbiome-directed local therapy that normalizes dysbiosis long term, is awaited.

Questions

1. Which of the following genes associated with AD/AD-like genetic disorders have function(s) in barrier formation?
   A. *DOCK8*
   B. *FLG*
   C. *CDSN*
   D. *WAS*
   E. All of the above

2. Which of the following genes associated with AD/AD-like genetic disorders have function(s) in immunity?
   A. *STAT3*
   B. *DSG1*
   C. *SPINK5*
   D. *FOXP3*
   E. All of the above

3. Which of the following immune responses are associated with AD?
   A. T helper type 1 (interferon-γ response)
   B. T helper type 2 (interleukin-4 response)
   C. T helper type 17 (interleukin-17 response)
   D. T helper type 22 (interleukin-22 response)
   E. All of the above

4. Which of the below AD-like genetic disorders are particularly prone to developing food allergy?
   A. Hyper IgE syndrome
   B. Wiskott-Aldrich syndrome
   C. SAM syndrome
   D. Peeling skin syndrome
   E. Netherton syndrome

5. Dysbiosis in lesional atopic skin is overrepresented by:
   A. *Pseudomonas aeruginosa*
   B. *Candida albicans*
   C. *Malassezia furfur*
   D. *Staphylococcus aureus*
   E. All of the above

Answers

1. B, C
2. A, D
3. A
4. C
5. D

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Contact Dermatitis

23

Stefan F. Martin and Thilo Jakob

Abstract

Our skin is exposed daily to a large number of chemicals in household products, cosmetics, in the environment and in the workplace. Many of these chemicals can cause irritant or allergic contact dermatitis. Allergic contact dermatitis is an inflammatory skin disease that is mediated by our immune system. In this chapter we summarize current methods for the diagnosis of contact dermatitis and treatment strategies. In addition we review our current understanding of the cellular and molecular pathomechanisms and its implications for the development of novel diagnostic and treatment strategies and of animal-free testing strategies for contact allergen identification.

Keywords

Allergic contact dermatitis • ACD • Antigen presenting cell • APC • Hypersensitivity • Damage-associated molecular pattern • T cell • Mononuclear cell • Local Lymph Node Assay • LLNA • Contact dermatitis • Heterologous innate immunity

Abbreviations

ACD	Allergic contact dermatitis
APC	Antigen presenting cell
CHS	Contact hypersensitivity
DAMP	Damage-associated molecular pattern
DNBS	2,4-dinitrobenzene sulfonic acid
DNCB	2,4-dinitrochlorobenzene
DNFB	2,4-dinitrofluorobenzene
DNTB	2,4-dinitrothiocyanobenzene
FLG	Filaggrin
HA	Hyaluronic acid
hTCPA	Human T cell priming assay
ICD	Irritant contact dermatitis
LLNA	Local Lymph Node Assay
LZT	Low zone tolerance
MAMP	Microbe-associated molecular pattern
MHC	Major histocompatibility complex
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PRR	Pattern recognition receptor
TCR	T cell receptor
TNBS	2,4,6-trinitrobenzene sulfonic acid
TNCB	2,4,6-trinitrochlorobenzene
TLR	Toll-like receptor
Treg	Regulatory T cell

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Key Points

- Allergic contact dermatitis affects 5–10 % of the general population and prevalence is increasing
- Contact dermatitis is the most important occupation-related skin disease
- Causative treatment strategies, development of new drugs and of *in vitro* assays to replace animal testing for contact allergen identification are needed and require a detailed mechanistic understanding of the pathomechanisms
- The innate immune response to contact allergens is mechanistically similar to anti-infectious immune responses
- Biomarker panels are being identified for improved diagnostics and distinction of different types of eczema

Allergic Contact Dermatitis: Prevalence, Clinical Presentation and Etiology

Allergic contact dermatitis (ACD) is an eczematous skin reaction to substances (mostly small chemical compounds, so-called haptens) in non-toxic concentrations that requires an immunological cell-mediated sensitization and usually occurs after repeated exposure to the substance, while the same substance does not elicit reactions in non-sensitized individuals.

ACD is caused by activation of the immune system by chemical allergens due to their ability to cause skin inflammation and, consequently, a chemical-specific T cell response. ACD shows increasing prevalence. Roughly 20 % of the general population is sensitized to at least one contact allergen and about 5–10 % develop ACD once per year [1, 2].

The more than 4,000 known contact allergens are found for example in household products, cosmetics, plants, jewelry, clothes and in the workplace. Sensitization does not necessary result in the development of ACD but once the disease has developed, it can become chronic. A major problem is the formation of memory T cells which cause eczema upon re-exposure to the eliciting contact allergen.

Occupational contact dermatitis is the most common occupation-related skin disease [3]. Here, chronic ACD may result from chronic allergen exposure leading to significant damage to the skin. Chronic ACD is difficult to treat and requires complete avoidance of the contact allergen. This often demands that the patients change profession. The socio-economic costs of ICD and ACD are very high and the treatment options are still limited [3–5].

The yearly hitlist of the clinically most relevant contact allergens as presented by the German working group for contact dermatitis (DKG)/German Information Network of Departments of Dermatology (IVDK) consortium has nickel in the first place for many years already, followed by fragrance mix and balsam of Peru [6]. The contact allergen of the year 2013 was the preservative methylisothiazolinone (MI) and sensitization to MI remains a very serious problem [7, 8]. MI is used in cosmetics but also in household products such as paint. Due to the replacement of other preservatives the use of MI and methylchloroisothiazolinone (MCI)/MI (Kathon CG) has increased, and the concentrations used seem problematic. Even airborne exposure to MI can cause severe allergic skin reactions as well as asthma symptoms [9]. Action is now taken to reduce the concentrations of MI in consumer products and to find replacements.

The clinical presentations of ACD can be classified according to the disease kinetics and duration (acute versus chronic ACD), the route of allergen exposure (direct skin exposure, airborne exposure, systemic exposure), the localization (localized e.g., hand, lower leg, face, eyelid, genito-anal, generalized, flexural etc.), the exposure conditions (occupational, accidental, iatrogenic) and the type of contact allergen (e.g., weak, strong, obligatory contact sensitizer).

The acute ACD is characterized by an onset of clinical signs and symptoms within 3–12 h after contact with the allergen. The kinetic of the response depends on the degree of the preexisting sensitization – the stronger the degree of sensitization the faster the onset of symptoms. The initial reaction is characterized by dermal edema, vasodilatation and a beginning perivascular infiltrate of mononuclear cells. Within 6–24 h the infiltrate becomes more prominent and is accompanied by epidermal changes with spongiosis and increasing exocytosis of mononuclear cells into the epidermis. Depending on the type of contact allergen and degree of sensitization the reaction peaks at 24–48 h with a dense dermal mononuclear infiltrate, intra-epidermal blister formation, loss of the granular layer (stratum granulosum) and signs of parakeratosis. The resolution of the acute ACD reaction usually starts around 48–72 h and is characterized by reduction of epidermal spongiosis and blister formation, acanthosis of the epidermis and gradual reduction of the dermal and epidermal mononuclear infiltrate. Clinically, ACD is characterized by infiltrated erythematous pruritic patches and papules with or without vesicular reactions. The histological changes are reflected by the symptoms of acute ACD that initially present as pruritic erythema (stadium erythematosum), pruritic palpable infiltration, with or without formations of small and sometimes confluent larger blisters (stadium vesiculosum), subsequently crust formation (stadium crustosum) and a resolution with eczematous fine lamellar scaling (stadium squamosum). Chronic ACD is mostly characterized by a less prominent spongiosis and a more prominent acanthosis, hyper- and parakeratosis and a less prominent mononuclear infiltrate mostly in the upper

dermis. Clinically, chronic lesions are typically characterized by pruritus, lichenification, erythema, scaling, fissures and excoriations.

Neither acute nor chronic ACD present with histological or clinical changes that definitely allow the differentiation of the type of insult that caused the dermatitis. The only clinical signs that are suggestive of ACD are disseminated small papular eczematous skin reactions that extend beyond the actual area of contact to the allergen. In contrast, irritant contact dermatitis (ICD) is usually sharply demarcated and restricted to the area of direct skin contact with the irritant.

Clinical manifestations of ACD can vary according to the anatomical location in which the contact allergen was encountered. Frequent locations are hands (in particular in occupational ACD), face and eyelids (often associated with use of cosmetics), lower legs (in particular in patients with topical treatment of leg ulcers), and perianal region (in particular in patients with topical treatment of pre-existing perianal dermatosis).

Clinical manifestations of ACD also vary based on the route of allergen exposure. The classical ACD usually begins in the area of direct skin contact. Since some of the contact allergens are volatile components, ACD may also primarily present in areas of airborne exposure such as the face, the neck and extremities. Examples of airborne contact allergens are composite plants allergens (*Asteraceae*), formaldehyde, expoxy resins, isothiazolones, fragrances, drugs and others [10–12].

Also systemic exposure to contact allergens either via enteral or via parenteral application can trigger ACD in sensitized individuals. Clinical examples of systemic ACD are patients sensitized to drugs (e.g., via epicutaneous exposure at the work place) that develop generalized dermatitis upon oral or parenteral application. This often presents with a characteristic clinical pattern as sharply defined symmetrical erythema of the gluteal/perianal area, and/or V-shaped erythema of the inguinal/perigenital area and in flexural or intertriginous folds. Due to the prominent involvement of the buttocks this presentation had previously been designated "Baboon syndrome", a term that was more recently suggested to be replaced by the less derogative and more descriptive acronym SDRIFE, symmetric drug-related intertriginous and flexural exanthema [13]. Allergens reported to induce this drug-induced systemic ACD include betalactam antibiotics such as aminopenicillins, cephalosporins, but also a wide range of other drugs such as clindamycin, macrolides, mitomycin, cimetidine, naproxene, and pseudoephedrine [13]. Other examples of contact allergens that have been reported to induce ACD include oral intake of nickel, chromium and cobalt salts, inhalation of mercury vapours, and-ingestion of balsam of Peru. Clinically systemic ACD presents either as dermatitis flare-up in areas of previous contact with allergen, flare-up of previous positive patch test sites, or as dermatitis in previously unaffected skin [14].

Finally, ACD can also develop to substances that only in combination with exposure to ultraviolet radiation act as allergens/haptens. The mechanism of this photoallergic contact dermatitis involves photochemical reactions that lead to the generation of haptens or full allergens which in turn induce allergic sensitization and classical T cell-mediated hypersensitivity reactions upon subsequent exposure [15]. Examples of photoallergens include systemic drugs such as non-steroidal anti-inflammatory drugs (NSAID), diphenhydramine, phenothiazine and topically applied substances such as halogenated aromatic hydrocarbons (e.g., salicylanilides), hexachlorophene, and chemical components of sunscreens such as 2-hydroxy-4-methoxybenophenone, 4-isopropyl dibenzoyl methane, or para-amino benzoic acid. Photoallergic reactions differ from phototoxic reactions in which exposure to UV radiation leads to the generation of phototoxic substances that cause damage to epithelial, endothelial and immune cells in the vicinity without involving allergic sensitization and hapten/allergen-specific T cell responses. Classical examples of phototoxic substances include furocumarines (psoralens), amiodarone, sulfonamides and certain dyes such as acridine orange. Clinically, both photoallergic and phototoxic reactions are characterized by occurrence in skin areas that are exposed to UV radiation, and sparing of areas that are naturally less exposed such as the skin behind the ear lobes or under the chin. While phototoxic reactions are usually restricted to the area of exposure to the photosensitizer (e.g., bullous dermatitis pratensis induced by giant hogweed) photoallergic reactions may extend beyond the area of exposure.

Conflicting data exist concerning atopy as a potential predisposing factor for ACD [16–19]. The skin barrier defect and bacterial colonization may favor ACD due to enhanced penetration of chemical allergens and enhanced basal inflammation [19, 20]. Infection or tissue damage may facilitate sensitization by providing danger signals that activate the innate immune system (Fig. 23.1, 3) [21]. These danger signals are essential for ACD [22] and such heterologous innate immune stimulation may amplify the contact allergen-dependent, autologous danger signaling or even replace it (Fig. 23.2) [23].

Diagnosis of Allergic Contact Dermatitis

The diagnosis of ACD is ideally based on the patient's history, the clinical presentation and a positive patch test reaction to the suspected contact allergen. In every day practice this ideal situation is rarely present. The clinical presentation, the affected areas and the distribution may be suggestive of ACD, while morphological skin changes and histology

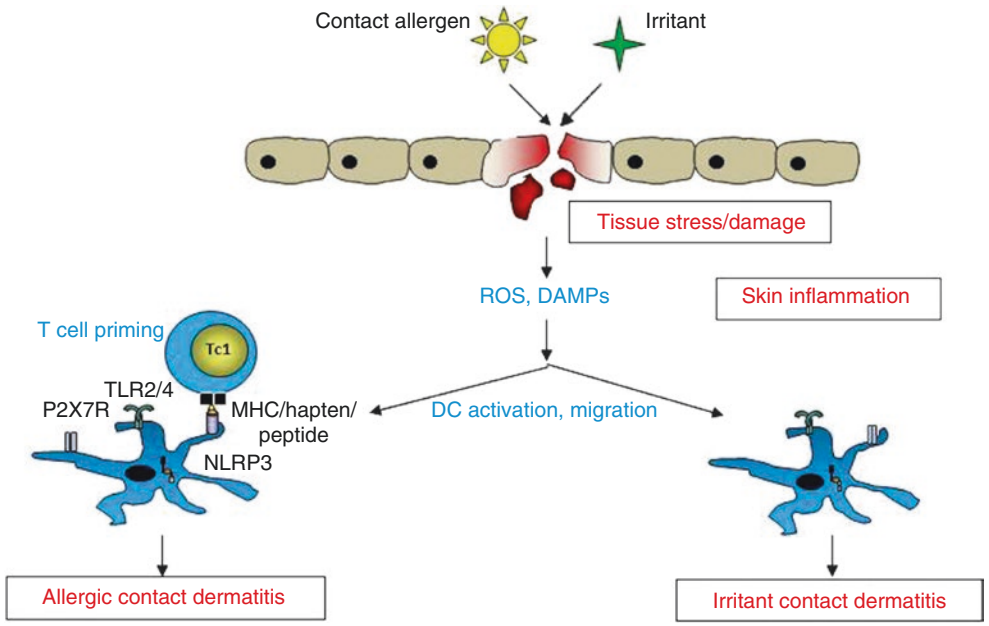

Fig. 23.1 Contact allergens and irritants cause tissue stress and damage. ROS are induced and stressed or damaged cells release and or/produce danger signals such as PRR-activating DAMPs. Additional danger signals are derived from the extracellular matrix. Consequently skin inflammation results and DCs are activated and migrate from the epidermis to the dermis and then to skin draining lymph nodes. Due to the chemical modification of proteins contact allergens form T cell epitopes that are presented on activated DCs in the lymph node and prime contact allergen-specific T cells. This concludes the sensitization phase of ACD. The recruitment of activated effector/memory T cells to the skin upon repeated contact with the contact allergen leads to eczema. Irritants cause eczema without induction of an allergen-specific T cell response

do not allow a definite differentiation between ACD and other forms of dermatitis, such as ICD, atopic dermatitis or seborrheic dermatitis. For the identification of relevant contact allergens a detailed patient history is of utmost importance. This must include detailed information on the work environment, recreational activities, medication, use of cosmetics, emollients, detergents and other substances that the patient has been exposed to prior to the onset of the dermatitis.

The mainstay for the diagnosis of ACD is the patch test in which under standardized conditions contact allergens are applied to the healthy skin of the patient with the goal to reproduce the allergic eczematous skin reaction. Prerequisite for patch testing with suspected contact allergens is that skin reactions during acute or chronic ACD have been sufficiently controlled or even better resolved by topical or systemic anti-inflammatory treatment. Patch testing during ongoing ACD produces increased numbers of false positive patch test reactions and thus should be avoided.

The standard procedure of the patch test involves application of the contact allergen in the corresponding vehicle (vaseline for lipophilic, aqueous solution for hydrophilic allergens) in a Finn chamber to the skin (usually skin of the back) of the patient for 24 h or 48 h. The first reading is taken at 48 h, a second reading is recommended at 72–96 h after initiation of the test. Standardized criteria for reading the patch test reactions have been developed by the DKG and allow a grading and interpretation of the reaction (Table 23.1). Like all test systems the patch test has also a number of pitfalls and limitations and may generate false positive or false negative results. False positive results may occur in patients in whom the ACD has not sufficiently been treated or in patients that display a very strong sensitization. In these situations multiple chemically non-related allergens can induce false positive reactions. This phenomenon is described as "angry back" or excited skin syndrome and most likely reflects the fact that the skin displays reduced thresholds to the irritative capacity of these unrelated allergens.

The majority of contact allergens has an intrinsic capacity to activate mechanisms of the innate immune response and in this sense can act as irritants. Since this effect is concentration-dependent, using the optimal test concentration of the contact allergen is crucial for the interpretation of the test results. To optimize the performance, test conditions for the most common contact allergens have been standardized. Since relative allergenic and irritative potential of contact allergens can vary a great deal, attempts have been made to classify allergens into those that have a higher irritative than allergenic capacity and those that have a high allergenic and little irritative potency. Calculation of the reaction index (RI) which analyzes the relationship between number of positive patch test reactions and number of questionable or irritant reactions was suggested by the IVDK a parameter to assess the quality of patch test preparations [24, 25]. Similarly, an

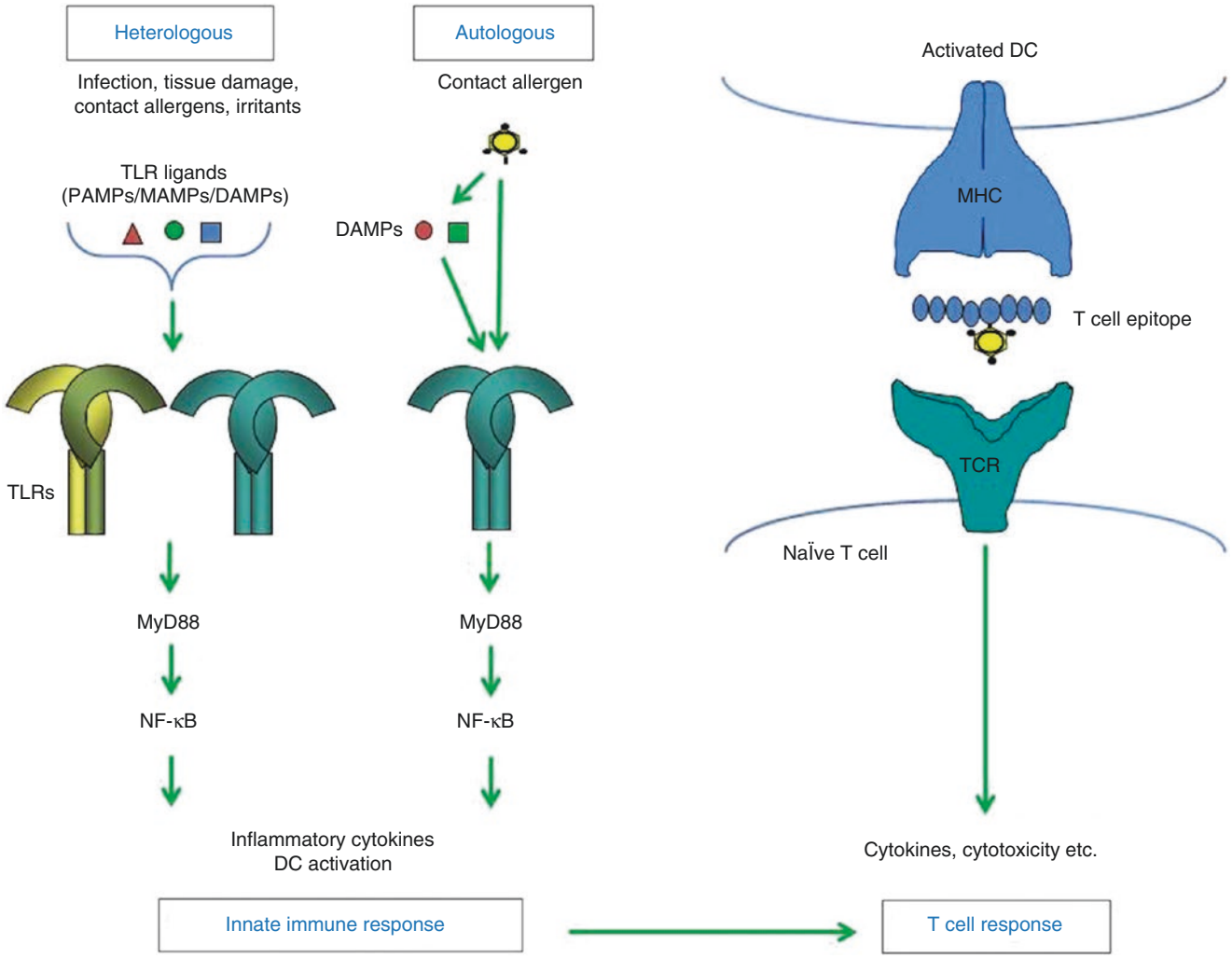

Fig. 23.2 Heterologous innate immune stimulation. Contact allergens can directly or indirectly provide autologous innate immune stimulation for example by triggering TLRs leading to activation of NF-kB and production of inflammatory cytokines and DC activation and migration. Heterologus innate immune stimulation can be provided for example by infection (e.g., PAMPs), tissue damage (DAMPs), irritants and other contact allergens that trigger the same signaling pathways via the same or different TLRs. As a result autologous innate immune stimulation can be amplified or – when absent – be substituted by heterologous innate immune stimulation. Due to T cell epitope formation by the contact allergen a T cell response is induced by activated DC when the innate immune stimulation is sufficiently strong. Upon repeated allergen contact contact allergen-specific effector/memory T cells enter the skin and exert effector function such as cytokine production and cytotoxicity

Table 23.1 Grading of patch test reactions according to the German Working Group for Contact Dermatitis (DKG)

Grading	Morphology	Interpretation
–	No changes	Negative
ir	Sharply demarcated erythema, blister, erosion, necrosis, ulcer	Irritative
?	Only erythema (allergic or irritative)	Questionable
F	Follicular papules, and/or pustules	Questionable
+	Erythema, palpable infiltrate, discrete papules	Positive
++	Erythema, palpable infiltrate, papules, vesicles	Positive
+++	Erythema, palpable infiltrate, confluent vesicles	Positive

Morphological skin changes induced by patch testing form the basis for the grading and interpretation of patch test results

additional parameter, the positivity ratio (PR) which is defined as the frequency of + reactions among the total number of positive patch test reactions (+ to +++), was developed to be used in combination with the RI to identify problematic allergens [26]. Even though subsequent evaluation of both parameters by a Danish group has challenged the general

applicability of this concept [27] it clearly demonstrates that interpretation of the patch test reactions very much depends on the type of allergen tested.

Similarly, false negative reactions may be related to low allergen concentrations in the test preparation or reduced or altered skin permeability. In particular, weak contact allergens that may elicit ACD in sensitive skin areas such as the eyelids, may not be detected when tested on the back skin of the patient. Similarly, weak contact allergens may elicit ACD in areas with a preexisting damage of the barrier function, while they are not strong enough to elicit a reaction on the intact skin of the back. Since the epidermal barrier is crucially involved in determining the level of allergen penetration, modification of the patch test by tape stripping the upper layers of the epidermal stratum corneum prior to allergen application may be used to simulate particularly sensitive or damaged skin areas. Even though this approach has recently been standardized and shown to increase test sensitivity in particular to weak contact allergens [28, 29] interpretation needs to be done very carefully in particular in relation to irritative test reactions which occur more frequently under these conditions.

Both positive or negative test reactions may be re-evaluated by a repeated open application of the suspected substance [30, 31]. Often positive test reactions are observed that are of questionable relevance. They may simply indicate that the patient is sensitized but not necessary allergic to the substance. In this case the substance of interest can be applied twice daily to a 2×2 cm area of the cubital skin for consecutive 7 days after which ACD should develop provided that the contact sensitization is clinically relevant. The same approach can be taken when a strongly suspected substance gives a negative patch test reaction or is not available or suitable for occlusive patch testing.

Finally, when photoallergic reactions are suspected a modified patch (photopatch) test may be applied in which two identical panels of allergens are applied to the back skin for 24 h and subsequently one panel is irradiated with UVA (5–10 J/cm^2), while the other panel is protected from UV radiation. The test readings are performed at 48, 72 and 96 h according to the same criteria as the standard patch test and allow addressing the role of UV light for the elicitation of the ACD [32].

Treatment of Allergic Contact Dermatitis

Since in the majority of the cases ACD presents as a localized skin reaction and the inflammatory skin infiltrate is accessible to topical treatment, the use of topical glucocorticosteriods applied to the affected skin areas is the mainstay of ACD treatment. In cases of extended skin involvement or generalized ACD short term systemic therapy with glucocorticosteriods may be considered. The topical treatment of ACD corresponds to treatment of other forms of dermatitis. The vehicles used for topical glucocorticosteriod treatment should be adapted to the clinical presentation and state of the eczematous skin reaction. Acute dermatitis requires treatment with hydrophilic creams and/or lotions that provide a cooling and anti-inflammatory effect and help to dry acute weeping or blistering skin lesions. In contrast, chronic dermatitis is usually characterized by dry and brittle skin with lichenification and hyperkeratosis and thus requires more lipophilic vehicles such as lipophilic creams or ointments. In cases of prominent hyperkeratosis in particular on palms and soles the keratolytic effects of salicylic acid or urea may be used to reduce the hyperkeratosis and thus allow better access of the topical anti-inflammatory agents. In case of dermatitis with bacterial superinfection, anti-infective agents such as octinidine or polyhexanide may be used. Supportive measures should include minimizing irritative insults to the skin such as chronic exposure to wet conditions at the work place or repeated hand washing. Since allergic sensitization and elicitation of ACD requires for a variety of allergens some sort of adjuvant irritative effect or skin damage as cofactor to fully develop, reduction of this kind of aggravating cofactors will help to improve the skin condition and to avoid relapses. The mainstay of the treatment of ACD is the anti-inflammatory therapy with topical glucocorticosteriods in the correct vehicle. Topical calcineurin inhibitors such as tacrolimus or pimecrolimus can also be effective, and due to an almost absent induction of skin atrophy may be considered in chronic patients that already have steroid-damaged skin. In patients in whom steroid therapy is not appropriate, UVB or PUVA phototherapy may be an effective alternative. In particular in patients with localized chronic hand dermatitis crème PUVA therapy has proven to be a valuable and well standardized therapeutic option.

However the treatment can only be successful if the offending allergen is avoided. So great care should be taken to identify the relevant allergen and large efforts must be taken to explain the necessity of allergen avoidance. In particular in work related contact allergies this may mean leaving the workplace for good and/or changing the profession. The best prophylaxis of ACD is the complete avoidance of the contact allergen, which in many cases is not possible. In addition, risk factors to develop ACD, such as chronic skin damage that leads to a reduced skin barrier function, should be avoided and/or treated. In the same line of thought, consequent basic skin care using rehydrating lipophilic creams or ointments should be recommended to prevent impairment of barrier function. In addition, the use of barrier creams can be recommended for some allergens and irritants that prevent or reduce penetration of the offending agent.

Finally, animal experiments suggest that in ACD specific tolerance can be induced. Attempts to induce contact allergen

specific tolerance in humans have been reported, however only for certain plant allergens like poison ivy [33]. Overall the effects were rather transient and the clinical benefit was not convincing. Similarly oral tolerance induction to nickel has been reported that caused an amelioration of skin manifestation, a reduced skin test reactivity to nickel and a reduced T cell reactivity upon nickel restimulation [34]. Again effects were rather moderate and transient in nature. In summary, so far no effective and lasting allergen specific tolerance induction has been established in humans.

Risk Factors Predisposing to ACD

Many factors may contribute to the susceptibility to ACD. Up to now, genetic studies have not revealed an association of ACD with specific HLA alleles. However, several gene polymorphisms have been identified [35]. These are found in genes regulating skin barrier function, detoxification, innate inflammatory immune responses and T cell responses. Several polymorphisms in the stratum corneum protein filaggrin (FLG) have been found to impact its function and to impair the barrier function of the skin [36]. FLG mutations have been associated with increased susceptibility to atopy [37, 38] but also to ICD and ACD [39, 40]. FLG-deficient mice exhibit increased antigen penetration and exacerbated CHS responses triggered by the irritant croton oil or the contact allergen DNFB [41].

Chemistry and Contact Dermatitis

Contact allergens are low molecular weight chemicals that share one characteristic feature: they are protein-reactive. Due to their small size the chemicals *per se* cannot be recognized by the immune system and are therefore also designated haptens (half-antigens). Their protein binding is essential for their immunogenicity and antigenicity. Organic chemical allergens can covalently bind to proteins, and metal ions form complexes with proteins. Some contact allergens are not reactive haptens but are pre-haptens which require oxidation or pro-haptens which require metabolic conversion to full haptens. Such chemicals are highly problematic in terms of contact allergen identification in patch testing and in *in vitro* assays since an adduct and not the parent compound causes sensitization and ACD [42].

A detailed understanding of the relation between the physico-chemical properties, structure and reaction mechanisms and their biological activity is used to develop *in silico* prediction methods to identify potential contact sensitizers in so-called quantitative structure-activity relationship (QSAR) approaches. Grouping according to so-called mechanistic domains [43, 44] is being analysed with respect to allergenicity and allergenic potency. In a recent study allergenic potency as determined in the mouse local lymph node assay (LLNA) could be correlated with mechanistic domains. The results of that study showed that the more potent contact allergens triggered a broader range of signaling pathways than the less potent ones [45]. These data are encouraging further investigations using QSAR and mechanistic domains to promote our understanding of the relation of the chemistry of contact allergens and its impact on the immune system.

Functional Consequences of Protein Modification by Contact Allergens

The most fascinating question that remains to be solved is how the chemical reactivity of contact allergens is translated into biological responses that can lead to the development of ACD. The interaction of chemicals with biomolecules can alter their function. Since contact allergens are generally chemically reactive electrophiles or complex-forming metal ions, it is to be expected that this reactivity is responsible for their action as allergens. In fact, neutralizing the reactivity of the strong contact allergen 2,4-dinitrochlorobenzene (DNCB) by coupling it to lysine abrogates its ability to induce CHS (our unpublished data). Hypothetically, contact allergens may mimic or interfere with conventional post-translational protein modifications [46].

The chemical reactivity of contact allergens regulates immunity at two levels: the first level is the induction of signaling cascades due to chemical protein modification and the second, resulting level is the regulation of gene expression. Information regarding the identity of the functionally relevant chemically modified proteins is scarce. In one study it was demonstrated that treatment of the human monocytic leukemia cell line THP-1 with the contact allergen DNFB does not modify all cellular proteins but only some which are not necessarily the most abundant proteins [47]. Thus, there is selectivity in the targeting of proteins by contact allergens. New studies begin to analyse contact allergen-modified proteins in 3D skin models. In a recent study high resolution magic angle spinning (HR-MAS) nuclear magnetic resonance (NMR) spectroscopy was used to identify protein modifications by a ^{13}C-labeled electrophile in reconstructed human epidermis (RHE) [48]. Compared to *in vitro* modification of human serum albumin (HSA) which took several days to be detectable, the *in situ* protein modification in RHE was detectable after less than 24 h. The predominant lysine modification of HSA, as observed *in vitro*, was not detected in RHE. This method will be useful to identify contact allergen-modified adducts formed in the skin. Eventually this technique may promote the identification as well as quantitative and qualitative analysis of the contact allergen-modified proteome.

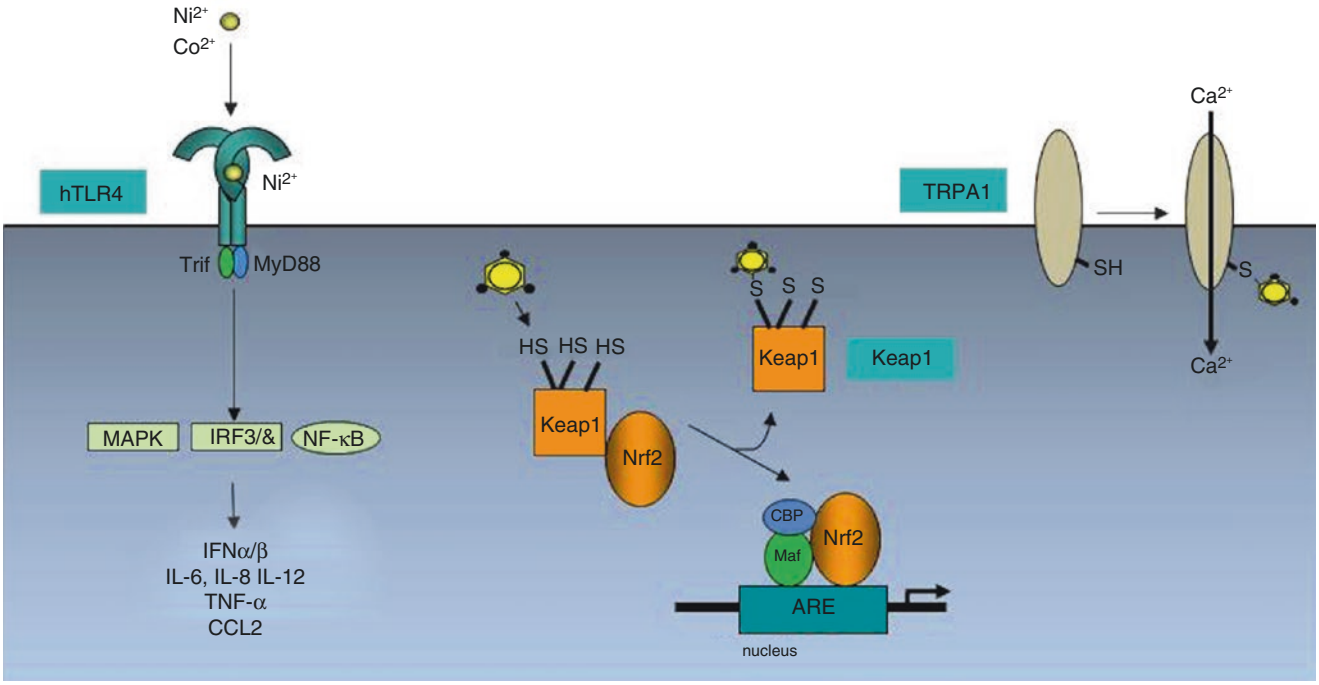

Fig. 23.3 Direct activation of signaling cascades by contact allergens. The metal ions nickel and cobalt form complexes with conserved histidines in human TLR4 resulting in TLR4 dimerization and signaling via NF-kB and MAP kinases and production of inflammatory mediators. Organic chemical allergens such as TNCB or DNCB bind covalently to cysteine residues in the cytosolic protein Keap1. This leads to release of the transcription factor Nrf2, its nuclear translocation and transcriptional activation of the expression of genes containing antioxidant response elements (ARE). These contact allergens can also bind to cysteines in the calcium channel protein TRPA1. Calcium influx activates pathways involved in itch and inflammation

Up to now, only very few proteins, whose modification by contact allergens induce a biological response, have been identified (Fig. 23.3). Human TLR4, the receptor for lipopolysaccharide (LPS, endotoxin) from the cell wall of Gram-negative bacteria, is complexed and dimerized by the metal ions Ni^{2+}, Co^{2+} [49, 50]. Pd^{2+} also interacts with human TLR4 [51]. This results in signal transduction for production of inflammatory mediators in the absence of LPS. The cytosolic, cysteine-rich sensor protein for oxidative or electrophilic stress, Keap1, contains cysteine residues that can be modified by contact allergens. This triggers expression of anti-oxidant phase 2 response- and immune genes due to their activation by the Keap1-regulated transcription factor Nrf2. The promoters of these genes harbor an antioxidant response element (ARE). This pathway limits contact allergen-triggered inflammation. Mice lacking Nrf2 have a much lower sensitization threshold for contact allergens, most likely due to an increased level of pro-inflammatory oxidative stress [52, 53]. A third target for modification by electrophilic contact allergens is the ion channel transient receptor potential ankyrin 1 (TRPA1). TRPA1 also harbors cysteines that are attacked by contact allergens such as cinnamic aldehyde or DNCB [54, 55]. Extracellular Ca^{2+} can then enter the cell. TRPA1 is expressed on a subset of nociceptive nerve fibers, also in the skin, but also on keratinocytes and endothelial cells. The TRPA1 channel is involved in pain but also in itching in inflammatory immune responses [56]. For atopic dermatitis it has been shown that TSLP produced by Th2 cells acts on sensory neurons in the skin to cause itch in a TRPA1 dependent manner [57]. TRPA1-deficient mice show reduced edema, inflammation and itching in cinnamic aldehyde-induced edema in mouse ear skin or in CHS to oxazolone and urushiol [58]. Interestingly, one of the endogenous agonists of TRPA1, 4-hydroxy-2-nonenal (HNE), is produced as a consequence of ROS-mediated oxidation of membrane phospholipids [59]. ROS are important inflammatory mediators in CHS [60].

Innate Immune Responses in Allergic Contact Dermatitis

The induction of ACD requires the priming of contact allergen-specific T cells. This depends on two crucial events: activation of the innate immune system and formation of T cell epitopes (Fig. 23.1). It is a peculiarity of contact allergens that they have this dual function [46]. The activation of the innate immune system results in skin inflammation. The most important outcome of this innate inflammatory response in the sensitization phase is the activation and polarization of

skin DCs allowing their migration to the draining lymph nodes and presentation of contact allergen to T cells in the context of MHC molecules. Naïve, contact allergen-specific T cells are then primed and polarized towards a Tc1/Th1, Tc17/Th17 phenotype. In the elicitation phase, the innate inflammatory response results in the secretion of cytokines and chemokines, up-regulation of adhesion molecules on endothelial cells and eventually the recruitment of effector or memory T cells into the skin where they exert their effector function to produce ACD.

Recent studies in the mouse CHS model have uncovered mechanistic details of the cellular and molecular innate immune response [22, 61, 62]. The emerging picture clearly indicates that the immune system reacts to contact allergens as if they were infectious agents. This is due to the fact that contact allergens activate the same anti-infectious immune response mechanisms as viruses and bacteria.

The innate cellular response to contact allergens is initiated in the epidermis by activation of keratinocytes and Langerhans cells. Here, the innate inflammatory response is essential to abrogate the naturally tolerogenic milieu in the skin as a major immunologic barrier. As a consequence the normally tolerogenic Langerhans cells are switched to an immunogenic phenotype allowing lymph node migration and T cell priming [63, 64]. Despite the fact that Langerhans cells are dispensable for CHS in some experimental models which usually use saturating contact allergen concentrations, they most likely play a role in ACD under physiological conditions, especially at low contact allergen concentrations and absence of spreading to the dermis [65–67]. Further analysis of the orchestration of the cellular innate immune response has identified mast cells as important initiators of skin inflammation [68]. Mast cell deficiency or mast cell depletion before sensitization significantly reduces CHS in mice. This is due to a role of histamine from mast cells in increasing the permeability of the blood vessels in the skin. The absence of mast cells decreases the infiltration of neutrophils and the emigration of DCs from the skin. Neutrophil depletion efficiently abrogates CHS as does the depletion of subsets of skin DCs [66, 67, 69–71]. Both the sensitization and elicitation phase of CHS depend on the presence of neutrophils [71]. The fact that depletion of one or the other cell type has a similar outcome underlines the essential collaboration of the different innate immune effector cells. The same principle is seen in the orchestration of the molecular mechanisms of contact allergen-induced innate immune responses [46].

Pathogens are recognized by the innate immune system via so-called pattern recognition receptors (PRRs) which are triggered by pathogen- or microbe-associated molecular patterns (PAMPs, MAMPs). Examples for families of PRRs are the Toll-like receptors (TLRs), transmembrane proteins in the plasma membrane or endosomal membranes, the cytosolic NOD-like receptors (NLRs), the RIG-I like receptors (RLRs) and C-type lectin receptors (CLRs) in the plasma membrane [72].

PAMPs/MAMPs can be bacterial cell wall components, flagellin, viral and bacterial nucleic acids, lipids and carbohydrates. These are perceived as danger signals by PRRs and induce innate anti-infectious immune responses. Contact allergens can also trigger PRRs by direct or indirect mechanisms [61]. Nickel- and cobalt ions bind to conserved histidine residues of human TLR4 inducing dimerization and signaling [49, 50]. The murine TLR4 lacks these histidines and therefore is not triggered by Ni or Co. This results in resistance of mice to CHS to these metal ions. However, replacement of the murine TLR4 by the human TLR4 in transgenic mice renders these mice susceptible to CHS [49]. Up to now this is the only known case where contact allergens are directly activating a PRR. For other contact allergens, organic molecules such as 2,4,6-trinitrochlorbenzene (TNCB) or 2,4-dinitrofluorobenzene (DNFB) or oxazolone an indirect activation of PRR has been demonstrated [60, 73, 74]. These contact allergens induce endogenous danger signals. A rapid induction of ROS and release of ATP from skin cells has been revealed [60, 74]. Moreover, the degradation of the extracellular matrix (ECM) component hyaluronic acid (HA) results in fragments that can activate TLR2 and TLR4 [60]. Constitutive overexpression or induction of expression of human hyaluronidase 1 in mouse skin under control of the K14 promoter resulted in lack of CHS when expression was induced before sensitization, most likely due to DC depletion from the skin. CHS was enhanced when overexpression was induced at the time of sensitization [75]. HA breakdown resulted in the emigration of DCs from the skin. The HA effects were dependent on TLR4. These data show a TLR4-dependent role of HA fragments for the mobilization of DC from the skin. The pro-inflammatory role of HA breakdown is evident in Shar-Pei dogs who accumulate HA over time due to an overexpression of hyaluronan synthetase (HAS) 2. Periodic breakdown of accumulated HA results in a periodic fever syndrome in these dogs [76]. These findings illustrate the important role of the ECM in innate immunity [77].

The endogenous danger signal high mobility group box 1 (HMGB1) is involved in the *in vitro* induction of IL-18 in the human keratinocyte cell line NCTC2544 [78]. HMGB1 was released upon treatment of the cells with the contact allergens para-phenylene diamine (pPD), DNFB or citral.

While ATP triggers the activation of the cytosolic NLPR3 inflammasome that induces the maturation of immature pro-IL-1β and pro-IL-18 via caspase-1, ROS contribute to skin inflammation for example by triggering oxidative HA breakdown and by promoting TLR signaling and inflammasome activation. Nickel also activates the NLRP3 inflammasome [79].

A recent study [80] demonstrated that mice lacking MyD88 or CARD9 are resistant to CHS to TNCB. Irritant CHS to SLS was normal. While MyD88 is an adaptor protein involved in TLR/IL-1R signaling, CARD9 is an adaptor that plays a role in the signaling of CLRs. The study clearly showed that the contact allergens TNCB/TNBS, DNFB and oxazolone can trigger ITAM-Syk-Card9/Malt10 signaling in DCs *in vitro* resulting in IL-1α and IL-1β production. Signaling was dependent on the ITAM containing adaptor protein DAP12. Syk phosphorylation and CARD9/Bcl-10 mediated NF-kB activation resulted in the production of immature pro-IL-1α and -IL-1β. ROS formation and ROS mediated NLRP3 inflammasome activation for the production of mature IL-α and IL-1β in DCs was Syk-dependent but independent of CARD-9/Bcl-10. The DC-mediated priming and differentiation of contact allergen-specific IFN-γ and IL-17-producing effector T cells was dependent on IL-1β and MyD88. It remains to be determined if and which receptor (e.g., a CLR), couples to this signaling pathway and how it is activated by contact allergens. Moreover, the molecular mechanisms of crosstalk of this signaling pathway with the TLR pathway remain to be determined.

Further important signaling pathways have been identified by genomic or proteomic profiling studies using human cell lines. Two prominent pathways are the aryl hydrocarbon receptor (AhR) and the Keap1/Nrf2 pathway [81]. The AhR is a transcription factor that regulates not only detoxification pathways but also immune processes such as Langerhans cell function and Th17 differentiation. Dietary and endogenous ligands have been identified that modulate immune function [82] and evidence for contact allergen-mediated activation of the AhR has been provided [83].

Disturbed epidermal homeostasis involving keratinocyte death promotes skin inflammation [84] Here, the NF-kB pathway plays an important regulatory role. Mice with an epidermis-specific loss of the NF-kB subunit RelA did not develop spontaneous inflammation. However, DNFB- and oxazolone-induced allergic CHS was aggravated while croton oil-induced irritant CHS developed as in wild type mice [85]. The loss of RelA leads to up-regulation of the small calcium-binding proteins S100A8/A9. These proteins are up-regulated in inflammatory and autoimmune diseases and can have pro-inflammatory, but also anti-inflammatory activity by acting as ligand for TLR4 [86–88]. Increased keratinocyte apoptosis and up-regulation of XIAP associated factor 1 (XIAF1) were observed for contact allergens and croton oil. XIAF1 blocks the anti-apoptotic function of X-linked inhibitors of apoptosis (XIAPs). It was speculated that the selective aggravation of contact allergy may be due to an effect of the contact allergen-specific T cell response on the proliferation of keratinocytes. The nuclear hormone receptor peroxisome proliferator activated receptor (PPAR)-α is involved in the regulation of keratinocyte proliferation and differentiation as well as inflammation. PPAR-α is expressed in keratinocytes but also in Langerhans cells, mast cells and T cells. Its activation can inhibit NF-kB activation [89]. Topical treatment of mice with PPAR-α ligands such as clofibrate counteracts keratinocyte hyperproliferation [90] and reduces TPA-induced irritant CHS and oxazolone-induced allergic CHS. This correlated with a reduction in the levels of TNF-α and IL-1-α [91]. PPAR-α-deficient mice had exacerbated CHS which was associated with impaired IL-2 production in lymph nodes and a decrease in regulatory T cell (Treg) numbers and function [92]. Interestingly, an endogenous PPAR-α ligand, palmitoyl ethanolamide (PEA), is up-regulated along with PPAR-α by contact allergens [93]. These findings underline the importance of dysregulated keratinocyte homeostasis for inflammatory skin diseases.

Many of the mechanisms described above for contact allergens can also be triggered by chemically reactive drugs or drug metabolites. *N*-acetyl-*p*-benzo-quinoneimine (NAPQI), the toxic metabolite of acetaminophen is involved in drug-induced liver injury. It can damage hepatocytes. These release self-DNA which acts as DAMP and activates TLR9 on sinusoidal endothelial cells. ROS and ATP then contribute to activation of the NLRP3 inflammasome [94, 95]. Moreover, NAPQI can also activate TRPA1 [96].

The polarization of the cytokine profile secreted by skin DCs is an essential step in the development of ACD. Polarization of T cells towards a Th1/Tc1 phenotype requires IFN-γ and IL-12 or IL-18, IL-21 and IL-27 whereas the polarization of Tc17/Th17 cells requires IL-6 and TGF-β or IL-6, IL1-β and IL-23 [97]. The innate immune response is certainly instrumental in this polarization process. However, there is also evidence for chemical-intrinsic properties that contribute to that [98, 99]. It was observed that the contact allergens DNFB and DNCB induced a type 1 cytokine profile (IFN-γ^{hi}, IL-4/-5/-10^{lo}) in the skin draining lymph nodes following topical exposure of Balb/c mice. In contrast, the respiratory allergen trimellitic anhydride (TMA) as well as FITC and DNBSCl induced a type 2 profile (IL-4/-5/-10^{hi}, IFN-γ^{lo}). Interestingly, these cytokine profiles correlated with the modification of proteins. *In vitro* studies using human U937 monocytes showed preferential modification of cellular proteins by DNFB and DNCB, but preferential modification of serum proteins by the other chemicals [100].

A modulation of the epigenetic regulation of gene expression has also been discussed in the context of T cell polarization [101]. A first study analyzed genome wide changes in the methylation of DNA from skin draining lymph nodes of Balb/c mice exposed to DNCB or TMA [102]. Characteristic changes were found for both chemicals with differently methylated regions in various pathways including cytokine and chemokine genes. Future studies using T cells or DCs are needed to identify potential methylation signatures that are specific for contact or respiratory allergens.

Mechanisms of Irritant Contact Dermatitis

Unlike ACD, ICD is caused by chemicals that are not covalently binding to proteins or form complexes with proteins like metal ions do. Chemicals such as detergents, acids, bases and solvents with a variety of physico-chemical properties cause a toxic-irritant skin eczema that may evoke pathologically relevant stress or damage to the skin barrier (Fig. 23.1) [103]. Innate inflammatory immune responses also play a role in ICD but adaptive immunity is not involved. The underlying cellular and molecular mechanisms are not well understood. From the CHS model it is known that ICD to the irritant croton oil is absent in mice lacking TLR4 and IL-12Rβ2 or TLR2 and TLR4 (our unpublished data) but is normal in mice lacking P2X7R [74]. SLS induced CHS is normal in mice lacking MyD88 or CARD9 [80]. Addition of croton oil to sub-sensitizing doses of TNCB or oxazolone is not able to compensate the lack of sufficient innate immune stimulation in the CHS model [104]. On the other hand, addition of SLS to the tolerogen/weak contact allergen restores IL-1β production and prevents 2,4-dinitrothiocyanobenzene (DNTB)-mediated tolerance induction to DNFB [105]. These data suggest that there are contact allergen-specific signaling pathways that cannot be triggered by some irritants. In addition, other pathways are triggered by irritants and there may also be irritant-specific pathways not triggered by contact allergens. Due to the essential irritant effect of contact allergens that is required to induce skin inflammation, it is not surprising that there is an overlap of cytokine and chemokine profiles induced by irritants and contact allergens [106, 107].

Differences between contact allergens and irritants are for example the selective up-regulation of CXCR4 on Langerhans cells by contact allergens. This leads to chemokine-selective migration of LCs to the dermis: LC migration in response to contact allergens is driven by CXCL12 while migration in response to irritants is driven by CCL2 and CCL5 derived from dermal fibroblasts [108, 109]. A recent study revealed a role for basophils in attracting eosinophils in a mouse model of croton oil-induced ICD [110]. Eosinophil-deficient mice had impaired, IL-5-transgenic mice exacerbated ICD. In an *in vitro* co-culture model basophils secreted IL-4 and TNF-α, and promoted CCL11 expression from fibroblasts. These data suggest a role for basophils in the maturation and attraction of eosinophils to the skin in ICD. Their contribution to production of pro-inflammatory ROS was discussed. Interestingly, basophils and eosinophils are found in other inflammatory human skin diseases including ACD [111–113].

These findings highlight common principles for the immune response to chemicals. Contact allergens, protein-reactive drugs or their reactive metabolites and irritants cause tissue stress and damage. This leads to oxidative stress and the formation of DAMPs, release of DAMPs from stressed and damaged cells and the activation of downstream signaling events that are in part mediated by PRRs. The chemical reactivity of contact allergens and protein-reactive drugs causes the chemical modification of proteins which can result in the direct activation of signaling cascades as is the case for human TLR4, Keap1 and TRPA1 and in in the formation of T cell epitopes. The former leads to innate immune responses resulting in xenoinflammation which is essential for the subsequent activation of the adaptive immune system and the generation of contact allergen- or drug-specific effector and memory T cells [22].

Heterologous Innate Immunity

Contact allergens and irritants are rarely encountered as pure, single substances. Consumer products such as cosmetics, household products or occupational chemicals such as paints or metal cutting fluids often contain combinations of irritants and contact allergens with other chemicals. This combination is of great relevance. The interaction of the different chemicals may result in enhanced skin penetration or augmentation of sensitization and challenge reactions. Facilitated sensitization may be the result [114–116]. Examples are the augmentation effects by combinations of irritants and contact allergens or several contact allergens [117, 118]. Mechanistically, this can be explained based on the specificity of the innate immune response. Due to the activation of identical signaling pathways by different TLRs or other PRRs, a given contact allergen can generate signals for example via TLR4 and these may be amplified by irritants or other contact allergens that also trigger TLR-dependent inflammation. The result is a T cell response and ACD to this contact allergen. If the autologous innate signals triggered by the contact allergen that elicits the T cell response are too weak, augmentation by heterologous innate immune stimulation by irritants or other contact allergens is possible (Fig. 23.2). Moreover, such heterologous innate stimuli may replace missing autologous stimulation. Heterologous innate immune stimulation can also be provided by infections. This may even break tolerance or abrogate genetically based resistance to contact allergy as shown in the CHS model. Here mimicking an infection renders CHS-resistant TLR4/IL-12Rβ2 deficient mice susceptible to CHS. Injection of contact allergen-modified DCs from these mice fail to induce sensitization in wildtype mice. Stimulation of their TLR9 with CpG-oligodeoxynucleotides (CpG-ODN) *in vitro* restores their sensitizing potential [73]. Likewise, CpG-ODN injection of the CHS-resistant mice at the time of sensitization with contact allergen also abrogates resistance (our unpublished data). Combining nickel which does not trigger mouse TLR4 with LPS allows to efficiently sensitize mice

for CHS to nickel [119] and amplifies patch test reactivity [120]. These data highlight the importance of heterologous innate immune stimulation as a process that significantly impacts the outcome of immune responses to chemicals depending on their context [23].

Adaptive Immune Responses in ACD

ACD develops in two phases. The first, sensitization phase is initiated upon skin contact with a chemical allergen. The contact allergen penetrates into the skin and due to its reactivity binds to extracellular, plasma membrane-associated and intracellular proteins. T cells specific for organic chemical contact allergens such as TNCB recognize hapten-modified peptides on MHC molecules [121]. Metal ions such as nickel are recognized by T cells due to complex formation of the metal ion with histidine residues in the MHC molecule and the T cell receptor. For some T cell clones one coordination site is a histidine residue in the peptide bound to the MHC molecule, for others this not the case [122, 123]. The effector T cells in ACD are CD4+ and CD8+ T cells. In the mouse CHS model the effector T cells are usually cytotoxic CD8+ T cells that produce IFN-γ. Moreover, CD8+ IL-17 producing T cells play a role in ACD [113, 124, 125]. In the mouse CHS model a role for dendritic epidermal T cells (DETC) as producers of IL-17 has been described [126] and the ASK1/p38 MAP kinase pathway was shown to be involved in IL-17 production in the elicitation phase of CHS [127]. In human ACD there is some evidence for early infiltrating CD8+ T cells that may cause initial damage. This is similar to results from atopy patch tests [128]. CD4+ T cells are then also detected later. Other effector cells in ACD are infiltrating NK cells that amplify the response due to their IFN-γ production [129]. In the CHS model evidence for contact allergen-specific NK cell responses and solely NK cell-mediated CHS-like reactions in T-cell deficient mice has been provided [130, 131]. However, these reactions seem to be quite different from T cell-mediated CHS [132].

Down-regulation of the immune response in ACD is determined not only by effector T cell death but also by regulatory immune cells such as Treg. In the CHS model ICOS + CD4 + CD25 + Foxp3+ Treg have been identified and a critical role for Langerhans cells in Treg induction has been identified [63, 133]. Invariant NKT cells (iNKT cells) also have regulatory function in CHS [134]. More recently, PU1 + CD4+ Th9 cells have been isolated from ACD skin biopsies of nickel allergic patients [135]. They may have a regulatory role in ACD by acting on Th1 cells directly or via enhancement of IL-4 production by Th2 cells. Interestingly, IL-9 was increased after skin exposure to nickel, rubber and fragrance in a gene expression profiling study of skin biopsies from allergic patients [136].

Tolerance Induction to Contact Allergens

Induction of allergen-specific tolerance is a major goal of the immunotherapy of allergic diseases. Hyposensitization can re-establish allergen tolerance for some years in type I allergies for example to insect venoms, house dust mite and pollen allergens. Immunotherapy (IT) involves the application of increasing doses of the allergen via the subcutaneous (SCIT) or sublingual (SLIT) route. Allergen peptides or recombinant allergens are now also used [137]. The underlying mechanisms involve a shift in the balance between allergen-specific Th2 cells and Treg as well as other regulatory cells such as regulatory B cells, including the *de novo* induction of such regulatory cells [138]. For ACD there are up to now no established protocols to induce contact allergen-specific tolerance and studies in the CHS model show successful tolerance induction only before sensitization. The clinical problem is therefore not solved, yet [139]. In the CHS model, low zone tolerance (LZT) has been studied for many years. LZT is induced before sensitization and results from the repeated application of contact allergen at doses 100- to 1000-fold below the dose required for sensitization. Recent work has revealed that tolerogenic CD11c + DCs induce contact allergen-specific CD8+ Treg [140]. IL-10 producing CD4 + Foxp3+ Treg were essential for LZT induction and rendered CD11c + DC tolerogenic by direct cell-cell contact via gap junctions. In addition, in the skin draining lymph node, DCs produce TNF-α, which induces the death of effector T cells [141].

The tolerogenicity of contact allergens can be dose-related as in the case of LZT or intrinsic as in the case of DNTB which is a very weak contact allergen and used as a tolerogen. The common principle is most likely the lack of induction of a productive innate immune response. This fails to overcome the homeostatic immunoregulatory default which maintains tolerance and induces active contact allergen-specific tolerance involving DCs and regulatory T cells. The latter occurs due to the fact that T cell epitopes can still be formed by low dose contact allergen or weak contact allergens/tolerogens such as DNTB. The contact allergen is then presented on immature/tolerogenic DCs which results in the induction of CD4+- or CD8+ Treg and the induction of effector T cell anergy and death [63, 140, 141]. The central importance of the innate immune response in shifting the balance between tolerance and immunity was demonstrated by the fact that the irritant SLS was able to prevent tolerance induction by DNTB [105]. The combination of SLS, a heterologous innate immune stimulus [23], with DNTB induced IL-1β. DNTB alone failed to do so.

These findings clearly show that the magnitude of the innate inflammatory immune response is a critical determinant of tolerance and immunity and, most likely, of allergenic potency [142]. It remains to be tested whether tolerogenic

adjuvants may be successful as negative heterologous innate stimuli in the re-establishment of tolerance to contact allergens [23]. A major issue is the tolerization of effector and memory T cells. A combination of anti-inflammatory therapies and strategies targeting effector/memory T cells and inducing contact allergen-specific regulatory T cells should be promising.

Biomarker Identification, Gene Signatures

The identification of changes in gene and protein expression induced by contact allergens and irritants will provide important information regarding the mechanisms of action of these chemicals. Pathway analysis can then be used to validate the pathologically relevant pathways and to identify drug targets for new, causative therapies. In addition, characteristic gene signatures can be identified that allow identification of contact allergens and their discrimination from irritants. The classification of chemicals based on physico-chemical and reaction-mechanistic characteristics will reveal whether the gene and protein expression profiles segregate with these characteristics. Dose-response studies will also be important in this context in order to understand the regulation of the balance between immunity and tolerance by contact allergens.

A recent study has provided such initial results from the genomic profiling of patch test biopsies for nickel, fragrance and rubber [136]. One hundred forty-nine genes were commonly regulated by all contact allergens compared to petrolatum as control. Differences between the allergens were observed for their efficiency in the induction of innate immunity and Th1/Th2/Th17/Th22 responses. Another recent genomic profiling study focused on the intra-individual comparison of skin lesion for psoriasis and non-atopic or atopic eczema in patients with both diseases, but also provided data on nickel-induced contact dermatitis [143]. Induced ACD could be differentiated from naturally occuring eczema by the selective down-regulation of late epidermal differentiation markers such as late cornified epithelial (LCE)1 and LCE2 family members, selective up-regulation of adhesion molecules such as ICAM-1 and of extracellular matrix associated HAS3 and epithelial-stromal interaction 1 (EPSTI1) the expression of which is modulated by inflammatory cytokines. Moreover, inflammasome components and neutrophil attracting chemokines were up-regulated in ACD. These studies mark the beginning of future genomic and proteomic studies that will hopefully identify chemical class-specific biomarker signatures for the identification of drug targets, improvement of diagnostics and development of *in vitro* assays for the identification of contact allergens.

A genome-wide association study (GWAS) with volunteers exposed to the irritants SLS and nonanoic acid revealed differential expression of 883 genes for the two irritants. Only 23 genes were commonly regulated by both chemicals [144].

These data highlight the importance to consider chemical-specific mechanisms. Especially in the case of irritants, it should be rewarding to classify them according to their physico-chemical properties and to analyse their mechanism of action by global technologies as there is little mechanistic understanding regarding the signaling pathways triggered by different irritants. Such studies will not only provide potential therapeutic targets, they will also help to understand the clinically relevant interaction of irritants with contact allergens which may lead to an augmentation of sensitization and of the clinical response, for example due to heterologous innate immune stimulation [23, 117].

In Vitro Assays for Contact Allergen Identification

A great challenge is the replacement of the LLNA (OECD guideline 429) by *in vitro* assays that identify contact allergens. The LLNA measures the proliferation of mouse lymph node cells following repeated topical application of a test substance or the solvent to the ear skin. Stimulation indices are calculated and effective chemical concentrations needed to give an SI = 3 (EC3 values) are used to classify chemicals including the determination of relative allergenic potency.

The current roadmap for the development of such assays is the so-called adverse outcome pathway (AOP) for skin sensitization [145]. It describes the key steps in the sensitization process of ACD that should be addressed by *in vitro* assays. Given the complexity of sensitization, it is clear that a combination of different assays in an integrated testing strategy (ITS) is the most likely strategy for the identification of sensitizing chemicals. Different assays have been developed and are tested for their sensitivity, specificity and accuracy [146]. A recent study demonstrated the advantages of the combination of selected *in vitro* assays covering different mechanistic aspects of the sensitization process of ACD in an ITS [147].

Eventually, the identification of contact sensitizers must also include assessment of their allergenic potency which is currently not possible in *in vitro* assays and remains a major advantage of the LLNA. Potency assessment is relevant to determine concentrations of contact allergens such as fragrances that can be used safely in consumer products or in the workplace. Recently, 131 substances have been categorized solely based on relative human skin sensitizing potency data. Such datasets should be more critical for judging the performance of non-animal methods that aim at measuring allergenic potency than a comparison with LLNA

EC3 values [148]. Eventually, safety levels must be determined for contact sensitizers used in consumer products or in the workplace and the impact on clinical outcome has to be monitored. A comprehensive review of testing strategies and preventive measures and their impact on clinical outcome was published by Thyssen et al. [149–151].

Future Clinical and Research Challenges

Our mechanistic understanding now allows us to develop strategies that decrease or prevent sensitization. Already at the level of the chemistry, steps for primary prevention can be undertaken. For example, the introduction of a methyl group into the allergenic hair dye pPD reduces its skin sensitizing potency [152]. Chemical alteration of epoxy resin monomers can also reduce their skin sensitizing potency [153]. Such efforts reduce the risk of ACD. Moreover, interference with innate immune responses may prevent sensitization or even elicitation as shown in the CHS model [60, 61, 74, 80] and aid in the induction of tolerance. Targeted therapies to blunt the effector/memory T cell response and to induce regulatory T cells are needed. Global technologies will help to identify biomarker profiles for contact allergens and irritants that should improve diagnostics and promote the identification of relevant signaling pathways and novel drug targets as well as the development of mechanistically-based assays for the *in vitro* identification of contact allergens.

Questions

1. What is the outcome of the sensitization to contact allergens?
   A. The primary activation of contact allergen-specific T cells
   B. The recruitment of T cells from the blood into the skin
   C. The induction of skin inflammation by T cells
   D. The reactivation of memory T cells

2. What is a hallmark of contact allergens?
   A. Contact allergens are proteins
   B. Contact allergens have enzymatic activity
   C. Contact allergens bind to proteins covalently or by complex formation
   D. Contact allergens bind to NF-κB

3. Which standard test is used for the diagnosis of ACD?
   A. The Prick test is the current standard test
   B. The Local Lymph Node Assay is the current standard test
   C. The Patch test is the current standard test

4. How do the metal ions nickel, cobalt and palladium activate the human innate immune system?
   A. These metal ions induce penetration of bacterial TLR ligands into the skin
   B. These metal ions bind to and dimerize human TLR4
   C. These metal ions induce extracellular matrix degradation
   D. These metal ions destroy the skin barrier

5. ACD is a T cell-mediated skin disease. Which T cell subsets are the main effector cells of ACD?
   A. CD4+ Th2 cells
   B. Invariant NKT cells
   C. CD4+CD25+ regulatory T cells
   D. CD4+ Th1 and Th17 cells, CD8+ Tc1 and Tc17 cells

Answers

1. A
2. C
3. C
4. B
5. D

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Immunology of Acne

24

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Abstract

Activation of the immune system is a central event in the development of acne, and our understanding of this process is rapidly expanding. In this chapter, we describe how *P. acnes*, sebaceous glands, and keratinocytes contribute to inflammation in acne and how current acne treatments modulate this immune response. Specifically, we address the role of anti-microbial peptides, Toll-Like Receptors (TLRs), sebaceous lipids as well as cytokines and the role of the inflammasome as driving inflammation in acne. The anti-inflammatory activities of retinoids, tetracyclines and other therapies are discussed.

Keywords

Acne • P. acnes • Inflammation • Innate immunity • Immunology • Cytokines • Inflammasome • Sebaceous gland • Keratinocytes • Anti-microbial peptides

Acne

Clinical Presentation and Etiology

Acne is one of the most prevalent skin conditions encountered by dermatologists, affecting nearly 85 % of the people between the ages of 12 and 24 years including 40–50 million people in the United States each year [1, 2]. Acne has a significant psychosocial and economic impact with an estimated average cost per episode of $690 dollars and a total burden of $12 billion dollars per year in the United States [3–7].

Acne is a disease of the pilosebaceous unit (PSU) and begins with the formation of the microcomedone. The severity of acne is determined by the type, number and distribution of lesions and the presence of scarring. Acne is a multifactorial disease that results from the interaction of four main pathogenic factors: (1) the production of sebum by androgen-mediated stimulation of sebaceous glands, (2) abnormal hyperkeratinization of the follicles leading to comedone formation, (3) colonization of the PSU by *Propionibacterium acnes (P. acnes)*, and (4) inflammation, which is trigged by the interaction of immune cells, keratinocytes, and sebocytes with *P. acnes* after follicle disruption.

P. acnes, a gram positive, anaerobic, pleomorphic diphtheroid is the predominant organism in the follicular flora, although aerobic *Staphylococcus epidermidis* may also be present [8, 9]. *P. acnes* relies on sebaceous lipids, specifically triglycerides, as a nutrient source and metabolizes these into free fatty acids [10].

Experimental Models for Acne Vulgaris

Acne research is limited by the lack of a model system that mimics all aspects of acne pathogenesis. Cell culture, whole organ culture and animal models (mouse, hamster and rat) are used to study the individual features of acne, such as sebum production and hyperkeratinization. However, animal models for inflammation in acne, in which *P. acnes* plays a major role, are not available because *P. acnes* does not

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colonize the PSU. One attempt to mimic the microenvironment of acne lesions was done by T. Nakatsuji and colleagues, in which a sebocyte-filled tissue chamber implanted into ICR mice was injected with *P. acnes* and the immune response was measured in the chamber fluids [11]. Thus, studies of acne inflammation rely on *in vitro* methods and acne patient populations.

Activation of the immune system is a central event in the development of acne, and our understanding of this process is rapidly expanding. In this chapter, we describe how *P. acnes*, sebaceous glands, and keratinocytes contribute to inflammation in acne and how current acne treatments modulate this immune response.

Innate and Acquired Immune Systems in Acne

Anti-microbial Peptides (AMPs): Good or Bad?

Increasing evidence indicates that AMPs, produced by neutrophils and epithelial cells, play a pivotal role in acne pathogenesis. The most important skin-derived AMPs are beta-defensins, cathelicidins and S100 proteins. For each of these, both pro-inflammatory and anti-inflammatory activities are reported. In general, the presence of *P. acnes* triggers the production of AMPs, which leads to the direct killing of the microbe and reduction in bacterial burden. However, AMPs also have immunomodulatory properties such as promoting chemotaxis and cytokine release, leading to the outstanding question of whether or not AMPs themselves substantially contribute to inflammation in acne.

Beta-Defensins (BD)

Both human BD-1 (hBD-1) and hBD-2 are expressed within the PSU and hBD-2 is significantly increased in acne vulgaris and hidradenitis suppurativa [12–14]. hBD-2 expression is increased in areas of high sebum secretion including the forehead, nose and chin when compared to the cheeks [15]. *P. acnes* triggers hBD-2 expression in keratinocytes and sebocytes [16, 17]. hBD-2 has antimicrobial activity against *P. acnes* and could limit the *P. acnes*-induced inflammatory response [18].

However, hBD-2 itself likely contributes to inflammation. It induces chemotaxis of immune cells and triggers the release of pro-inflammatory cytokines, histamine and prostaglandins [19]. In addition, hBD-2 enhances keratinocyte proliferation and migration, implying that hBD-2 contributes to possible comedone formation in acne [20].

Cathelicidin

Cathelicidin (LL-37) is increased in acne lesions as well as in hidradenitis suppurativa [21]. The functional LL-37 peptide is present within human sebaceous glands and *P. acnes* induces expression in sebocytes *in vitro* [22]. Similar to hBDs, cathelicidin peptides have direct antimicrobial activity against *P. acnes,* but also initiate cytokine production and inflammation in the host organism [22, 23]. In rosacea, elevated LL-37 expression causes increased inflammation characterized by increases in IL-8, neutrophilic infiltrate and erythema [24]. A similar process may occur in acne.

S100 Proteins

S100A7 (also known as psoriasin) is increased in the overlying epidermis and sebaceous duct of acne lesions and hidradenitis suppurativa [14, 21]. *In vitro*, S100A7 is bactericidal against *P. acnes* and is even more effective when combined with cathelicidin [22]. As with other AMPs, S100A7 may be partially responsible for inflammation in acne, as it is a powerful chemokine for neutrophils and lymphocytes.

Other AMPs

Lactoferrin, lysozyme, RNase 7, and both splice variants of koebnerisin, (S100A15L and S100A15S) are all elevated in inflammatory acne lesions compared to non-lesion control skin [21]. Granulysin, RANTES (CCL5), perforin, CXCL9, substance P, chromogranin B, and dermcidin are not elevated in acne lesions in this study [21], but are reported as elevated in others [13]. The presence of all these AMPs is likely protective and simultaneously pro-inflammatory.

Toll-Like Receptors (TLR)

TLR2 is expressed within sebaceous glands as well as basal and infundibular keratinocytes [25]. Studies have demonstrated increased TLR2 expression within acne lesions and hidradenitis suppuritiva compared to normal skin [26, 27]. Moreover, patients with acne have increased TLR2 expression on peripheral monocytes compared to those unaffected [28]. TLR4 expression is also increased in epidermal keratinocytes of acne skin compared to healthy skin [17].

TLR2 activation by *P. acnes* is an important step in the pathogenesis of acne [27–29]. Activation of TLR leads to activation of NFkB transcription factor and up-regulation of numerous cytokines and chemokines that trigger inflammation through recruitment of immune cells and AMP production. For example, through TLR2, *P. acnes* induces IL-12 and IL-8 cytokine production in monocytes, which likely triggers neutrophil chemotaxis [27].

Sebaceous Glands and Lipids

Sebocytes play a key role in the pathogenesis of acne and actively participate in regulation of the immune system [30]. They express TLRs and have been demonstrated to produce various inflammatory cytokines in response to *P. acnes* [27–29]. Additionally, the synthetic pathways for

the production of prostaglandins and leukotrienes from arachidonic acid are intact within sebaceous glands and sebocytes *in vitro*. These lipid mediators affect lipid synthesis, neutrophil chemotaxis and cytokine production [31].

Increased sebum production, altered sebum and skin surface lipid composition also contribute to inflammatory acne. Triglycerides within sebum are hydrolyzed to free fatty acids (FFA) by *P. acnes* lipase enzymes. These FFA including oleic, palmitic and lauric acids, act as "damage associated molecular patterns (DAMPs)" and can activate TLR2 and TLR4 resulting in inflammatory cytokine and AMP production [32]. The levels of lineolic acid (C18:2) are lower in acne patients compared to controls which may cause changes in keratinization of the follicle and increased neutrophil activity [33–35].

Sphingolipids and ceramides may also play a role in acne. Acid Sphingomyelinase (ASMase) is one enzyme responsible for catalyzing the breakdown of sphingomyelin on cell membranes to ceramide; ceramide, in turn, can induce apoptosis, differentiation and proliferation. Nakatsuji and colleagues demonstrated that *P. acnes* and its release of CAMP factor in combination with host ASMase activity was cytotoxic to keratinocytes *in vitro* and increased inflammation *in vivo* [36]. Interestingly, retinoid treatment of keratinocytes suppressed the expression of ASMase and numerous genes involved in ceramide biosynthesis [37], perhaps explaining some of its potency in the treatment of acne.

Sebaceous lipids are not all bad, some have potent antimicrobial effects. Lauric acid (C12:0) has strong direct antimicrobial activities against *P. acnes* [18]. In addition, lauric acid, along with palmitic acid (C16:0) and oleic acid (C18:1, *cis*-9), enhanced the expression of hBD2 in human sebocytes, suggesting indirect killing of *P. acnes* through hBD-2 [18]. Both palmitoleate (C16:1) and oleic (C18:1), produced by stearoyl-CoA desaturase-1 (SCD1) in the sebaceous gland in a TLR2 dependent manner, are bactericidal against gram-positive, but not gram-negative, organisms [38], although their activity on *P. acnes* has not been exclusively demonstrated.

Cytokines and T-cells

Gene expression studies demonstrate inflammatory acne lesions have increased levels of TNFα, IL-1β, IL-10 and IL-8 as well as increased levels of neutrophils and lymphocytes compared to non-acne skin [13, 39]. Additional cytokines involved in the pathogenesis of acne include IL-6, IL-12, IL-17, IFNγ, TGFα and epidermal growth factor (EGF).

IL-1 Family of Cytokines

Release of IL-1β propagates and perpetuates the inflammatory signal [40–43]. IL-1β release is also mediated by TLR2 activation. Inhibiting monocyte TLR2 activity reduces the secretion of IL-1β by half after stimulation with *P. acnes* [40]. IL-1β has been shown to induce the production of IL-6 and IL-8, a powerful neutrophil attractant, in sebocytes [29, 40, 41, 44]. IL-1β and IL-6, together with Transforming Growth Factor β (TGFβ), induce naïve helper T cells to undergo differentiation into T_h17 cells which can amplify inflammatory signals through the production of IL-17 (See below) [45].

IL-1α is constitutively produced by sebocytes and keratinocytes, but levels increase rapidly following *P. acnes* stimulation early in the formation of microcomedones. High levels of IL-1α results in hypercornification of the follicular infundibulum, mimicking that seen in microcomedones, and may promote keratinocyte terminal differentiation through upregulation of small proline rich protein 1 [43, 46, 47]. IL-1α has been found in high concentration in open comedones, and the production of the cytokine is increased with FGFR2 mutations [30]. FGFR2 mutations underlie Apert Syndrome, a key feature of which is acne [48, 49]. In one study, an uncommon single nucleotide polymorphism (SNP) in the IL-1α gene, a substitution of serine for alanine called the T allele, may be associated with more severe acne [49].

The Interleukins

IL-6 is a marker of activated monocytes, and participates in directing T_h17 differentiation [27, 45]. IL-8 acts to attract neutrophils, whose arrival and degranulation can promote rupture of the PSU [29, 43]. The release of both IL-6 and 8 is promoted by activation of the TLR2, by sebocytes following IL-1β stimulation, and may play a role in stress- induced acne [27, 30, 40]. Corticotropin-Releasing Hormone (CRH) levels rise during stress, and CRH receptor activation on sebocytes promotes IL-6 and 8 secretion [30, 43]. IL-12 is a powerful driver of differentiation toward T_h1 axis responses, is a principal inflammatory mediator in the response to gram positive organisms, and *P. acnes* induces increased secretion of IL-12 via TLR2 binding [27, 43]. IL-17 is involved in the T_h17 axis and will be discussed in depth separately, but promotes the release of TNFα, IL-6, and matrix metalloproteinases [45]. *P. acnes* derived proteases can also stimulate the secretion of IL-1α, IL-8 and TNFα by activation of the protease-activated receptor-2 (PAR2) on the cell membrane of keratinocytes [43].

TNFα, IFNγ, EGF, TGF

Among other roles, TNFα may increase keratinocyte expression of adhesion molecules in the follicular infundibulum and thus may contribute to the formation of the microcomedone through abnormal desquamation [46, 50]. Furthermore, one report suggests that a SNP in the TLR2 gene, Asp299Gly, may increase TNFα secretion following bacterial activation. SNPs in the TNFα promoter region have been studied to varying results, but some hold these polymorphisms may drive acne by increasing production of TNFα in response to NFkB activation [49]. IFNγ is produced by monocytes in response to *P. acnes* and drives differentiation of T cells toward the T_h1

axis [45]. EGF and TGFα together appear to promote rupture of the pilosebaceous unit [46]. TGFβ induces the differentiation of T_{reg} cells in the presence of IL-2 [51].

The *P. acnes* induced cytokine cascade may explain the early perivascular and perifollicular infiltration of lymphocytes seen at the initiation of the microcomedone, prior to clinical evidence of inflammation, and preceding the arrival of neutrophils [46].

T_h1 and T_h17 Cells

Acne is a T_h1/T_h17 associated disease. T_h1 and T_h17 cells are detected within acne lesions [45, 52] and the corresponding cytokine profiles are induced by *P. acnes*.

T_h17 cells are powerful drivers of inflammation and are thought to play a critical role in the development of several immunologically mediated diseases such as multiple sclerosis, psoriasis, and rheumatoid arthritis [45, 51]. This subset of helper T-cells produces IL-6, IL-17, IL-21 and IL-22 which act on tissues to promote degradation of the extracellular matrix and maintain intense chronic inflammation. T_h17 cells are identified by the production of IL-17 family cytokines (IL-17A and IL-17 F) and by the presence of cell markers RORα and RORc. *P. acnes* drives the production of IL-1β and IL-6 through mechanisms discussed above which appear to then promote the differentiation of naïve helper T-cells to T_h17 cells. The bacterium promotes the production and secretion of IL-17, as well as RORα and RORc.

The Inflammasome

Recently elucidated patterns of disease, termed autoinflammatory conditions, manifest from dysfunctional upregulation of innate immunity and several of these disorders share acne as a distinctive feature. Characterization of the inflammasome, an intracellular multi-protein complex capable of activating innate immunity, and its role in auto-inflammatory disease has informed several recent advancements in our understanding of acne [42, 48, 53].

Structure and Function of the Inflammasome

Inflammasome signaling begins with the activation of a Nucleotide-Binding Domain Leucine-Rich Repeat-Containing Protein, also referred to as a Nucleotide Oligomerization Domain-like Receptor Protein (both abbreviated NLRP) and culminates with the secretion of mature, active IL-1β, IL-6 and IL-18 which propagate inflammation [40–42, 53]. The NLRPs are cytosolic, and thought to be non-adaptable pattern recognition receptors, much like TLR's [41, 42, 54]. However, one study has questioned whether NLRPs respond to pattern molecules directly, respond to indirect signals or perhaps both [42]. Four NLRPs are well described: NLRP1, NLRP3 (also termed cryopyrin or NALP3), NLRC4 (also termed IPAF) and AIM2 [41, 42, 54]. These proteins can respond to various threats such as bacterial Pathogen Associated Molecular Patterns (PAMPs), viral and fungal pathogens, and Reactive Oxygen Species (ROS) [40–42, 54]. NLRP activation results in the recruitment of pro-caspase 1 through a Caspase Recruitment Domain (CARD) [41, 42]. In the case of NLRP3, the protein lacks a CARD and instead relies on the Apoptosis-associated Speck-like protein containing a Caspase recruitment domain (ASC) to bind pro-caspase 1 [42, 54]. Binding of pro-caspase 1 results in proteolytic cleavage into active caspase 1 subunits which subsequently convert the precursor of IL-1β into its active form [40, 42, 54]. Caspase 1 has also been shown to increase secretion of IL-1α [41].

P. acnes Activates the NLRP3 Inflammasome

The role of the inflammasome in acne vulgaris is currently being elucidated. First, *P. acnes* has been shown to induce the production of IL-1β and caspase 1 in human monocytes, as well as increasing the production of NLRP1 and NLRP3 [40]. Interfering with the production of NLRP3, but not NLRP1, results in dramatic decreases in the production of IL-1β, as does inhibition of caspase 1 [40, 55]. The production of AIM2 and NLRC4 (IPAF) are not affected by exposure to *P. acnes* [40, 54]. This information suggests that *P. acnes* stimulates the production of IL-1β via activation of the NLRP3 inflammasome in human monocytes *in vitro*, and *in vivo*. Though *P. acnes* is an extracellular pathogen, phagocytosis transmits the bacterium or its products to the cytosol where NLRPs reside [40, 55]. This internalization of bacteria appears to be necessary for NLRP3 inflammasome-dependent IL-1β production [55]. Bacterial muramyl dipeptide has been implicated as an activator of NLRP3, and *P. acnes* produces this molecule [40]. Direct binding of muramyl dipeptide and activation have not been demonstrated in acne.

A recent study shows that sebocytes not only express the constituents of the NLRP3 pathway, but that this pathway is activated upon exposure to *P. acnes*. Exposure to *P. acnes* led to increased levels of active caspase 1 and IL-1β in an NLRP3 dependent manner. The stimulated IL-1β production was dramatically impaired by the presence of N-acetyl cysteine, which the authors suggest as evidence for ROS mediating the activation of NLRP3 in some manner [54]. Other authors have suggested that NLRP3 can be activated by thioredoxin-interacting protein, which is itself released in the presence of ROS.

Autoinflammatory Disorders Involving Acne

The role of the inflammasome in the development of acne is suggested by PAPA (Pyogenic Arthritis, Pyoderma gangrenosum and Acne) syndrome [48, 53]. PAPA syndrome is a monogenic autoinflammatory disorder that involves severe nodulocystic acne as a hallmark feature. The syndrome

develops from a mutation in the Proline-Serine-Threonine-Phosphatase-Interactive-Protein 1 (PSTPIP1) [41, 48, 53]. In PAPA syndrome, the aberrant PSTPIP1 protein develops an increased binding affinity for the protein pyrin, which results in increasing rates of complexation of ASC with the inflammasome and thus recruitment of pro-caspase 1. It is unclear whether PSTPIP1 and pyrin binding activate pyrin to stimulate ASC complexation, or whether PSTPIP1 disinhibits inflammasome assembly by sequestering pyrin [41, 48, 53]. Regardless, as a result of PSTPIP1's increased affinity for pyrin, increased levels of IL-1β and Tumor Necrosis Factor α (TNFα) have been observed in patients with PAPA syndrome, and treatments antagonizing IL-1 or TNFα have been successful in ameliorating disease symptoms [48, 53]. The increase in ASC recruitment to inflammasomes in PAPA syndrome may implicate NLRP3, which appears to play a role in the inflammatory response to *P. acnes* [40, 42, 54]. NLRP3 mutations also underlie the cryopyrin associated periodic fever syndromes such as Familial Cold Autoinflammatory Syndrome, Muckle-Wells Syndrome, NOMID and CINCA syndromes [41, 42].

Other inflammasome components have been implicated in the pathogenesis of pustular autoinflammatory disorders. Pyoderma gangrenosum, Acne and Suppurative Hidradenitis (PASH) syndrome, another disorder involving severe cystic acne, is being evaluated as a separate abnormality of PSTPIP1 that may upregulate inflammasome activity [41, 53]. CARD14 mutations are responsible for certain forms of pustular psoriasis [53].

The Impact of Acne Treatments on Inflammation

The available spectrum of acne treatments impacts acne lesion development at every step. However, most therapies have been shown to have some role in tempering the host's immune response to *P. acnes*. These immunomodulatory effects are crucial, as clinical symptoms arise from host response to the bacterium [28].

Retinoids

The activity of retinoids in the treatment of acne was discovered serendipitously during study of the medication for application in icthyoses [56]. Retinoids are a structurally diverse family of molecules that share the ability to bind retinoic acid receptors and activate the retinoid system [51, 56]. This system influences the expression of an impressively broad array of genes, including those which regulate the immune response [51, 56, 57]. So powerful is the retinoid influence on immunity, that retinoids and their analogues are even showing promise as therapies for autoimmune diseases such as systemic lupus erythematosus [51].

Retinoids bind to nuclear hormone receptors of the steroid-thyroid hormone family which regulate transcription upon activation [46, 51, 56, 57]. These receptors belong to one of two types: the Retinoic Acid Receptors (RARs) and the Retinoid X Receptors (RXR), each of which has three isotypes: α, β and γ. In the inactive state, RAR is sequestered by a co-repressor protein and complexed with thyroid hormone. Upon retinoid binding, RAR is released from its repressor protein, and is obligated to bind with an RXR as a heterodimer to participate as a transcription factor. RXR faces less stringent requirements, and can dimerize with another RXR, or with thyroid hormone, vitamin D3 or peroxisome proliferator-activated receptors. Once the dimer is formed, the complex is capable of binding to Retinoic Acid Response Elements (RAREs) of select genes and modulating their transcription. The activated retinoid receptors may also function to antagonize other transcription factors independent of RARE binding. Retinoids commonly used as therapy in acne include tretinoin (*all-trans* retinoic acid; ATRA), isotretinoin (13-cis retinoic acid), adapalene and tazarotene. The latter 2 are 3rd generation retinoids, also termed arotinoids [46].

Treatment with retinoids, particularly isotretinoin, has been demonstrated to decrease the expression of TLR2 in monocytes, and reduce the levels of IL-1β, IL-6, IL-10 and IL-12 produced in response to *P. acnes* stimulation [28, 46, 56]. This effect is durable and has been shown to persist 6 months after treatment [28]. Though ATRA may play a role in the differentiation of regulatory helper T-cells (T_{regs}), systemic retinoid therapy does not seem to affect the cytokine profiles of peripheral T cells [28]. It appears that retinoids are able to blunt or perhaps normalize the immune response to *P. acnes* by reducing the expression and response of TLR2 to the bacterium.

As discussed above, T_h17 cells are a class of helper T-cells that maintain chronic inflammation and direct tissue mediated destruction of the extracellular matrix. A studies have revealed the ability of *P. acnes* to direct naïve T-cell differentiation to T_h17 cells. ATRA (along with vitamin D3, whose receptor can complex with RXRs) has been shown to inhibit the expression of genes necessary for T_h17 differentiation [28, 45, 51]. These genes include IL-17, RORα and RORc, but isotretinoin had no impact on IL-17 receptor level expression [45]. Retinoids are also shown to promote and maintain the development of $T_{regs.}$ T_{regs} are a class of helper T-cells characterized by FOXP3 expression which inhibit cytotoxic effector T-cells and promote self-tolerance through the secretion of IL-10. ATRA acts to inhibit mRNA expression of IL-2 and IFNγ [51].

In addition to modulating cytokine production, isotretinoin also normalizes expression of many AMPs that are increased in inflammatory acne lesions, including hBD2, cathelicidin, S100A7 [21].

Isotretinoin is able to induce apoptosis in sebocytes both *in vitro* and *in vivo*, which logically reduces the production rate of sebum [44]. Evidence suggests that this process occurs

through up-regulation of the protein NGAL [62]. Though this mechanism is not clearly delineated in sebocytes, NGAL induces apoptosis in some cell lines by altering iron metabolism through the binding of siderophores [44]. NGAL levels rise rapidly during the induction of isotretinoin therapy and precede reductions in *P. acnes* colonization and sebum production. As NGAL is not directly toxic to *P. acnes*, colonization density is likely reduced following nutrient deprivation due to reduced sebum production. NGAL is toxic to gram negative bacteria through the binding of bacterial siderophores [44]. This effect on bacterial iron metabolism probably underlies isotretinoin's activity in gram negative folliculitis.

Knowledge of retinoid action as anti-inflammatories continues to expand. Studies in cancer suggest that retinoids can influence the activity of NFkB, a downstream participant in the TLR2 pathway [51]. Activated retinoid receptors can antagonize AP-1, a proinflammatory transcription factor [56].

Tetracyclines

Tetracyclines act at the 30s subunit of bacterial ribosomes to reduce *P. acnes* burden and thus indirectly inhibit inflammatory pathways. The versatile antibiotics, which include tetracycline, doxycycline, and minocycline among others, also play a direct part in reducing inflammatory cascades. By virtue of ribosomal interference, tetracyclines can reduce the production of bacterial proteins (e.g. lipase) which may serve as neutrophil chemoattractants [50]. This translational interference occurs even at sub-antimicrobial dosing [50, 58].

Recruitment of neutrophils contributes to the development of inflammatory acne lesions. Tetracyclines can inhibit this process in a few ways. First, tetracyclines are well-documented to chelate calcium [58]. Tetracycline sequestration of intracellular calcium prevents proper assembly of microtubules which are critical to neutrophil mobility [50, 58]. Bacterial lipase is itself an attractant to neutrophils, in addition to its ability to cleave free fatty acids. Tetracyclines reduce production of lipase via ribosomal inhibition, and thus reduce the protein's ability to serve as a chemoattractant. IL-8 is a critical cytokine for the attraction and activation of neutrophils. Tetracyclines are shown to reduce IL-8 production in certain study models [50].

There does not appear to be consensus in the way tetracyclines reduce the secretion of cytokines, but studies show clear inhibition of TNFα, IL-1β and IL-8 production. The influence of tetracyclines may be via post-translational modification, as one study presented reduced levels of active cytokines while mRNA levels remained unaffected by tetracycline application [50]. Some posit that tetracyclines inhibit the action of matrix metalloproteinases, which are important in the maturation of certain cytokines to active forms, such as TGFβ [50, 58].

ROS are an important part of immune mediated bacterial killing, but the radicals can damage host tissue and propagate inflammation by many mechanisms including lipid peroxidation and inflammasome activation [40, 43, 50]. Tetracyclines can inhibit the expression of inducible nitric oxide synthase, the products of which can promote free radical formation. Tetracyclines may directly scavenge free radicals, and they also interfere with activation of calmodulin, a mediator in ROS formation and release [50, 59]. Minocycline appears to be the most potent anti-oxidant of the antibiotic class [50]. It is important to note that in other cases, tetracyclines can promote radical formation (such as in doxycycline's phototoxic response), indicating that this anti-inflammatory pathway may need more study.

As discussed above, lipid mediators derived from arachidonic acid can participate in inflammatory signaling. All relevant members of the tetracycline class can inhibit phospholipase A2, the enzyme that cleaves precursors to arachidonic acid. Minocycline can inhibit 5-lipoxygenase by preventing transport of the enzyme to the nuclear membrane [50].

Other Therapies

Benzoyl peroxide has long been a cornerstone in acne therapy. The compound is derived from coal tar, and rapidly penetrates the stratum corneum to reach the follicle where it converts to benzoic acid [60]. Benzoyl peroxide is directly toxic to *P. acnes* and rapidly kills the organism through oxidation of cell membrane components [46, 60]. Five days of treatment with benzoyl peroxide can reduce *P. acnes* populations by 95 %, greatly relieving bacterial influence on inflammation [60]. Benzoyl peroxide is also toxic to neutrophils in clinically relevant doses and weakly inhibits protein kinase C [59].

Dapsone, a sulfonamide antibiotic, has application to a number of pustular disorders. It inhibits myeloperoxidase, hampering the formation of ROS, and decreases production of IL-8 [60].

Summary

Our understanding of the pathogenesis of acne has evolved dramatically through research. Continued discovery has demonstrated the immune response to *P. acnes* as a primary player in the development of acne, rather than subordinate spectator. Once, it was believed that follicular keratinization changes and abnormal sebum metabolism precipitated acne lesions, and immunologic activation was secondary to these physical changes. Now it is evident that immune activation is an early, if not the earliest, event in comedogenesis.

Initial studies of *P. acnes* showed that the organism could elicit the production of cytokines and matrix metalloproteinases from leukocytes. We now know that keratinocytes and sebocytes respond to the organism as well. However, the precise mechanisms of activation were elusive for some time,

and many have questioned how *P. acnes* could influence immunity from within the immune-privileged PSU [61]. How exactly can *P. acnes* activate the immune cells surrounding the follicle if the organism is contained within the intact PSU?

Exciting studies have drawn back the curtain on this mystery. We now know that *P. acnes* can exert its influence through the activation of pattern receptor molecules, specifically TLR2 and the NLRP3 inflammasome. By interacting with these receptors on or in keratinocytes, sebocytes or leukocytes, *P. acnes* can stimulate the production of cytokines. These chemical messengers then activate and recruit immune cells, alter keratinocyte adhesion biology, and influence the behavior of lipid molecules of the PSU. *P. acnes* does not need to escape the PSU to initiate immune signaling, as keratinocytes and sebocytes both express these pattern recognition molecules which are activated independent of leukocytes. Perhaps it is the activation of TLRs and inflammasomes within follicular keratinocytes and sebocytes and the subsequent cytokine production that attracts the lymphocytic infiltrate described in early microcomedones. Once these leukocytes are present, they may then amplify and perpetuate the inflammatory call to arms. As we have seen above, the released cytokines can influence changes in the PSU. IL-1 produced by inflammasome activation may underlie Guy and Kealy's observation that IL-1-like activity can produce follicular hyperkeratinization [47]. Furthermore, the IL-1 and IL-6 produced from pattern receptor molecule activation may help drive Th17 axis differentiation and activation, a recently recognized important feature of acne. Future studies are needed to address whether or not cytokine production by keratinocytes and sebocytes initiate microcomedome formation.

Acne is a complex, multifactorial disease, with inflammation as a key driver of pathology. The rapid and potent immune response to *P. acnes* appears to influence each stage of acne's development, and amplification of the inflammatory cascade results in a self-perpetuating cycle. Bacterial PAMPs activate innate pattern receptors, which produce AMPs and cytokines to recruit inflammatory cells, which themselves produce more cytokines in response to *P. acnes*. With each new advance or discovery of this process comes ever more exciting opportunities to develop targeted therapies that may help interrupt this impressive cycle.

Questions

1. How is *P. acnes* responsible for activation of the immune system ?
   A. Anti-microbial peptides
   B. Tollo Like Receptors
   C. Cytokines
   D. Inflammasome
   E. All of the above
   F. None of the above

Correct Answer: (E) All of the above

2. What role does sebum play in inflammation?
   A. Cleaved triglycerides can act as damage associated molecular patterns for TLR activation
   B. Anti-microbial lipids can inhibit P. acnes
   C. P. acnes sphingomyelinase can augment inflammation
   D. A + C only
   E. All of the above

Correct Answer: (E) All of the above

3. Which of the above statement(s) are true concerning how current acne treatments affect inflammation?
   A. Retinoids decrease TLR2-mediated inflammation
   B. Retinoids decrease P. acnes burden
   C. Dapsone is cytotoxic to P. acnes
   D. Tetracycline increases epithelial differentiation
   E. All of the above

Correct Answer: (A) Retinoids decrease TLR2-mediated inflammation

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Adverse Medication Reactions

25

Roni P. Dodiuk-Gad, Wen-Hung Chung, and Neil H. Shear

Abstract

Cutaneous adverse drug reactions (ADRs) are among the most frequent adverse reactions in patients receiving drug therapy. They have a broad spectrum of clinical manifestations, are caused by various drugs, and result from different pathophysiological mechanisms. Hence, their diagnosis and management is challenging.

Severe cutaneous ADRs comprise a group of diseases with major morbidity and mortality, reaching 30 % mortality rate in cases of Toxic Epidermal Necrolysis.

This chapter covers the terminology, epidemiology, pathogenesis and classification of cutaneous ADR, describes the severe cutaneous ADRs and the clinical and laboratory approach to the patient with cutaneous ADR and presents the translation of laboratory-based discoveries on the genetic predisposition and pathogenesis of cutaneous ADRs to clinical management guidelines.

Keywords

Cutaneous adverse drug reactions • Classification • Pathogenesis • Clinical approach • Laboratory tests • Human leukocyte antigen (HLA)

Terminology

The World Health Organization defined an adverse drug reaction (ADR) in 1972 as "a response to a drug that is noxious and unintended and occurs at doses normally used in man" [1]. Edwards and Aronson [2] proposed a different definition in 2000: "an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product."

The terms 'adverse reaction' and 'adverse effect' are interchangeable, except that an adverse reaction is seen from the point of view of the patient and adverse effect is seen from the point of view of the drug. However, both terms must be distinguished from 'adverse event'. An adverse event is an adverse outcome that occurs while a patient is taking a drug, but is not or not necessarily attributable to it [2].

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Differentiating between serious ADR and severe ADR is imperative. Serious ADR is a legal term applied to any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect [3]. Conversely, the term 'severe' is a clinical term used to describe the intensity (severity) of a medical event, as in the grading 'mild', 'moderate', and 'severe'; thus, a severe skin reaction need not be serious [2].

Epidemiology

ADRs are associated with significant morbidity and mortality and have considerable economic implications. Clinical manifestations of an ADR are variable and may include cutaneous and or systemic features [4].

When analyzing the type of ADRs most encountered, two major groups emerge; **common-mild reactions** and **rare-severe reactions**. Common-severe reactions are not approved for clinical usage and rare-mild reactions are usually not noticed or reported. Cutaneous ADRs are among the most frequent adverse reactions in patients receiving drug therapy [5]. They accounted for 65 % of all reported ADRs in a 4-year retrospective study in Taiwan [6].

The prevalence and incidence of cutaneous ADRs vary greatly among different populations [7–11]. In the USA, a 7-year prospective study found that the prevalence of cutaneous ADRs was 2.2 % in hospitalized patients [7]; and an 11-year retrospective study found the annual incidence of cutaneous ADRs to be 2.26 per 1,000 persons [11]. In Denmark, in a 1-year cross-sectional study the prevalence of cutaneous ADRs was 0.33 % in in-patients and 0.14 % in out-patients [8]. In southern China, in an 8-year retrospective study, the prevalence of cutaneous ADRs was 0.14 % in hospitalized patients [9]. In India, in a 12-month prospective study, the primary incidence of cutaneous ADRs was 2.05 per 1,000 persons [10].

The need to survey ADRs in clinical practice is universally recognized. Various methods may be employed: spontaneous surveillance, prescription-event monitoring (PEM), linkage analysis, case-control surveillance and cohort studies [5]. In 1963, the 16th World Health Assembly reaffirmed the need for early detection and rapid dissemination of information on adverse reactions due to medications. This affirmation led to the creation of the World Health Organization (WHO) Programme for International Drug Monitoring, under whose auspices systems have been created in member states for the collection and evaluation of individual case safety reports (ICSRs) [12]. In 1978 the WHO set up its international drug monitoring programme in Sweden at the Uppsala Monitoring Centre (UMC) http://www.who-umc.org. The US Food and Drug Administration (FDA) provides several options for reporting adverse events. One such option is MedWatch, the FDA Safety Information and Adverse Event Reporting Program http://www.fda.gov/safety/MedWatch/default.htm, founded in 1993 as a system for both consumers and healthcare professionals to report adverse events. MedWatch is intended to detect safety hazard signals for medical products; in the event a signal is detected, the FDA can issue medical product safety alerts or order product recalls, withdrawals, or labelling changes to protect the public health [13].

A number of international research groups are investigating severe cutaneous ADRs (SCARs): the RegiSCAR network, an international registry of SCAR established in 2003, the Japanese Research Committee, J-SCAR, the Asian SCAR consisting of Japan and Taiwan SCAR groups (J-SCAR and T-SCAR) established in 2010, and the Southeast Asia network, SEA-SCAR, with ten member countries: Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, Singapore, and Vietnam. The International Serious Adverse Event Consortium (iSAEC), a non-profit organization formed in 2007, is a pharmaceutical industry- and FDA-led international consortium that focuses on identifying and validating DNA variants useful in predicting the risk of rare drug-induced serious adverse events [14].

Pathogenesis

Immunologic Versus Non-immunologic Mechanisms

Immunological Mechanisms

Mechanisms of adverse drug reactions (ADRs) can be classified into immunologic and non-immunologic etiologies. There are two common types of immune-mediated drug reactions: immediate-type hypersensitivity (Type I hypersensitivity) and delayed-type hypersensitivity (Type IV hypersensitivity).

1. Immediate-type drug hypersensitivity: Immediate-type drug hypersensitivity reactions usually occur minutes to hours after drug exposure, with clinical manifestations including pruritus, urticaria, angioedema, and bronchospasm to anaphylaxis. The reaction is mediated mainly by drug-specific IgE, the most common causative agents being penicillins, cephalosporins and neuromuscular blocking agents. IgE-mediated reactions to drugs are usually thought to be an immune response to a hapten/carrier complex. In the primary drug sensitization, drug-specific IgE is formed when plasma cells transformed from activated B cells interact with T cells. In an allergic reaction, drug allergens bind to mast cells with high-affinity Fc receptor, to which drug-specific IgE is bound, causing

mast cells to release mediators, such as histamine, leukotrienes, prostaglandins and cytokines [15].

2. Delayed-type drug hypersensitivity: Delayed-type drug hypersensitivity reactions usually take several days to weeks following drug exposure, with variable clinical presentations that may include Maculopapular Eruption (MPE), Fixed Drug Eruption (FDE), Acute Generalized Exanthematous Pustulosis (AGEP), Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN) and Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS). T cell receptor (TCR), CD4+ and CD8+ T cells are involved in different delayed-type drug hypersensitivity reactions [16].

Drug Recognition by T Cells in Delayed-Type Drug Hypersensitivity

Drugs are low molecular weight and usually considered not able to bind to TCRs to activate adaptive immunity. In the case of drug allergy, drug interactions with TCRs may involve a drug-peptide complex presented by human leucocyte antigen (HLA) molecules of antigen-presenting cells (APCs). This process is known as the hapten concept; an example is β-lactams that covalently bind to lysine residues [17].

Drugs can also interact directly with TCRs without binding to the peptide/HLA of the APC in what is known as the P-i concept (pharmacological interaction of drugs with immune receptors) [18]. For example, carbamazepine is not able to bind covalently to peptides or proteins, but can associate with low affinity to TCRs and provoke T cell activation [19].

Immunohistologic Characteristics and Functions of Drug-Specific T Cells in Delayed-Type Drug Hypersensitivity

The immunohistologic characteristics of delayed type drug hypersensitivity are summarized in Table 25.1. The skin of MPE is infiltrated by numerous mononuclear cells (CD4 and CD8 T cells, monocyte/macrophages) and some eosinophils. Typically, interface dermatitis is seen with a predominance of CD4+ T cells. These cells are located mainly in the perivascular dermis, and both CD4+ and CD8+ T cells are located at the dermoepidermal junction [20].

Skin manifestations of DRESS may vary from MPE-like to exfoliative dermatitis and are characterized by a heavy infiltration of CD4+ and CD8+ T cells, monocyte/macrophages and eosinophils [21]. MPE and DRESS share many pathological features, but DRESS exhibits more severe dyskeratosis (keratinocyte death in epidermis) and a greater extent of systemic involvement and eosinophilia [26].

Immunohistology of skin lesions in AGEP reveals intraepidermal pustules with infiltration of neutrophils surrounded by IL-8 producing T cells [22].

Despite very diverse clinical presentations, constant features of delayed-type drug hypersensitivity are the presence of high numbers of drug-specific CD8+ cytotoxic T cells and low numbers of innate NK lymphocytes [20, 27, 28].

CD8+ T cells of cutaneous ADRs have classic cytotoxic functions: lysis of autologous lymphocytes or keratinocytes in an MHC class I–restricted and drug-dependent manner [28].

Cytotoxic Immune Cells in SJS/TEN

Drug-induced SJS and TEN are severe cutaneous ADRs in which cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells are activated, and subsequently carry out the cellular immune reactions directed at keratinocytes in a major histocompatibility class (MHC) I-restricted manner. Upon activation of these immunocytes, various cytotoxic signals, including granulysin, perforin/granzyme B, Fas/Fas ligand, and cytokines/chemokines, are relayed to the skin lesions to mediate the disseminated keratinocyte death [23–25]. It is noteworthy that the number of granulysin-positive cells in fixed drug eruptions was found to be similar to that observed in SJS/TEN [27].

Non-Immune-Mediated Hypersensitivity

Non-immune-mediated hypersensitivity is commonly referred to as pseudoallergic reactions because they do not involve a specific immune mechanism – neither IgE-mediated (Type I) nor delayed (Type IV) hypersensitivity. Clinical manifestations, which range from milder erythematous to urticarial reactions to severe lethal anaphylaxis, may be indistinguishable from immune system-mediated hypersensitivity reactions. Common non-immune-mediated hypersensitivity can be caused by contrast media, vancomycin, non-steroidal anti-inflammatory drugs (NSAIDs), opiates, plasma expanders, and drugs used in general anesthesia [29].

NSAIDs-induced pseudoallergic reactions have been attributed to cyclooxygenase-1 inhibition and overproduction of leukotrienes, and may require higher drug doses than are needed for true IgE-mediated reactions [30]. Mast cell

Table 25.1 Summary of immunohistologic characteristics of delayed-type drug hypersensitivity [16, 20–25]

Phenotypes	Major immune cells	Major cytokines or cytotoxic mediators
MPE	CD4+ > CD8+ T cells	IFN-γ, TNF-α, IL-4, IL-5, perforin/granzyme B
DRESS	CD4+ > CD8+ T cells, eosinophils	IFN-γ, TNF-α, IL-4, IL-5, TARC/CCL17
SJS/TEN	CD8+ T cells, NK cells	IFN-γ, TNF-α, Fas-FasL, perforin/granzyme B, granulysin
AGEP	Neutrophils	IL-8

MPE maculopapular drug eruption, *DRESS* drug reaction with eosinophilia and systemic symptoms; *SJS/TEN* Stevens-Johnson syndrome/toxic epidermal necrolysis, *AGEP* acute generalized exanthematous pustulosis

degranulation is involved in some of these pseudoallergic reactions.

The Role of Cytokines or Inflammatory Mediators

Drug-specific T cells mediate skin inflammation in variable clinical presentations of delayed-type drug hypersensitivity through the release and induction of different cytokines and chemokines (Table 25.1) [31]. The heterogeneous cytokines include Th1 cytokines (interferon-γ) and Th2 cytokines (IL-4, IL-5) [22]. Increased expression of IL-5, which is a key cytokine for activation of eosinophils, is commonly seen in delayed-type drug hypersensitivity [32]. The activation of eosinophils can be further enhanced by the chemokines eotaxin and RANTES [33]. Thymus and activation-regulated chemokine (TARC/CCL17) has been reported to be a DRESS specific cytokine [34]. In addition to Th1 and Th2 cytokines, a recent study demonstrated the involvement of IL-17A-producing Th17 in DRESS and SJS/TEN [35]. Elevated expression of the neutrophil-attracting IL-8 has been known to be the key cytokine involved in AGEP.

There are several cytokines involved in SJS/TEN. Numerous studies have shown tumor necrosis factor alpha (TNF-α) strongly expressed in SJS/TEN lesions and correlated with disease severity [24, 36, 37]. TNF-α is a potent cytokine that induces cell apoptosis, cell activation, differentiation, and inflammatory processes [38, 39]. Interferon gamma (IFN-γ) is a common cytokine involved in delayed-type drug hypersensitivity, including SJS/TEN. IFN-γ was intensely expressed in the superficial dermis and epidermis of SJS/TEN lesions [36, 37]. IFN-γ is also known to promote antigen presentation and thus stimulate the cell-mediated immunity by upregulation of MHC molecules [40–42]. In addition to TNF-α and IFN-γ, several cytokines and chemokine receptors that are responsible for trafficking, proliferation, and activation of T-cells and other immune cells have been found elevated in the skin lesions, blister fluids, blister cells, PBMCs, or plasma of SJS/TEN patients. These cytokines/chemokines include IL-2, IL-5, IL-6, IL-10, IL-12, IL-13, IL-15, IL-18, CCR3, CXCR3, CXCR4, and CCR10 [24, 36, 37, 43–45].

Immune Mediators for Cell-Mediated Cytotoxicity in SJS/TEN

The central hypothesis proposed to explain the severe mucocutaneous lesions of SJS/TEN is the CD8+ cytotoxic T cell and natural killer (NK) cell-mediated cytotoxic immune reactions. Three major cytotoxic signals from cytotoxic cells are reported to be involved in the extensive skin necrosis of SJS/TEN, including the Fas–FasL interaction, perforin/granzyme B, and granulysin, which can induce keratinocyte apoptosis [23, 28, 46].

Granulysin is not only a cytotoxic protein; it is also a chemoattractant and proinflammatory activator that can promote monocyte expression of CCL20 [47], and is capable of promoting antigen-presenting (dendritic) cells and leukocyte recruitment, and activating specific immune responses, such as IL-1b,IL-6, IL-10, TNF-a [48].

Genetic Predisposition

Genetic Factors in Delayed-Type Drug Hypersensitivity

Reports on the familial occurrence of severe drug hypersensitivity and cases occurring in identical twins suggest genetic links [49–52]. The HLA genes show strong association with drug hypersensitivity. Examples of strong associations of HLA alleles with specific drug-induced hypersensitivity reactions include abacavir, nevirapine, carbamazepine, and allopurinol (Table 25.2).

The view that HLA alleles are the main genetic determinants of SJS/TEN was first proposed by Roujeau et al. [61], who reported the weak associations of HLA-A29, B12, and

Table 25.2 Recently reported HLA associations with drug hypersensitivity reactions

Drug	HLA association	Hypersensitivity reactions	Reference
Carbamazepine	B*1502	SJS/TEN	Chung et al. [53]
Allopurinol	B*5801	SJS/TEN/DRESS	Hung et al. [54]
Abacavir	B*5701	MPE/DRESS	Mallal et al. [55]
Flucloxacillin	B*5701	Hepatotoxicity	Daly et al. [56]
Lumiracoxib	DRB1*1501, DQB1*0602, DRB5*0101, DQA1*0102	Hepatotoxicity	Singer et al. [57]
Dapsone	B*1301	MPE/DRESS	Zhang et al. [58]
Nevirapine	DRB1*0101	MPE/DRESS	Martin et al. [59]
Methazolamide	B*5901	SJS/TEN	Kim et al. [60]

MPE maculopapular drug eruption, *DRESS* drug reaction with eosinophilia and systemic symptoms, *SJS/TEN* Stevens-Johnson syndrome/toxic epidermal necrolysis

DR7 in sulfonamide-related TEN, and HLA-A2, B12 in oxicam-related TEN in Europeans [61]. Following the immunological hypothesis, the most striking evidence of genetic susceptibility to SJS/TEN was provided by the findings that HLA-B*15:02 is strongly associated with carbamazepine-induced SJS/TEN [53], HLA-B*58:01 with allopurinol-induced SJS/TEN or DRESS [54], and HLA-B*5701 with abacavir hypersensitivity [62].

The HLA association to specific drug-induced hypersensitivity can be ethnic and phenotype-specific. The strength of HLA associations with specific drug-induced hypersensitivity in different populations has been found related to the prevalence of the susceptibility allele in the ethnic population. The association of HLA-B*15:02 with carbamazepine-induced SJS/TEN was replicated in other Asian countries, including Thailand, Hong Kong, Malaysia, China, Vietnam, Cambodia, Reunion, Philippines and Indian ethnicities, which carry high HLA-B*15:02 allele frequency, but not in Europeans, which carry low HLA-B*15:02 allele frequency (<1 %) [63]. In contrast, the strong association of HLA-B*58:01 with allopurinol-induced SJS/TEN is more universal, being found in Han Chinese in China, Thai populations, Korean, Japanese, and European populations; HLA-B*58:01 is the allele common to all these populations [64]. The phenotype-specific characteristics are exemplified by carbamazepine hypersensitivity. While HLA-B*15:02 is strongly associated with carbamazepine-induced SJS/TEN, it is not associated with carbamazepine-induced DRESS; in an international study, HLA-A*31:01was strongly associated with carbamazepine-induced DRESS, but not with carbamazepine-induced SJS/TEN [65].

Phenytoin – an aromatic antiepileptic drug structurally related to carbamazepine – also frequently causes SJS/TEN and DRESS [66, 67]. HLA-B*15:02 has been associated with phenytoin-related SJS/TEN in Asians, although the association is much weaker than that found for carbamazepine-related SJS/TEN [68]. A recent genome-wide association study by Chung WH et al. turned up cytochrome (CYP) 2Cvariants, including CYP2C9*3, that showed a strong association with phenytoin-related SCAR. The significant association between CYP2C9*3 and phenytoin-related severe cutaneous ARDs was replicated in different Asian populations [69].

Genetic Factors in Immediate-Type Drug Hypersensitivity

Similar to delayed-type drug hypersensitivity, genetic predisposing factors have been reported in immediate-type drug hypersensitivity. β-lactam allergy was reported associated with gene variants of IL13, IL4, and IL4RA [70–73]. Several genetic predisposing factors, including gene polymorphisms in cysteinyl leukotriene receptor type 1 (CysLTR1) and leukotriene C4 synthase (LTC4S) [74] and high-affinity IgE receptor (FcepsilonR1) [75], were associated with aspirin.

Classification

Cutaneous ADRs may be classified in terms of their presumed mechanism, severity of the reaction, histological findings, and cutaneous morphological manifestations.

Mechanism of ADRs

The modern pharmacological classification of ADRs differentiates two basic types of reactions; type A, predictable reactions, and type B, unpredictable or idiosyncratic reactions. Type A reactions ('augmented') are dose-dependent, common and predictable based on the pharmacology of the drug; about 80 % of all ADRs are type A. Type B reactions ('bizarre') do not occur at any dose in most patients, but may be dose dependent in susceptible individuals. They are uncommon, affecting a small number of patients based on an individual predisposition that depends on both genetic and environmental factors [76, 77].

The pathogenesis of Type A reaction was described in the sixteenth century by Paracelsus, the Swiss German Renaissance physician who founded the discipline of toxicology: "All things are poison, and nothing is without poison; only the dose permits something not to be poisonous" [78]. The pathogenesis of Type B reaction was designated in the first century BC didactic poem, De rerum natura (On the Nature of Things), by the Roman poet and philosopher Lucretius: "One man's meat is another man's poison" [79].

Type B reactions can be categorized into different subtypes according to Gell and Coombs' classification system [80]. The effector phase of the allergic reaction is classified into four types: Type I mediated by drug-specific IgE antibodies, Types II and III mediated by drug specific IgG or IgM or IgA antibodies, and Type IV induced by drug-specifc T lymphocytes [81]. This classification system may be helpful in daily clinical practice as a guide to diagnostic and therapeutic decisions.

In addition to the basic classification of Type A and B reactions, further types of reactions were subsequently added; Type C- dose and time-related, 'Chronic'; Type D-time-related. 'Delayed'; Type E- withdrawal effects, 'End of use'; and Type F- unexpected failure of therapy, 'Failure' [2].

Severity of Cutaneous ADRs: Skin only (Simple) Versus Skin and Systemic Involvement (Complex)

The diagnosis of a cutaneous ADR must be followed by differentiation between a simple reaction involving only the skin and a complex reaction that includes systemic involvement of organs in addition to the skin [82]. Systemic

involvement should be explored even in a mild cutaneous eruption due to a drug since the severity of skin manifestation does not necessarily mirror the severity of the systemic involvement. Systemic involvement is evaluated by assessing the patient's symptoms, including fever, facial edema, malaise, chills, dyspnea, cough, palpitations, nausea, vomiting, diarrhea, sore throat and arthralgia. Further investigation is based on the patient's symptoms. Basic laboratory screen, conducted in cases of suspected systemic involvement, includes a full blood count, liver and renal function tests, and urine analysis [83].

Histological Classification of Cutaneous ADRs

Skin biopsy is an invaluable diagnostic modality in the assessment of drug eruptions. Histologically, drug eruptions can elicit a variety of inflammatory disease patterns in the skin and panniculus, and overlapping reaction patterns. Ackerman et al.'s basic patterns of inflammatory skin diseases [84] (Table 25.3) are a helpful guide. The most common pattern of drug eruptions is the perivascular type, while psoriasiform and granulomatous patterns are rarely reported [85]. Drug eruptions may also mimic specific skin diseases such as lupus, lichen planus or lymphoma [85]. A single drug may cause a wide range of reaction patterns and no reaction pattern is specific for a particular drug [88]. While the histological changes are not distinctive in many cases of drug eruption, a few important histopathological clues may aid in the diagnosis: (1) Overlapping histological patterns in one specimen (e.g., lichenoid and spongiotic). (2) Presence of eosinophils (although not mandatory); although eosinophils are an important tell-tale sign of a drug-induced reaction, they may also be conspicuous in skin rashes devoid of a drug association and sparse or absent in some drug exanthems. (3) Apoptotic keratinocytes. (4) Mismatch between clinical and histomorphological features [85, 86, 88].

In a study assessing the histological pattern of 104 cases of diagnosed drug eruption during a 5-year period in one institution [89], the majority of the cases (94 %) were morbilliform-type rashes. The most common histological pattern was superficial perivascular and interstitial with interface changes. Eosinophils were present in only 50 % of cases, and approximately half (53 %) of the cases exhibited epidermal-dermal interface changes [89].

In view of the large diversity of cutaneous drug reactions, it is helpful to approach them as clinicopathologic entities and to base the diagnosis on a combination of clinical, histo-

Table 25.3 Pattern analysis of the main types of cutaneous ADRs according to Ackerman et al.'s classification of inflammatory skin diseases [84–87]

Perivascular	Superficial perivascular	Mixed infiltrate	Spongiotic	Psoriasiform	Interface pattern
	Purpuric drug eruption	Urticarial drug eruption	Pityriasis rosea–like eruption Photosensitive drug eruptions: Phototoxic reaction Photoallergic reaction	Psoriasiform drug eruption	Vacuolar: EM SJS TEN FDE Morbilliform drug eruption Lupus erythematosus-like eruption Chemotherapy-induced interface dermatitis Lichenoid drug eruption
Nodular and diffuse	*Lymphomatous*	*Neutrophilic*	*Granulomatous drug eruptions*		
	Pseudolymphomatous drug reaction	Drug-induced Sweet syndrome	Interstitial granulomatous drug reaction (IGDR) Drug-induced accelerated rheumatoid nodulosis Drug-induced granuloma annulare Drug-induced sarcoidosis		
Vesiculobullous	Drug-induced linear IgA bullous dermatosis	Drug-induced pemphigus	Drug-induced bullous pemphigoid	Drug-induced pseudoporphyria cutanea tarda	
Pustular	AGEP				
Vasculitis	Drug-induced vasculitic reaction				
Folliculitis and perifolliculitis	Acneiform drug eruptions	Drug-induced eosinophilic pustular folliculitis (Ofuji's disease)			
Fibrosing dermatitis	Sclerodermoid drug reaction				
Panniculitis	Drug-induced panniculitis				

EM erythema multiforme, *SJS* Stevens-Johnson syndrome, *TEN* toxic epidermal necrolysis, *FDE* fixed drug eruption, *AGEP* acute generalized exanthematous pustulosis

logical and disease course data [89]. Heightened awareness of the possible mimicry of other skin diseases and of the suspicious histopathological clues pointing to drug etiology are key elements to the appropriate histological diagnosis of drug reactions in the skin [85, 88, 89].

Morphological Classification of Cutaneous ADRs

A widely accepted approach to diagnosing the type of drug eruption is a simplified method based on the morphology of the primary lesions. The four main categories are maculopapular, urticarial, pustular and blistering [82]. The diagnosis of the drug eruption can be challenging since the same cutaneous morphology can be manifested in a simple reaction involving only the skin and in a complex reaction including systemic involvement in addition to the skin. Therefore, there are two major steps in diagnosing drug eruptions: determine the morphology and assess systemic involvement [90].

Maculopapular Eruptions – MPE (Synonyms: Morbilliform, Exanthematous)

Terminology The term 'maculopapular' is descriptive. Morbilliform means measles-like, the rash of measles consisting of macules and papules that tends to confluence. The etymon of 'exanthema' is the Greek 'exanthema', which means 'a breaking out'. Thus exanthema merely means 'rash', and 'exanthematous rash' literally means 'rash-like rash'. Therefore, the terminology is redundant [89].

Skin Signs Polymorphous pink-to-red macules and or papules usually in a symmetric distribution that may coalesce to form plaques (Fig. 25.1) [91]. The eruption begins on the trunk and upper extremities and progressively becomes confluent. In addition, purpuric lesions may appear on the ankles and feet [90]. The drug eruption can also manifest in a scarlatiniform pattern of pinpoint-sized pink-red papules coalescing and giving the skin the texture of sandpaper [92].

Maculopapular Eruptions – MPE – Simple (Skin Only)

Frequency The most common drug-induced eruptions, occurring in 1–5 % of first-time users of most drugs [91].

Lag Period 7–14 days [90].

Symptoms Pruritus and low-grade fever are common [91].

Common Sites of Involvement The eruption usually begins on the trunk and becomes generalized. Palms and soles are often involved; mucous membranes are usually spared [90].

Histology Nonspecific changes consisting of mostly superficial but also deep perivascular and interstitial infiltrate of lymphocytes. Eosinophils and epidermal-dermal interface changes appear in approximately half the cases [89].

Differential Diagnosis viral exanthems, scarlet fever, toxic shock syndrome, acute graft versus host disease (GVHD), Kawasaki disease, juvenile idiopathic arthritis [90].

Treatment Identifying and discontinuing the causative drug are the most important steps in management. Symptomatic treatment with antipruritic agents and potent topical glucocorticoids may be helpful [91]. A decision can be made to continue the drug and offer symptomatic treatment if the drug is of paramount importance, but the risk: benefit ratio of this option has to be carefully weighed, and the evolution of the eruption must be meticulously monitored [90].

Prognosis The eruption often fades within 7–14 days of discontinuation of the offending drug and scaling and desquamation may follow. Re-challenge may lead to reappearance of the reaction within a few days [90].

Offending Drugs The most common classes of drugs implicated are penicillins, sulfonamides, cephalosporins, and antiepileptics [90].

Maculopapular eruptions – MPE – Complex (skin + systemic involvement): DRESS – See Severe Cutaneous Adverse Drug Reactions.

Urticarial Eruption

Terminology The term 'urticaria', first introduced by William Cullen in the eighteenth century, is derived from urtica urens (common European stinging nettle). One of the earliest descriptions of urticaria comes from China, and is more than

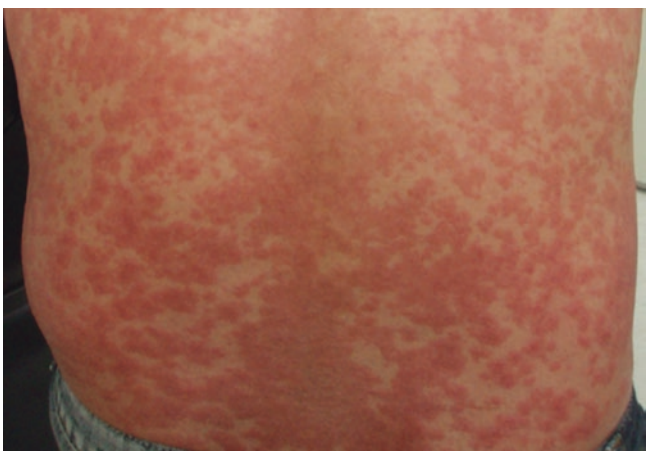

Fig. 25.1 Erythematous macules and papules coalescent into ill-defined plaques on the trunk – maculopapular morphology of cutaneous ADR

2,000 years old. In the Huangdi Neijing, written around 200 BC, urticaria is referred to as Feng Yin Zheng ('wind type concealed rash'). In ancient Latin medical literature, urticaria was called 'uredo' (urere means 'to burn'), and in the old Persian medical texts, 'essera' (meaning 'elevation') [93].

Skin Signs Urticaria is induced by superficial dermal swelling due to plasma leakage and vasodilation triggered by activation of mast cells. The skin manifestations of this process include erythematous and edematous papules and plaques (wheals) of various sizes that may coalesce to form large plaques [94]. Wheals may be characterized by pink or pale center and assume a figurate or polycyclic configuration. Linear lesions can be seen with dermatographism [92, 94].

Urticarial Eruption – Simple (Skin Only)

Frequency Drug-induced urticarial eruptions are the second most common type of cutaneous drug eruption and account for approximately 5 % of all cutaneous drug eruptions [85].

Lag Period Urticaria occurs within minutes to days of drug administration [94].

Symptoms A major clinical feature is pruritus, the lack of which should put the diagnosis in doubt. The lesions can also be painful if they occur on the soles, over joints, or in areas where the skin is tightly adhered to subcutaneous tissue [94]. A single lesion lasts less than 24 h and upon resolution leaves normal skin. However, new lesions may continue to arise for various periods of time. Acute urticaria is defined when a bout of hives lasts less than 6 weeks; when it lasts longer, it is defined as chronic urticaria [95].

Urticaria may be associated with angioedema [93]. Angioedema is defined as a deep, dermal, subcutaneous and/or mucous swelling that may involve the intestinal lining and the upper respiratory tract. Symptoms include slight heat, burning, pain and sensation of pressure or tightness. However, pruritus is minimal or absent. Swelling of gastrointestinal tract mucosa can induce abdominal pain, vomiting and diarrhea. Edema of the respiratory tract may induce various symptoms including life-threatening asphyxia. Drug-induced angioedema is associated with urticaria in approximately 50 % of cases. Some drugs may induce angioedema without urticaria [96].

Common Sites of Involvement Lesions of urticaria can appear anywhere on the skin, including the palms, soles and scalp, but not on mucosal surfaces [94]. Angioedema most commonly occurs in the head, neck and hands, but can occur anywhere and frequently involves mucosal tissue. Swelling may be more prominent in areas of looser skin, such as the scrotum, labia, lips, and eyelids [94].

Histology Urticarial drug reactions are characterised by dermal edema and a superficial and deep perivascular and interstitial dermatitis. The mixed inflammatory infiltrate comprises lymphocytes, histiocytes, mast cells, eosinophils and neutrophils. The presence of neutrophils and deep vascular plexus involvement may be a clue to the drug-induced nature of the urticaria [86].

Differential Diagnosis The wheals with central red halo of urticaria may resemble the target lesions of erythema multiforme. Four clinical signs of urticaria can help distinguish it from erythema multiforme: (1) The central zone consists of normal skin, whereas in erythema multiforme, skin is dusty, bullous or crusted. (2) Each lesion is transient, lasting less than 24 h, whereas erythema multiforme lesions are 'fixed' for a few days. (3) New lesions appear daily and in erythema multiforme all lesions appear within the first 72 h. (4) There may be associated swelling of face, hands and feet and in erythema multiforme there is no edema [97]. Differential diagnosis of urticaria includes also bullous pemphigoid, urticarial vasculitis and serum sickness-like reaction (SSLR). Drug-induced urticaria needs to be differentiated from cases of urticaria induced by other etiologies, such as food, environmental allergens, insects, systemic illness, physical stimuli, genetic and idiopathic [94].

Urticaria and angioedema are the most common symptoms of anaphylaxis (88 % of cases), and are one of the clinical criteria of the National Institute of Allergy and Infectious Disease (NIAID) and the Food Allergy and Anaphylaxis Network (FAAN) for the diagnosis of anaphylaxis [98]. Therefore, all cases of sudden acute urticaria and angioedema should be evaluated for indications of the anaphylactic type of reaction: presence of respiratory compromise, decreased blood pressure, and end-organ dysfunction (collapse, syncope, incontinence) [98].

Treatment The most important step in the management of drug induced urticaria with or without angioedema is withdrawal of the causative agent. In most cases of acute urticaria, when the trigger is removed the rash quickly resolves. H1-receptor blockers are the mainstay of treatment for patients with only cutaneous symptoms. Systemic glucocorticoids are indicated in all cases with upper airway edema and should be considered in cases with extensive cutaneous involvement. Epinephrine is reserved for angioedema with upper airway involvement [94]. The presence or absence of any airway involvement should be specifically investigated.

Prognosis Both urticaria and angioedema fade without visible sequelae. Following resolution, there should be no residual pigmentary changes unless excoriated [94].

Offending Drugs Many drugs can induce acute urticaria, and do so by both immunologic and non-immunolgic mechanisms. The major drugs responsible for immunologically based urticaria are antibiotics, especially penicillins

and cephalosporins [90]. The major drugs triggering mast cell release (non-immunolgic mechanisms) are aspirin, nonsteroidal anti-inflammatory drugs (NSAIDS), opioids and radiocontrast media [90]. Viral infections or connective tissue diseases may induce or augment urticarial drug reactions [86].

Urticarial Eruption – Complex (Skin + Systemic Involvement)

- **Anaphylaxis**

The National Institute of Allergy and Infectious Diseases (NIAID) and the Food Allergy and Anaphylaxis Network (FAAN) defined anaphylaxis as a systemic reaction resulting from the sudden release of multiple mediators from mast cells and basophils, often life threatening, and usually unexpected. The World Allergy Organization (WAO) has divided anaphylaxis into immunologic (further divided into immunoglobulin E [IgE]-mediated and non-IgE-mediated), non-immunologic, and idiopathic causes. Drugs are the second most common cause of anaphylaxis after food, which constitutes 20 % of triggers [98]. Common medications associated with anaphylaxis include penicillins, NSAIDs, and biologic response modifiers [99]. The NIAID/FAAN definition of anaphylaxis has been translated into clinical diagnostic criteria that include an acute onset of illness (minutes to hours) and involvement of the dermatologic, respiratory, cardiovascular, or gastrointestinal systems [98]. Epinephrine is the only first-line treatment for anaphylaxis and is the sole effective treatment for an acute reaction. Delays in administration have been associated with fatalities. Supportive treatment with oxygen, fluids and additional drugs are also necessary according to the cardiopulmonary resuscitation (CPR) anaphylaxis algorithm [98].

- **Serum sickness-like reaction (SSLR) – See Severe Cutaneous Adverse Drug Reactions**.

Pustular Eruptions

Terminology The term pustule originates in classical Latin in which pustule means a blister [100].

Skin Signs Pustular drug eruptions are characterized by monomorphic eruption consisting of erythematous papules (mostly follicular) and pustules at the same location lacking comedones.

Pustular Eruptions – Simple (Skin Only)

Acneiform Drug Eruptions (Acne Medicamentosa)

The term acneiform is applied to eruptions that resemble acne vulgaris.

Frequency Varies, depending on the drug. The highest incidence involves epidermal growth factor receptor inhibitors (EGFRIs), affecting 60–100 % of patients [101].

Lag Period The eruption begins after a variable delay; corticosteroids may induce an acneiform eruption from shortly after their introduction (2–4 weeks) to several months [101]. Acneiform eruptions induced by EGFRIs usually appear after 1–2 weeks of treatment but can also occur after only a few days [102].

Symptoms Pruritus, tenderness and pain may occur. In cases of chemotherapy-related side effects, their appearance and severity are part of the criteria used for the classification of the ADR [103].

Common Sites of Involvement Lesions may be located in and beyond the seborrheic areas, such as the arms, trunk, lower back and genitalia [104].

Histology Drug-induced acneiform eruptions show histopathologic features similar to acne vulgaris. Early lesions most commonly have a corneocytic plug within a widened infundibulum, accompanied by infundibular spongiosis, perifollicular edema, with sparse perivascular and perifollicular infiltrates of neutrophils and lymphocytes. Larger older lesions show similar findings but the infiltrate is denser, with more neutrophils around the involved follicles, and infundibular rupture [85, 88]. In a review of the histological findings of acneiform eruptions induced by EGFRIs [105], all ten cases showed a superficial, predominantly neutrophilic suppurative folliculitis with ectatic infundibula and a rupture of the epithelial lining.

Differential Diagnosis The main differential diagnosis is acne. The following clinical characteristics of acneiform drug eruptions may aid in differentiating between the two entities: (1) Clinical presentation: monomorphic pattern, lack of comedones and cysts and localization on areas beyond the seborrheic area. (2) Patient characteristics: age of onset before or after the teens, and absence of past history of acne. (3) Resistance to conventional acne therapy. (4) Time relationship: onset after recent drug introduction, improvement after drug withdrawal, and recurrence after drug reintroduction [101]. The differential diagnosis also includes folliculitis, rosacea, perioral dermatitis, demodicosis, acne cosmetic, acne mechanica, chloracne, acne necrotica and acneiform presentation of cutaneous lymphomas [104].

Treatment The main treatment is withdrawal of the offending drug and the application of topical treatments as needed (benzoyl peroxide topical antibiotics and topical retinoids) [90]. The management of acneiform eruptions associated

with chemotherapy differs from all other types of acneiform drug euptions, as acneiform eruption is an expected outcome and discontinuation of the medication is not an option in a patient who is responding to therapy [102, 103, 106, 107]. In fact, continuation of EGFRI therapy in these patients may be especially favourable in view of studies that have shown an increased survival with increasing severity of rash [102]. The cutaneous reaction serves as an important clinical tool for determining tumor response and survival [102]. The National Cancer Institute developed a scale for defining the degree of rash and laid down management guidelines for each stage [103]. Other management protocols were suggested by Bachet et al. [107], who recommended that unless contraindicated, a tetracycline should be routinely prescribed for the prevention of acneiform eruption in patients treated with an EGFRI for more than 6 weeks. Chiang et al. [106] reported successful treatment with isotretinoin for high grade and refractory cases.

Prognosis In most patients with acneiform drug eruption, the rash resolves upon discontinuation of the offending drug and the use of topical treatment. In EGFRI-induced acneiform eruption, prophylactic administration of a tetracycline was associated with significantly lower incidence of grade 2–3 folliculitis and improved quality of life of patients [107].

Offending Drugs The drugs responsible for acneiform eruptions include [101]:

- Hormones: corticosteroids and corticotropin – adrenocorticotropic hormone (ACTH), androgens and anabolic steroids, hormonal contraceptives; other hormones – thyroid-stimulating hormone, danazol.
- Neuropsychotherapeutic drugs: tricyclic antidepressants, lithium, antiepileptic drugs, aripiprazole, selective serotonin reuptake inhibitors.
- Vitamins: B1, B6, B12, D2.
- Cytostatic drugs: dactinomycin – actinomycin D, azathioprine, thiourea, thiouracil.
- Immunomodulating molecules:cyclosporine, sirolimus.
- Antituberculosis drugs: isoniazid, rifampin, ethionamide.
- Halogens: iodine, bromine, chlorine.
- Targeted therapies: EGFRIs (cetuximab, panitumumab), multitargeted tyrosine kinase inhibitors (gefitinib, erlotinib, lapatinib,sorafenib, sunitinib, imatinib), vascular endothelial growth factor inhibitor (bevacizumab), proteasome inhibitor (bortezomib), tumor necrosis factor-a inhibitors (lenalidomide,infliximab), histone deacetylase inhibitor (vorinostat).
- Miscellaneous: dantrolene, quinidine, antiretroviral therapy antibiotics.

Drug-Induced Eosinophilic Pustular Folliculitis (Ofuji's Disease)

Few cases of drug-induced eosinophilic pustular folliculitis have been reported [88, 108–111]. Drugs reported include chemotherapy (cyclophosphamide, methotrexate, and 5-fluorouracil) [108], minocycline [109], carbamazepine [110], and allopurinol with timedium bromide [111]. Clinical presentation includes pruritic follicular papules and pustules on the face, scalp, trunk and arms [88]. Histological findings include spongiosis of the follicular epithelium, and an intra- and perifollicular lymphohistiocytic infiltrate with numerous eosinophils that form microabscesses within the follicular epithelium [88]. Topical steroids are the first line of treatment [108].

Pustular eruptions – Complex (skin + systemic involvement)

Acute generalized exanthematous pustulosis (AGEP) – See Severe Cutaneous Adverse Drug Reactions.

Bullous Eruptions

Bullous Eruptions – Simple (Skin Only)

Pseudoporphyria

Terminology The term pseudoporphyria was coined in 1975 by Korting to describe patients with chronic renal failure and a bullous disease resembling porphyria cutanea tarda (PCT) [112].

Frequency The incidence of pseudoporphyria is unknown. However, in a 6-month prospective study, 12% (9/74) of children taking naproxen for juvenile idiopathic arthritis developed pseudoporphyria [113].

Lag Period The skin lesions appear following drug intake combined with exposure to light. Various time durations were reported, weeks to months [114–116].

Skin Manifestations The clinical features of pseudoporphyria may be identical to those of PCT; both exhibit vesicles, bullae, milia, and scarring on sun-exposed skin. In contrast to PCT, however, hypertrichosis, hyperpigmentation, sclerodermoid changes, and dystrophic calcification are rarely reported in pseudoporphyria [117]. Often, fragility and bruising may be the only clinical signs [116]. In children, facial scarring resembling erythropoietic protoporphyria (EPP) may be found [117].

Symptoms Skin fragility and photosensitivity [116].

Common Sites of Involvement The lesions appear on sun-exposed skin, particularly the hands and feet, but also on the face and extensor surfaces of legs [116].

Histology the histological features are identical to those seen in PCT. The blisters are subepidermal and the floor of the blister is typically lined by well-preserved dermal papillae (festooning). There is usually no significant inflammatory component although a light perivascular lymphocytic infiltrate may occasionally be seen in the superficial dermis. Thickening of the superficial vessels (highlighted by a PAS stain) and dermal sclerosis with elastosis may be apparent. In both pseudoporphyria and PCT, direct immunofluorescence reveals granular deposits of IgG and C3 at the basement membrane zone and in the perivascular region [115].

Differential Diagnosis While pseudoporphyria and PCT share clinical and histologic features, they can be differentiated by several features. Most important, by definition, biochemical porphyrin abnormalities are absent in pseudoporphyria. Epidemiologically, pseudoporphyria affects mainly women while there is a male predilection in PCT. Clinically, hypertrichosis, hyperpigmentation, sclerodermoid changes, and dystrophic calcification are frequently evident in PCT and conspicuously absent in pseudoporphyria [117]. The differential diagnosis also includes other types of cutaneous porphyria that manifest with blistering, epidermolysis bullosa acquisita, polymorphous light eruption, and other photosensitive dermatosis [117].

Treatment Treatment entails discontinuation of suspected agents and sun protection, especially against UVA wavelengths, for several months following withdrawal of the drug [114].

Prognosis Blisters may continue to appear for weeks-months after discontinuation of the offending drug [117].

Offending Drugs The most common group of drugs causing pseudoporphyria are NSAIDS [117]. Other groups are antibiotics, diuretics and retinoids. Additional culprits are hemodialysis, renal failure, tanning beds and excessive sun exposure [117].

Fixed Drug Eruption (FDE)

Terminology Fixed drug eruption (FDE) was first reported by Boums in 1889 [118], and the term was coined by Brocq in 1894 [119].

Frequency The incidence is not known, but is suspected to vary greatly by geographic region [120].

Lag Period After initial use of the offending agent, a variable refractory period of weeks, months or years may pass before the lesions first appear on the skin of a sensitized individual [121]. Repeated exposure to the agent typically results in acute lesions within 30 min to 8 h. A refractory phase may occur following an acute flare in which exposure to the offending drug will not exacerbate the lesion for weeks to months [121].

Skin and Oral Membrane Manifestations In its classical form, FDE typically presents round or oval, sharply demarcated, red to livid, slightly elevated plaques ranging from several millimeters to over 10 cm in diameter. Vesicles or even blisters can develop [122]. Usually only a single lesion appears. Sometimes, multiple lesions are present and even lead to generalized FDE characterized by multiple, sharply defined, deep red macules distributed bilaterally and often symmetrically. Generalized bullous FDE is characterized by flaccid blisters arising on these macules. Mucosal lesions are usually bullous and may appear with or without involvement of other areas of the skin [122].

Symptoms Patients often complain of burning and itching in the lesions. General symptoms such as fever, nausea, dysuria, abdominal cramps and diarrhea are rare [122]. Pruritus and burning may be the only manifestations of reactivation in a postinflammatory hyperpigmentation lesion [121].

Common Sites of Involvement The eruption can occur anywhere on the body, but the lips, palms, soles, genitalia (especially male genitalia), groin and occasionally oral mucosa are favored sites [121]. The diagnostic hallmark of FDE is the reappearance of the lesions precisely over the previously affected sites. Studies investigating the predilection areas indicate that some specific kind of drugs cause FDE predominantly at specific sites: examples are tetracycline and location on the male genital area, and naproxen and FDE on the lips [122]. In rare cases, FDE manifests in old trauma sites such as BCG vaccination, burn scar, venipuncture site or insect bite. With each recurrence, additional sites may be affected. The presence of numerous lesions is referred to as generalized FDE [122].

Histology Histologically, the acute phase is characterized by marked basal cell hydropic degeneration, with lymphocyte tagging along the dermoepidermal junction and individual keratinocyte necrosis. Marked pigmentary incontinence is typical, and may be the sole histological finding in late lesions [121].

Differential Diagnosis Skin lesions can imitate various dermatoses, including lichen planus, erythema multiforme, erythema annulare centrifugum, and pityriasis rosea. In generalized FDE, residual pigmentation in healed lesions may be reminiscent of erythema dyschromicum perstans. Involvement of oral and genital mucosa raises the possibility of herpes simplex, pemphigus vulgaris, aphthous stomatitis, Behçet syndrome, and erosive lichen planus [122]. Generalized bullous FDE may resemble SJS/TEN. The following typical

clinical features of generalized bullous FDE may aid in differentiating between conditions: (1) Blistering usually affects only a small percentage of body surface area, and between the large blisters there are sizable areas of intact skin. (2) Erosive mucosal involvement is rare, and when it does occur is rather mild. (3) Patients usually do not feel sick or have fever, and generally are in much better overall health than those with SJS/TEN. (4) Most patients report a history of a similar, often local reaction [123].

Treatment For mild lesions, topical corticosteroids usually suffice. In severe involvement, especially generalized bullous FDE, systemic corticosteroids may be indicated. Strict avoidance of the causative drug and cross-reacting substances is essential for prophylaxis. Successful desensitization was reported [122].

Prognosis The prognosis of localized FDE is good and the lesions fade within a few days to leave a post-inflammatory brown pigmentation [122]. Generalized bullous FDE does not have this benign nature and the mortality rate was 22 % in a recent case control study of 58 patients [120].

Offending Drugs The most common groups of drugs implicated are antibiotics, analgesics, antiphlogistics and hypnotics [122]. There is usually only one causative drug (monosensitivity), but sometimes several drugs can induce FDE in the same patient (multisensitivity). It has also been claimed that recurrences of FDE can be induced in nonspecific fashion by mast cell degranulators such as food, acetylsalicylic acid, bacterial toxins, or physical stimuli [122].

Bullous Eruptions – Complex (Skin + Systemic Involvement)

- **Drug-induced/triggered autoimmune blistering dermatosis (pemphigus, bullous pemphigoid (BP)) and linear IgA bullous dermatosis (LABD)**

Terminology Pemphigus Two Italian dermatologists, Caccialanza and Bellone, were the first to imply activation of pemphigus by a drug (penicillin) in 1951 [124]. However, Degos's publication in 1969 of penicillamine-induced pemphigus in a patient with Wilson's disease is considered the first report of drug-induced pemphigus [125].

BP Bean et al. reported the first case of drug-induced BP in 1970 [126].

LABD Baden et al. reported the first case of drug-induced LABD in 1988 [127].

Cases of autoimmune blistering dermatosis resulting from exposure to drugs present clinical, histologic and immunopathologic features identical or very similar to those seen in idiopathic disease, but are induced by systemic ingestion or local use of certain drugs. There appear to be two main types: drug-induced autoimmune blistering dermatosis proper, the acute and self-limiting type with rapid resolution after withdrawal of the offending agent; and drug-triggered autoimmune blistering dermatosis in which the role played by the drug is only secondary to hereditary and immunologic factors. The drug stimulates a predisposition (hidden susceptibility) to develop the disease and is considered the chronic type in which the disease persists despite withdrawal of the offending agent [128, 129].

Frequency Unknown.

Lag Period Pemphigus Weeks to months [130, 131]
BP Days to weeks [132, 133]
LABD Days to weeks [134, 135]

Symptoms/Common Sites of Involvement/Histology Similar to the idiopathic type of autoimmune blistering dermatosis.

Differential Diagnosis There are no distinctive clinical features that enable differentiation between drug-induced/triggered and idiopathic autoimmune bullous dermatosis. It is obvious that spontaneous remission following withdrawal of the offending drug points to a drug-induced autoimmune blistering dermatosis. However, other clinical findings may also be suggestive of a drug origin in cases of pemphigus and BP: (1) Patients are younger than those with idiopathic disease. (2) Mucous membranes are frequently involved. (3) Combined clinical and immunohistologic features of various immunobullous diseases may exist. (4) Severe general status may appear including high fever. (5) In cases of drug-induced pemphigus, features of pemphigus foliaceus are more common than those of pemphigus vulgaris [130, 133, 136]. Of note, drug-induced LABD patients tend to be older than idopathic type patients [134, 135].

The polymorphic nature of the eruption may mimic other bullous diseases and or drug-induced bullous diseases such as SJS, TEN, and FDE [136].

Treatment Treatment consists of discontinuing the offending agent, and, depending on the severity of the disease, systemic immunosuppressive treatment [129].

Prognosis Drug-induced autoimmune blistering dermatosis remits after the offending drug is withdrawn, while drug-triggered autoimmune blistering dermatosis may persist despite withdrawal of the offending agent and chronic immunosuppressive treatment may be required [129, 130].

Offending Drugs Pemphigus Two major groups of chemical structures were found in the drugs or their metabolites implicated in pemphigus: sulfhydryl radical drugs (thiol drugs or SH drugs) such as penicillamine, and phenol drugs such as aspirin [128, 137, 138].

BP Many drugs were reported [129, 132, 136], the most frequent being NSAIDS, cardiovascular

agents and penicillin-derived antibiotics [136]. In addition, external use of skin and mucous membrane preparations has been documented to provoke cases of either BP or cicatricial pemphigoid [136].

LABD Of the various drugs reported, vancomycin is the most common [134, 135, 139].

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) – See Severe Cutaneous Adverse Drug Reactions.

Severe Cutaneous Adverse Drug Reactions

Drug reaction with eosinophilia and systemic symptoms (DRESS), Drug-induced hypersensitivity syndrome (DIHS), Drug-induced delayed multiorgan hypersensitivity syndrome.

Epidemiology

The incidence of DRESS remains to be determined because of variable presentations and lack of universally accepted diagnostic criteria [140]. The estimated risk at first or second prescription of an aromatic antiepileptic drug was 1–4.5 in 10,000 [141]. A slight female predominance was found in the RegiSCAR study (male/female 0.8) [142].

Etiology

The drugs most commonly inducing DRESS are anti-convulsants (mainly aromatic anti-convulsants such as carbamazepine), allopurinol, sulfonamides (the anti-infective sulfamethaxazole-trimethoprim, and the anti-inflammatory sulfasalazine), and antibiotics (such as vancomycin and minocycline) [142]. Numerous other drugs have been reported [140, 143, 144].

The role of human herpesvirus (HHV) reactivation in the development of this adverse drug reaction is well recognized, especially HHV-6 [145]. HHV-6 reactivation is among the diagnostic criteria of the Japanese consensus group for DRESS/drug-induced hypersensitivity syndrome [146]. The reactivation of other herpesviruses, including HHV-7, cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpes simplex virus was also reported [147].

DRESS is considered to result from complex interactions between genetic predisposition, exposure to drug and viral reactivation [148].

Lag Period

Delayed onset of 2–8 weeks after drug administration followed by a stepwise development of manifestations. Rechallenge can result in a reaction within hours to days [26]. The lag period differs between drugs; carbamazepine tended to show a longer latency (median 29 days) than allopurinol (median 20 days) in the RegiSCAR study [142].

Clinical Features

DRESS has multi-organ involvement with cutaneous, mucosal, hematological and solid organ manifestations.

Skin The cutaneous involvement in DRESS is typically extensive and symptomatic (pruritus, burning and pain) [142, 143]. Various dermatological features were reported. Walsh et al. [143] proposed a classification system based on four distinct patterns: (1) urticated papular exanthema, the most common, (2) morbilliform erythema, (3) exfoliative erythroderma, and (4) erythema multiforme-like (EM-like), which was prognostic of more severe hepatic involvement. The extent of skin involvement varies between studies: it exceeded 50% of the body surface area in most of the patients (79%) according to the RegiSCAR study [142]; head and neck edema observed in most patients [26, 142]; and pustules reported in various studies, predominantly in a facial distribution of the edema [142, 143].

Mucous Membranes Mild mucosal involvement was recorded in 56% of patients with DRESS (66/117 cases) in the RegiSCAR study [142]. Most frequent were oral lesions including lips, oral cavity and throat [142]. The manifestations of oral lesions in DRESS include cheilitis, erosions and dysphagia that may appear before skin lesions, and oropharaynx is considered the first site of herpesvirus reactivation in DRESS [149]. Involvement of eyes and genitalia were also reported in the RegiSCAR study [142].

Systemic Involvement Multi-organ involvement is common in DRESS and may include a wide variety of systems. High-grade fever (38–40 °C) is a typical early manifestation that may last for several weeks; it often precedes the cutaneous eruption by several days [142]. Lymphadenopathy is common and has two distinct types: a benign pattern of lymphoid hyperplasia and a pseudolymphoma pattern [150]. Hematologic abnormalities are frequent and diverse, the most common being marked leukocytosis, eosinophilia and atypical lymphocytes [142]. However, neutrophilia, monocytosis, thrombocytopenia, anemia, pancytopenia and hemophagocytic syndrome were also reported [140, 142, 143, 151]. Hypereosinophilia and activated neutrophils, if persistent, can contribute to organ damage [142]. The liver is the most frequently affected visceral organ in DRESS; hepatitis with isolated elevation of liver enzymes is common and usually anicteric and without cholangitis. However, severe acute hepatitis with liver failure may result and is the primary cause of mortality in DRESS [150]. Renal involvement is common [150]. Involvement of the following organs was also reported: lungs, muscle, heart, pancreas, colon, thyroid, joints, parotid

gland and brain [150]. The type of organs involved was found to be related to the eliciting drug [152].

Histology

The most common pathological changes found in a study of 32 patients with DRESS were basket-weave hyperkeratosis (94%), dyskeratosis (97%), lymphocytic exocytosis (91%), spongiosis (78%), papillary edema (66%), perivascular lymphocytic infiltration (97%), eosinophilic infiltration (72%), and interface vacuolization in the dermoepidermal junction (91%) [26]. The presence of severe dyskeratosis was correlated with a greater extent of systemic involvement [26]. In a different study assessing the histological findings of 27 cases with DRESS [143], the predominant pathological pattern was spongiotic dermatitis with superficial lymphocytic infiltrate (59%); necrotic keratinocytes were noted in 33% of cases, and were associated with a worse hepatic involvement [143].

Diagnostic Criteria

The diverse presentations in DRESS have hampered efforts to define diagnostic criteria. Three diagnostic criteria have been proposed: Bacquet et al. [153], the Japanese study group of severe cutaneous adverse reactions to drugs (J-SCAR) [146], and the RegiSCAR network [154].

Treatment

The first step in the management is immediate withdrawal of the culprit drug. The treatment is tailored according to the severity and extent of systemic involvement, and the diagnosis of viral reactivation of herpesviruses (mostly HHV-6) [150, 155, 156]. Management protocol for DRESS based on the consensus of experts was designed by the French Society of Dermatology [156], and includes four visceral involvement severity categories and corresponding treatment: (1) No severe systemic involvement: topical corticosteroids (potent or very potent), emollients, H1-antihistamines. (2) Severe systemic involvement (transaminases >5 times normal, renal involvement, pneumonia, hemophagocytosis, cardiac, etc.): systemic corticosteroids equivalent to 1 mg/kg/day of prednisone and multidisciplinary evaluation. (3) Life-threatening signs (hemophagocytosis with bone marrow failure, encephalitis, severe hepatitis, renal failure, and respiratory failure): systemic steroids with intravenous immunoglobulin (IVIG) at a dose of 2 g/kg over 5 days. The IVIG should not be used without associated steroids. The treatments are to be conducted under multidisciplinary supervision. (4) Severe systemic involvement and confirmation of a major viral reactivation: combining steroids and antivirals (such as ganciclovir) and/or IVIG.

Counselling both the patient and his family members about drug avoidance is necessary. First-degree relatives have a higher risk of developing the same drug reactions [90]. Increased knowledge of HLA susceptibility genes enables screening patients with DRESS for several high risk drugs [148, 157].

Prognosis

Symptoms are usually present for several weeks even after discontinuation of the offending agent and appropriate treatment [155]. Late complications include the appearance of autoimmune diseases such as lupus erythematosus and autoimmune thyroiditis, with laboratory evidence of autoantibodies [144]. Systemic corticosteroids were found beneficial in the prevention of autoimmune disease. However, this effect needs to be counterbalanced against the higher risk of viral reactivation and infection. [144]. In a 1-year follow-up study of 52 affected patients with DRESS in Taiwan, the overall cumulative incidence of long-term sequelae was 11.5%; four developed autoimmune diseases (Graves disease, type 1 diabetes mellitus and autoimmune hemolytic anemia); and the other two developed renal failure and required lifelong hemodialysis. The author concluded that the sequelae of DRESS can be divided into two major types that appear in different age groups: young patients tend to develop autoimmune diseases; elderly patients are more vulnerable to end-organ failure [158].

Mortality in DRESS has been estimated at 10%, with most patients dying from liver failure [159]. Pancytopenia, leukocytosis, tachycardia, tachypnea, coagulopathy, gastrointestinal bleeding and systemic inflammatory response syndrome were associated with a poor outcome in DRESS patients [159, 160].

Serum Sickness-Like Reaction (SSLR)

Epidemiology

The incidence of SSLR is unknown. Epidemiology studies in children suggest that the overall frequency induced by cefaclor is 0.024–0.2% per course of the drug [76]. Most reactions were reported in children under 5 years old, mainly during the second and third courses of therapy [161].

Etiology

Cefaclor is the most common cause of SSLR in children, inducing 84.1% of cases [162]. Other drugs implicated include other cephalosporins, [163] penicillins, [164] minocycline, [165] insulin, [166] and infliximab [167].

Lag Period

Usually 7–14 days (range 0–20 days) [162, 168].

Clinical Features

Skin The skin is the most frequent finding in SSLR, including erythema that progresses to urticarial lesions (pruritic and migratory), urticarial wheals with dusty to purple centers ('purple urticaria') that morphologically resemble erythema multiforme (EM) [161] and other cutaneous manifestations including morbilliform or scarlatiniform eruptions [82].

Mucous Membranes Mucous membranes are not involved [161].

Systemic Involvement Joint involvement may be prominent, presenting with edema, decreased range of motion, warmth, pain, and difficulty walking. Polyarticular involvement is often observed, with involvement mainly of the wrists, ankles, hips and knees [169]. Some authors suggested that joint involvement may be related in part to increased fluid in the skin around affected joints due to urticarial eruption rather than arthritis [161]. Fever, malaise, myalgia and lymphadenopathy were also reported. Neurologic involvement, gastrointestinal symptoms and renal complications were rarely documented [163]. Notable laboratory abnormalities include elevated erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and leukocytosis [163, 170].

Histology

The histological findings of SSLR appear to be in the spectrum of urticaria with no vasculitis [171]. Histology can be helpful in differentiating SSLR from acute hemorrhagic edema of infancy, which is characterized by vasculitis [171].

Diagnostic Criteria

There are no diagnostic criteria. The diagnosis is based on clinical findings [161].

Treatment

Withdrawal of the offending agent and symptomatic treatment with oral antihistamines and topical corticosteroids are usually sufficient. A short course of oral corticosteroids may be required in patients with severe symptoms [82].

Prognosis

The disease course is benign and resolves in a few days. However, a few cases lasting several weeks have been described [170]. No long-term morbidity has been reported [172].

Acute Generalized Exanthematous Pustulosis (AGEP)

Epidemiology

The estimated incidence of AGEP is 1–5 cases per million per year [173]. Female predominance was reported in several studies [174–176].

Etiology

The majority of cases appear to be related to drugs (>90 %), mainly antibacterials [4]. In a large multinational case-control study (the EuroSCAR study), the following agents were highly suspected drugs for AGEP: prestinomycin, ampicillin/amoxicillin, quinolones, (hydroxy)chloroquine, anti-infective sulfonamides, terbinafine and diltiazem [176].

Lag Period

Latent periods fall into two categories, according to the offending drug: median duration of 1 day, associated with antibiotics (including sulphonamides), and median duration of 11 days for all other associated drugs [176]. Longer periods of months were reported in a few AGEP cases with an underlying malignancy [177].

Clinical Features

AGEP is a severe acute pustular cutaneous reaction characterized by a rapid clinical course [174].

Skin The typical morphology of AGEP is an acute edematous erythema with burning and or itching sensation, followed by dozens to hundreds of small (pinhead sized) non-follicular sterile pustules with a predilection for the big folds, or with widespread distribution (Fig. 25.2). Sometimes confluence of pustules may mimic a positive Nikolsky's sign [176, 178]. Additional cutaneous manifestations include marked edema of the face, purpura, blisters and target-like lesions [173, 174, 179], all of which overlap with manifestation of AGEP and TEN [180, 181], and acute localized exanthematous pustulosis (ALEP) [179, 182].

Mucous Membranes Mild, nonerosive mucous membrane involvement of one location (mostly oral) occurs in about 20 % of cases [183].

Systemic Involvement Fever (above 38 °C) and leukocytosis with neutrophilia are almost always apparent. Lymphadenopathy, myalgia, headache, mild eosinophilia, elevated CRP, slight reduction of creatinine clearance, and mild elevation of aminotransferases were also reported [173, 175]. A 10-year retrospective review of 58 patients with AGEP [184] turned up 10 patients (17 %) with at least one systemic involvement in the acute phase, 7 with abnormal hepatic function test, 6 with renal insufficiency, two with acute respiratory distress and one patient with agranulocytosis. Mean peripheral neutrophil counts and mean C-reactive protein levels were elevated significantly in patients with systemic involvement [184].

Histology

Biopsy specimen should be obtained from an early pustular lesion [183]. A histopathological study of 102 AGEP cases [185] found the following histopathological features: (1) All cases demonstrated pustules (sub/intracorneal and or intraepidermal). (2) The main epidermal features were spongiosis (80 %), neutrophil exocytosis (77 %) and necrotic keratinocytes (67 %). (3) The main dermal features were mixed superficial (100 %), interstitial (93 %) and mid/deep-dermal infiltrates (95 %) containing neutrophils (100 %) and eosinophils (81 %).

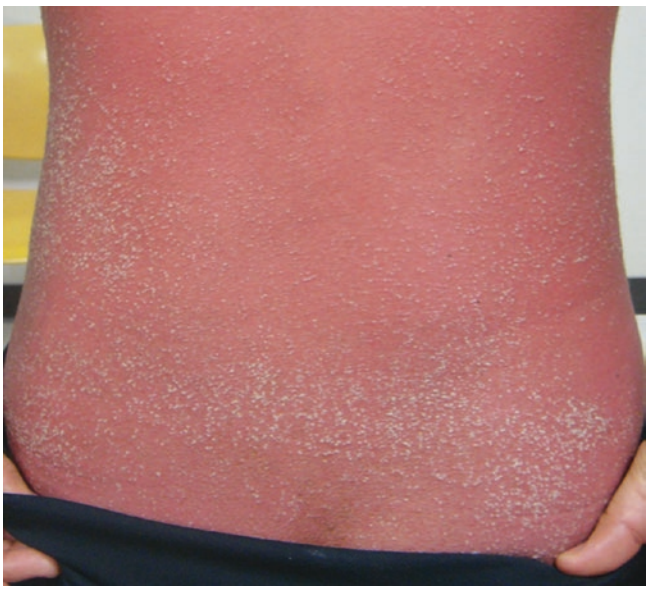

Fig. 25.2 Multiple, pin-head sized, non-follicular pustules on erythematous skin on the trunk in a patient with AGEP

Diagnostic Criteria

The AGEP validation score developed by the Euro- SCAR study group is a standardized scoring system made up of data related to clinical features (morphology and clinical course) and histopathology. Based on this score, AGEP cases can be categorized as no AGEP, possible AGEP, probable AGEP, and definite AGEP [173].

Treatment

Treatment consists of discontinuation of the causative drug and supportive treatment. Although, specific treatment is generally unnecessary, topical and systemic steroids were reported [174, 175]. The treatment of overlapping AGEP and TEN cases is not yet established [180], although successful treatment with infliximab was documented [181].

Prognosis

After elimination of the causative drug, pustules usually spontaneously disappear in a few days with desquamation, and the reaction fully resolves within 15 days [183]. The overall prognosis is good, although high fever or superinfection of skin lesions can sometimes lead to life-threatening situations in patients of old age or poor general condition [173]. The mortality rate is about 5 % [4].

Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)

Epidemiology

The annual incidence of SJS and TEN is 1.2–6 and 0.4–1.2 per million individuals, respectively [186, 187]. The annual incidence of SJS and/or TEN in HIV patients is estimated at 1–2 per 1000 individuals, approximately 1000-fold higher than that of the general population [188]. The incidence of SJS/TEN increases with age; children less than 15 years of age account for only 10 % of the samples in most studies [189]. Women are two times more likely to be affected by SJS/TEN than men in the adult population, while the male to female ratio is about equal in children [189].

Etiology

Drug exposure is the most common cause of SJS/TEN [190], with more than 200 drugs identified [191]. The groups of medications associated with high risk of inducing SJS/

TEN vary according to the population. In the general population in Europe, high risk drugs for SJS/TEN include allopurinol, carbamazepine, cotrimoxazole and other anti-infective sulfonamides, lamotrigine, nevirapine, oxicam-NSAIDS, phenytoin, phenobarbital and sulfasalazine [192]. In the pediatric population in Europe, they include anti-infective sulfonamides, phenobarbital, carbamazepine and lamotrogone [189]. In Africa, they include antibacterial sulfonamides, nevirapine, tuberculosis drugs, NSAIDs, antiepileptics, aminopenicillin, analgesics and allopurinol [193]. Non-medication triggers, implicated mainly in SJS, include infections, contrast media and vaccinations [194–196]. ALDEN is an Algorithm for the Assessment of Drug Causality in SJS/TEN developed by the RegiSCAR study group and consists of 6 parameters according to which the drug causality is classified as very unlikely, unlikely, possible, probable and very probable [197].

Lag Period

Usually 4–28 days. The median latency was longer (above 30 weeks) for drugs with no associated risk [192].

Clinical Features

SJS and TEN represent different degrees of a severe, acute and life-threatening mucocutaneous reaction. We will refer to this disease spectrum as a single entity, namely SJS/TEN. The classification of SJS/TEN, defined by Bastuji-Garin et al. [198], is based on the extent of epidermal detachment and the findings of characteristic skin lesions (Table 25.4). It should be emphasized that only necrotic skin, which is already detached (e.g., blisters, erosions), or detachable skin (positive Nikolsky sign whereby slight rubbing of the skin results in exfoliation of the outermost layer) should be included in the evaluation of the extent of epidermal detachment [190].

Skin The characteristic skin morphology of SJS/TEN consists of 'flat, atypical target lesions' and 'spots/macules', which are defined as follows. Flat, atypical target lesions are round lesions, with only two zones and/or a poorly defined border, nonpalpable with the exception of potential central blister. 'Spots/macules' are nonpalpable, erythematous or purpuric macules with irregular shape and size, often confluent [198]. Epidermal necrosis, the hallmark process of SJS/TEN, induces flaccid blisters with positive Asboe-Hansen sign (lateral extension of bullae with pressure), erosions, positive Nikolsky sign, and in severe cases extensive skin sloughing [199]. At least 1 % of epidermal detachment is required for the diagnosis of SJS/TEN [83]. In rare instances, extensive epidermal necrosis occurs with only widespread erythema and no evidence of 'flat, atypical target lesions' or 'spots/macules'; these cases were classified as 'TEN without spots' (Table 25.4). A characteristic sign of SJS/TEN is severe pain and tenderness of the skin [83].

Mucous Membranes Mucosal involvement is evident in most of the cases with erythema, erosions and ulceration, due to necrosis of the epithelial lining [199]. SJS/TEN involve more than 2 mucosal sites in 17–71 % of cases [200]. Most common sites are oral (Fig. 25.3), ocular and genital mucous membranes, although any mucous membrane may be involved, such as respiratory, gastrointestinal and urethral [199]. Fuchs syndrome is a unique type of SJS that involves the mucosa without skin lesions and was reported to be associated with mycoplasma pneumoniae, mostly in children and adolescents [201].

Table 25.4 Classification of erythema multiforme major (EMM), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) according to Bastuji-Garin et al. [198]

	EMM	SJS[a]	SJS-TEN overlap	TEN	
				TEN with spots	TEN without spots/TEN with widespread erythema
Skin detachment (%) detached skin, blisters, erosions and/or detachable skin – positive Nikolsky sign[b]	<10 %	<10 %	10–30 %	>30 %	>10 %
Typical target lesions individual lesions less than 3 cm in diameter with a regular round shape, well defined border, and at least three different zones	+	–	–	–	–
Atypical target lesions Round lesions with two zones and/or a poorly defined border *Raised* – edematous, palpable lesions *Flat* – nonpalpable with the exception of potential central blister	Raised	Flat	Flat	Flat	–
Spots/macules with or without blisters nonpalpable, erythematous or purpuric macules with irregular shape and size, often confluent	–	+	+	+	–

[a]At least 1 % of epidermal detachment is required for the diagnosis of SJS
[b]Slight rubbing of the skin results in exfoliation of the outermost layer

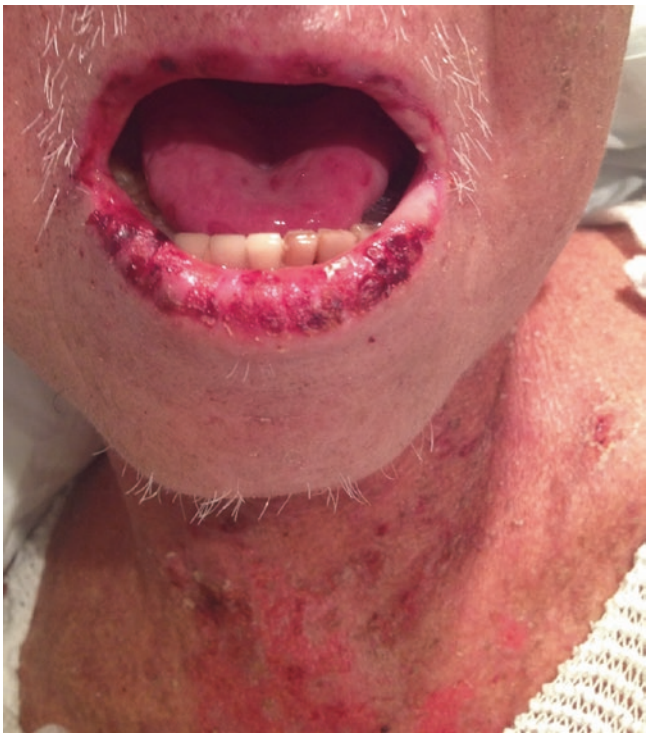

Fig. 25.3 Erosions with hemorrhagic crust on the lips, tongue and skin in a patient with TEN

Systemic Involvement Systemic findings in SJS/TEN include: (1) Flu-like symptoms (malaise, fever, anorexia) that are usually the initial signs of the disease in the prodromal phase prior to the cutaneous involvement. (2) Epidermal barrier breakdown-related symptoms including hypothermia, dehydration and sepsis. (3) Organ involvement induced by necrosis of epithelial lining, including respiratory distress syndrome, colitis, hepatitis and nephritis [199].

Histology

Characteristic histologic features include extensive keratinocyte destruction via apoptosis with separation of the epidermis from the dermis at the dermoepidermal junction. A paucicellular, dermal mononuclear infiltrate has been commonly described. Lymphocytes cross the dermoepidermal junction with moderate infiltration of the epidermis. EM and SJS often demonstrate less keratinocyte destruction on a background of extensive dermal mononuclear inflammation [202]. In a retrospective analysis of the clinical records and histologic material of 37 patients with TEN, the histologic spectrum ranged from sparse to extensive dermal mononuclear inflammation, the extent of which predicts clinical outcome approximately as well as SCORTEN. Increased inflammation correlated with a worse prognosis; a mean cell count of dermal mononuclear >215 cells per high-power field predicted a worse prognosis (65 %) vs 24 % mortality in those with <215 cells in patients with 30 % or more total body surface area sloughing [202]. However, in a retrospective study analyzing clinical records and skin biopsy of 108 patients with SJS, SJS/TEN overlap and TEN, dermal infiltrate severity was not associated with day-1 SCORTEN or hospital death, but full-thickness epidermal necrosis was associated with mortality [203].

Diagnostic Criteria

Diagnostic criteria based on integration of the major clinical characteristics of skin and mucous membrane findings, pathology assessment, lag period and systemic signs remain to be defined.

Treatment

The management of SJS/TEN consists of a multidisciplinary approach that includes the following important aspects:

1. **Identification and withdrawal of the culprit drug**: documenting the medication history during the previous 2 months and withdrawal of all suspected and unessential medications [123].
2. **Transfer of the patient to intensive care, burn unit or other specialty unit**: supportive care including thermoregulation, fluid replacement, nutritional support, monitoring for infection, sedation and pain management, and psychological support [204].
3. **Assessment of skin, mucous membranes and systemic involvement and the SCORTEN score**: Type of lesions in the skin, extent of epidermal detachment, and mucous membranes and systemic involvement. All patients should be evaluated by an ophthalmologist promptly following the diagnosis and at regular follow-up intervals to minimize potential long-term ocular sequelae [205]. Possible acute manifestations include the eyelids, conjunctiva and cornea, and result in the classification of ocular involvement as mild, moderate or severe [206]. Bringing other specialists in on the patient's care is decided in accordance with the relevant findings. The SCORTEN system, a severity-of-illness score for Toxic Epidermal Necrolysis, developed to stratify severity of illness and predict mortality in patients with TEN, includes seven independent risk factors: age, malignancy, tachycardia, initial body surface area of epidermal detachment, serum urea, serum glucose, and bicarbonate [207].
4. **Skin treatment**: There are no clinical guidelines for the skin care of patients with SJS/TEN. Debridement of the necrotic epidermis was recommended in past publications

[187, 204]. Recent publications advise avoiding debridement, which may cause hypertrophic scars, and recommend considering the detached epidermis as a natural biological dressing that favors reepithelialization [14, 205, 208]. Various topical treatments reported include bioactive skin substitutes, semi-synthetic and synthetic dressings, and topical antimicrobials [187, 204]. A recent report on the management of SJS/TEN in an experienced French referral center described the following treatment; wound care once a day with minimal manipulation to prevent skin detachment, including a bath containing a solution of chlorhexidine 1/5000 (morphine is given prior to the bath and/or equimolar mix of oxygen and nitrogen monoxide during the bath); if bathing is not possible, the chlorhexidine solution is sprayed 2–3 times daily on the skin, blister fluid is aspirated while maintaining the blister roof, vaseline is systemically applied over all detached skin areas, topical sulfa-containing medications are avoided, and hydrocellular or absorbent nonadhesive dressings are applied at least once daily to cover pressure points [205].

5. **Mucous membranes treatment**: Specialized care is essential to prevent lifelong complications [208]. Although there is no standardized care for ocular management, the following supportive local treatment is advised: tear replacement solutions, removal of pseudomembranes, lysis of symblepharon, debridement of loosened epithelium, topical antibiotics to prevent secondary infection, topical corticosteroid to prevent scar formation, and cycloplegic drops to relieve pain, photophobia and ciliary spasm [206]. Amniotic membrane transplantation was found effective in the acute and chronic stages of SJS/TEN [209, 210]. A 'Triple-TEN' protocol for severe ocular cases was recently reported [211], comprised of the following: (1) Subconjunctival triamcinolone (Kenalog 20 mg) administered into each of the fornices to curb the local inflammatory response without compromising systemic immunity. (2) Placement of amniotic membrane tissue mounted on a polycarbonate skirt (ProKera) over the corneal and limbal regions to facilitate reepithelialization of the ocular surface. (3) insertion of a steeply curved acrylic scleral shell spacer (Technovent, SC21) to vault the lids away from the globe and provide a barrier to symblephara formation. This treatment offers an effective therapeutic option, without the need for microsurgical equipment, microscope, or sutures in the critical care setting.

   Oral- The mouth should be rinsed several times a day with an antiseptic or anifungal solution and the lips lubricated with an ointment such as dexpanthenol [123].

   Genital- Wet dressings or sitz baths and lubrication with emollient are recommended to avoid adhesions and strictures of genital erosions in females [123, 205].

   A specialist is required in case of involvement of other mucous membranes: respiratory, gastrointestinal and/or urethral.

6. **Systemic immunomodulatory treatment**: The optimal therapeutic regimen has yet to be established, but according to recent publications, the following conclusions can be drawn: the use of IVIg does not yield survival benefits in SJS/TEN [212]; cyclosporine decreased the death rate and the progression of detachment (dosage of 3 mg/kg/day for 10 days) [213]; systemic corticosteroids were associated with clinical benefit according to the Euroscar-study [214] and were reported to be the most common treatment for SJS/TEN in a recent survey of 50 drug hypersensitivity experts from 20 countries [14]. One of the suggested protocols is IV dexamethasone 1.5 mg/kg pulse therapy (given for 30–60 min) for 3 consecutive days [215]. Treatment with anti-TNF biologic treatment was reported to be beneficial [216–218]. A prospective, randomized, open-label trial currently underway in Taiwan [14] comparing etanercept versus systemic corticosteroids in patients with SJS/TEN, reported that the average duration to reach maximal skin detachment and complete skin healing was shorter in the etanercept group. In vitro investigations demonstrated that etanercept, steroids or thalidomide significantly decreased granulysin expression of blister cells. Etanercept did not, however, increase the cytotoxic effect to keratinocytes found with thalidomide [14].
7. **Causality assessment and communication with the patient and his/her family, health-care providers and regulatory agencies**: Recent discoveries of specific HLAs that predict genetic susceptibility to SJS/TEN offer a simple, fast, safe and reliable method for establishing clear causality between a drug and a disease [148]. The HLAs are specific to a drug and an ethnic background [148]. Since these tests are available only for certain drugs and a negative test does not exclude the drug as the offending agent, additional clinical and laboratory methods are available for assessing causality. (**See Practical approach to the diagnosis and management of cutaneous ADRS and Clinical and laboratory assays in the diagnosis of cutaneous ADRs**.) For information on communication with the patient and his/her family, health-care providers and regulatory agencies **see Practical approach to the diagnosis and management of cutaneous ADRS**.

Prognosis

The mortality rates of SJS/TEN are variable. That of TEN may approach 30% [191], and that of children with SJS/TEN is approximately 2–7.5% [189]. In a large-scale, population-based, 1-year follow-up study of 460 SJS/TEN patients, the

6-week in-hospital mortality rate was 23%, and the death rate from 6 weeks to 1 year was 14% [219]. The mortality rate at 1 year in this study was 24% for SJS, 43% for SJS and TEN overlap, and 49% for TEN. Several factors were found to affect mortality: age, severity of reaction, recent malignancy, pre-existing severe kidney or liver disorder, and recent infection. The last two factors were recognized for the first time in this study as being independent risk factors for death. All other factors are part of the SCORTEN [207]. The severity of the reaction was a major risk factor for death in the first few weeks, and severe co-morbidities and older age had major impact on mortality after 6 weeks [219]. Early and late physical complications are common among patients who survive SJS/TEN [219], with some 80% experiencing long-term sequelae [220]. Complications may affect multiple organ systems including skin, nails, hair, oral and genital mucosal sufaces, eyes, kidneys, gastrointestinal tract, and respiratory system [221]. Ocular complications, which can lead to blindness, are the major long-term morbidity [206]. A few studies have dealt with the quality of life of patients surviving SJS/TEN [221–223], which was found to be lower in every domain from before hospitalization to follow-up and a low rate of return to previous employment was documented [221]. Patients reported concerns about social interactions, fear of taking medications, and fear of contracting an illness necessitating medication [223]. Insufficient information and support for patients surviving SJS/TEN was also documented [221–223]. Unfortunately, because of the rarity of SJS/TEN, most physicians are not aware of the long-term complications of the diseases [220].

Practical Approach to the Diagnosis and Management of Cutaneous Adverse Drug Reactions

There are several methods to approach a patient with a cutaneous ADR. The following is the authors' protocol:

Clinical Assessment of Drug-Induced Skin Injury: 4Ds by Dr. Shear

Diagnosis of the Adverse Event

A cutaneous eruption in a patient taking a medication should immediately raise the suspicion of a cutaneous ADR. The physician must then determine whether the patient's clinical symptoms are signs of a cutaneous ADR or of another skin disease not related to a drug. The diagnosis of a cutaneous ADR is based on three key clinical elements (Fig. 25.4): (1) **Appearance**- the morphology of the cutaneous eruption according to four main categories of the primary lesion: maculopapular, urticarial, bullous and pustular (see section "Morphological Classification of Cutaneous ADRs"). (2) **Systemic**- extra-cutaneous signs (fever, dyspnea, lymphadenopathy, etc.) that distinguish between a simple reaction involving only the skin and a complex reaction that includes systemic involvement in addition to the skin (see section "Severity of Cutaneous ADRs: Skin only (Simple) Versus Skin and Systemic Involvement (Complex)") and (3) **Histology**- histopathology and direct immunofluorescence studies of skin biopsies to confirm the clinical impression and to distinguish between a drug-induced eruption and other skin diseases (see section "Histological Classification of Cutaneous ADRs").

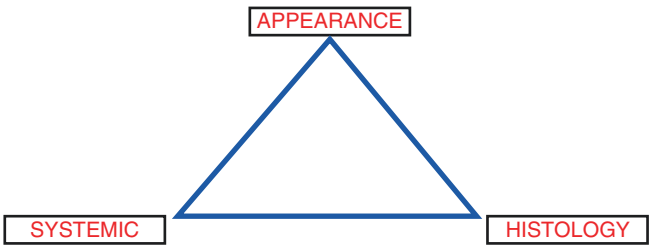

Fig. 25.4 Shear's Diagnostic Triangle of adverse cutaneous drug reactions

Differential Diagnosis

Establishing a differential diagnosis that takes into account all possible diagnoses is essential. Ranking the approximate likelihood of each condition is encouraged.

Drug Exposure (Timing)

All medications, regardless of route of administration, must be considered, especially new drugs taken in the 8 weeks prior to the skin reaction. Drugs taken intermittently, such as vitamins, sedatives, pain relievers, laxatives and natural products, must also be considered. Assessment of the lag period – the time between initiation of the drug and onset of the cutaneous reaction – is crucial in view of the different lag times for different cutaneous drug reactions. A recommended method for drug exposure analysis is to chart a timeline in order to visualize the chronology and facilitate comprehension of the event. The timeline includes the relevant information (starting day, dosage, and discontinuing day) for each drug and the signs and symptoms throughout the period in question [82].

Determine Probabilities

The most important challenge in assessing drug-induced skin injury is establishing whether there is a causal relationship between the suspected drug and the untoward clinical event. The following methods are helpful: (1) Patient history: the patient should be questioned about previous cutaneous reactions to drugs, and whether rechallenge with the drug improved the eruption [82]. These data should also be part of the above timeline. (2) Analysis of the literature: search for information regarding the frequency with which the type of reaction is related to a particular drug. (pubmed

http://www.ncbi.nlm.nih.gov/pubmed/, Litt's D.E.R.M. Drug Eruptions and Reactions Manual and database http://www.drugeruptiondata.com/). (3) Rate the reaction on Naranjo et al.'s adverse drug reaction probability scale, which classifies the drug reaction according to a probability category as definite, probable, possible or doubtful [224], and or on Edwards et al.'s causality assessment criteria [2]. (4) In vitro and in vivo diagnostic assessments including HLA genetic tests. (see section "Clinical and Laboratory Assays in the Diagnosis of Cutaneous ADRs").

Communication with the Patient and Family, Health-Care Providers and Regulatory Agencies

Patient and Family

Good communication strategies will aid in the interactions with the patient and family following a cutaneous ADR and decrease the likelihood of lawsuits, especially in cases of severe reactions such as SJS/TEN. Physicians are advised to follow these steps: (1) Express empathy and say "sorry" according to the "apology laws" in an honest and respectful fashion and in a way that protects the physician from having an apology used against him in case of legal action. http://www.sorryworks.net/. (2) Provide disclosure in a "disclosure meeting" planned according to the acronym CONES: Context – arrange the setting for a quiet, uninterrupted meeting and decide on the participants; Opening shot – the first sentence in the meeting explains the aim of the conversation; Narrative – lay out the facts; it is advised to avoid using the words "error" and "mistake" since the ADR is a result of multiple factors, particularly when the facts are not completely known; Emotions – provide an empathic environment; Summary (3) Provide the patient with clear information on his cutaneous ADR, the name of the offending drug, potential cross-reacting drugs, and drugs which can be safely taken as an alternative to the offending drug. In addition, advise the patient to wear a medic-alert bracelet. (4) Family counselling is part of the management plan since the predisposition to some cutaneous ADRs may be genetic [191].

Health-Care Providers

Information on the adverse event must be provided to the family physician and entered in the patient's records.

Regulatory Agencies

Report the cutaneous ADR to the manufacturer and regulatory agencies [225]. International reporting systems: MedWatch, the FDA Safety Information and Adverse Event Reporting Program http://www.fda.gov/safety/MedWatch/default.htm, and WHO Uppsala Monitoring Centre (UMC) http://www.who-umc.org.

Clinical and Laboratory Assays in the Diagnosis of Cutaneous ADRs

Pharmacogenomic Aspects of Drug Reactions

The strong associations found between HLA alleles and specific drug-induced hypersensitivity reactions have fostered pharmacogenetic testing to prevent the development of life-threatening drug-induced hypersensitivity reactions, such as SJS/TEN and DRESS. The usefulness of such testing is dependent on a number of factors, including the incidence and severity of the adverse event, the sensitivity and specificity of the predictive markers, and the availability of equally effective, alternative medications for individuals who test positive.

HLA-B*1502 Test for Prevention of Carbamazepine-Induced SJS/TEN

Although the incidence of SJS/TEN is relatively low, it is life-threatening and many patients who survive have long-term sequelae, such as ocular complications. HLA-B*1502 is a useful and strong predictive marker with high sensitivity and specificity for carbamazepine-induced SJS/TEN in Asian populations. This genetic association is strong enough that it prompted the USFDA and many countries to relabel the genetic information for carbamazepine, and to recommend screening for HLA-B*1502 before prescribing the drug for subjects of Asian descent. The HLA-B*1502 test for carbamazepine-induced SJS/TEN has very high sensitivity (near 100 %) and specificity (97 %). With the 0.25 % prevalence rate of carbamazepine-induced SJS/TEN among Chinese, the HLA-B*1502 test has a 7.7 % positive predictive value and 100 % negative predictive value for detecting [226]. In view of the serious consequences of SJS/TEN and the availability of alternative drugs, withholding carbamazepine from screened patients who test positive for HLA-B*1502 and switching to alternative antiepileptic drugs is reasonable and feasible in the high risk populations, including Chinese and South-East Asians.

HLA-B*5701 Test for Prevention of Abacavir Hypersensitivity

Abacavir is used in the treatment of HIV infection, and has been associated with drug hypersensitivity syndrome in 8 % of patients [227]. HLA-B*5701 is a strong and useful predictive marker with high sensitivity and specificity for abacavir hypersensitivity in Caucasians, prompting the USFDA and many other countries to recommend screening for it before prescribing the drug. The HLA-B*5701 test for immunologically-mediated abacavir hypersensitivity has very high sensitivity (100 %) and specificity (97.4 %) as well as positive predictive value (55 %) and negative predictive value (100 %) [55].

Other Potential Genetic Tests in Drug Hypersensitivity

HLA-B*5801 is a potentially useful predictive marker for allopurinol-induced SJS/TEN or DRESS, with 3 % positive predictive value and almost 100 % negative predictive value for detecting allopurinol-induced SJS/TEN or DRESS in Chinese (Table 25.2). This association was significant in Caucasian and other Asian populations as well. The recent American College of Rheumatology guidelines for the management of gout recommend HLA-B*5801 screening for populations with high frequency of the allele [228].

Other recently discovered HLA alleles related to drug hypersensitivity of potential usefullness in clinic practice are HLA-B*1301 for dapsone hypersensitivity [58], HLA-A*3101 for carbamazepine-related DRESS [65], and CYP2C9*3 for phenytoin hypersensitivity [69].

In Vitro Assessment

Lymphocyte Transformation Test (LTT) and Lymphocyte Activation Assays

The lymphocyte transformation test (LTT) is a widely used in vitro assay for the diagnosis and identification of offending drugs with T cell-mediated drug hypersensitivity [229]. LTT is based on the activation and proliferation of T cells from PBMC obtained from drug-sensitized patients after stimulation, and incubation with the culprit drug in vitro [230]. Following in vitro stimulation by specific drugs, drug-specific T cells are activated and release several cytokines that promote proliferation of T cells. This in vitro proliferation of specific drug-activated T cells can be detected by the incorporation of 3H-thymidine during DNA synthesis after 6 days of culture. The results of LTT are expressed as the stimulation index (SI): the relationship between the 3H-thymidine uptake in cells (counts per minute (c.p.m.)) with and without the drug antigen [229]. The general sensitivity of the LTT is 50–80 %, varying with different drugs and different phenotypes of delayed-type hypersensitivity reactions; thus, a negative result does not exclude the possibility of drug hypersensitivity. Extensive studies on LTT for beta-lactam drugs report even higher sensitivity [230–234]. The specificity of the LTT is 85–100 % in different studies [231–233, 235].

LTT for the diagnosis of drug hypersensitivity has limitations. Because it is measured by radioisotopes, the sensitivity can be very low and negative results are commonly observed for specific drugs (e.g., allopurinol, lamotrigine) and specific phenotypes (e.g., SJS/TEN) [236, 237]. Several non-radioactive methods have been developed for measuring lymphocyte proliferation or activation in in vitro tests for diagnosis of delayed-type drug hypersensitivity, including the use of carboxyfluorescein succinimidyl ester (CFSE) cell staining dye [238, 239], and measuring cytokines or cytotoxic proteins expression, such as INF-γ, IL-2, IL-4, IL-5, IL-13, granzyme-B, and macrophage migration inhibitory factor [240–244].

Basophil Activation Test (BAT)

Flow cytometry-assisted basophil activation test (BAT), which measures specific cell makers such as CD69 or CD203c to quantify basophil activation after antigen-specific stimulation, has been widely used in the diagnosis of immediate-type drug hypersensitivity [245]. BAT directly measures basophil responses instead of IgE sensitization. It has been applied to the diagnosis of different drugs implicated in immediate-type hypersensitivity, including beta-lactam antibiotics, neuromuscular blocking agents, aspirin, NSAIDs and radiocontrast media [246–248]. The sensitivity of BAT varies in different types of drugs: that for beta-lactam antibiotics ranged from 28.6 to 55 % [249, 250]; that for NSAIDs ranged from 30 to 70 % [251, 252].

Computational Analysis for HLA Alleles

Recent data have shown that the unique interaction between drug, T-cell receptor and HLA molecule is a key factor in the development of immune-mediated adverse reactions to drugs. The discovery of strong association of specific HLA alleles with specific drug-induced hypersensitivity (e.g., HLA-B*1502 to carbamazepine-SJS/TEN, HLA-B*5801 to allopurinol-SJS/TEN/DRESS, and HLA-B*5701 to abacavir hypersensitivity), and studies of the functional role of HLA-B* allele (e.g., HLA-B*1502) directly interacting with a specific drug (e.g., carbamazepine) and unique T-cell receptor support the hypotheses of the ‘pharmacological interaction with immune receptors’ (p-i) [18, 19, 253].

In recent years, bioinformatics and computer modeling have been applied to elucidate how drug molecules interact with specific HLA in drug hypersensitivity. HLA alleles have been associated with liver injury induced by different drugs (such as flucloxacillin). Using silico strategies to examine HLA haplotype relationships, and bioinformatics tools, Alfirevic et al. [254] demonstrated a connection between the different HLA alleles associated with drug-induced liver injury caused by therapeutically and structurally different drugs, suggesting a mechanism of peptide binding of one of the associated HLA alleles [254]. Computer modeling of the molecular interaction between HLA-B*1502 and carbamazepine predicted a favorable drug-binding position in the B pocket of the HLA-B*1502 protein, where the side chain of Arg62 could form a hydrogen bond with the ketone group of 5-carboxamide of carbamazepine (Fig. 25.5) [253].

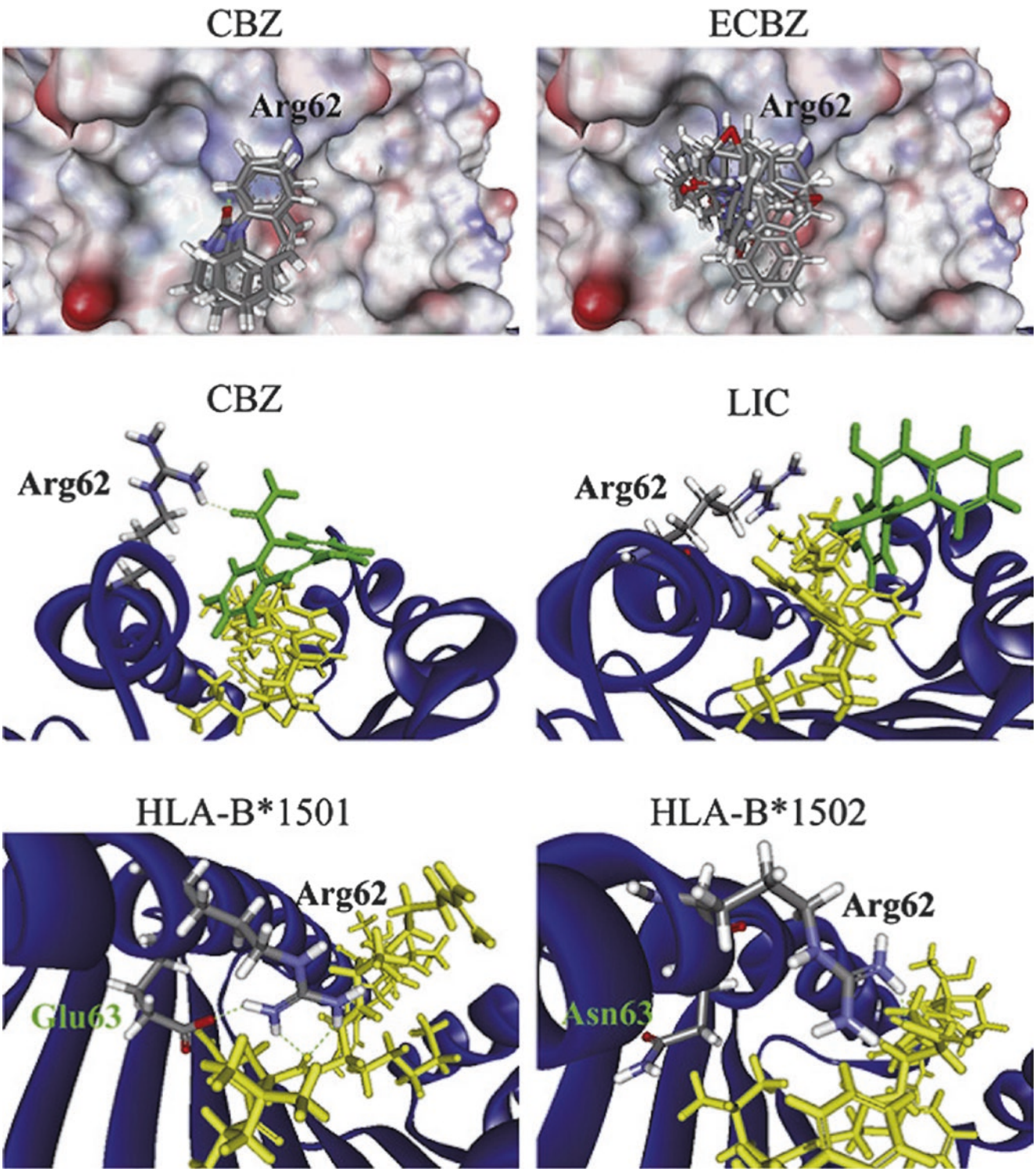

Fig. 25.5 Computer modeling of the molecular interaction between HLA-B*1502 and carbamazepine and its derivatives (Figure from Wei et al. [253])

Conclusion

Cutaneous ADRs have a wide spectrum of clinical manifestations that may be caused by multiple drugs and different mechanisms. In this decade, our understanding of the pathogenesis of cutaneous ADRs had progressed greatly. Understanding how a drug can possibly cause reactions in the skin has led to an understanding of the cellular immunology, cytokines and immunogenetics. These key insights can help mitigate the risk of reactions by testing for genetic factors, and to understand the treatment of drug reactions by better understanding the pathways involved. The future will depend on better genetic screening and directed approved therapies.

Review Questions

1. What is the major immune cell in acute generalized exanthematous pustulosis (AGEP)?
   A. CD4 T cells
   B. CD8 T cells
   C. Eosinophils
   D. Neutrophils
   E. NK cells

2. In which of the following delayed-type hypersensitivity reactions is interleukin (IL) 8 known to be involved?
   A. acute generalized exanthematous pustulosis (AGEP)
   B. drug reaction with eosinophilia and systemic symptoms (DRESS)
   C. maculopapular drug eruption (MPE)
   D. Stevens-Johnson syndrome (SJS)
   E. Toxic epidermal necrolysis (TEN)

3. The HLA gene B5701 and hypersensitivity reactions MPE/DRESS are associated with use of which of the following drugs?
   A. Abacavir
   B. Allopurinol
   C. carbamazepine
   D. Dapsone
   E. Nevirapine

4. What is the most common group of drugs causing pseudoporphyria?
   A. Antibiotics
   B. Diuretics
   C. NSAIDS
   D. Protease inhibitors
   E. Reverse transcriptase inhibitors

5. Which drug is most closely associated with fixed drug eruptions on the male genitalia?
   A. Allopurinol
   B. Carbamazepine
   C. Minocycline
   D. Tetracycline
   E. Vancomycin

Answers

1. D
2. A
3. A
4. C
5. D

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Cutaneous Vasculitis: A Clinical Approach

26

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Abstract

Vasculitis, an inflammatory condition of blood vessel walls, varies in severity from a self-limited disorder to a life-threatening disease, and can be caused by many different mechanisms. This condition can be an idiopathic primary process or a secondary manifestation of certain triggers such as infection, malignancy or systemic inflammatory conditions. Vasculitis can present many challenges to the clinician, including diagnosis and classification, necessary diagnostic studies and appropriate treatment. Depending on the organs and caliber of blood vessels involved, vasculitis can manifest with a wide spectrum of clinical and histopathologic findings. In cases of suspected vasculitis, the laboratory and histolopathologic studies yield invaluable information beyond the history and physical examination. Confirmation of vasculitis should prompt a search for potential underlying systemic disorders. Treatment for vasculitis is driven by the severity of symptoms and extent of organ involvement. Most cases of vaculitis are isolated occurrences and can be managed conservatively with rest and elevation of affected limb, and nonsteroidal anti-inflammatory drugs for symptom control. For disease that is recalcitrant or with evidence of systemic involvement, more aggressive therapy, including immunosuppressive agents is necessary. Traditional agents for treating vascultis include corticosteroids, cyclophosphamide, azathioprine, methotrexate, colchicine, dapsone and mycophenolate mofetil. Newer modalities include rituximab and TNF-α inhibitors. This chapter reviews the classification and diagnosis of cutaneous vasculitic conditions and current treatment options.

Keywords

Skin Disease • Cutaneous vasculitis • Blood vessel disease • Skin pathology • Direct immunofluorescence • Indirect immunofluorescence • Immunosuppressive agents

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Key Points

- Vasculitis can be caused by many different mechanisms.
- Skin biopsies for hematoxylin and eosin and direct immunofluorescence are the cornerstones of diagnosing vasculitis.
- Serology, histories, and physical examinations can help determine the type of vasculitis.
- Treatments can include traditional agents such as corticosteroids, cyclophosphamide, azathioprine, methotrexate, colchicine, dapsone and mycophenolate mofetil, as well as newer modalities that include rituximab and TNF-α inhibitors.

Vasculitis is defined as inflammation of blood vessel walls. With inflammation of the vasculature, there is resultant wall destruction and increased permeability, which can lead to aneurysm formation, extravasation of blood cells, and stenosis. Clinically, these processes present as hemorrhage, tissue ischemia, or infarction of the affected organ. Depending on the organs and caliber of blood vessels involved, vasculitis can manifest with a wide spectrum of clinical findings, from a benign, self-limiting course, to death. Vasculitis of any organ can be a primary process (idiopathic) or a secondary manifestation of other triggers such as trauma, infection, malignancy, systemic inflammatory conditions, connective tissue disease, and drug hypersensitivity.

The skin in particular is among the most common organs affected by vasculitis due to its rich vascular supply, exposure to cold temperatures and trauma, and predisposing hemodynamic conditions (e.g., venous hypertension, stasis in the lower extremities).

Because lesions of the skin are readily visible to the clinician, often they are the first indication of potentially life-threatening vasculitic processes occurring elsewhere in the body. As such, it is critical to develop a systematic and thorough approach to the patient with suspected vasculitis. Cutaneous signs are varied and are primarily a reflection of the size of the affected vessels. Involvement of small, superficial vessels results in erythema and purpuric macules, whereas deeper involvement of larger vessels presents with increasingly severe lesions including livedo, palpable purpura, vesicles, ulcers, urticaria, subcutaneous nodules, distal gangrene, and necrosis. In this regard, the morphology of lesions can be a clue to the underlying vasculitic process; however, multiple lesion types are often present, as a range of blood vessel sizes may be involved. While the majority of cases of vasculitis restricted to the skin are self-limited, the physiologic response to inflammation from blood vessels results in the release of chemical mediators that may give rise to a variety of systemic findings, such as malaise, fever, weight loss, arthralgias, arthritis, myalgia, night sweats, and laboratory abnormalities [1–3]. Therefore, a complete history, physical exam, review of systems, and laboratory workup are necessary to further classify suspected vasculitis and identify extracutaneous involvement. In all cases, biopsy and clinical histologic correlation provide the gold standard for diagnosis. Herein, features common to most, if not all, variants of cutaneous vasculitis are described, including workup, common etiologies, and specific presentations.

Cutaneous Vasculitides

Cutaneous vasculitis most often manifests as palpable purpura in dependent areas and under tight-fitting clothing [4]. These lesions may be asymptomatic, tender, or pruritic, and, depending on the disease process and the size of the affected vessels, there may be varying degrees of involvement of other organs such as the kidneys, gastrointestinal (GI) tract, or lungs [5]. While accompanying signs and symptoms may give hints to systemic disease, there are no pathognomonic indicators of extracutaneous involvement, although tools have been proposed that may identify patients with more extensive disease [6, 7]. Furthermore, because different vasculitic processes can present with the same skin findings, and because many conditions can mimic vasculitis, prognosis cannot be determined from physical examination alone [8–11].

The annual incidence of biopsy-confirmed cutaneous vasculitis has been reported from 40 to 60 cases per million [12, 13]. Approximately 40 % of vasculitides limited to the skin are idiopathic and typically self-limited [12–14]. Other etiologic factors have been described (Table 26.1), with infection and drug reaction being most common among these [12–14]. There is a slight predilection in women over men, and all ages can be affected (mean adult onset = 47 years, mean pediatric onset = 7 years), with 90 % of pediatric cases represented by HSP. [12]

Initial Workup of Cutaneous Vasculitis

In cases of suspected vasculitis, the laboratory results and the histologic workup provide invaluable information beyond the history and the physical examination in determining the degree of systemic involvement and uncovering underlying causes for the vasculitic process. While physical findings can afford initial clues as to the types of vessels involved, the diagnosis of vasculitis can only be confirmed histologically. As the histopathologic diagnosis does not lend information regarding the degree of extracutaneous involvement, a clinicpathologic correlation is necessary in the evaluation of these

Table 26.1 Causes of cutaneous vasculitis [12–14]

Causes	Frequency (%)
Idiopathic	40
Infection	22
Drug reaction	20
Connective tissue disease	12
Henoch-Schönlein purpura	10
Malignancy	<5
Systemic vasculitis	<5
Other systemic disease	<5

Table 26.2 Suggested laboratory workup for suspected vasculitis [13–15]

Standard workup[a]	Other tests[b]
CBC with differential	Renal biopsy
CMP	Nerve conduction studies
UA with microscopic evaluation	Hypercoagulability panel
Biopsy for H&E and DIF	Echocardiogram
Blood and urine cultures	
Rheumatoid factor	
C-reactive protein	
ANA	
ANCA	
Cryoglobulins	
Complement (C3, C4, CH50)	
Hepatitis panel	
Stool guaiac	
Chest x-ray	
HIV	
SPEP/UPEP	

[a]*CBC* complete blood count, *CMP* complete metabolic panel, *UA* urinalysis, *H&E* hematoxylin and eosin staining, *DIF* direct immunofluorescence, *ANA* antinuclear antibody, *ANCA* antineutrophil cytoplasmic antibody, *SPEP/UPEP* serum protein electrophoresis/urine protein electrophoresis

[b]Other tests to consider based on the individual case

patients. Recommended initial laboratory workup for all patients and other tests that should be considered on a case-by-case basis have been described (Table 26.2) [13–15]. Taken together, these data ultimately contribute to decisions regarding prognosis and treatment modalities. For example, serum complement is a known mediator of vascular inflammation, and low levels indicate excessive consumption, suggesting more extensive or systemic involvement [16]. An elevated erythrocyte sedimentation rate (ESR) has been described in approximately 50% of patients with limited cutaneous vasculitis [17]. An ESR of 40 mm/h or greater is associated with a high probability of systemic involvement [14].

Skin biopsies sent for both routine hematoxylin and eosin (H&E) staining as well as direct immunofluorescence (DIF) are the cornerstones in determining whether physical findings represent true vasculitis or other conditions that can clinically mimic vasculitis. Decisions as to which lesions should be biopsied have direct impact on the diagnostic information elicited. Tissue should be acquired from lesions between 24 and 48 h after their appearance, as sampling before or after this time range may result in false-negative results. Biopsies with thrombosis or perivascular lymphocytic inflammation are characteristic of older lesions. In cases where suspicion of vasculitis is high but histology does not correlate with clinical findings, biopsy should be repeated. Likewise, if a medium-vessel vasculitis such as polyarteritis nodosa (PAN) is suspected, the biopsy must be of sufficient depth to include the subcutaneous tissue where these vessels are found. In general, ulcerated lesions should be avoided [1].

The diagnosis of vasculitis is confirmed unequivocally by the presence of an inflammatory infiltrate around and within the walls of vasculature with fibrin deposition. These areas of fibrinoid necrosis are accompanied by swelling and necrosis of endothelial cells, as well as secondary changes such as erythrocyte extravasation and necrosis leading to purpura and infarction, respectively [1, 12]. Apoptotic cells are seen

Table 26.3 Agents commonly reported

Medication	
Minocycline	Famciclovir
Carbamazepine	Montelukast
Hydralazine	Infliximab
Isoniazid	Etanercept
Propylthiouracil	Rituximab
Allopurinol	Ciprofloxacin
Metformin	

Reported to cause vasculitis [28–40]

frequently as well as overlying ulceration. Determination of vessel size, type of cellular infiltrate, depth, and degree of involvement on H&E stains helps in classification and generation of differential diagnoses.

The pathogenic features of vasculitis in the skin are related to vessel wall injury, which can be toxin mediated, immune mediated, or from direct infection, and all three mechanisms can result in the histologic pattern of fibrinoid necrosis. It is critical to identify those patients in which pathogenesis is caused by antibody-mediated toxicity and immune complex formation, as they are more likely to have extracutaneous involvement [1].

Deposition of immune complexes leads to complement activation, further recruitment of inflammatory cells and cytokines, and expression of adhesion molecules such as E-selectin, P-selectin, and intercellular adhesion molecule 1 (ICAM-1) [12]. With endothelial cell retraction, there is vascular deposition of immune complexes, neutrophil infiltration, edema, hemorrhage, and thrombosis [12].

Immunofluorescence is an essential diagnostic tool in the evaluation of cutaneous vasculitis, especially in the small-vessel group. This technique consists of the detection of immunoglobulins (Igs) and complement deposited within tissue. Deposition of IgA, IgG, IgM, and C3 in or around vessels identified by DIF characterizes antibody and immune complex–mediated vasculitis, and the patterns of deposition further classify disease. Lesion age is critical to evaluation, as up to 30 % of immune-mediated vasculitides are negative on DIF by 72 h and only C3 is detected after this point [17, 18]. Tissue samples are transported in Michel's medium and stored at 4 °C prior to processing at specialized laboratories. Samples are washed, flash frozen, sectioned, and then stained to detect antibodies and complement in and around blood vessels. Certain diagnoses cannot be made without characteristic DIF patterns, which will be discussed with their associated conditions below.

Serologic testing has become routine in evaluation of vasculitis. In particular, antineutrophil cytoplasmic antibodies (ANCAs) have established clinical utility in dermatology [19]. Initially described in patients with rheumatoid arthritis, ANCA-associated vasculitides include small and medium-sized involvement such as Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and many drug-induced cases of vasculitis, as well as systemic inflammatory conditions and connective tissue diseases [20]. It is believed that vasculitides associated with ANCAs have a distinct mode of pathogenesis [20, 21]. Antibodies can be directed against cytoplasmic (c-ANCA) and perinuclear (p-ANCA) neutrophil-derived products. C-ANCA antibodies are directed against proteinase 3 (PR3) and p-ANCA antibodies are directed toward myeloperoxidase (MPO), elastase and lactoferrin [20, 21]. Inflammatory cytokines are believed to induce the translocation of these targets to the surface of neutrophils, allowing binding of ANCAs and adherence to endothelial cells, ultimately causing damage to vessel walls [22]. Titers may predict clinical relapse or disease activity, and serial testing is recommended, as transient elevations in ANCAs can be seen with acute infections [23, 24]. Because c-ANCA is represented by only one antigen (PR3), enzyme-linked immunosorbent assay (ELISA) is a more sensitive and specific assay. Since several antigens are responsible for the p-ANCA pattern, immunofluorescence is preferred.

Drug–Induced Vasculitis

Medications from virtually every pharmacologic class (including herbal supplements) have been linked to drug-induced vasculitis, resulting in a range of clinical presentations (Table 26.3) [25–40]. In a MEDLINE database search for published cases of drug-induced vasculitis by ten Holder et al. [41], vasculitis was more often associated with propylthiouracil, hydralazine, colony stimulating factors, allopurinol, cefaclor, minocycline, D-penicillamine, phenytoin, isotretinoin, and methotrexate. The onset of findings after exposure to the causative agent is typically 5–20 days [1], and while withdrawal is often sufficient to reverse the vasculitic process, there have been cases of fatal drug induced allergic vasculitis in previously healthy patients [42]. There have also been reports of cutaneous vasculitis stemming from vaccines [43]. Ironically, many

Table 26.4 Immunoglobulin A (IgA) vs. non–immunoglobulin A vasculitides [46–51]

IgA predominance?[a]	No IgA or IgG/IgM predominance?[a]
IgA vasculitis	Non-IgA vasculitis
Henoch-Schönlein purpura	Cryoglobulinemia II/III
	HUVS[b]
	Rheumatoid vasculitis
	Connective tissue disease
	Wegener's granulomatosis
	Churg-Strauss syndrome
	Microscopic polyangiitis
	Behçet's syndrome
	Paraneoplastic vasculitis

[a]Predominance seen on direct immunofluorescence
[b]*HUVS* hypocomplementemic urticarial vasculitis syndrome

of the medications used for the treatment of systemic inflammatory conditions have also been linked to the development of cutaneous vasculitis [37–39]. Although the precise pathogenic mechanisms are varied, there appears to be a combination of cell-mediated and humoral immune responses contributing to the development of the observed vasculitis. As such, a thorough medication history is critical in the initial evaluation of the patient with suspected vasculitis, and all recently added medications should be discontinued. In those patients with both p-ANCA and c-ANCA, as well as eosinophilic infiltrates, the notion of drug-induced vasculitis should be entertained [44, 45]. Finally, one should keep in mind that the vast majority of drug-induced small-vessel vasculitis falls into the category of cutaneous leukocytoclastic angiitis (hypersensitivity vasculitis) or IgA vasculitis (Henoch-Schönlein purpura).

Vasculitis Associated with Systemic Conditions

Cutaneous vasculitis may result from a number of systemic triggers such as infectious, inflammatory, autoimmune, and malignant diseases, as well as pregnancy, and be of varying severity (Table 26.4) [46–51]. The workup of patients may reveal an underlying condition. Vasculitis can present prodromally or at any time during the disease. Among malignant diseases associated with vasculitis, hematologic cancers are seen most frequently. Bachmeyer and colleagues [52] found that of 95 hospitalized patients with hematologic malignancies, 23 (24 %) had biopsy proven cutaneous vasculitis. Skin findings developed before (26 %), during (39 %), and after (35 %) the diagnosis of malignancy. A thorough history, physical, and laboratory workup often point to the underlying disease processes, and in cases where there is failure to respond to treatment, investigation for occult malignancy should be considered.

Treatment

If systemic conditions are excluded and potentially causative agents discontinued, treatment for vasculitis is driven by the severity of symptoms and extent of extracutaneous involvement. Many cases are isolated occurrences and can be managed supportively with rest, warming, compression and elevation of affected lower extremities. Symptomatic treatment for pain and inflammation can often be accomplished with nonsteroidal anti-inflammatory drugs, and pruritus with antihistamines. For recalcitrant disease or with evidence of systemic involvement, more aggressive therapy, including immunosuppressive agents, is necessary. Therapeutic decisions are based on the experience of the clinician and the details of the specific patient. Initially, systemic corticosteroids alone or in combination with steroid-sparing immunosuppressants are often employed. Long-term, steroid-sparing medications are used for control with systemic corticosteroids limited to disease flares, as to avoid the well known adverse effects of chronic corticosteroids. Each medication has certain adverse effects (i.e., bone marrow toxicity, impaired renal function and drug-induced bladder complications) and regular and frequent laboratory evaluations are necessary to screen for these. Corticosteroids, cyclophosphamide, azathioprine, methotrexate, colchicine, dapsone and mycophenolate mofetil have been reported to treat vasculitis [14].

Corticosteroids demonstrate great utility across the spectrum of autoimmune and inflammatory disorders by suppressing effects of cytokines (IL-1, TNF-α), T-cells and B-cells. Circulating T-cell depletion occurs from corticosteroid enhanced circulatory emigration, induction of apoptosis, inhibition of T-cell growth factors, and impaired release of cells from lymphoid tissues [53]. Higher corticosteroid doses render significant B-cell effect and reduced immunoglobulin production.

Cyclophosphamide is an alkylating agent first developed to treat malignancies and has been used in the treatment of certain autoimmune diseases. Having potent immunosuppressive effects it depresses B-cell function more than T-cell function. Cyclophosphamide is used often in combination with corticosteroid therapy, considered the first-line treatment for Wegener's granulomatosis [54]. Cyclophosphamide is also used for remission induction with less toxic medications (i.e., methotrexate or azathioprine) for maintenance therapy.

Azathioprine, initially used in transplantation medicine for immunosuppressant properties, is also often used for its anti-inflammatory qualities. Azathioprine's active metabolites are 6-thioguanine monophosphate and other metabolites [55]. The exact mechanisms by which these purine analogs lead to immunosuppressive and anti-inflammatory effects are not clear.

Methotrexate is an antimetabolite chemotherapeutic agent with immunosuppressive effects used for inflammatory and immune-mediated processes. This drug is a potent competitive antagonist of dihydrofolate reductase, thereby inhibiting DNA synthesis and cell division. The immunosuppressive effects of methotrexate stem from inhibition of DNA synthesis in immunologically competent cells, suppressing antibody responses [56]. The anti-inflammatory effects are predominantly mediated by adenosine [57].

Colchicine has both antimitotic and anti-inflammatory properties and has demonstrated utility in dermatologic diseases characterized by polymorphonuclear leukocyte infiltration. Within leukocytes, colchicine binds to the dimers of tubulin, preventing the assembly of tubulin subunits into microtubules, thereby leading to arrest of mitosis [58].

Dapsone is useful in treating many skin diseases, primarily those characterized by neutrophilic infiltrates in the skin. Dapsone has been thought to accomplish this by altering neutrophil chemotaxis and respiratory burst, thereby leading to reduced oxidative damage in tissues [59]. The exact mechanisms are not clear, however.

Mycophenolate mofetil has demonstrated efficacy in multiple types of autoimmune and inflammatory skin diseases. MMF is a prodrug which first must be converted to the active drug form mycophenolic acid (MPA). MPA inhibits inosine monophosphate dehydrogenase, thus depriving the T- and B-lymphocytes of purine metabolites necessary for growth and replication and subsequent immunosuppression [60].

Often, these treatment regimens are sufficient to control disease activity with few adverse effects when appropriate monitoring is performed. Some patients, however, may be refractory to these treatments or develop intolerable side effects from therapy. These factors have ushered in an era of alternative treatment modalities with less toxicity and greater efficacy. More recently, effective treatments with tumor necrosis factor-α (TNF-α) inhibitors [61, 62] and anti-CD20 antibodies have been described [63, 64]. Rituximab is a chimeric monoclonal antibody directed against the B-cell lineage specific CD20 antigen, yielding B-cell depletion activity and subsequent inhibition of antibody production. Originally developed for the treatment of B-cell non-Hodgkin's lymphoma, rituximab has increasingly been used to treat a variety of immune-mediated disorders.

Clinical Mimickers of Cutaneous Vasculitis

A variety of conditions are capable of clinically simulating cutaneous vasculitis and have been termed pseudovasculitides [65]. Many of these conditions can be excluded by biopsy, and are typically associated with conditions that cause hemorrhage or vessel occlusion, and should always be in the differential diagnosis for vasculitis. Vessel wall dysfunction or incompetence from infiltrative processes, nutritional deficits such as scurvy [66], infection, embolism, vasospasm, and trauma can all present with varying degrees of purpura, petechiae, ecchymoses, ulcers, and necrosis. Likewise, hypercoagulable states such as antiphospholipid syndrome and factor V Leiden can lead to similar clinical pictures and need to be excluded [8].

Classification of Cutaneous Vasculitis

Classification of vasculitis in the skin is typically based on the size of predominantly affected blood vessels and type of inflammatory response, which when interpreted with DIF examination and laboratory workup, correlate with disease etiology and affect the treatment decisions. Vessels of varying sizes are frequently involved, however, as vasculitic processes do not always recognize arbitrary boundaries of vessel size. Most texts refer to the classification schemes outlined by the Chapel Hill Consensus Criteria (Table 26.5) or the American College of Rheumatology, although it is often difficult to characterize individual patient variations [67–69].

Small blood vessels are ubiquitous in the skin. They include arterioles, capillaries, and postcapillary venules. They are typically 50 μm or less in diameter, and may not have a fully developed muscular layer. Clinical lesions of cutaneous small-vessel vasculitis (CSVV) are most commonly nonblanchable and purpuric, and are found in dependent areas (buttocks, back, lower extremities). When urticarial lesions are present, they are less pruritic, short-lived (<24 h), and can occur anywhere on the body. They include Wegener's granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis, Henoch-Schönlein purpura, essential cryoglobulinemic vasculitis, and cutaneous leukocytoclastic angiitis.

Table 26.5 Classification of cutaneous vasculitis according to the Chapel Hill Consensus Conference [67]

Large vessel vasculitis	Medium vessel vasculitis	Small vessel vasculitis
Takayasu arteritis	Polyarteritis nodosa	Microscopic polyangiitis
Giant cell arteritis	Kawasaki disease	Granulomatosis with polyangiitis[a]
		Eosinophilic granulomatosis with polyangiitis[b]
		Cryoglobulinemic vasculitis
		IgA vasculitis[c]
		HUVS (anti-C1q vasculitis)[d]

[a]Wegener's Granulomatosis
[b]Churg-Strauss syndrome
[c]Henoch-Schönlein purpura
[d]Hypocomplementemic urticarial vasculitis

Medium-sized blood vessels are larger than 50 μm, have fully developed muscular layers, and are located deeper within the dermis or subcutaneous fat. Clinically, these processes present with livedo, nodules, ulcerations, or digital infarcts. Wedge biopsy is typically needed for sufficient diagnostic yield, and biopsy of necrotic or ulcerated areas is of low yield. Included among this group are polyarteritis nodosa and Kawasaki disease. Vasculitis of these vessels is commonly referred to as necrotizing vasculitis, reflecting the hyalinization, coagulative necrosis, and degeneration of muscular layers, where it is more readily visible. Occasionally nerve or muscle biopsy can provide additional diagnostic information if histology is inconclusive.

Large-vessel vasculitides rarely have cutaneous manifestations and include giant cell (temporal) arteritis and Takayasu's arteritis, and will be mentioned only briefly.

Cutaneous Leukocytoclastic Angiitis

As defined by the Chapel Hill Criteria, cutaneous leukocytoclastic angiitis (CLA) is the term applied to patients with hypersensitivity vasculitis. Exogenous chemicals, infectious agents, cytokines, and circulating immune entities that do not strongly activate complement can cause CLA by inducing an inflammatory cascade in endothelium of CSVV. Most commonly triggered by infections or drugs, the onset is acute with both palpable and nonpalpable purpuric and urticarial lesions on the lower extremities appearing 5–20 days after initial exposure, and 2–4 days after repeat exposures (Fig. 26.1) [70]. These cases tend to be single episodes, and relapsing cycles can result from systemic inflammatory conditions, infection, and malignancy. Extracutaneous involvement is rare, with the exception of constitutional symptoms caused by mediators of inflammation released locally [71]. Serum sickness resulting from the injection of nonhuman serum can present with a similar picture, but is rarely seen in modern practice.

Routine laboratory tests are usually normal, as extracutaneous disease is rare. The ESR is elevated in up to 50% of cases, while complement levels and urinalysis are normal. There are no specific serologic markers for CLA, making it largely a diagnosis of exclusion, and normocomplementemic urticarial vasculitis is likely to be a clinical variant of this condition [72].

On histology, there is a neutrophil predominant vasculitis of superficial vessels with varying numbers of surrounding eosinophils (Fig. 26.2). Direct immunofluorescence is positive in roughly half of these biopsies, displaying mild to moderately intense granular IgM deposits with weak or absent C3. The lack of complement involvement may correlate with the relatively benign course of this condition and low level of systemic involvement. Although DIF is frequently negative, it is a key factor in discriminating CLA from IgA vasculitis, which shares the same triggers and clinical presentation [73, 74].

Up to half of the cases of CLA are idiopathic and resolve spontaneously. In cases with a known trigger, treatment consists of removal of the offending agent or resolution of underlying systemic condition. Immunosuppressive treatment is largely unnecessary in CLA, with the exception of the most severe cases, and is aimed at reducing constitutional symptoms and synovitis. Moderately dosed corticosteroids (0.5 mg/kg/day) are a reasonable option until symptoms resolve. Recalcitrant cases warrant more extensive investigation. Rituximab therapy has demonstrated efficacy in the treatment of cutaneous angiitis refractory to high dose corticosteroids and cyclophosphamide [75].

Henoch-Schönlein Purpura

Henoch-Schönlein purpura (HSP) is defined as an IgA-mediated syndrome presenting with a tetrad of purpura, abdominal pain, arthralgias, and hematuria [76]. It is most commonly seen in children, but there have been increasing reports of HSP in adults [76, 77]. HSP runs a more severe

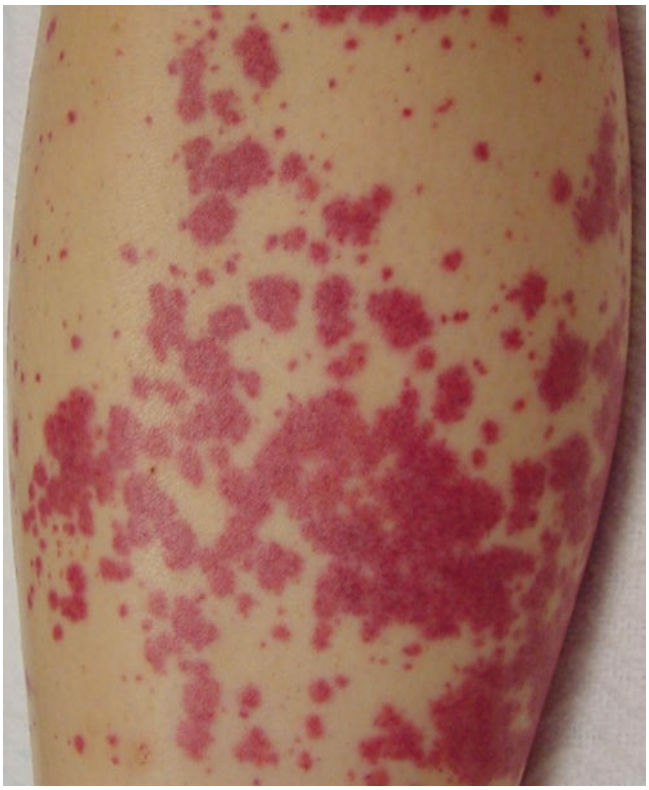

Fig. 26.1 Acute palpable purpura of cutaneous leukocytoclastic angiitis (hypersensitivity vasculitis)

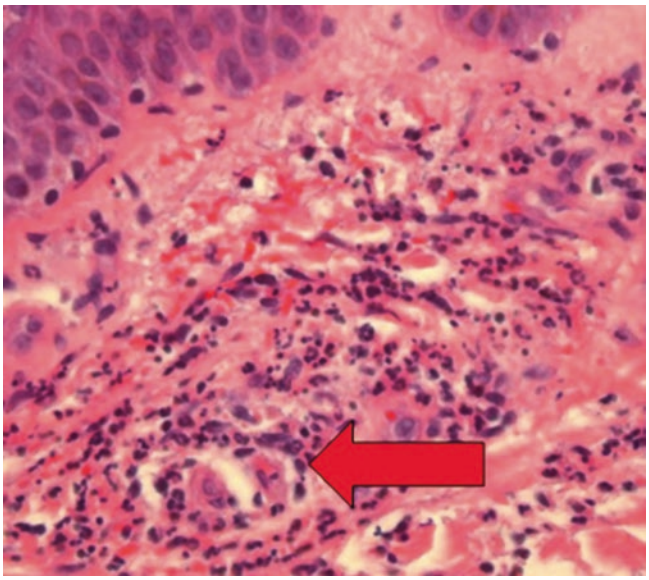

Fig. 26.2 Hematoxylin and eosin (H&E) staining of skin biopsy from a patient with cutaneous small-vessel vasculitis. This is a leukocytoclastic vasculitis involving small, superficial vessels, rich in neutrophils (*arrow*)

course and is more likely to cause long-term renal disease in adults compared to children [78, 79].

As with CLA, HSP is often preceded by medications or infection, most commonly upper respiratory, gastrointestinal (GI), and genitourinary (GU). When associated with GI and GU infections, it can be difficult to discern findings related to infection from those caused by the vasculitic process itself. Approximately 50 % of those affected develop systemic involvement such as nephritis, neuropathy, and GI symptoms. [80] Skin lesions are seen in all patients and are typically palpable purpura or urticaria of the lower extremities and buttocks that turn into purpura with annular configuration (Fig. 26.3). Koebnerization is known to occur in HSP. A subset of patients displays only urticarial lesions, and lesions above the waist have been associated with renal disease, as are elevated ESR, fever, and adult onset [81]. Women have an increased risk for the development of proteinuria or preeclampsia in future pregnancies [82, 83].

Extracutaneous involvement can appear in any organ, with the kidneys, GI tract, and joints being most common. Renal failure secondary to glomerulonephritis is the most serious complication of HSP.

Renal involvement occurs in about 33 % of children and 63 % of adults with HSP. [84] The risk of progression to renal insufficiency ranges from 5 to 15 % in children and seems to be much higher in approximately 30 % in adults [77]. Children with renal involvement have a higher incidence of hypertension and renal failure as adults, and 15 % of children on hemodialysis have renal failure secondary to HSP. [85] Urinalysis is an absolute requirement for the patient with suspected HSP, with hematuria providing the most sensitive measurement for renal involvement. Recent studies have revealed abnormalities of IgA1 glycosylation and formation of autoantibodies to aberrantly glycosylated IgA1 molecules with subsequent mesangial deposition and renal disease [86, 87]. New noninvasive disease activity markers (aberrantly glycosylated IgA1 and its immune complex) may aid in the diagnosis and help guide therapy. Gastrointestinal involvement can range from pain to hemorrhage and necrosis of the bowel from mesenteric vasculitis, and pulmonary hemorrhage has also been reported [88]. However, it is difficult to determine if involvement of these organs is the result of a prodromal infection or from the vasculitic process itself.

There is mounting evidence that acute hemorrhagic edema of infancy (AHEI) is a variant of HSP, although in contrast with HSP, extracutaneous involvement is rare and IgA deposition is rarely seen with DIF. [89] AHEI is frequently seen after bacterial infection, and lesions appear on the face and extremities. There are no specific serology associations with HSP, although up to 40 % of adult patients demonstrate some degree of IgA gammopathy [81]. When appropriate, additional workup should include serum and urine protein electrophoresis (SPEP and UPEP), total immunoglobulin quantitation, and immunofixation.

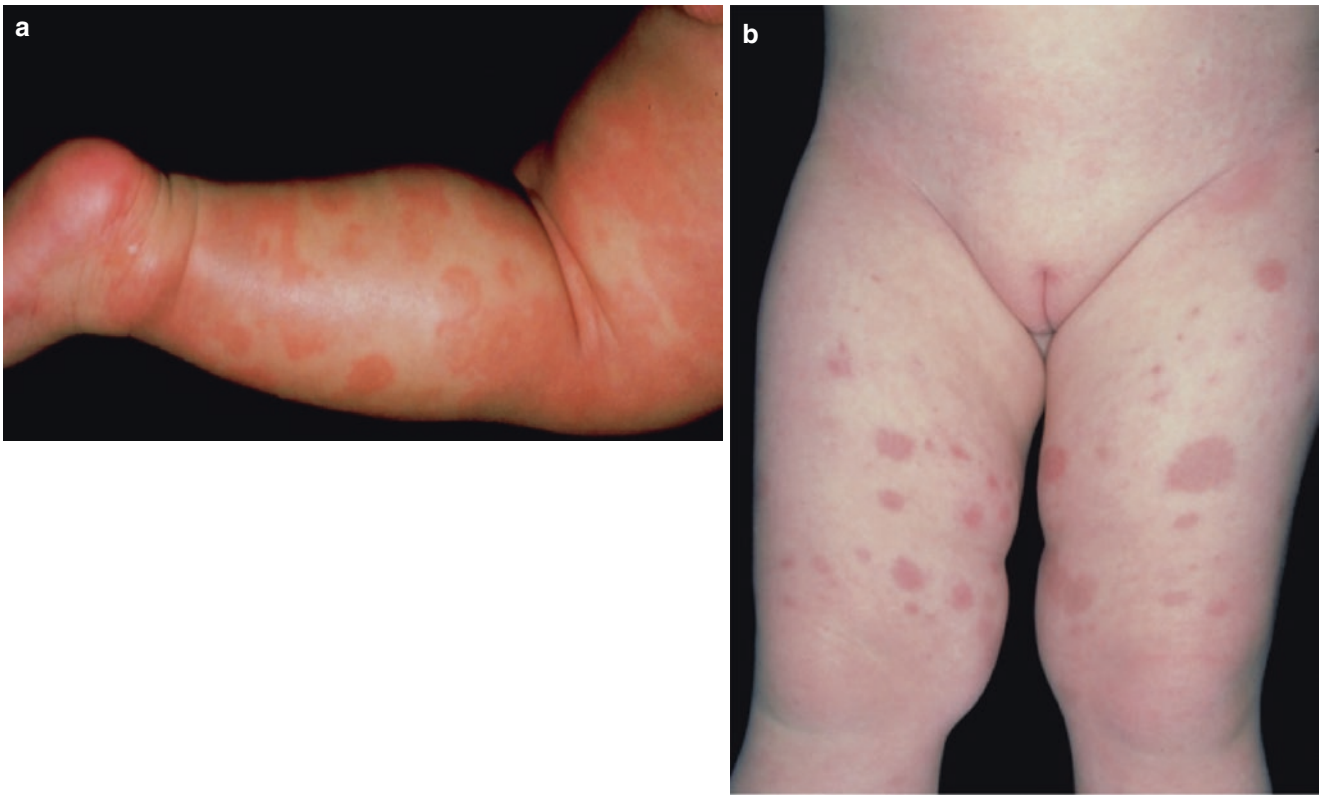

Fig. 26.3 (**a**, **b**) Infants with typical lesions of Henoch-Schönlein purpura. Lesions above the waistline are associated with a worse prognosis (Courtesy of Dr. M. Mercurio)

Routine H&E staining reveals a neutrophil-rich small-vessel vasculitis of the superficial dermis with leukocytoclasia and few eosinophils (Fig. 26.2). DIF provides the gold standard for diagnosis, with IgA deposits in small, superficial vessels found in virtually all biopsies. Granular deposits of other Ig classes are occasionally seen, with IgA being the most prominent [87].

As with any of the vasculitides, treatment decisions hinge on the degree of involvement and constitutional symptoms. While the cutaneous findings are largely refractory to therapy, synovitis and GI symptoms are quite responsive to moderate doses of oral corticosteroids. Skin lesions frequently have an initial response to steroids, followed by rapid relapse upon completion of treatment. Antimetabolites such as azathioprine and mycophenolate mofetil, as well as alkylating agents can be effective for treating glomerulonephritis. However, there is little evidence that any other immunosuppressive therapy beyond corticosteroids is effective in HSP nephritis [90].

Urticarial Vasculitis

Urticarial vasculitis presents clinically as urticaria of the trunk and proximal extremities, and histologically as vasculitis (Fig. 26.4). It occurs in two forms differentiated by serum complement levels. The normocomplementemic variant is a subset of CLA, while the hypocomplementemic type (HUVS) is a subset of systemic lupus erythematosus (SLE) [72, 91]. Patients with low levels of C3, C4, and total serum hemolytic complement (CH50) have dramatically increased rates of complement consumption. CH50 is a more sensitive predictor of HUVS since C3 and C4 are acute-phase reactants and may be normal in mild disease or early-stage disease. Thorough workup of patients with urticarial vasculitis

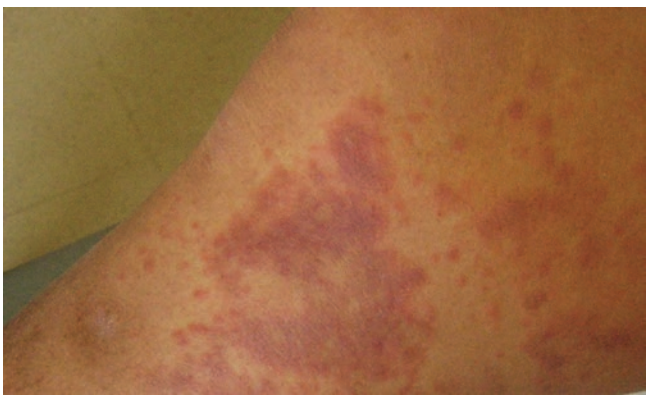

Fig. 26.4 Urticarial vasculitis. These lesions are difficult to distinguish clinically from traditional urticaria and can occur on the face and upper extremities (Courtesy of Dr. F. Tausk.)

should include the quantitation of C3a, C5a, and C3bi when available [72, 74].

Ninety percent of normocomplementemic urticarial vasculitis (NUV) are triggered by infection or medications, and are self-limited without indication of systemic involvement [73]. The clinical differential includes urticarial HSP, neutrophilic urticaria, Schnitzler's syndrome, and urticarial cryoglobulinemia II and III.

Neutrophilic urticaria, also known as polymorphonuclear predominant urticaria (PPU) is a subset of chronic urticaria [92]. Lesions are typically pruritic, and mild constitutional symptoms can be present. Histologic features of PPU can be confused with NUV, as perivascular neutrophilic infiltrates can be dense, with occasional karyorrhexis; however, there is no definite disruption of vasculature and DIF is consistently negative in PPU. Chronicity and no obvious underlying cause argue against NUV. Treatment consists of antihistamines, leukotriene antagonists, dapsone, or colchicine. In cases of PPU it is important to rule out Schnitzler's syndrome, which is characterized by NUV, fever, lymphadenopathy, hepatosplenomegaly, peripheral neuropathy, bone pain, and monoclonal IgM gammopathy [93]. In contrast to patients with NUV, the hypocomplementemic variant of urticarial vasculitis presents as a chronic, relapsing syndrome (HUVS) with signs and symptoms typically seen with SLE, including fever, arthralgias, and myalgias [73, 91].

In contrast to lesions of NUV, HUVS lesions tend to have a purpuric component upon careful examination. Synovitis, GI involvement, and scleritis are not uncommon associated findings. A subset of HUVS patients with more overt symptoms of SLE will display angioedema, thought to be mediated by a high rate of consumption of C1 esterase caused by autoantibodies to C1q. In both variants of urticarial vasculitis, histology reveals a dense neutrophilic infiltrate in and around the walls of small vessels that disrupts normal architecture. In HUVS, dermal edema and neutrophils extend to the dermal-epidermal junction (DEJ), resulting in vacuolar changes and clefting of the basement membrane zone (BMZ). The only serologic marker associated with NUV is an elevation in ESR in up to 70% of patients, while essentially all patients with HUVS have high ESR and an antinuclear antibody (ANA) titer greater than 1:320 at some point in their disease course [1].

As with CLA, 50% of patients with NUV have immune deposition within and around blood vessel walls seen with DIF. IgM is seen more frequently than IgG, and C3, if present, is weak and patchy. In contrast, essentially all patients with HUVS show significant IgG and C3 intravascularly and perivasculary within superficial dermal vessels extending to the BMZ (Fig. 26.5). It has been reported that this latter finding resembles the lupus band test, further linking HUVS with SLE [74, 94].

Treatment of NUV is similar to that for CLA— conservative and directed toward alleviation of mild symptoms. HUVS, however, frequently requires systemic immunosuppressive therapy such as corticosteroids, azathioprine (3–4 mg/kg/day), or mycophenolate mofetil (40–50 mg/kg/day); these medications are usually sufficient. Anti-inflammatory agents such as dapsone, colchicine, methotrexate, and calcineurin inhibitors are not typically effective, as they have no effect on the production of immune complexes or anti-C1q synthesis.

Cryoglobulinemia

Cryoglobulinemic vasculitis affects both small and medium-sized skin blood vessels. Cryoglobulins are antibodies that precipitate with cold, and three types of cryoglobulinemia exist as defined by the type of antibodies present. Type I is a monoclonal gammopathy that commonly presents as a hyperviscosity syndrome and thrombotic events in the context of myeloproliferative disorders, and does not represent true vasculitis. Type II is mixed, with an IgM monoclonal component (usually an IgM rheumatoid factor) in conjunction with polyclonal gammopathy. Type III consists of polyclonal cryoglobulins. Because types II and III readily form immune complexes, they are more likely to cause vasculitis, as opposed to the thrombotic vasculopathy seen with type I cryoglobulins [1, 95, 96]. Cryoglobulinemia type II has antibodies that form immune complexes with much higher avidity and levels of complement fixation, resulting in more significant clinical syndromes. Hepatitis C is by far the most common cause of type II cryoglobulinemia, representing essentially all cases previously labeled as essential mixed cryoglobulinemia (Fig. 26.6) [95, 96]. It is felt that the virus stimulates the immune system chronically, causing B-cell expansion and production of autoantibodies. Other associated infections include endocarditis, hepatitis B, and

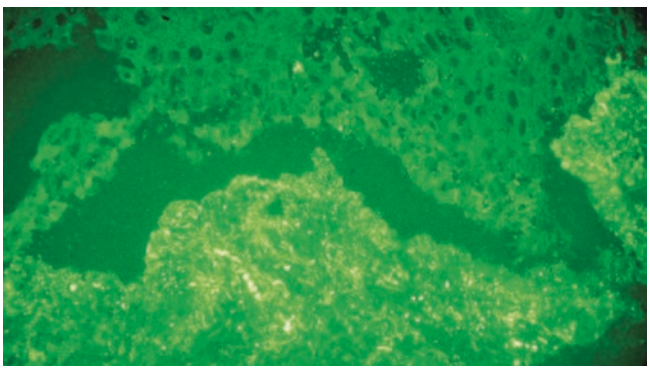

Fig. 26.5 Direct immunofluorescence of biopsy from a patient with hypocomplementemic urticarial vasculitis syndrome (HUVS) revealing granular immunoglobulin G (IgG) in and around superficial vessels

HIV. Connective tissue disease, other autoimmune conditions, and malignancy have been associated with both types II and III cryoglobulinemic vasculitis [97].

Clinically, skin lesions associated with cryoglobulinemia type I are indistinguishable from those seen with type II, and resemble the palpable purpura and urticarial findings seen with any CSVV. As with most vasculitides other than urticarial vasculitis, lesions most commonly appear on the lower extremities. Cryoglobulin-associated nonpalpable purpura can also present as the capillaritic eruption seen in Schamberg's disease. The benign hypergammaglobulinemic purpura seen in Waldenström's (lymphocytic vasculitis) is clinically, serologically, and histologically indistinguishable from that seen with cryoglobulinemia type III, and, as such, is generally believed to belong to the same spectrum of disease [97]. Pigmented purpura above the waistline or involving the soles of the feet favor cryoglobulinemic vasculitis over Schamberg's, as do lesions seen at different stages of evolution, ulcerative lesions, and constitutional symptoms. Ulceration signals the involvement of medium-sized vessels and can help differentiate from other CSVV. As mentioned, medium-sized vessels can also be seen with cryoglobulinemia.

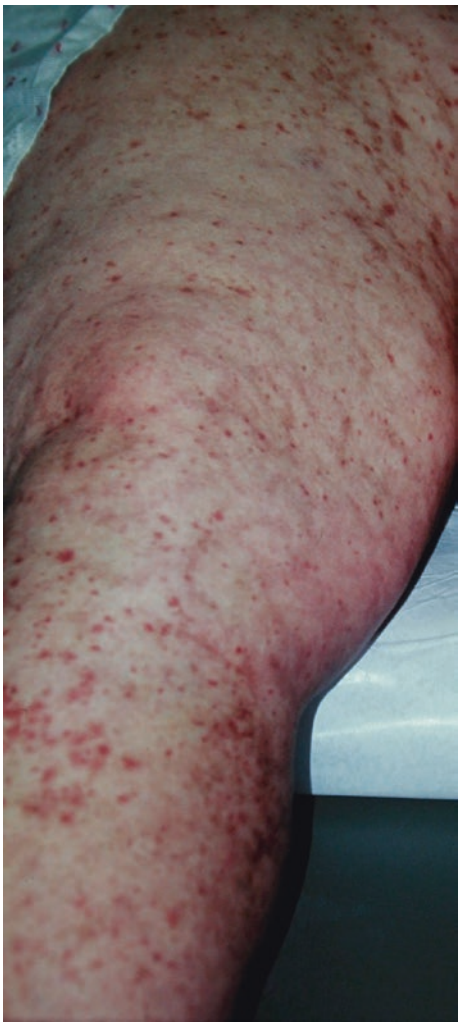

Fig. 26.6 Clinical image of patient with known hepatitis C presenting with the CSVV (purpura) and medium-sized vessel vasculitis (livedo) lesions associated with cryoglobulinemia type II

These patients also tend to have systemic symptoms such as nephropathy, neuropathy, arthralgias, and gastrointestinal involvement [1, 98].

Serologically, cryoglobulin type II patients demonstrate monoclonal IgM rheumatoid factor (RF) and polyclonal IgG. All patients have an RF greater than 1:320, and over 90 % have decreased C4 but essentially normal C3. Therefore, a negative cryoglobulin assay and negative RF in the setting of low C4 virtually excludes cryoglobulinemia type II, whereas a low titer cryoglobulinemia in the absence of vasculitis is common after many infections. A positive cryoglobulinemia with negative RF activity likely represents an incidental finding and not vasculitis. Cryoglobulinemia type III demonstrates a polyclonal gammopathy without any specific monoclonal spike, and low levels of both C3 and C4 [1].

Histology demonstrates findings consistent with CSVV or both CSVV and medium-sized vessel vasculitis (MSVV), but not MSVV alone. Some texts report the deposition of a nonspecific, homogeneous intravascular infiltrate associated with cryoglobulinemic vasculitis; however, this likely represents thrombotic vasculopathy seen with type I cryoglobulinemia and not true vasculitis [97].

Direct immunofluorescence in cryoglobulinemia type II usually reveals significant granular IgM and C3 deposition in and around small and medium sized vessels, while type III has both IgG and IgM in addition to C3. In practice, it is often difficult to distinguish between types II and III cryoglobulinemia by DIF.

Because types II and III cryoglobulinemias are difficult to distinguish histologically and clinically, treatment decisions should hinge on the serologic workup and be directed toward the underlying etiology (e.g., hepatitis C) as well as the vasculitis. Treatment of the latter is similar to that of other vasculitides and is based on combinations of steroids and steroid-sparing agents that are used in an effort to target antibody and immune complex–mediated inflammation. Differences do exist, however, when treating the underlying condition in type II as opposed to type III cryoglobulinemia. For instance, in the setting of hepatitis C–induced type II cryoglobulinemia with high titers of cryoglobulins and RF and low complement, antiviral treatment with interferon-γ could potentially result in massive formation of immune complexes, exacerbation of vasculitis, and multi-organ failure [98]. This group of patients should be treated with immunosuppressive agents of low hepatotoxicity for at least 6 months prior to antiviral therapy [99].

An additional difference in the management of type II versus type III cryoglobulinemia is with plasmapheresis aimed at removing pathogenic immunoglobulins. Because antibodies in type II cryoglobulinemia are intravascular as opposed to the intra- and extravascular deposition seen in type III cryoglobulinemia, plasmapheresis is much more effective in the prior condition, as extravascular antibodies are not affected by plasmapheresis. Rituximab is a newer therapeutic option that has demonstrated its utility by its B-cell depletion activity and subsequent inhibition of antibody production such as that which occurs in cryoglobulinemia. Studies have demonstrated the efficacy of rituximab as monotherapy in the treatment of cryoglobulinemia vasculitis, or when used in combination with corticosteroids [100]. Rituximab plus corticosteroids appears to provide better efficacy in patients with relapsing disease or disease refractory to other therapies [101, 102].

Antineutrophil Cytoplasmic Antibody (ANCA)–Positive Vasculitis

ANCAs are antineutrophil cytoplasmic antibodies and are found in varied autoimmune disorders. In the setting of vasculitis, they are associated with three conditions: Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and Churg-Strauss syndrome (CSS). Skin findings of these three conditions are those of CSVV or cutaneous medium-vessel vasculitis (CMVV), with palpable purpura being most common. [103, 104] Papules and nodules are occasionally seen on extensor surfaces and can be ulcerated or necrotic, occasionally with overhanging borders as seen with pyoderma gangrenosum [105, 106]. However, primary cutaneous disease in these conditions is essentially nonexistent, as all patients with WG, MPA, and CSS have varying degrees of extracutaneous involvement. Wegener's granulomatosis has significant associated extracutaneous involvement including upper and lower respiratory, renal, and nervous system manifestations, and almost all of these patients are c-ANCA positive. Churg-Strauss syndrome is hard to distinguish from WG except for the presence of significant tissue and blood eosinophilia, higher levels of p-ANCA, and associated atopy in CSS [107]. The diagnosis of CSS requires eosinophil predominance in a mixed infiltrate. Some patients with CSS describe pruritus as a symptom preceding the development of vasculitic lesions, and this is thought to be related to the high number of eosinophils. Microscopic polyangiitis is the most common cause of vasculitis associated with pulmonary-renal syndrome, and almost all of these patients are p-ANCA positive [71, 108].

Histologically, lesions from the ANCA-associated vasculitides demonstrate classic findings of vasculitis or Churg-Strauss (cutaneous extravascular necrotizing) granulomas (CENG) [105, 109]. The latter is a misnomer, as the blood vessels in these infiltrates demonstrate clear evidence of vasculitis. Originally observed in CSS, but later seen in several vasculitides, CENG lesions contain four components: (1) a central area of degenerated extracellular substance surrounded by (2) a palisaded mononuclear infiltrate; (3) variable polymorphonuclear interstitial infiltrate; and (4) variable degrees of vasculitis or vasculopathy of small or medium sized vessels [106, 109, 110]. This fact helps differentiate CENG seen in systemic vasculitides from the palisaded granulomas of rheumatoid nodules. Both the histologic and clinical appearances are similar to those of rheumatoid nodules. Oscillating titers of pathogenic antibodies cause sustained damage to the endothelial lining, leading to slow death and degeneration of the extracellular matrix, resulting in a palisading inflammatory reaction. Because extensor surfaces of the extremities are prone to trauma and injury, they tend to be a common site for their location. Whether typical vasculitis or CENG is present, marked levels of eosinophils seen on biopsy (>60 %) are pathognomonic for CSS. Direct immunofluorescence in all types of ANCA vasculitis is positive in approximately 80 % of cases for IgG and IgM deposition, but not C3. Cutaneous extravascular necrotizing granuloma is also referred to as CSG, Winkelmann's granuloma, interstitial granuloma, palisaded neutrophilic and granulomatous dermatitis of connective tissue disease, superficial ulcerating rheumatoid necrobiosis, and rheumatoid papule [1, 107, 109].

Because ANCA-associated vasculitis occurs in the setting of extracutaneous disease, treatment of the primary vasculitis treats the cutaneous findings. Standard management involves combinations of steroids and steroid-sparing immunosuppressive agents [20, 22, 23].

Cyclophosphamide and glucocorticoids have been standard therapy for remission induction in severe ANCA-associated vasculitis but they are associated with high rates of adverse events and death. Rituximab was thought to be a potentially more effective and safer treatment than cyclophosphamide. Two recent randomized control studies, RITUXVAS (rituximab-based regimen versus cyclophosphamide in ANCA-associated renal vasculitis) [111] and RAVE (rituximab for ANCA-associated vasculitis) [112], have demonstrated similar efficacy of rituximab to cyclophosphamide for the induction of remission in ANCA-associated vasculitis. Results also revealed that sustained-remission rates were high in both groups, and the rituximab-based regimen was not associated with reductions in early severe adverse events, compared to cyclophosphamide. These findings could suggest that the nature of the disease and prolonged high-dose glucocorticoid therapy and are the main contributors to the adverse events.

Remission ANCA-associated vasculitis is a relapsing condition, for which cyclophosphamide has been used for

prolonged periods to maintain remission. Given the potential adverse effects of high cumulative cyclophosphamide toxicity (bladder cancer and myeloproliferative disease) safer maintenance therapeutic options are needed. In a prospective multi-center study, Pagnoux et al. [113] randomized 126 patients to receive either methotrexate or azathioprine as maintenance therapy after successful induction of remission with cyclophosphamide. The authors found no difference in efficacy and safety of these two agents. Mycophenolate mofetil (MMF) is an alternative to azathioprine for remission maintenance therapy in AAV. Results from a small pilot study in 11 patients were promising, with only one relapse occurring after 14 months of follow-up [114]. However, the IMPROVE randomized study comparing MMF and azathioprine as maintenance agents after cyclophosphamide induction in 174 patients, found an increased hazard ratio of 1.7 for relapse in the MMF group, and a shorter time to relapse [115]. Both treatments had similar adverse event rates. MMF may have greatest utility in azathioprine intolerant renal failure patients for whom methotrexate is contraindicated.

The utility of TNF blocking agents has been mixed. Etanercept was shown not effective for the maintenance of remission in patients with Wegener's granulomatosis when added to glucocorticoids and cyclophosphamide or methotrexate [116]. Further, a high rate of serious adverse events and an excess of solid organ tumors were seen. Infliximab demonstrated effectiveness for inducing and maintaining remission when used with conventional therapy, although there was an increased rate of severe infections [117].

Connective Tissue Disease–Associated Vasculitis

Essentially any cutaneous vasculitic syndrome can be triggered by connective tissue disease (CTD). Furthermore, these patients are more prone to infections and drug exposure, resulting in higher rates of CLA or IgA-mediated vasculitides. Three vasculitic entities in particular are associated with CTD: CTD-associated vasculitis, HUVS (discussed above), and lymphocytic vasculitis (LV) [118]. Connective tissue disease–associated vasculitis is most frequently seen with SLE, but can also happen with other CTDs such as Sjögren's syndrome, dermatomyositis, systemic sclerosis, and mixed connective tissue disease [118, 119].

Clinically, these diseases can present as CSVV of the lower extremities. Although they may resemble the lesions of cryoglobulinemia, CTD vasculitides may present as a pure CMVV without small vessel involvement, whereas small vessels will always be involved in cryoglobulinemia. Systemic involvement is present in essentially all patients, with the kidneys being the most affected organ. Central and peripheral neuropathies and GI and cardiac involvement can also occur at varying rates.

Serologically, high titers of antinuclear antibodies (ANAs) and low complement are present. Different combinations of ANA are commonly seen including anti–double-stranded DNA (anti-dsDNA), ribonucleoprotein (RNP), Ro, and Sm, further increasing the chance for vasculitis.

Histologically, CTD-associated vasculitis shows CSVV or CMVV without any distinguishing features. Lymphocytic vasculitis is rare and more commonly seen in SS and SLE; however, it can occur in association with other CTDs as well. Lymphocytic vasculitis is also known as benign hypergammaglobulinemic purpura (BHP) of Waldenström. Most experts believe that both LV and BHP of Waldenström are type III cryoglobulins associated with CTDs [118, 119].

As opposed to cryoglobulinemia, however, DIF reveals IgG in and around small and medium-sized vessels as the predominant immunoglobulin. C3 is usually very strongly deposited. Interestingly, in vivo ANA is very commonly present. This reflects the high titer of ANA, especially RNP in keratinocytes and dermal cells in these patients [1].

As the development of vasculitis in CTD is often associated with an ominous prognosis, aggressive treatment of the underlying CTD is mandatory. Combinations of corticosteroids, with high doses of antimetabolites or alkylating agents and occasionally plasmapheresis, are necessary to control the progression of this disease.

Rheumatoid Vasculitis

Rheumatoid vasculitis (RV) is a rare but severe complication in patients with advanced, usually otherwise quiescent seropositive rheumatoid arthritis (RA) [120]. It can affect virtually any sized vessel, with palpable purpura being the most common presenting sign, but ulcerations, livedo reticularis, digital infarcts, or cutaneous nodules can also be seen. Isolated, fluctuating periungual splinter hemorrhages, known as Bywater's lesions, are caused by digital small-vessel vasculitis. Cutaneous medium-vessel vasculitis involvement typically presents as deep geographic ulcers at the malleoli. As with cryoglobulinemia, these patients frequently have accompanying mononeuritis multiplex. [98]

Serologically, high titers of RF often with low levels of both C3 and C4 are characteristic of RV. This often helps in distinguishing RV from cryoglobulinemia since RF is elevated in both, and, even in RA patients with higher titers of RF, low levels of cryoglobulins are not uncommon. Thorough exclusion of other causes of CMVV in RA patients with cryoglobulins is mandatory (e.g., viral hepatitis, lymphoma, HIV) [120–122].

Histologic findings in RV are indistinguishable from those in other CSVVs and CMVVs. Direct immunofluorescence is indistinguishable from cryoglobulinemia, with heavy granular IgM and variable degrees of C3 in and around small and medium vessels [120].

The aim of therapy in RV is to reduce IgM immunocomplexes with combinations of corticosteroids with antimetabolites or alkylating agents. Methotrexate is effective for the synovitis and T-cell–mediated symptoms of RA, but is not effective for RV. Because of the IgM RF, plasmapheresis in combination with steroids and alkylating agents is a good choice in patients with life- threatening disease (as with cryoglobulinemia type II) [122]. Bywaters lesions alone do not necessitate aggressive systemic therapy.

Evidence supporting the use of biologic agents consists of case reports and case series. Puéchal et al. [123] described nine patients with rheumatoid vasculitis refractory to cyclophosphamide treatment that were subsequently treated with anti-TNF therapy. Remission was achieved in two-thirds of patients, with a significant decrease in prednisone dose. Severe infection occurred in three patients. Other reports describe patients intolerant or refractory to cyclophosphamide therapy with successful treatment of rheumatoid vasculitis with infliximab [124, 125]. An additional benefit of anti-TNF therapy is the treatment of synovitis, which is not contained with cyclophosphamide. Rituximab improves articular symptoms in rheumatoid arthritis and has been shown to be effective in ANCA-associated vasculitis. In a recent study of 17 patients with RA-associated vasculitis, nearly three-fourths of patients receiving rituximab achieved complete remission with a significant decrease in daily prednisone dosage and an acceptable toxicity profile [126]. Other reports also describe the successful use of rituximab to treat RA vasculitis [127, 128].

Polyarteritis Nodosa

Polyarteritis nodosa (PAN) is the prototype of a pure medium-sized vessel vasculitis (Fig. 26.7), often presenting as a systemic illness with multi-organ involvement. It is a diagnosis of exclusion when other causes of CMVV such as RV, ANCA vasculitis, and cryoglobulinemia have been ruled out. While the etiology of PAN is unknown, the hepatitis B virus has been implicated as an etiologic agent. Guillevin et al. [129] found that over a 30-year period the frequency of HBV-PAN was roughly 50 % to about 17 %. The authors attributed the decline to factors such as widespread use of the hepatitis B vaccine and increased safety of blood transfusions and products. HBV-related PAN is an acute disease, occurring shortly after infection and sharing the characteristics of classic PAN.

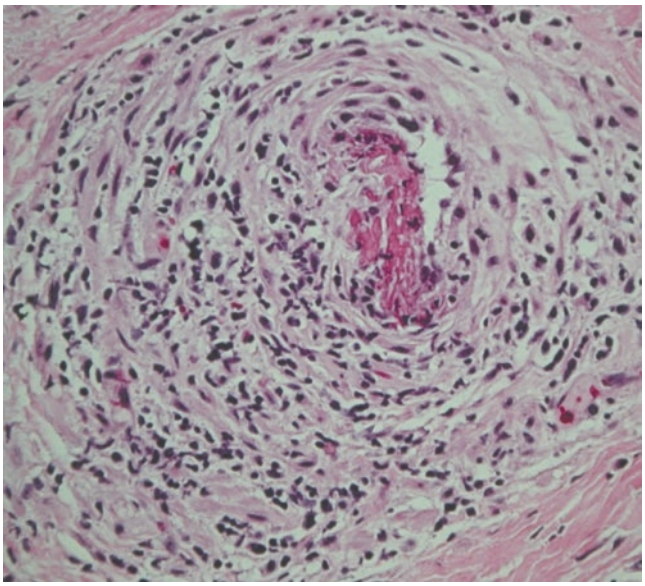

Fig. 26.7 H&E staining of a skin biopsy from patient with polyarteritis nodosa. Note involvement of medium-sized vessel

The most common presentation is with painful cutaneous nodules and ulcerations with a predilection for the malleoli [130], but since PAN is a pure CMVV, other lesions can include nodules, ulcers, livedo (Fig. 26.8), and digital infarcts (Fig. 26.9), and less frequently papulonecrotic lesions on extensor surfaces (CENG) [131]. Ulcerations heal with stellate, atrophic, ivory-colored scars or hyperpigmentation, designated as atrophie blanche or livedoid vasculitis in the past, or even Degos' like lesions. They are often accompanied by neuropathic pain resulting from involvement of the vasa nervorum. Focal synovitis and arthralgia may be present in the joints close to areas of cutaneous involvement, particularly the ankle, as opposed to erythema nodosum, where generalized arthralgias may be present. Disease limited to the skin occurs in less than 10 % of those affected, and PAN can occur in children after streptococcal infection [132].

Differential diagnosis for nodular lesions includes erythema nodosum, nodular vasculitis, and erythema induratum [133–135]. The latter two are most likely variants of this disorder, with a prominent component of panniculitis present. Infectious causes of ulceration, pyoderma gangrenosum, calciphylaxis, and other causes of medium-sized vessel vasculitis must be included in the differential. Patients with nodules limited to around the ankles may have cutaneous PAN, and even these patients require close and long-term follow-up for progression to systemic disease [136, 137]. Systemic disease is present in virtually all patients with extensive nodulo-ulcerative disease. Synovitis, hypertension, and mononeuritis multiplex are the most common systemic findings.

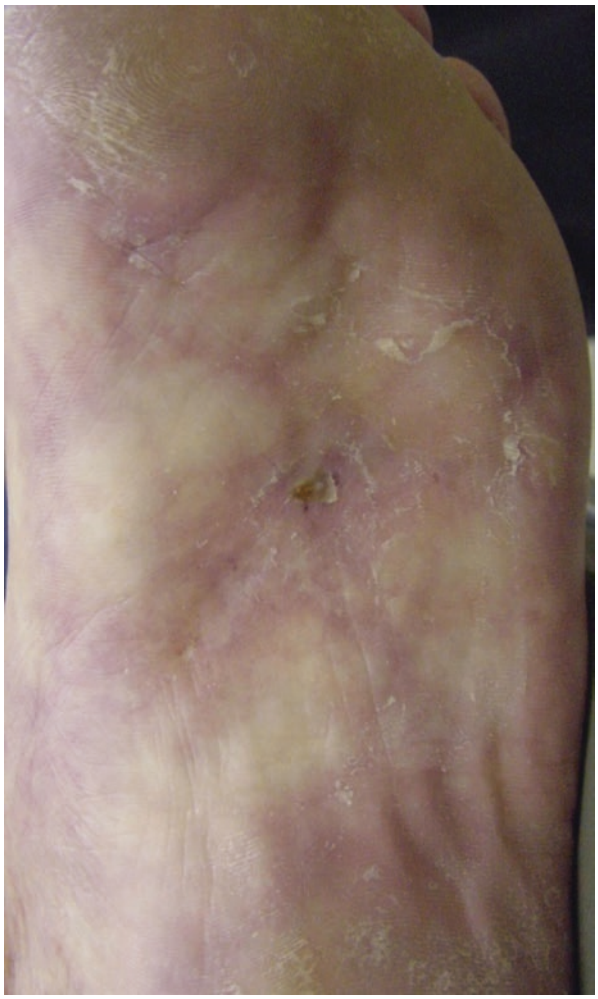

Fig. 26.8 Livedo reticularis in a patient with polyarteritis nodosa

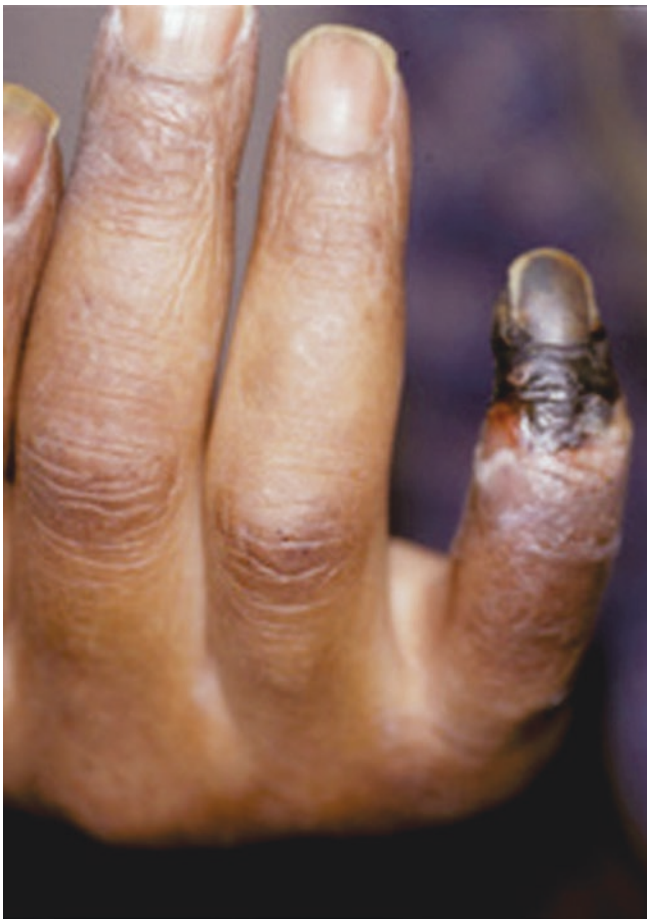

Fig. 26.9 Digital infarct in a patient with poyarteritis nodosa

Kawasaki's disease is an equivalent of PAN in children with a predilection for the coronary arteries, leading to coronary aneurysms and myocardial infarction [132, 136–139].

Serologic testing may reveal elevated ESR with hypocomplementemia. ANCA, ANA, and RF are typically negative, but may be present at insignificant titers.

By definition, small-vessel involvement should rule out the diagnosis of PAN. Because PAN is a pure CMVV, shallow biopsies that do not sample deeper skin vessels will lead to frequent misdiagnosis. Therefore, multiple, deep biopsies are recommended. The diagnostic yield in PAN is the following: nodules (90–100 %), ulcers (50–80 %), livedo (0–20 %), and digital infarcts (0–5 %). Direct immunofluorescence shows granular IgM in and around medium-sized vessels and weak C3 in approximately 60 % of cases [1].

The approach to treatment of PAN requires consideration of disease severity and whether the patient is infected with HBV. Corticosteroids alone may be sufficient for mild nodular disease, while ulcerative disease without significant systemic involvement may respond to prednisone in combination with azathioprine. Severe cutaneous disease with digital infarcts and systemic involvement should be treated with corticosteroids and alkylating agents. Plasmapheresis is not effective in PAN since IgM immune complexes and RF do not play a prominent role in this disease in contrast to the high titers of these factors seen in RV and cryoglobulinemia [131]. In HBV-associated PAN, the potential risk of accelerating viral replication complicates the use of corticosteroids and cytotoxic agents. In this patient population, studies have shown favorable outcomes with a combination of short-term steroid therapy, antiviral agents, and plasma exchanges [129, 140].

Cutaneous Large-Vessel Vasculitis

Cutaneous large-vessel vasculitis (CLVV) is rare, as there simply are not large vessels found in the skin. These disorders typically target the great vessels of the body such as the aorta and its major branches and include giant cell (temporal) arteritis and Takayasu's arteritis. Giant cell arteritis is more likely in Caucasian patients over 50 years,

with a predilection for the extracranial branches of the carotid artery, while Takayasu's arteritis is more common in persons of Far Eastern descent under age 50, affecting the thoracic aorta and branches supplying the upper extremities [2]. Involvement of large vessels can occasional manifest as lesions of the scalp and tongue [134].

Paraneoplastic Vasculitis

Cutaneous vasculitis is most often associated with relatively benign conditions, but can be part of a paraneoplastic syndrome. The incidence of paraneoplastic vasculitis is unclear, as most information has come from case reports and small series of patients. Difficulty in distinguishing between vasculitis from a malignancy or other causes (chemotherapy, infection), are factors which can confound determination of epidemiologic data. Recommended guidelines have been suggested that would better establish a vasculitis as being part of a paraneoplastic process. These include establishment of a temporal relationship between vasculitis and malignancy and a relationship between effective treatment for malignancy and vaculitis [141]. The reported frequency of cutaneous vasculitis associated with malignancy varies in the literature from about 2 to 5 %, with the vast majority affecting adults [142–144].

The pathogenesis for the development of paraneoplastic vasculitis remains unknown. Tumor antigen induced antibodies directed at normal vessel endothelium, cytokines causing endothelial injury, and impaired clearance of immune complexes injuring endothelial cells are thought to be contributing factors [141, 145].

Paraneoplastic vascultis is seen more commonly with hematologic malignancies (multiple myeloma, T-cell leukemia), as compared with solid tumors [143, 146]. The cause for this stronger association between vasculitis and hematological malignancies is unclear. Solid tumors more commonly associated with vasculitis include those arising from the urinary tract, lung, prostate, colon, renal, breast, and head and neck cancer [143, 144, 147].

The signs and symptoms of paraneoplastic vasculitis will vary depending on the type of vasculitis and the organ system(s) affected. Cutaneous small vessel vasculitis generally occurs with palpable purpura mainly in the dependent areas of the body. Polyarteritis nodosa (PAN) predominantly affects medium-sized arteries and can appear clinically as livedo reticularis, ulcers, and subcutaneous nodules. Other clinical features of the vasculitides also are dependent on the size of the vessel affected, but can include constitutional symptoms, arthralgias, myalgias, paresthesias, abdominal pain and hematuria. Leukocytoclastic angiitis is the most common type of paraneoplastic vasculitis [143, 144]. Malignancy-associated cryoglobulinemic vasculitis, Henoch-Schonlein purpura, polyarteritis nodosa, and giant cell arteritis have also been described [142, 144]. There is no variation in the histologic features between paraneoplastic vasculitis and vasculitis without an associated malignancy.

Vasculitic skin manifestations may represent the initial sign of a neoplastic disease [148]. Most often vasculitis precedes the diagnosis of malignancy, but can be found simultaneously or after the malignancy has been found [149]. In patients with unexplained vasculitis, a malignancy should be considered, particularly in those with advanced age, and when vasculitis becomes chronic, recurrent or is no longer effective to treatment. Infection, medication or connective tissue diseases are much more common causes of cutaneous vasculitis and should be ruled out. Work-up should include a comprehensive medical history and physical exam, as well as appropriate cancer screening. Persistent constitutional symptoms should raise the clinician's index of suspicion for malignancy. Enlarged lymph nodes or viscera palpated on physical exam should necessitate the search for malignancy. In the setting of hematologic abnormalities, a peripheral blood smear and bone marrow biopsy should be considered. Serologies remarkable for anti-neutrophilic or ds-DNA antibody suggests potential presence of a connective tissue disease. Appropriate imaging should be performed, such as a chest radiograph or computed tomography scan to exclude lung cancer. Electrophoresis should be performed to evaluate the presence of abnormal immunoglobulins in serum or urine. In the presence of hematuria, kidney cancer should be excluded. A positive hemoccult or anemia could be an indicator of blood loss from the gastrointestinal tract, requiring endoscopy to rule out a malignancy.

Treatment and prognosis of paraneoplastic vasculitis is generally related to the underlying malignancy. Often times, treatment of the underlying malignancy leads to complete resolution of the vasculitis [145, 147]. Paraneoplastic vasculitis may also require treatment with glucocorticoids alone or in combination with immunosuppressive agents, particularly when a curative treatment of the neoplasm is not possible [145, 147]. A flare of vasculitis can mark the return or progression of the malignancy [144].

Conclusion

Vasculitis, or inflammation of the vasculature, can be caused by many different mechanisms that ultimately result in varying degrees of vessel wall destruction, hemorrhage, ischemia, or infarction of affected organs. Any sized vessel in any organ can be affected and will determine the clinical and pathologic findings, with fibrinoid necrosis being the pathognomonic feature. The patient with vasculitis can present initially to dermatologists,

rheumatologists, or primary medical service, as a wide range of initial presenting signs occur. Because even mild cutaneous disease does not rule out severe and significant systemic involvement, thorough workup including history and physical, serology, histology, and immunofluorescence is mandatory for these patients.

Questions

1. Which of the following statements is true regarding Henoch-Schonlein purpura (HSP) and renal disease?
   A. End-stage renal disease (ESRD) occurs in 50% of patients
   B. Pupura above the waist is associated with the development of renal disease
   C. Azathioprine is not an effective treatment of nephritis
   D. Renal insufficiency is more likely to occur in children than in adults

2. Which of the following regarding Polyarteritis nodosa is correct?
   A. Hepatitis B virus has been implicated as an etiologic agent
   B. Shallow biopsies that sample superficial dermis are sufficient for diagnosis
   C. Is the prototype small-vessel vasculitis
   D. Plasmapheresis is the mainstay of therapy

3. Each of the following distinguishes Churg-Strauss syndrome from Wegener's granulomatosis except
   A. Eosinophilia
   B. Associated atopy and asthma
   C. Higher levels of p-ANCA
   D. Granulomatous inflammation

4. The following therapeutic agents have been shown effective for induction of remission in ANCA-associated vasculitis except
   A. Corticosteroids
   B. Rituximab
   C. Cyclophosphamide
   D. Dapsone

5. All of the following characterize Cryoglobulinemic vasculitis except
   A. Cryoglobulin type I represents a true vasculitis
   B. Palpable purpura of the legs is the most common lesion seen
   C. Cryoglobulin type II patients demonstrate monoclonal IgM rheumatoid factor and polyclonal IgG
   D. Cold enhances most of the lesions

Answers

1. B
2. A
3. D
4. D
5. A

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Allergic Urticaria

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Abstract

Urticaria, commonly known as hives, is characterized by the episodic appearance of pruritic, erythematous papules or plaques with superficial swelling of the dermis. Urticaria is classified as either acute or chronic based on symptom duration. Acute urticaria, which is defined as having hives for less than 6 weeks, is estimated to occur in 15–23 % of the population, although cases are likely to be underreported due to the short-lived nature of the disease. Chronic urticaria, defined by symptom duration of greater than 6 weeks, can be further classified as physical urticaria or chronic idiopathic urticaria (CIU). Physical urticarias are comprised of many subtypes in which a specific trigger can quickly induce hives, while the majority of hives in CIU do not occur as a result of a known trigger. The prevalence of physical urticaria is not well-established, but it is thought to account for 20–35 % of all cases of chronic urticaria. CIU, which has been renamed as chronic spontaneous urticaria (CSU) in recent guidelines, occurs in approximately 0.1–3 % of the population and, like many forms of chronic urticaria, has a female predominance.

Keywords

Urticaria • Angioedema • Basophils • Mast cells • Physical urticarial

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Key Points

- Urticarial lesions, commonly known as hives, are produced in the skin by the degranulation of mast cells.
- Urticaria can be acute or chronic (lasting greater than 6 weeks), and may be allergic (mediated by immunoglobulin E) or nonallergic (mediated by pharmacologic effects of drugs such as aspirin).
- There are a multitude of causes of urticaria, including food or medication allergies and infections.
- There are subsets of chronic urticaria that are caused by physical factors such as exposure of the skin to pressure, vibration, cold, or even water (aquagenic urticaria).
- In most cases of chronic urticaria, it is not possible to identify an etiology. One theory is that a subset of these cases may have an autoimmune basis.
- Urticaria can be a presenting sign of vasculitis. Urticaria may also be associated with autoimmune diseases such as Hashimoto's thyroiditis.
- Treatment is based on identifying a trigger factor. Antihistamines are a cornerstone of treatment. For more severe cases, immune modulating drugs or biologic agents such as omalizumab (Xolair) may be necessary to control signs and symptoms of this process.

Overview of Urticaria

Urticaria, commonly known as hives, is characterized by the episodic appearance of pruritic, erythematous papules or plaques with superficial swelling of the dermis (see Fig. 27.1). Urticaria is classified as either acute or chronic based on symptom duration. Acute urticaria, which is defined as having hives for less than 6 weeks, is estimated to occur in 15–23 % of the population, although cases are likely to be underreported due to the short-lived nature of the disease [1]. Chronic urticaria, defined by symptom duration of greater than 6 weeks, can be further classified as physical urticaria or chronic idiopathic urticaria (CIU). Physical urticarias are comprised of many subtypes in which a specific trigger can quickly induce hives, while the majority of hives in CIU do not occur as a result of a known trigger [2]. The prevalence of physical urticaria is not well-established, but it is thought to account for 20–35 % of all cases of chronic urticaria [3, 4]. CIU, which has been renamed as chronic spontaneous urticaria (CSU) in recent guidelines, occurs in approximately 0.1–3 % of the population and, like many forms of chronic urticaria, has a female predominance [1, 5–7].

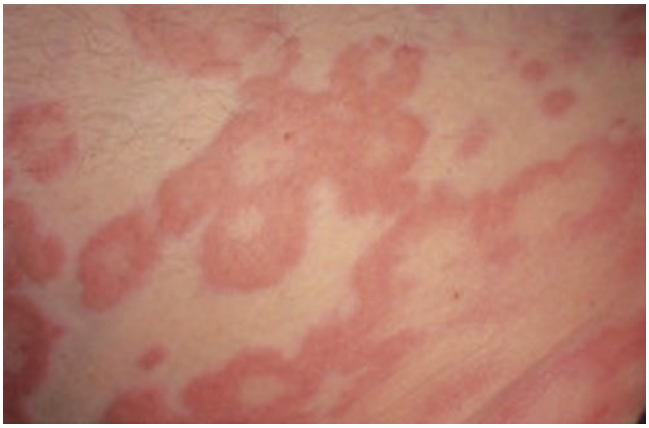

Fig. 27.1 Urticarial lesions (By permission of B Cohen and CU Lehmann, DermAtlas, Johns Hopkins University, 2000–2007)

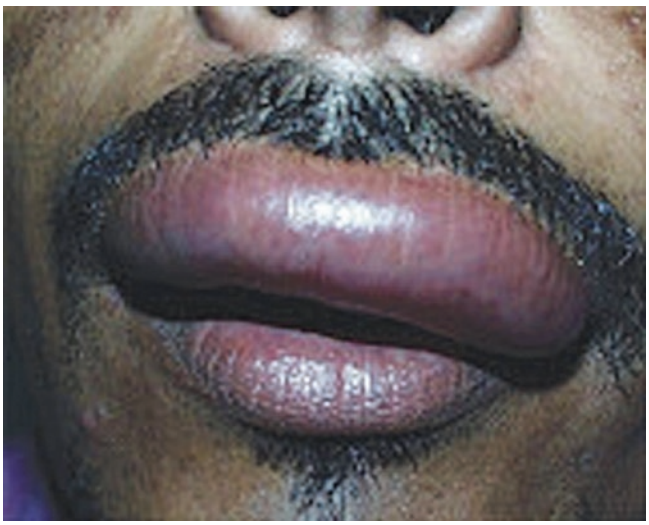

Fig. 27.2 Angioedema (By permission of B Cohen and CU Lehmann, DermAtlas, Johns Hopkins University, 2000–2007)

Clinical Features of Urticaria

Clinically, urticarial lesions are intensely pruritic and can appear anywhere on the body, typically appearing quickly and lasting 1–24 h [8]. Unlike other pruritic skin diseases such as atopic dermatitis, patients with urticaria find relief from rubbing the skin versus scratching, making excoriated skin a less common finding in CSU [9]. Lesions can vary in size and can be confluent (see Fig. 27.1). The swelling observed with urticaria results from the movement of plasma from small blood vessels into adjacent connective tissue [4]. Angioedema often coexists with urticaria, and develops from a deeper, swelling of the dermis, subcutaneous, and submucosal tissues. Angioedema is described as painful or burning in quality, and is rarely pruritic [10]. It frequently involves mucous membranes with common locations being the face, lips, tongue, pharynx, and extremities (see Fig. 27.2) [9].

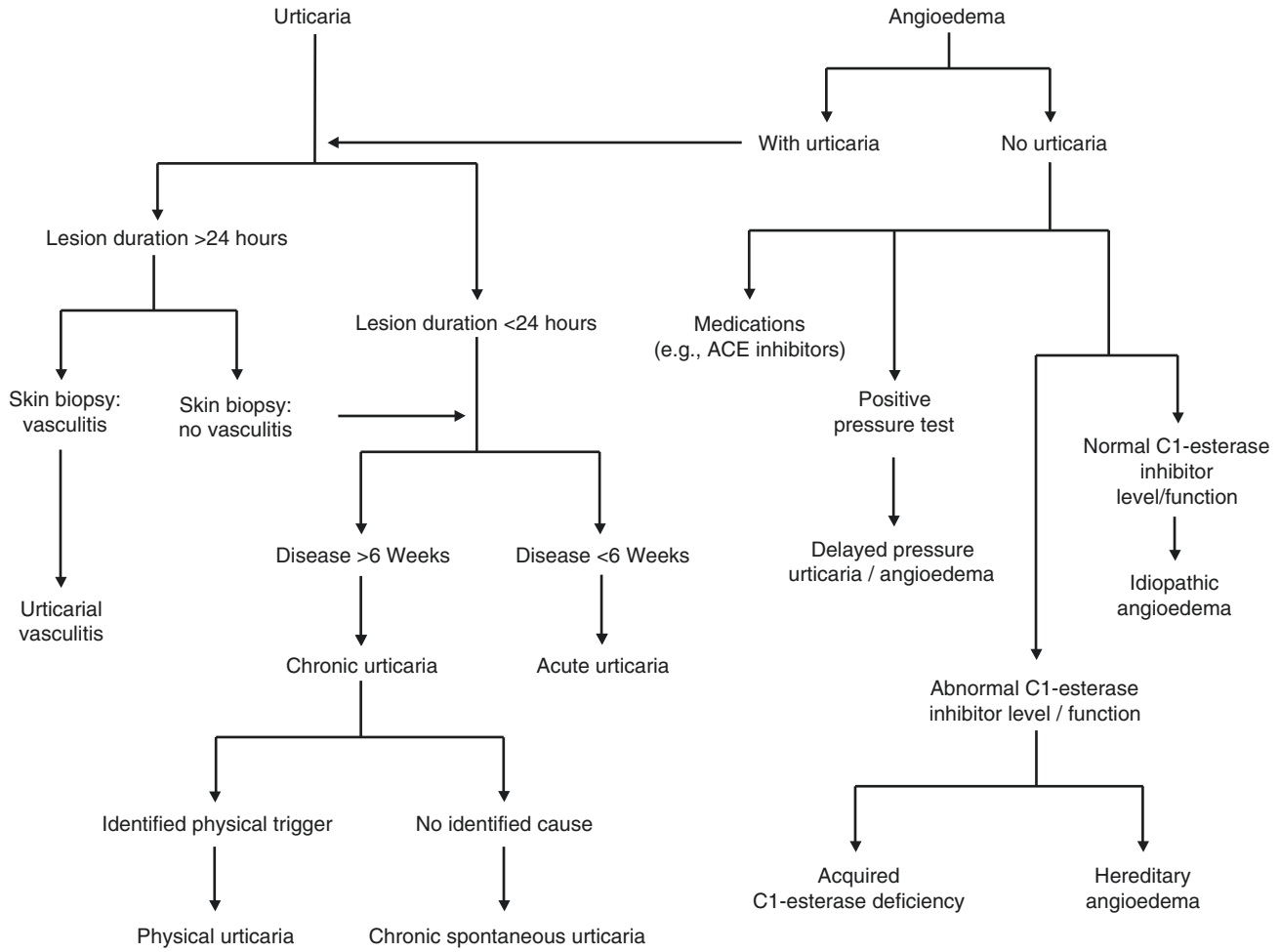

Fig. 27.3 Diagnosis algorithm for urticaria

In contrast, urticaria, both acute and chronic, rarely involves mucosal surfaces [10]. Angioedema typically has a slower resolution time than hives, often greater than 24 h and larger areas may take longer to resolve [9, 10].

History and Physical Exam

In diagnosing either acute or chronic urticaria, a good history is the most critical element (see Fig. 27.3). It is important to assess the time of onset of hives, as some patients may experience diurnal variation, as well as the temporal association with certain environmental exposures or stressors. The most important aspect of history-taking is to identify the inducing agent for the patient's urticaria and/or angioedema. This involves questions regarding recent use of medications, including antibiotics, nonsteroidal anti-inflammatory agents, and aspirin. A history of food ingested shortly prior to symptom occurrence, recent changes in environment, and possible insect stings should also be sought.

Patients should provide a description of lesions including elements such as shape, size, distribution, color, pigmentation, and the quality of pain or itch. Urticarial lesions are typically pruritic and usually demonstrate complete resolution without skin pigment changes. A clinician must also assess for the presence of angioedema in association with urticaria. In patients with isolated angioedema without urticaria, family history is critical to assess for hereditary angioedema (HAE), an autosomal dominant condition resulting from a defect in C1 esterase inhibitor function [10].

Additional questions should include recent infections, thyroid disease symptoms, surgical history of implantable devices, and for females, the relationship of hives to their menstrual cycle or pregnancy. Further evaluation of exposures, including patient's work, may assist in understanding the timing or exacerbation of lesions. It is also important to evaluate how the patient is coping and what therapeutics, including nonprescription medications and dietary regimens, are being used and if they are providing any relief.

Along with the detailed history, the physical exam in a patient with urticaria can be helpful in excluding other

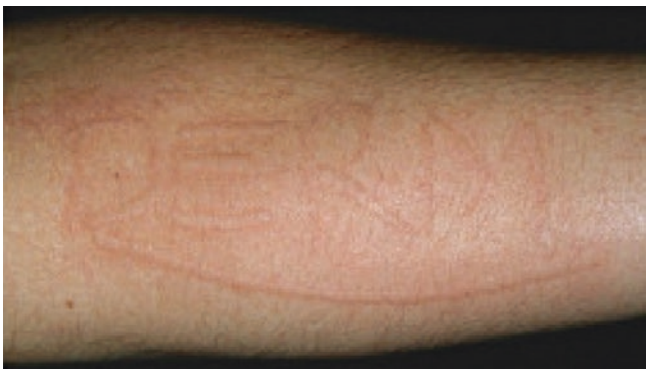

Fig. 27.4 Dermatographism (By permission of B Cohen and CU Lehmann, DermAtlas, Johns Hopkins University, 2000–2007)

disorders. It is important to do a complete exam as well as assess for dermatographism, wheals brought out by stroking of the skin (see Fig. 27.4). The size, distribution, and color of lesions should be noted. Wheals are characteristically pink or red due to histamine-induced dilatation of vessels in the skin while vasculitic lesions have a darker red or purple appearance resulting from vascular damage and leakage [6]. Urticarial lesions can be easily blanched. The physician should also inspect mucous membranes for the presence of angioedema (see Fig. 27.2). Patients should also be assessed for thyroid gland abnormalities on physical exam. Physical urticarias may require additional maneuvers, such as an ice-cube test to evaluate cold urticaria, and should be tailored to the suspected type of urticaria (see Fig. 27.5 and Table 27.1) [1]. No specific laboratory testing is needed for all patients with urticaria and should be dependent on the subtype of urticaria: acute, chronic idiopathic/spontaneous, or physical [11].

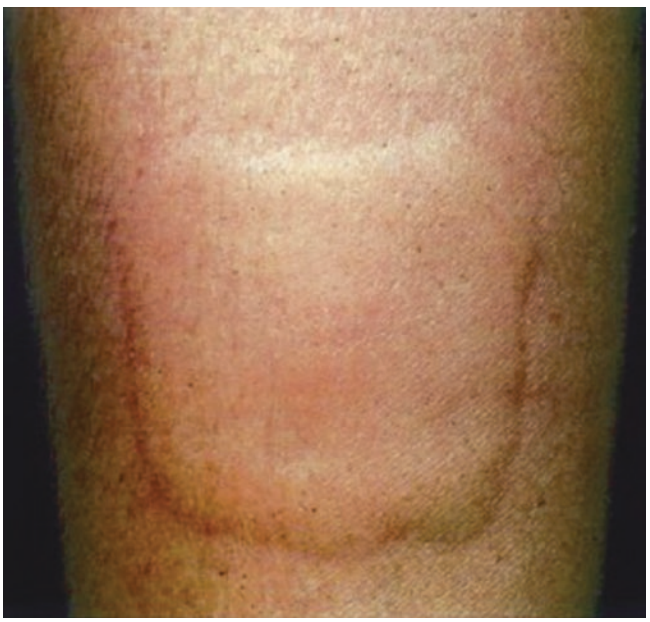

Fig. 27.5 Ice-cube test for cold urticaria (By permission the Department of Dermatology, University of Iowa College of Medicine)

Acute Urticaria

Acute urticaria is classified as hives of less than 6 weeks duration and accounts for up to 56 % of cases of urticaria [12]. Lesions are short-lived, lasting less than 24 h but can return [12]. Clinically, acute urticaria cannot be distinguished from chronic urticaria by physical exam alone. No routine laboratory testing should be done unless used to identify the trigger, such as in suspected food or drug reactions.

Acute urticaria can be classified as allergic (IgE-mediated) or nonallergic. IgE-mediated urticaria may occur in allergic responses to foods, insect stings, and drugs. Non-IgE mediated urticaria may occur in response to a "pseudoallergen" like aspirin, or result from other immunologic responses as seen with blood transfusions, serum sickness, and febrile illnesses [7].

Urticaria related to an IgE-mediated food allergy should occur within 30 min to 2 h following exposure. Testing for food allergies is appropriate in acute urticaria if the patient's history reveals urticaria associated with other symptoms such as nausea or vomiting, however food allergies are not a common cause of acute urticaria in adults. In children, food allergy plays a larger role in acute urticaria, such as with milk, egg, soy, peanuts, and wheat [13].

Drugs can also be responsible for acute urticaria, through either IgE or non-IgE mediated processes. Drugs have been implicated as a cause of acute urticaria in 9.2–27 % of cases [12]. Penicillin is an example of an allergen, which can elicit urticaria through an IgE-dependent mechanism, while acetyl salicylic acid (aspirin) is a frequent non-IgE mediated stimulus for histamine release [12]. ACE inhibitors have also been implicated as triggers for angioedema through effects on the bradykinin pathway [10].

Viral infections are the most common reason for acute urticaria with onset of hives typically occurring a few days after the start of viral symptoms. The coexistence of acute urticaria and upper respiratory infections reported between 28 and 63.4 % [13–16]. Surprisingly, no identifiable trigger is found in 30–50 % of cases of acute urticaria [12].

Chronic Urticaria

Chronic urticaria by definition is wheals occurring at least 2 days per week of at least 6 weeks duration [17]. In contrast to acute urticaria, chronic urticaria is unlikely to be the result of IgE-mediated allergic reactions [18, 19]. Chronic urticaria can be divided into two subtypes: chronic idiopathic/spontaneous urticaria and physical urticaria. Chronic spontaneous

Table 27.1 Classification of physical urticaria

Type	% of all urticaria	Pathogenesis	Features	Reaction times	Provocation test	Differences on biopsy	Treatment
Dermatographic	7–10 %	Unknown, but elevated plasma histamine levels and positive passive transfer experiments, indicate an IgE-mediated process.	Mechanical shearing forces lead to lesions. Koebner's phenomenon, a wheal and flare reaction at pressure sites (e.g. waistband), is present. Higher frequency in young adults.	(1) Immediate – within 2–5 min; lasting 30 min (2) Intermediate – within 30 min to 2 h; lasting 3–9 h (3) Late onset (rare) – within 4–6 h; lasting 24–48 h	Stroke forearm or back with tongue blade	Scant leukocytic infiltrate in upper dermis.	Nonsedating H_1 blocker.
Delayed pressure	3–5 %	Unknown	Constant application of pressure to skin results in erythema and superficial and deep swelling. Pressure areas commonly affected. Male predominance.	Within 3–12 h; lasting up to 48 h	Application of weight to one area for minimum 10 min	Infiltrate typically located in mid- to lower dermis. Neutrophils may be seen in lower dermis.	Nonsedating H_1 blocker. Short course of corticosteroids, leukotriene antagonists may also be used.
Vibratory	Rare	Unknown, but elevated histamine levels and mast cell degranulation reported following application of stimuli. Familial cases have autosomal dominant inheritance.	Occurs after vibratory stimuli (e.g. lawn mowing or motorcycling).	Within minutes; lasting up to 24 h	Challenge with vibratory stimuli (e.g. vortex mixer) for 5 min		Avoidance of trigger.
Familial cold		Gene alteration at CIAS1 on chromosome 1q.44. Autosomal dominant inheritance.	Immediate type characterized by burning papules or macules and systemic symptoms such as arthralgias and fever. Delayed type follows cold exposure.	Delayed occurs within 9–18 h of cold exposure, lasting 2–3 days.		(1) Immediate – Polymorphonuclear infiltrates. (2) Delayed – Mononuclear infiltrates.	

(continued)

Table 27.1 (continued)

Type	% of all urticaria	Pathogenesis	Features	Reaction times	Provocation test	Differences on biopsy	Treatment
Cold contact	3–5 %	IgE-mediated histamine release with IgM and IgG antibodies reported.	Occurs with skin cold exposure. May have angioedema. Rare cases of anaphylaxis after total body exposure to cold.	Within 2–5 min as skin re-warms.	Ice cube placement to area for 10–20 min	Loose lymphocytic and leukocytic infiltrates. Platelet infiltrates and vascular changes may also be present.	Nonsedating H_1 blocker. Leukotriene antagonist may be added.
Heat contact		Unknown, but complement system is afffected.	Occurs at sites of heat application. Divided into immediate nonfamilial and delayed familial.	(1) Immediate – within 5 min; up to 1 h (2) Delayed – within 6–18 h; lasting 12–24 h	Local contact with hot water or object		Avoidance of trigger.
Solar	Rare	Unknown, but thought to be IgE-mediated with increased serum histamine levels, mast cell and eosinophil degranulation, and photoallergen production. Secondary solar urticaria is characterized by a porphyrin metabolism abnormality leading to complement activation.	Induced by sunlight or indoor lighting exposure (wavelengths 280–760 nm). Most common in third and fourth decades of life, commonly affecting young adults.	Within 2–3 min; lasting 3–4 h	Expose to UV light		Avoidance of sun exposure and skin protection. Nonsedating H_1 blocker.

Type	% of all urticaria	Pathogenesis	Features	Reaction times	Provocation test	Differences on biopsy	Treatment
Cholinergic	2–7 %	Unknown, but acetylcholine, released by exercise, can release histamine. Elevated histamine and eosinophil and neutrophil chemotactic factors reported.	Due to a rise in core body temperature. Usually start on face and neck, then spreading. The pruritic wheals are small (1–5 mm) with "fried egg" appearance. More common in young adults.	Within minutes; lasting less than 1 h	Physical activity (e.g. running in place for 5 min)	Loose lymphocytic and leukocytic infiltrates in upper dermis.	Nonsedating H_1 blocker. Increase dose if necessary. In addition, danazol can be used.
Aquagenic	Rare	Unknown, but a proposed mechanism is that water induces formation of histamine-releasing substances.	Contact with water induces small hives similar to those seen in cholinergic urticaria. More common in young adults.	Within 2 min; lasting up to 1 h	Apply water compress for 30 min	Mast cell degranulation noted in challenged skin.	Avoidance of trigger.
Contact		May be IgE-mediated.	Inciting triggers are plants, foods, drugs, and chemicals.	Within minutes; lasting less than a few hours.			Avoidance of trigger.
Adrenergic		Unknown	Pin-sized wheals elicited by stress.	Within minutes; lasting less than 1 h			Propanolol

Source: Data from Zuberbier [6], Kaplan [10], Haas et al. [46], Kontou-Fili et al. [211]

urticaria (CSU) usually lacks an identifiable and consistent trigger, whereas physical urticaria is categorized into subtypes based on the specific trigger(s) which directly elicit hives within minutes. Lesions in physical urticaria generally do not last longer than a few hours, while CSU lesions typically are present for at least 6–8 h [4, 9, 17]. Some crossover exists between CSU and physical urticarias with 40 % of CSU patients displaying dermatographism [20].

Physical Urticaria

Physical urticaria is defined by the ability of a physical stimulus to reproducibly elicit urticarial lesions and accounts for 20–35 % of all cases of chronic urticaria [4]. Physical urticaria can be further divided into many subtypes depending on the physical stimulus (Table 27.1), and more than one subtype can exist simultaneously in one individual [17]. Evaluation of physical urticaria is strongly guided by the patient's history and provocative testing.

Mechanical shearing forces, such as rubbing or scratching the skin, are the catalyst for *dermatographic urticaria*, the most frequent type of physical urticaria, where lesions arise a few minutes following application of the trigger [2, 7]. Features of physical urticarias can be common in the general population with dermatographism reported in approximately 2–5 % of subjects with no history of chronic urticaria [21]. *Delayed pressure urticaria* and angioedema result after vertical pressure is applied to the skin with lesions, often painful, appearing several hours after application and persisting for up to 48 h; this subtype of urticaria predominantly affects the palms, soles, buttocks, and back and has a male predominance [7, 22]. *Vibratory urticaria* is instigated by vibratory forces, is quite rare, and can be described as familial with autosomal dominant inheritance or may occur in sporadic pattern [21, 23].

Familial cold urticaria is an autosomal dominant disease mainly affecting young adults and is due to gene alteration CIAS1 at chromosome 1q.44. This gene locus is also involved in Muckle-Wells syndrome and autosomal dominant periodic fever syndrome, both are diseases that demonstrate cold-sensitivity [24, 25]. *Cold contact urticaria*, which occurs after direct contact of skin to a cold object or air, also occurs predominantly in women, younger adults, and cold climates. This subtype can be idiopathic or can be incited by bacterial or viral infections [22]. *Heat contact urticaria* is rare and results after the skin directly comes in contact with a hot object or warm air [21, 22]. Ultra violet light is the trigger for *solar urticaria*, an IgE-dependent subtype that occurs at wavelengths of 280–760 nm and more commonly affects females and young adults [21, 22].

Cholinergic urticaria is aggravated by a rise in body temperature and thus is triggered by exercise, bathing, emotions, and less with alcohol or spicy food consumption [21, 22]. Cholinergic urticaria typically involves young adults and is characterized by small, pin-sized wheals (1–5 mm diameter), sometimes with a white halo, that last for less than an hour. The symptoms of cholinergic urticaria can be so mild that an estimated 80 % of people with this urticarial subtype do not seek medical advice [7, 22]. Cholinergic urticaria should be distinguished from exercise-induced anaphylaxis which involves the development of systemic symptoms [21]. *Adrenergic urticaria* is also described as pin-sized wheals but unlike cholinergic urticaria, it is elicited by stress and can by treated with propanolol [21, 22]. *Aquagenic urticaria*, which is elicited by any exposure to water regardless of its temperature, mimics cholinergic urticaria in the appearance of its lesions, has a female predominance and more often affects young adults [21, 22].

Contact urticaria can be IgE-mediated or non-IgE-mediated, with the inciting triggers ranging from plants such as grass, foods like peanut, drugs, cosmetics, chemicals, and textiles. Contact urticaria has a short-lived duration like cholinergic and adrenergic urticarial subtypes, occurring within minutes of exposure, and resolving within a few hours [21]. Systemic symptoms can be present with contact urticaria, especially if IgE-mediated [22].

Chronic Spontaneous Urticaria

CSU accounts for approximately 80 % of all cases of chronic urticaria [11]. CSU is episodic and persistent in nature with typical disease duration of 3–5 years [26]. One prospective study of CSU subjects demonstrated that at 6 months, 94 % of patients were still active, 75 % at 12 months, 52 % at 24 months, 43 % at 36 months, and 14 % at 5 years [3]. Likewise, 18.5 %, 54 %, and 67.7 % of children were in remission at 1, 3, and 5 years, respectively, after the onset of symptoms [27]. Disease duration was directly correlated to the presence of severe disease, angioedema, and the presence of anti-thyroid antibodies [3]. Angioedema coexists with urticaria in approximately 40–50 % of CSU patients [26, 28]. The most common and bothersome symptom for patients with CSU is pruritus, which frequently adversely affects their sleep. Fatigue and gastrointestinal symptoms have been previously described with exacerbations, however respiratory complaints and arthralgia also noted [9, 29].

CSU can be a socially and financially disabling disease with an impact on quality of life, comparable to that of coronary heart disease [30]. One study of 170 chronic urticaria patients reported individuals with CSU alone experience a moderate impairment in their quality of life as measured by the Dermatology Life Quality Index (DLQI), while those with CSU and angioedema and/or delayed pressure features had significantly greater quality of life impairment [31]. Compared to other debilitating dermatological diseases, the quality of life impairment in chronic

urticaria is similar to patients with severe atopic dermatitis and worse than patients with psoriasis, acne, and vitiligo [31]. Part of the frustration patients experience results from the lack of an identifiable trigger for urticarial exacerbations, leading to an unpredictable disease. CSU also carries an economic burden for patients with multiple medications, medical evaluations, work absence, and use of the emergency department [32, 33].

Aspirin and nonsteroidal anti-inflammatory agents (NSAIDs) can substantially aggravate urticaria and angioedema in CSU subjects via inhibition of prostaglandin synthesis. Selective cyclooxygenase-2 (COX-2) inhibitors lead to less symptoms than aspirin and traditional NSAIDs and provide an alternative choice for analgesics in CSU patients [34]. One study of chronic urticaria patients challenged with aspirin demonstrated that approximately 20 % of CSU subjects had a reaction, while those with physical urticaria were unaffected [35]. Patient history of suspected aspirin sensitivity correlates very well with reactions during aspirin challenges, as one study found that 92 % of patients with a history of reaction reacted during an aspirin challenge [36]. Patients should be advised that aspirin and traditional NSAIDs have the potential to exacerbate their disease, and should be informed that salicylates may be an occult ingredient in medications or supplements. Studies to assess the role of salicylates in foods demonstrated inconclusive results, and "low salicylate" diets are not generally recommended [11].

There is a higher prevalence of thyroid disease in CSU than in the general population. Hashimoto's thyroiditis and, less commonly, Graves' disease are the only reported systemic diseases with a correlation to CSU [37–40]. It has been reported that 27 % of CSU subjects have thyroid autoantibodies, nearly twice the rate observed in the normal population. Typically, the majority of those with thyroid autoantibodies are euthyroid and some may be hypothyroid, but it is rare for these individuals to be hyperthyroid [40–42]. Currently, these autoantibodies are not thought to be pathogenic in urticarial [43] but lend support to an autoimmune basis for a subset of CSU [44]. Studies of thyroid hormone replacement in patients with concomitant CSU and thyroid autoantibodies have yielded mixed results [43, 45]. The current recommendation is to screen patients for underlying thyroid disease and to treat any underlying thyroid disease, but no clear evidence exists for the use of thyroid hormone in euthyroid patients who present thyroid autoantibodies.

Histology

The classic wheal observed in both acute and chronic urticaria represents dermal edema with dilatation of postcapillary venules and lymphatic vessels (see new Fig. 27.6a, b) [46]. In biopsies, the leukocyte infiltrate is characteristically perivascular and classically consists of lymphocytes, neutrophils, macrophages, basophils, and occasionally eosinophils (see Fig. 27.6a, b) [47, 48]. Degranulated mast cells are also noted in urticarial lesions [48]. Histologically, CSU shares lesional features with acute urticaria consisting of a lymphocytic-predominant perivascular infiltrate. Occasionally, neutrophils are seen within capillary or post-capillary venule walls, but unlike the neutrophilic infiltrate seen in urticarial vasculitis, there is no evidence of vascular damage, nuclear debris, or red cell extravasation (Fig. 27.6b, c) [1]. The presence of intradermal CD3+, CD4+, CD8+, and CD25+ T-cells, as well as eosinophils, neutrophils, basophils, and macrophages is significantly higher in CSU skin lesions as compared to skin of nonatopic individuals [48]. A survey of basophil numbers in the skin biopsies from 24 skin diseases revealed that CSU had among the highest numbers of infiltrating basophils [49].

Although the cellular infiltrate of CSU resembles that seen in allergen-induced late phase skin biopsies, the cytokine profile in CSU is T_H1 and T_H2 with higher expression of IFN-γ, as well as IL-4 and IL-5 in the skin [48]. By immunohistochemistry, TNF-α and IL-3 protein expression are increased in acute and chronic urticarial lesions [50]. TNF-α is a cytokine produced by epithelial cells, leukocytes, and mast cells [51, 52]. TNF-α can induce mast cell mediator release and increase endothelial adhesion molecule expression, thus an increase of this cytokine in urticaria may contribute to the leukocyte infiltrate observed [51, 53, 54]. IL-3 is a cytokine produced by mast cells, T-cells, monocytes, and granulocytes, and can increase expression of the endothelial adhesion molecule P-selectin [55–59]. P-selectin expression is elevated in both the skin and serum of chronic urticaria patients [60]. Two endothelial adhesion molecules, integrins ELAM-1 and ICAM-1, were upregulated in acute and chronic urticarial lesions [61]. The persistent expression of ELAM-1 and ICAM-1 in lesions older than 6 h may help to explain the long duration of urticarial wheals [61].

Pathophysiology of Urticaria

Whereas the pathophysiology of allergic urticaria supports a specific allergen interacting with IgE bound on skin mast cells, chronic urticarias do not appear to involve IgE binding to allergen [4]. The pathophysiology of both physical urticaria and CSU is unknown. Although mast cell degranulation is a clear component in some subtypes of physical urticaria (dermatographic, cholinergic, cold, and solar urticaria), serum IgE may also play a role as demonstrated by passive transfer experiments [9, 22, 42]. An acute rise in serum histamine and PGD2 levels has been observed in cold urticaria following cold exposure [62]. In CSU, there is also evidence for mast cell degranulation observed on skin biopsies [48]. Mast cell number appears to be similar in nonlesional and

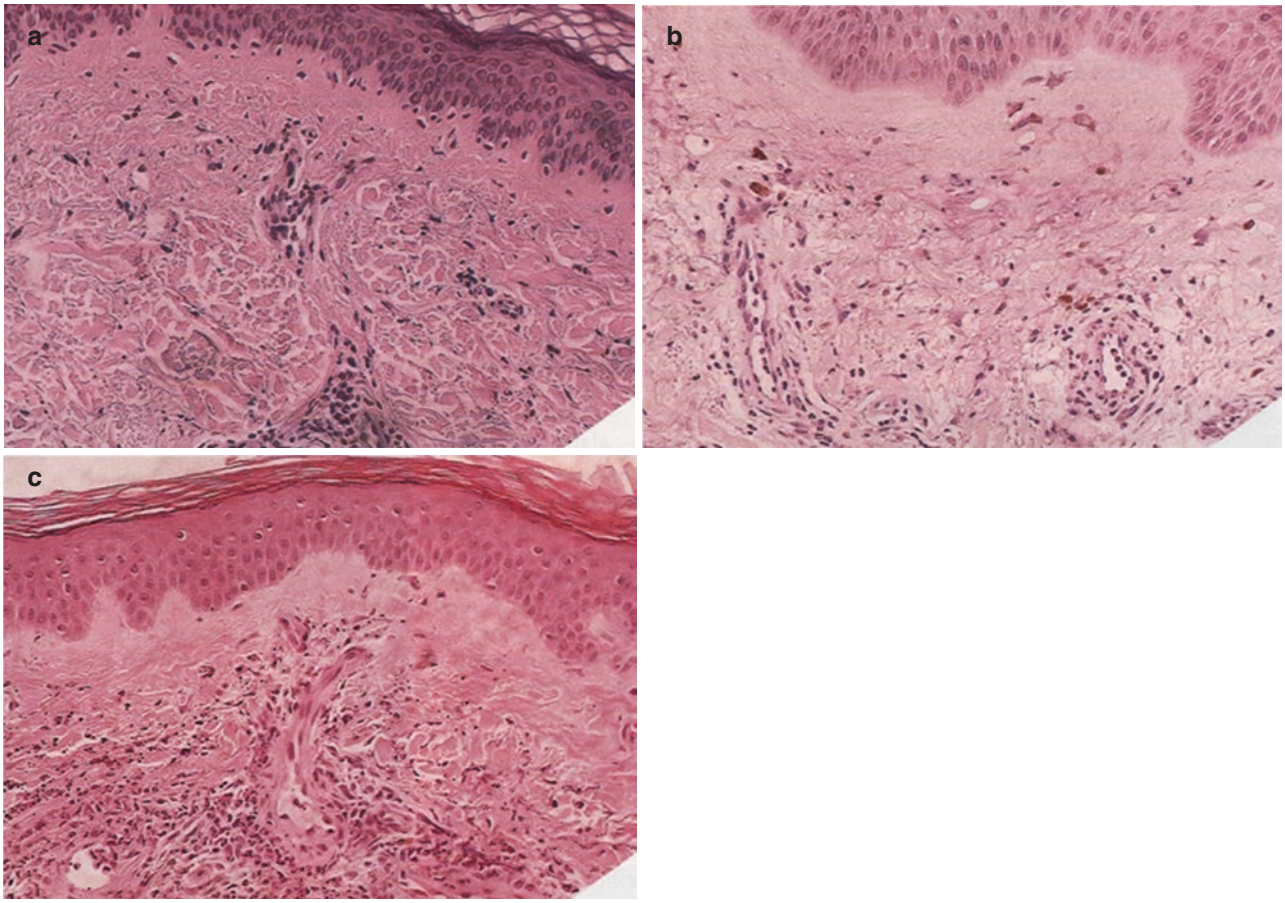

Fig. 27.6 Biopsy of urticarial lesion. (**a**) Representative of acute urticaria with dermal edema and a perivascular mononuclear infiltrate. (**b**) Chronic urticaria is may also have infiltration of neutrophils and/or eosinophils without evidence of vasculitis. (**c**) Urticarial vasculitis is shown with a large perivascular infiltration of neutrophils, and even some neutrophilic damage (By permission of Fireman and Savin [212])

lesional CSU skin and normal skin, using tryptase and chymase staining [63]. Although mast cell number is not altered, increased mast cell releasability has been demonstrated in patients with CSU with active disease, which resolves with disease remission, suggesting that the mast cell alteration is reversible [64]. Cultured peripheral blood mast cells from CSU patients have been found to have higher levels of spontaneous histamine release compared to healthy controls [65]. In contrast, another study found higher levels of total tryptase (a reflection of the body burden of mast cells) in the sera of chronic urticaria patients compared to controls, but no difference was found in mature tryptase levels (reflects mast cell degranulation) [66].

There has been accumulating evidence that basophils play a role in CSU [67]. Basophils have been reported in both lesional and nonlesional CSU skin using BB1 staining [48, 49, 68]. Blood basopenia is a feature of active CSU disease, and the reduction in basophil number correlates inversely with disease severity [69, 70]. As seen with allergen late-phase reactions, basophils that have migrated to the skin may play a role in duration as well as magnitude of urticarial lesions [71]. Select basophil surface markers correlate to basophil activation and have been measured in CSU, in particular, CD63, CD69, and CD203c [72–75]. CD63 is a member of the transmembrane-4 superfamily is rapidly mobilized onto the basophil surface by IL-3, allergen, anti-IgE and other stimuli of degranulation while CD69 is slowly induced following IL-3 stimulation [72, 76]. CD203c, also known as ectonucleotide pyrophosphatase, is unique to basophils, mast cells, and their progenitors and is mobilized by allergen, anti-IgE, and IL-3 [77–81]. CSU subjects demonstrate enhanced basophil surface expression of CD63, CD69, and CD203c when compared to non-allergic controls [49, 75].

Furthermore, CSU basophils demonstrated a decreased functional response to IgE receptor stimulation but not to other stimuli [67, 82–85]. Early studies showed that histamine release mediated via the basophil high affinity IgE receptor, FcεRI, was diminished in subjects with CSU versus controls [84, 85]. This finding is paradoxical since anti-histamines are used to treat CSU, and pruritus, the predominant symptom in CSU, is histamine-dependent. A later study has shown that

basophils in CSU have altered FcεRI-mediated histamine release and have found two patterns of histamine release within subjects with active CSU disease [83]. Approximately 50% of subjects with CSU tested were found to be "responders," releasing greater than 10% of cellular histamine content when triggered with a polyclonal anti-IgE stimulus, and 50% were "nonresponders", releasing less than 10% of cellular histamine content [83]. Also, these two basophil phenotypes appear to be stable over time as subjects maintain active disease, but this altered basophil function improves with disease remission [84, 86].

In a subset of CSU referred to as "autoimmune urticaria," histamine-releasing autoantibodies have been reported and hypothesized to be pathogenic, although this remains controversial [44, 87–92]. These IgG autoantibodies are thought to activate mast cells and basophils via complement and act by C5a to potentiate histamine release [93]. These autoantibodies are detected in 30–40% of patients with CSU, and the majority of these autoantibodies are directed against the alpha subunit of the high affinity IgE receptor, FcεRI, while the remainder directly target IgE [29, 89]. In favor of autoimmune mechanisms in CSU is the increased incidence of thyroid autoantibodies, specifically anti-microsomal and anti-thyroglobulin, as well as reports of higher frequencies of certain HLA class II alleles (DR4, DQ8) in CSU patients [37, 38, 40, 41, 94]. The autoantibody theory is also supported by the observation that autologous serum injected into a CSU patient's skin can result in a wheal-and-flare response [95, 96]. However, a recent study demonstrated that healthy controls and patients with allergic rhinitis can also have a positive ASST, raising doubt about the usefulness of the ASST in the diagnosis of urticaria [11, 97, 98].

Since the presence of an ASST response alone does not prove that autoantibodies are present, further studies were done and demonstrated that sera from ASST-positive CSU subjects also had histamine-releasing activity (HRA) from healthy donor basophils [89, 99]. A large study found that 40% of CSU subjects' sera exhibit HRA when performed *in vitro* on donor basophils [88]. Although HRA is reported to indicate the presence of autoantibodies, the presence of HRA and the presence of autoantibodies by Western blotting in CSU subjects do not agree [100, 101]. The presence of serum HRA had no effect on the character of the leukocyte infiltrates of new (<4 h) or established (>12 h) skin lesions even though autoantibody presence has been previously linked to increased disease severity [48, 101, 102]. In addition, serum HRA has been demonstrated in healthy patients' sera, and there is no correlation between HRA and CSU basophil FcεRI function [83, 103].

Another issue with the autoantibody hypothesis is that anti- FcεRIα autoantibodies are also found at a similar frequency in other autoimmune diseases like systemic lupus erythematous (SLE) and dermatomyositis as well as in normal subjects [104]. Similar frequencies of positive results have been found between CSU and SLE patients using a commercial test which measures the ability of a patient's serum to trigger histamine release from donor basophils [105]. This lack of diagnostic specificity of the HRA assay raises concern about its usefulness in determining disease pathogenesis in CSU patients [103]. Also, anaphylaxis is rarely seen in CSU, which is a skin-limited disease, but would be expected to occur more often if autoantibodies directed against basophils and mast cells IgE receptor are present.

Laboratory Evaluation

In most cases of urticaria, history and physical examination are sufficient for diagnosis [6], and a thorough history may identify potential triggers such as medications. Patients with chronic urticaria who are resistant to conventional therapies or experience atypical lesion features (i.e., hyperpigmentation, bruising, prolonged duration) may need further evaluation to exclude secondary causes for hives. Laboratory testing should be limited because previous studies have shown extensive testing provides low yield [11, 106]. Patients who have symptoms or a family history of thyroid disease would warrant thyroid screening by measuring thyroid stimulating hormone and possibly thyroid autoantibodies.

For physical urticarias, patients are unlikely to benefit from further laboratory testing [107–109]. The physical exam can assist substantially in defining the type of physical urticaria that is present based on provocative tests (see Table 27.1). Patients with significant angioedema without urticaria should have complement levels checked along with C1 inhibitor testing (functional and quantitative levels) to screen for *hereditary and acquired angioedema* due to C1 esterase inhibitor deficiency which would result in low complement C4 levels [10].

Infections have been implicated in urticaria. As previously mentioned, viral infections, especially viral upper respiratory infections are a common cause of acute urticaria. A study in a Japan, which has a high prevalence of hepatitis C, demonstrated that this infection may manifest with chronic urticarial lesions, but further work in areas with lower prevalence has not supported this finding [110, 111]. It is recommended that individuals with risk factors for infectious hepatitis should have the appropriate testing. In recent years, Helicobacter pylori has been implicated to CSU but controlled studies have not been able to establish this link [112–115]. Parasitic infections are a rare cause of urticaria that should be investigated by stool examination for ova and parasites. Bacterial cultures are rarely necessitated as bacterial infections are not a common cause of urticaria [116].

Allergy testing may be of limited use in acute urticaria. Serum specific IgE testing (ImmunoCAP) or skin testing may be beneficial when a Type-I hypersensitivity reaction is strongly suspected. Skin testing is often difficult to perform in chronic urticaria patients due to the high prevalence of dermatographism and delayed pressure features in this group as well as their dependence on anti-histamines. ImmunoCAP testing is not affected by medications. A careful history can help to identify which allergens to test. Extensive food or inhalant allergy testing should not be performed blindly in patients with chronic urticaria as this does not improve patient outcomes and is not a cost-effective approach [11].

Another skin test used in the past to define autoimmune urticaria is the autologous skin serum test (ASST). Since the presence or absence of the ASST or autoantibodies does not alter treatment options in patients, the ASST should not be used as a diagnostic tool for chronic urticaria [7, 11]. Newer basophil-based assays to check for serum histamine releasing activity (HRA) also lack specificity and validation for clinical use [103, 105, 117, 118].

Other Conditions Associated with Urticarial Lesions

Urticarial vasculitis (*UV*) must be excluded in patients with chronic urticaria unresponsive to conventional therapy. UV is a Type-III hypersensitivity reaction mediated by antigen-antibody complexes deposited on vascular endothelium, and may be precipitated by infections, medications or neoplasms [119]. The duration of UV lesions is characteristically greater than 24 h and may be painful and purpuric, leaving residual skin changes [120]. These patients may also complain of systemic symptoms and have laboratory findings consistent with an inflammatory process, such as an elevated ESR or C-reactive protein or low complement levels [120]. To rule out urticarial vasculitis, the definitive test is a skin biopsy. The features of urticarial vasculitis on biopsy are leukocytoclasia, extravasation of red blood cells, fibrin deposition, leukocyte invasion of the vascular endothelium, and endothelial edema, of which the latter three features may also be observed in chronic urticaria (see Fig. 27.6) [1, 120].

There are other diseases associated with urticarial lesions, and patients presenting with appropriate symptoms should be evaluated for these disorders. *Urticaria pigmentosa* (*UP*), a subtype of mastocytosis, may mimic urticarial lesions with the exception that these are pigmented and typically longer-lasting lesions than those in urticaria. These lesions typically urticate with scratching (Darier's sign), however, the diagnosis must be confirmed by biopsy and histology in each case [121, 122]. UP is often limited in children, as there is generally a spontaneous regression of the condition by puberty [123]. Adult mastocytosis, however, is an aggressive disease, and all adults with UP should have a total tryptase level measured and undergo a bone marrow biopsy [121].

Bullous pemphigoid (*BP*) is an autoimmune blistering skin disease which, in its early stages, may also present with urticarial lesions. The primary lesions of BP result from IgG autoantibodies are directed primarily against the BP 230 and BP 180 antigens, which are components of the hemidesmosome adhesion complex and allow the basal cells of the epidermis to adhere to the basement membrane. BP primarily affects older patients, and should be considered in urticaria develops in elderly individuals [120].

Subacute cutaneous lupus erythematosus (*SCLE*) can present with urticarial-like lesions which can be mistaken for CSU. SCLE is characterized by nonscaring lesions that typically burn more than they itch. These lesions tend to fluctuate in appearance and occur predominantly on sun-exposed areas of the body, often hours to days following significant sun exposure [120].

The presence of systemic symptoms along with urticaria should raise concern for additional conditions. *Schnitzler's syndrome* is associated with recurrent urticarial lesions and dermatographism, fever, arthralgia, bone pain, lymphadenopathy, a high ESR, and IgM gammopathy. Patients with Schnitzler's syndrome generally have a pale rose or red eruption consisting of macules papules, and plaques which tend to occur in crops, last <24 h, and are usually not significantly pruritic [124]. Angioedema is not a common is not feature of this condition. There is a delay in diagnosis (usually 5 years or longer) because most patients are instead labeled as having antihistamine-unresponsive CSU, and patients with these symptoms warrant a serum protein electrophoresis [120].

Sweet's syndrome (acute febrile neutrophilic dermatosis) was first described in 1964 by Robert Sweet [125]. It is characterized by fever, neutrophilia (with blood polymorphonuclear leukocyte level greater than 10,000/mm^3), and painful, erythematous papules or plaques (dense dermal neutrophilic infiltrate) on the extremities, face, and neck [125]. Classic Sweet syndrome is associated with infection of the respiratory or gastrointestinal tract, or may occur after vaccination. There is also a paraneoplastic variant of Sweet syndrome which is most commonly associated with lymphoproliferative disorders, and occasionally associated with solid malignant tumors [126].

Also presenting with fever and urticaria are *serum sickness* (*SS*), *serum sickness-like reactions* (*SSLR*), and *urticaria multiforme*. Both SS and SSLR present with fever, rash and arthritis 7–21 days after exposure to the offending antigen, which is most often a medication. It is critical that a medication history be elicited from these patients, and that

suspected medication be promptly discontinued. Unlike classic SS, SSLR does not have detectable circulating immune complexes or hypocomplementemia, and its pathogenesis remains poorly understood. Urticaria multiforme primarily affects children, and involves urticarial plaques with a hemorrhagic pattern which appear 1–3 days following an acute viral illness [119].

Treatment

Acute Urticaria

For acute urticaria the most important step is to eliminate the trigger if identified. Patients can use antihistamines to help with symptom alleviation until resolution of the episode. Approximately 44–91 % of all urticaria patients treated with H1-antagonists found a benefit [127, 128]. Typically, non-sedating H1-antagonists are utilized although classic sedating H1-antagonists, such as diphenhydramine or hydroxyzine, may be used. The major risk associated with administration of first-generation H1-antagonists is somnolence [12]. For severe cases of acute urticaria, a short course of systemic corticosteroids may provide more prompt control [12]. One study reported that acute urticaria exacerbations lasted only 3 days in approximately 94 % of studied patients compared to only 66 % of patients not receiving steroids [129]. Topical steroids play no role in the treatment of urticaria since application would involve a large area of skin with minimal benefit [7].

Chronic Urticaria

The treatment of CSU is a greater challenge than that of acute and physical urticarias. The physician should explain to the patient the natural history of CSU and the lack of a cause as this may lessen patient frustration. Patients should avoid triggers, but these are rarely identified in CSU. Avoidance of certain potential triggers such as aspirin, nonsteroidal anti-inflammatory drugs, ACE inhibitors, and codeine-containing products that can directly stimulate skin mast cells is also recommended [7].

Second-generation antihistamines are first-line therapy for chronic urticaria [130]. H1-antagonists are used to control the intense pruritus associated with the CSU. One study reported that 94 % of patients with CSU had some itch relief with use of H1-antagonists, with the majority on the sedating type [131]. In patients with CU who do not respond adequately to second-generation antihistamines at FDA-approved doses, doses can be provided at higher than licensed doses, often with additional therapeutic benefit [130]. Similarly, the treatment of physical urticaria involves second-generation antihistamines and avoidance of triggers [132–137]. Some past studies in CSU patients demonstrated an improvement in dermatographism and pruritus with the simultaneous use of an H1-antagonist and H2-antagonist versus a H1-antagonist alone. *Doxepin*, a tricyclic antidepressant with some H1- and H2-receptor antagonist properties, may be useful for nighttime itch and has been shown to be more effective than diphenhydramine in the treatment of CSU [138]. Doxepin should not be used with MAO inhibitors due to a risk for Long QT Syndrome [7]. However, approximately 50 % of chronic urticaria patients do not respond to antihistamines and require additional agents to achieve symptom control [139, 140].

Success with *leukotriene receptor antagonists* has been reported for physical urticaria of the cold-induced and delayed pressure types, aspirin-sensitive urticaria, and food-induced acute urticaria [141–143]. However, there is conflicting results regarding the efficacy of leukotriene receptor antagonists in CSU. Various randomized, placebo-controlled studies reported that montelukast provided symptom alleviation in CSU patients when used as a monotherapy or in conjunction with cetirizine or desloratidine, but another placebo-controlled study demonstrated that montelukast plus desloratidine was equal to desloratidine alone while montelukast monotherapy offered no benefit over placebo [141, 144–146]. Another leukotriene receptor antagonist, zafiruklast failed to show benefit over placebo in CSU [147]. Some studies have only shown a benefit with leukotriene receptor antagonists in a subset of CSU patients with a positive ASST [148, 149]. Overall, leukotriene receptor antagonists may be added to anti-histamines in urticaria patients as a limited trial and have a side effect profile similar to placebo.

Systemic corticosteroids also have a role in severe, antihistamine-resistant CSU when rapid control is warranted or with episodes of significant angioedema. The mechanism for disease alleviation with corticosteroids is not known, but CSU patients treated with steroids have a transient rise in peripheral basophil counts perhaps suggesting decreased recruitment of basophils to the skin [70]. The use of systemic corticosteroids should be sparse due to the side effect profile with prolonged use including a greater risk for osteoporosis, peptic ulcer disease, diabetes, and hypertension, to name a few [150].

More recently, anti-inflammatory drugs have been used for the treatment of antihistamine-unresponsive and steroid-dependent CSU. In various case reports, *sulfasalazine* has shown some benefits in CSU as well as delayed pressure urticaria [151–154]. One recent study of patients with antihistamine-unresponsive CSU reported that 74 % of

patients treated with sulfasalazine had significant improvement in disease and with an additional 21 % showing minimal improvement, and in additional all patients treated either discontinued or decreased their steroid use [155]. Although the mechanism of action is unknown, it is hypothesized that sulfasalazine may alter IgE-mediated mast cell histamine release, with one study demonstrating reduced release in mast cells and two prior studies demonstrating enhanced release in mast cells as well as basophils [156–158]. *Dapsone*, which exhibits anti-inflammatory properties and inhibits neutrophil function, showed promising results in one open-label study and may be beneficial for patients with neutrophil-predominant infiltrates on skin biopsy [159, 160]. More recently, a small randomized placebo controlled trial showed benefit in patients with antihistamine-refractory CSU [161]. This medication should be avoided in individuals with G6PD deficiency, which places patients at risk for hemolytic anemia [150].

Hydroxychloroquine has also shown some benefit in chronic urticaria. A randomized, double-blind, placebo-controlled study showed a significant improvement in quality-of-life scores in patients treated with hydroxychloroquine, but only a marginal change in urticaria activity scores [162]. Hydroxychloroquine has a number of immunologic effects, which include inhibition of endosomal TLR signaling resulting in reduced B-cell and dendritic-cell activation, as well as inhibition of antigen presentation [163]. *In vitro* studies have shown that hydroxychloroquine can decrease the production of TNF-α, IL-6, and IFN-γ by mitogen-stimulated peripheral blood lymphocytes [163, 164]. When compared with other immunomodulatory agents, antimalarials have a favorable safety profile [163]. Although rare, there is a risk of retinopathy with prolonged use (>5 years) of hydroxychloroquine [150].

Cyclosporine, a calcineurin inhibitor, has shown some success in small series and randomized control trials in CSU patients [165–167]. Cyclosporine reduces the Th1 lymphocyte response, inhibits antibody formation, and inhibits basophil and mast cell anti-IgE induced histamine release [165, 168]. One of these studies reported that cyclosporine treatment did not change the presence of the ASST in patients who entered drug remission [167]. Blood pressure, kidney function, and liver function should be monitored regularly during the treatment period, which is generally 3–6 months [150, 169]. After the treatment course has been completed, remission may last up to 9 months in about 50 % of patients [150, 170].

Methotrexate is an anti-inflammatory agent with immunosuppressant activity, and has a number of actions ranging from increasing adenosine levels, inducing apoptosis in activated $CD4^+$ T cells, and decreasing neutrophil chemotaxis [171]. The most frequent adverse effects are nausea and vomiting (both dose-related) [172]. Methotrexate is also a known abortifacient and teratogen, and should be avoided in women capable of conceiving [172]. Small studies and case reports involving the use of methotrexate for CSU have shown conflicting results [173, 174]. One retrospective review reported a beneficial role for methotrexate for the treatment of steroid-dependent chronic urticaria [175]. However, a prospective study showed that a 3 month regimen of methotrexate (15 mg weekly) failed to provide any additional benefit over H1-antagonists [173].

Mycophenolate mofetil functions as a reversible inhibitor of inosine monophosphate dehydrogenase, which is involved in the synthesis of purines and unique to lymphocytes [139]. In a study of 9 CSU patients, mycophenolate provided a significant improvement in the individual urticarial activity scores at the end of a 12-week treatment period [139]. *Azathioprine*, which inhibits lymphocytes by also suppressing purine nucleotide biosynthesis, has been investigated in chronic urticaria in a limited number of cases with success [176].

Small case reports of *cyclophosphamide* have shown a potential role for these agents in CSU [177, 178]. Cyclophosphamide results in a differential cytotoxicity to various lymphoid cell populations, with selective suppression of B cells [172, 179]. Because of the risk of bladder toxicity, gonadotoxicity, myelosuppression, and malignancy, some recommend that its use be reserved for serious and potentially life-threatening disorders in which the benefit outweighs the risks [172].

Rituximab, a monoclonal anti-CD20, has had success in the treatment of SLE, and due to CD20's role in B cell development, it may decrease autoantibodies thus making it a potential therapeutic for CSU [180, 181]. This treatment has been associated with a number of adverse events, including supraventricular arrhythmias, acute coronary syndrome, interstitial lung disease, myelosuppression, infections, sepsis, anaphylaxis, and neoplasms [140]. Although promising, further controlled studies including needed to determine the safety and efficacy of this treatment.

A therapeutic option recently FDA-approved for CSU (ages 12 and above) is *omalizumab* (*Xolair*), a monoclonal antibody directed against IgE. Omalizumab is known to alter mast cell and basophil anti-IgE induced histamine release as well as decrease free IgE levels and FcεRI expression on mast cells and basophils [182]. A number of controlled studies have demonstrated that a significant improvement in disease activity occurs much earlier than the expected decline in skin mast cell IgE receptors [140, 183–185]. The response rate to omalizumab is approximately 65 %, when one includes only those patients who cannot be managed satisfactorily with high-dose antihistamines [140]. Similar response rates have been observed between in CSU subjects independent of autoimmune status [183, 184]. Taken together, these studies demonstrate an

excellent side effect profile with mild side effects (in 1–10 % of patients) such as local injection site reactions (swelling, redness, itching), sinusitis, headache, arthralgia and upper respiratory tract infections [140]. Despite its success, the mechanism of action of omalizumab in CSU is currently not clear.

TNF-α inhibitors, which have known anti-inflammatory properties, may be a potential therapeutic for CSU as they have been successfully used in autoimmune diseases such as rheumatoid arthritis and inflammatory skin diseases like psoriasis [186, 187]. There are two methods for neutralizing the activity of TNF-α: a neutralizing antibody (infliximab, adalimumab) and a fusion protein of a TNF-α receptor (etanercept) [186]. Among 20 patients treated with either adalimumab or etanercept, 12 obtained complete or almost complete resolution of symptoms and 3 received a partial response; however 6 patients experienced side effects [188].

Other therapies, although less studied, have also been used in chronic urticaria. *Plasmapheresis* has been used with the intended purpose of removing autoantibodies with temporary effects on disease activity [9]. One study in HRA-positive CSU patients demonstrated a reduction in ASST in patients treated with plasmapheresis, but these patients continued to have a positive ASST during clinical remission [189]. *Intravenous immunoglobulin* (*IVIG*) infusions were studied in patients with reported functional anti-FcεRI or anti-IgE autoantibodies and had a reported benefit [190, 191]. However, a subsequent study demonstrated that the improvement with IVIG was temporary, indicating that IVIG is likely not an effective therapy for CSU [192]. *Androgen therapy* with danazol was shown to be beneficial in one randomized controlled trial 17 male patients with cholinergic urticaria [193]. The anti-inflammatory effects of androgens may stem from their ability to interfere with endogenous sex steroid production and suppress leukocyte activation [178, 194, 195], but such agents should be avoided or used with extreme caution in females due to their strong androgenizing effects [178]. *Zileuton*, a 5-lipoxygenase inhibitor, inhibits leukotriene B4 and C4 production and has shown success in a small case study of chronic urticaria patients [196, 197]. Oral *ketotifen*, which has both antihistamine and mast cell stabilizing properties, has been widely used in Europe, Canada, and Mexico but is not approved by the FDA for use in the United States [198]. *Warfarin* therapy was studied due its ability to alter proteases of the complement, kinin, clotting or fibrinolytic systems which may potentially be activated in CSU, leading to vasoactive mediator release in CSU [199]. *Heparin* and *tranexamic acid* therapy may be effective in treatment-resistant chronic urticaria patients with elevated d-dimer levels [150, 200]. *Psoralen UVA or UVB phototherapy* has not proven beneficial in CSU in past studies, although PUVA is known to reduce skin mast cell number [201–205]. However, a more recent study of PUVA-and narrow band UVB treatment, demonstrated a significant decrease in symptoms in both treatment groups [206]. *Colchicine* has been suggested as a therapeutic option for delayed-pressure urticaria [207]. A recent retrospective study of 36 chronic urticaria patients treated with colchicine found that 56 % reported as complete or partial response [208]. *Interferon-alpha* has also been studied and does not appear to be an effective therapy [209, 210].

Conclusion

Urticaria is produced by the degranulation of mast cells in the skin, and has immunologic and nonimmunologic mechanisms. Chronic urticaria is a troublesome condition that can adversely affect the quality of life of this affected. Evaluations to identify and remove trigger factors are critical to the management of this condition. Treatments range from the H1 receptor antagonists with or without H2-antagonists, leukotriene receptor antagonists, tricyclic antidepressants, and systemic corticosteroids. In severe cases, immunosuppressive drugs and biologic agents have been successfully used.

Questions

You see a 43-year-old female with chronic hives that occur daily and disrupt her sleep. She has no other systemic disease aside from hypertension. She has failed updosing with cetirizine (20 mg bid) montelukast, and required 2 steroid courses in the last 4 months. Her physical exam is consistent with urticarial. Routine clinical labs are within normal (CBC WESR, Chemistries, and TSH

1. Which of the following add-on agents has regulatory approval for the treatment of chronic idiopathic urticaria refractory to H1 antihistamines?
   (A) Cyclosporine
   (B) Hydroxychloroquine
   (C) Omalizumab
   (D) Dapsone

   Answer: (C) Among the listed choices, only omalizumab and cyclosporine have been studied in randomized controlled trials. In 2014, the US Food and Drug Administration approved the use of omalizumab, a monoclonal antibody against immunoglobulin E, in adolescents and adults (12 years of age or older) with chronic idiopathic urticaria refractory to H1 antihistamines

   Given the evidence of hypertension, omalizumab therapy is a better choice given the side effect profile of cyclosporine

2. After discussion with you and reading further about omalizumab, the patient agrees to proceed with omalizumab therapy. Which of the following assessments prior to starting treatment would be most appropriate?
   (A) Pulmonary function tests
   (B) Blood or skin prick tests for environmental allergy
   (C) Total IgE level
   (D) Urticaria activity score
   (E) None of the above

   Answer: (E) Rationale for correct answer: At present, no testing for IgE or specific IgE levels is needed for chronic hives patients. In contrast, such IgE testing is needed prior to ordering omalizumab for asthma. Likewise, measuring PFTs or the urticaria activity score is not needed. Dosing and treatment response for omalizumab in chronic idiopathic urticaria are not dependent on serum IgE level or atopic history. Approved dosing for omalizumab for chronic urticarial are as follows: omalizumab 150 or 300 mg subcutaneously every 4 weeks

3. Approximately what percentage of patients with chronic idiopathic urticaria can have concomitant angioedema?
   (A) 5 %
   (B) 10 %
   (C) 20 %
   (D) 40 %

   Answer: (D) 40 %. Patients with CIU can have concomitant angioedema that occurs either simultaneously or separately from episodes of urticaria. The incidence has reported to be 40–80 % in different series. Swellings may involve the face or extremities. Life-threatening laryngeal edema is rare

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Vitiligo

28

Jillian M. Richmond and John E. Harris

Abstract

Vitiligo is an autoimmune disease of the skin that affects approximately 1 % of the population. It is mediated by self-reactive $CD8^+$ T cells that target and kill melanocytes in the basal epidermis. Patients present with patchy depigmentation, which commonly appears on the face, hands, feet and genitals, but may affect any part of the body. Depigmentation is typically progressive and chronic, slowly developing over the life of the patient. There are currently no FDA-approved treatments for vitiligo, and while some therapies are effective for disease, they primarily work through general immunosuppression. Like most autoimmune diseases, both genetic and environmental factors contribute to the risk of developing vitiligo. These factors promote immune dysregulation, which then initiates depigmentation and disease progression. Genes that confer susceptibility to vitiligo have been identified in Genome-Wide Association Studies (GWAS), implicating both innate and adaptive immunity. Environmental triggers for vitiligo include a number of common household commercial products that contain chemical phenols, which act as tyrosine analogs and induce stress responses in the melanocyte. Several recent studies connecting cellular stress and innate immune activation have shed light on possible mechanisms by which vitiligo is triggered through exposure to these environmental insults. Studies of the "effector" phase of vitiligo reveal that disease is driven by IFN-γ and its target genes, including chemokines. Here we will outline the clinical presentation and treatment options, immunopathogenesis, basic and translational research strategies, and future prospects for the treatment of vitiligo.

Keywords

Chemokine • Vitiligo • HLA • Human leukocyte antigen • MHC • Major histocompatibility • DC • Dendritic cell

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Key Points

- Vitiligo is an autoimmune response against melanocytes that results in patchy depigmentation
- Autoimmune responses in vitiligo can be triggered by combinations of genetic and environmental factors
- Melanocyte-intrinsic defects confer susceptibility to vitiligo and may make melanocytes more susceptible to environmental triggers
- Treatment options are limited to narrow-band UVB and topical steroids, though current research favors the development of more targeted immune suppression combined with factors that promote melanocyte regeneration

Clinical Presentation

Symptoms/Signs

Vitiligo is a disfiguring disease of the skin that results in prominent white patches, or depigmentation (Fig. 28.1a). It is due to a gradual loss of the melanin-producing cells, melanocytes, from the epidermis. Depigmentation in vitiligo most commonly affects the face, genitals, hands and feet, followed by the trunk. Vitiligo is a lifelong disease, such that progression can be observed for decades. Lesions begin as small, pinpoint macules (Fig. 28.1b), and grow into large patches that often coalesce with the potential to cover the entire skin surface, usually in a symmetrical distribution (Fig. 28.1c). Hairs are often spared from depigmentation (Fig. 28.1d), but may also be affected (Fig. 28.1e). Most patients are asymptomatic, while ~20 % report itching in affected skin [1], and small numbers experience redness and scaling, a subtype described as "inflammatory" vitiligo [2, 3] (Fig. 28.1f). While melanocytes are present in the epidermis, hair, eyes, inner ear, and brain, depigmentation is almost always limited to the epidermis and hair, with involvement of the hair less common than epidermis. Very rarely, patients experience inflammation in multiple organs, a variant labeled Vogt-Koyanagi-Harada Syndrome. Such patients are highly symptomatic, complaining of meningitis (nausea, vomiting, photophobia) from brain involvement, tinnitus and hearing loss from inner ear involvement, and eye pain, visual changes, and loss of vision from eye involvement [4].

A subset of patients have segmental vitiligo, which is characterized by depigmentation only on one side of the midline (Fig. 28.1g). During embryonic development, keratinocytes (ectoderm) and melanocytes (neural crest) emerge from the dorsal midline primitive streak, proliferate (keratinocytes) or migrate (melanocytes) ventrally, and generally meet at the midline, without crossing to the contralateral side [5]. This unilateral pattern of skin formation in the embryo is readily apparent in diseases of somatic mosaicism, where affected skin is unilateral and limited by the midline [5–8]. T cells, which are bone marrow-derived and recruited to the skin from the circulation, are thought to move freely through tissues, unlimited by the midline (reviewed in [9]). Therefore, the unilateral nature of segmental vitiligo should most likely be attributed to skin cells, rather than immune cells, and a form of somatic mosaicism could induce a unique susceptibility of melanocytes within a unilateral, focal segment to autoimmune attack. A recent study compared patterns of segmental vitiligo to those of known diseases of somatic mosaicism, and found that patterns of segmental vitiligo most resembled those of melanocytic origin [10, 11].

Disease Course

While it has a primarily progressive course, vitiligo can wax and wane, or even arrest altogether. Trauma to the skin may induce depigmentation at the site, a phenomenon known as koebnerization (Fig. 28.1h). Rapid spread of disease may occur, which has been associated with the trichrome variant in which three colors are evident, including the normal skin, depigmented skin, and an intervening zone of hypopigmentation that typically progresses to depigmentation (Fig. 28.1i). The inflammatory variant described above also typically marks rapidly progressive disease. Arrest of depigmentation, either spontaneous or induced, enables melanocytes to regenerate from pigmented hair follicles, a potential reservoir of melanocyte stem cells (Fig. 28.1j). Over time, these melanocytes can repigment the epidermis and erase any evidence of its existence [12] (Fig. 28.1k). In the segmental variant, depigmentation is usually limited to a focal, unilateral area, but may rarely spread beyond this initial site, a variant described as "mixed" vitiligo (Fig. 28.1l) [13].

Epidemiology and Diagnosis

Vitiligo affects approximately 0.5–1 % of the general population [14], although isolated populations where their genetics are more homogenous report higher rates [15]. The average age at diagnosis is 24 years [16], and half the patients who develop vitiligo do so before the age of 20. The segmental variant of vitiligo comprises ~30 % of childhood disease and 10–15 % of adult vitiligo, because childhood onset is more likely to be segmental [17]. The inflammatory variant of vitiligo is uncommon, and Vogt-Koyanagi-Harada Syndrome is very rare, although it appears to be most prevalent in Japan.

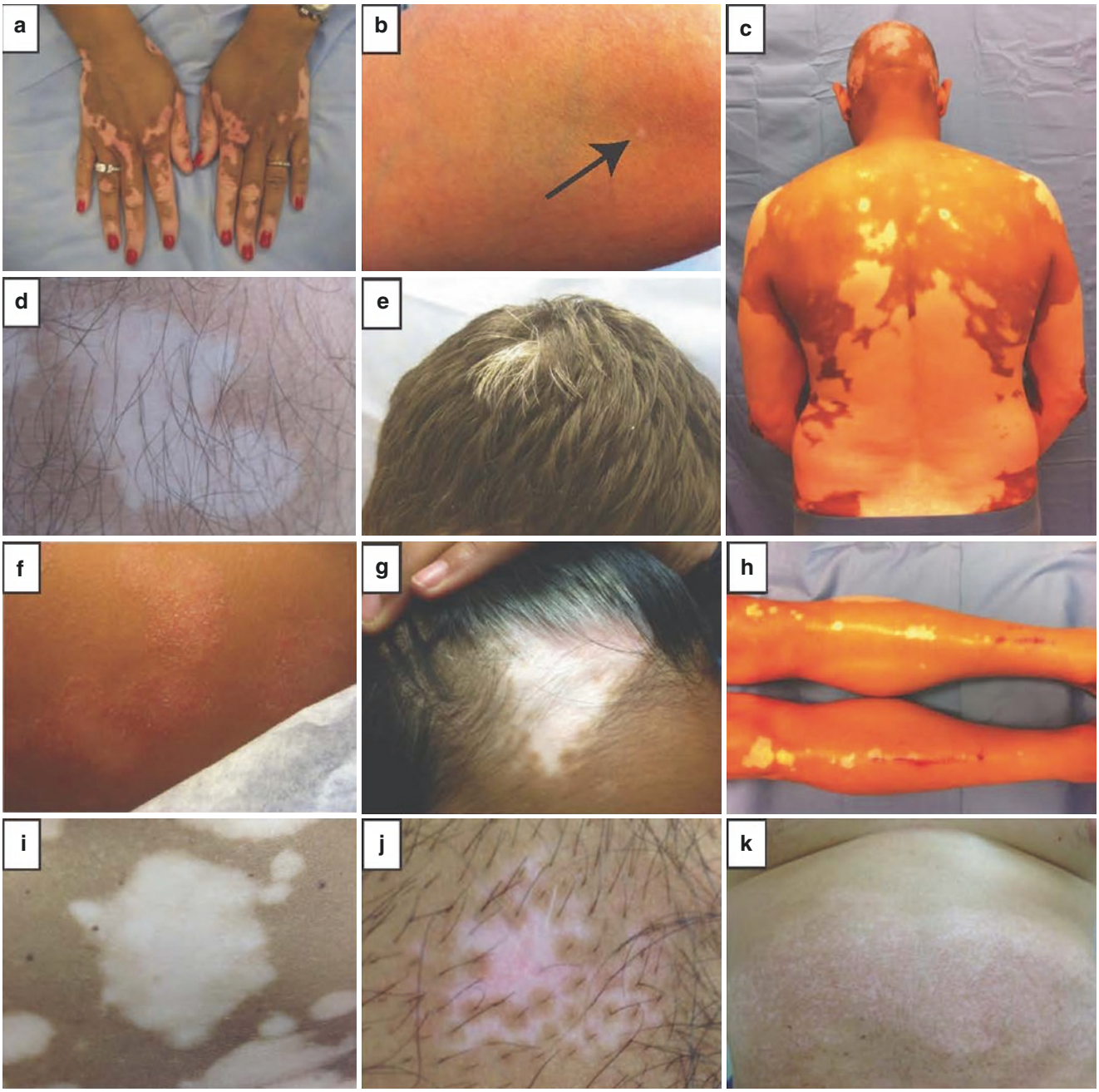

Fig. 28.1 Clinical manifestations of vitiligo. (**a**) Patchy depigmentation on the hands. (**b**) Early macule of vitiligo on the arm. (**c**) Large, coalescing patches of vitiligo on the trunk. (**d**) Epidermal depigmentation on the knee, with sparing of the hair. (**e**) Poliosis from vitiligo on the scalp. (**f**) Erythema and scaling in a depigmented patch in a young girl with inflammatory vitiligo. (**g**) Unilateral depigmentation on the forehead of a young woman with segmental vitiligo. (**h**) Depigmentation on the shins of an avid mountain-biker, reflecting koebnerization of skin trauma. (**i**) Trichrome pigmentation in a patch of vitiligo that includes normal skin and depigmented skin with a hypopigmented border. (**j**) Perifollicular repigmentation from pigmented, but not depigmented, hairs on the abdomen of a patient receiving nbUVB phototherapy. (**k**) Near-complete repigmentation in a large patch on the abdomen of a patient receiving nbUVB phototherapy. (**l**) Mixed vitiligo with depigmentation in a well-defined unilateral segment on the right chin as well as remote areas of depigmentation on the left chest and right hip

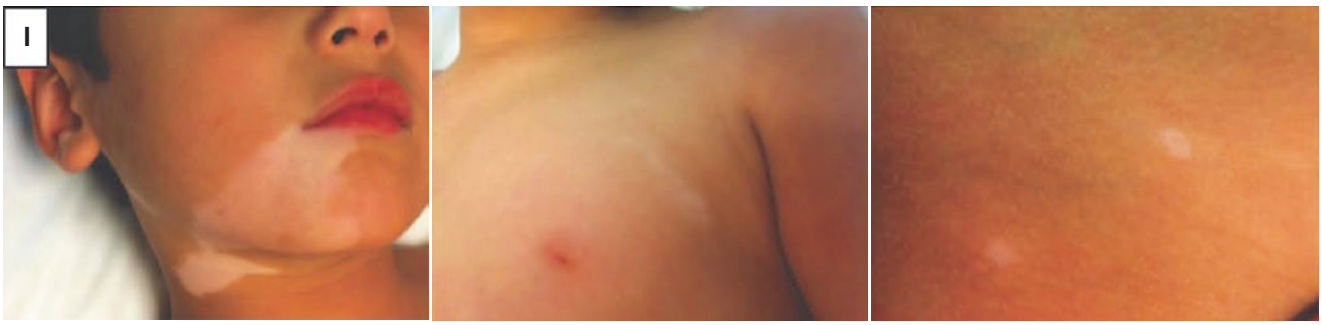

Fig. 28.1 (continued)

Vitiligo is typically a clinical diagnosis, and Woods lamp examination helps to distinguish the depigmentation of vitiligo from hypopigmentation of other conditions. The differential diagnosis of depigmentation is limited, and includes nevus depigmentosus, piebaldism, idiopathic guttate hypomelanosis (IGH), and trauma-induced depigmentation (burns and other scars). Nevus depigmentosus and piebaldism are present at or shortly after birth and grow with the child. IGH is characteristically limited to macules of small size on sun-exposed surfaces, and trauma-induced depigmentation is typically geographic-appearing and often recalled by the patient. If the diagnosis is not clinically clear, the lack of melanocytes can be confirmed through biopsy of the lesion using normal skin for comparison, although biopsy is rarely necessary. The differential diagnosis of hypopigmentation is extensive, and thus distinguishing depigmentation from hypopigmentation via Woods lamp examination is very helpful. Important hypopigmented conditions not to miss include hypopigmented mycosis fungoides due to the need for more intensive monitoring, lichen sclerosus et atrophicus (LSetA) due to the need for aggressive treatment to avoid permanent sequelae, as well as tinea versicolor, pityriasis alba, and progressive macular hypomelanosis due to simple alternative treatment strategies.

The risk of developing vitiligo in first-degree relatives of patients is 6.1 %, and in an identical twin is 23 % [16], implying a moderate *genetic* contribution to disease. The remainder of the risk has been described as *environmental* [18], which will be discussed below. A third possible explanation for twin disconcordance in vitiligo and other autoimmune diseases that is often not considered is a *stochastic* contribution, or risk conferred by chance during development. Stochastic influences occur during cell replication, differentiation, and migration during embryogenesis [19], the generation of T cell receptors through VDJ recombination and nucleotide insertion/deletion [20], and central tolerance induction in the thymus [21].

Psychological Impact

Vitiligo is a disfiguring condition, as skin color is an important component of identity. The porcelain-white lesions are brandished on the skin as if a public announcement of disease. Maybe for this reason, or for its similarity in appearance to tuberculoid leprosy (hypopigmented patches), vitiligo sufferers in India are frequently bypassed as candidates for arranged marriage [22]. Psychological consequences of vitiligo are severe, leading to depression, anxiety, sleep disturbances, sexual dysfunction, feelings of discrimination, and even suicidal thoughts and attempts. These emotional disturbances are comparable to those suffering from psoriasis and eczema [1, 23]. Public concerns over transmissibility of vitiligo make understanding its pathogenesis not only critical for the development of effective treatments to help our patients, but also for public education and acceptance of those who have been ridiculed and ostracized from their communities, beginning over 3500 years ago and continuing to this day [22].

Current Treatments

Treatments for vitiligo can be very effective for some patients, although they are all used "off-label", as there are currently no treatments that are FDA-approved to promote repigmentation of vitiligo. Options include topical steroids, topical calcineurin inhibitors, and narrow-band ultraviolet light B (nbUVB) [24].

Studies reveal that nbUVB is the most effective single therapy for vitiligo, followed by ultrapotent topical steroids and calcineurin inhibitors [25]. Combined therapy with nbUVB and topicals has greater efficacy then either one alone. In a head-to-head comparison, nbUVB showed similar efficacy to psoralen with ultraviolet light A (PUVA), however nbUVB resulted in a better color match of repigmented skin to normal skin [26], and long-term treatment with nbUVB appears to be safe, while PUVA increases the risk of

skin cancer [27]. Therefore, PUVA therapy is best avoided for vitiligo in favor of nbUVB. The frequency of treatment for nbUVB is most efficacious at 2–3 visits per week, as once weekly treatment has only modest efficacy. It appears that visits 3 times weekly induces repigmentation more rapidly than twice weekly, however over the long-term both schedules appear to have equal efficacy. Therefore after 2–3 months of treatment, those receiving therapy 3×/week typically have more repigmentation, while after six months of therapy either 2 or 3×/week have similar repigmentation. If possible for the patient, we often treat 3×/week for the first three months, and then decrease to 2×/week for the remainder of treatment.

Ultrapotent topical steroids appear to have slightly better efficacy than topical calcineurin inhibitors; however, they also have increased risk of epidermal atrophy, striae, and steroid-induced acne. Tacrolimus ointment does not induce atrophy, but may burn shortly after application and, in some patients, results in flushing of the skin after alcohol consumption. Burning seems to go away with repeated treatment. Skin flushing in public may be avoided by taking a small amount of alcohol to induce the flushing in a private environment, which then resolves and may not reappear with additional consumption. We often suggest patients treat with an ultrapotent topical steroid twice daily for one week, followed by tacrolimus ointment twice daily for one week. The patient then alternates treatment with steroid and calcineurin inhibitor each week, which appears to have good efficacy with significantly reduced risk of side effects.

In select cases where depigmentation is stable, autologous melanocytes can be harvested from uninvolved skin and transplanted to depigmented skin, resulting in repigmentation, a procedure called melanocyte-keratinocyte transplantation (MKTP) [17]. This approach may be curative for patients with highly stable disease, and is most useful for those with segmental vitiligo, a variant in which depigmentation is highly stable.

Avoidance of environmental triggers may prevent or reduce the severity of vitiligo. This includes avoiding exposure to phenol-containing products such as hair dyes, cleaning products, adhesives, and treated rubber products. Patient history can be helpful to identify exposures, though difficulty with recall and the large number of common household products involved can make this approach difficult. One study reported that patients with chemical-induced vitiligo often present with confetti-like macules of depigmentation, which may help to identify those patients in whom a detailed exposure history should be taken [28].

Monobenzyl ether of hydroquinone, or monobenzone, was first implicated as a risk factor for vitiligo through occupational exposure in a group of factory workers in 1939 [29]. It is now the only FDA-approved treatment for vitiligo, used for removing the remaining pigment in a patient with widespread disease. Its mechanism is through the exacerbation of vitiligo, described below. For clinical use, the chemical is compounded as a 20 % ointment and should be applied daily to pigmented skin. Successful depigmentation can take up to 12 months, and should be considered irreversible. In addition, pigmented skin remote from the application site may also depigment, so this strategy should not be used for localized depigmentation. A subset of those who use this approach exhibit allergic contact dermatitis to the chemical, which may be treatment-limiting. The majority of those who successfully undergo depigmentation with monobenzone topical therapy are very pleased with the results [30].

Pathophysiology of Vitiligo in Humans

Historically it has been debated whether vitiligo is an autoimmune disease or a melanocyte-intrinsic degenerative syndrome [31]. Like most other autoimmune diseases, vitiligo is multifactorial, requiring both genetic and environmental factors for melanocyte destruction. Melanocytes from vitiligo patients are difficult to grow *in vitro*, are more susceptible to oxidative stress, and have elevated cellular stress responses [32]. Autoimmunity plays a key role in pathogenesis as well, evident by the fact that the majority of genes that confer susceptibility to vitiligo play central roles in immune responses, and "adoptive transfer" of vitiligo has been observed in patients receiving bone marrow/stem cell transplants from donors with vitiligo [33–35]. In this section we will outline the pathophysiology of vitiligo with an emphasis on the recent literature.

Melanocyte Stress

Melanocytes are particularly susceptible to cellular stress, due to their exposure to UV irradiation and other environmental stressors, as well as the production of melanin, which generates reactive oxygen species (ROS) and activates the unfolded protein response (UPR). Electron microscopy of cellular substructure revealed that the endoplasmic reticulum (ER) in melanocytes from vitiligo patients was dilated compared to healthy controls [36], a characteristic that implicates cellular stress. Elevated levels of H_2O_2 and oxidative byproducts are present in vitiligo patients' epidermis compared with controls, suggesting uncontrolled generation of reactive oxygen species (ROS) [37]. In addition the enzyme catalase, which reduces H_2O_2 to O_2 and H_2O and thereby relieves oxidative stress, is

decreased in lesional skin [38], which may be either a cause or an effect of increased H_2O_2. Treatment of vitiligo with topical exogenous catalase has been attempted, with variable results [39].

Tyrosinase facilitates a number of steps that convert the amino acid tyrosine to melanin. Tyrosine is a phenol, containing a hydroxyl group attached to a benzene ring. Monobenzone was the first chemical reported to induce vitiligo in a group of factory workers exposed to the chemical through acid-cured rubber gloves. Distant spread of depigmentation to areas beyond the exposure sites (hands and forearms) suggested that monobenzone is not simply directly cytotoxic to melanocytes, but induces autoimmunity during exposure. Monobenzone treatment of vitiligo patients, used to depigment their skin to induce an even tone, also induces depigmentation at sites remote from the site of application, supporting this concept. Like tyrosine, monobenzone and other depigmenting agents are phenols, which act as tyrosine analogs but bind irreversibly to tyrosinase and thereby interrupt the melanin synthesis pathway [40]. This induces cellular stress through the induction of ROS and activation of the UPR, which then results in activation of innate immunity to induce inflammation [40–42]. Many household products contain other chemical phenols, including hair dyes and cleaning products, which have both been reported to induce chemical leukoderma, or chemically-induced vitiligo [28]. It is likely that phenols used as ingredients in these products act in a similar way as monobenzone, producing cellular stress through interaction with tyrosinase.

Initiation/Innate Immune Activation

There is evidence that the initiation of vitiligo requires innate immune activation. Several studies have implicated innate immune sensing of cellular stress, including dendritic cell (DC) activation by stress-induced release of exosomes [40], the heat shock protein HSP70i [43] and an apparent increased recruitment of natural killer (NK) cells into the skin of vitiligo patients [44]. Melanocyte stress is therefore linked to innate immune activation, and thus initiation of inflammation that may lead to adaptive autoimmunity; however, more studies will be required to identify the precise signaling pathways involved [45]. Innate immune gene polymorphisms in vitiligo have been identified through genome-wide association studies, though their functional roles in disease are still being defined (see section "Genetic Contributions"). Environmental triggers that induce melanocyte stress have been identified, and patients may be counseled to avoid these exposures (see section "Melanocyte Stress").

Adaptive/Autoimmune Response

Vitiligo is driven by $CD8^+$ T cell-mediated killing of melanocytes. This has been demonstrated *ex vivo* using a human skin culture system in which melanocyte-specific T cells isolated from affected skin migrated into an unaffected skin explant from the patient and induced melanocyte apoptosis. $CD8^+$ T cells were both necessary and sufficient for this effect [46]. Increased frequencies of melanocyte-specific $CD8^+$ T cells in the blood and skin correlate with disease severity [47]. The antigen specificities of the T cell receptors (TCRs) in vitiligo have been identified and are shared with melanoma, including MART-1, gp100, tyrosinase, and tyrosinase-related proteins 1 & 2 (TRP1 & 2) [48]. These T cells produce IFNγ which, in addition to inducing T cell recruitment, may be directly cytotoxic to melanocytes [49]. It is possible that vitiligo results from an overzealous antitumor response [50]. Patients with vitiligo are at a three-fold lower risk for melanoma, and those with melanoma who spontaneously regress or who are cured following treatment frequently develop vitiligo [51].

$CD4^+$ helper T cell responses may also play a role in vitiligo via IFNγ production, although functional evidence for their role is lacking [52, 53]. There is growing evidence that vitiligo is dependent on IFNγ and downstream genes including IFNγ-inducible chemokines [52, 54]. There are several case studies of patients who were treated with IFNα for hepatitis who developed vitiligo [55–64], suggesting that type I interferons can induce disease, though it is unclear what their role is in spontaneous vitiligo. Several studies have questioned whether T_H17 cells and IL-17 drive pathogenesis, but their functional role in vitiligo remains unclear. It is possible that a subset of vitiligo patients, such as those with inflammatory vitiligo, also have an IL-17 component in their disease pathogenesis, but studies to date have not classified T_H1 versus T_H17 in different vitiligo subtypes. Autoantibodies against melanocytes have been identified in patients [65], though it is uncertain whether they play a direct role in pathogenesis or are merely a biomarker of disease. A role for regulatory T cells (Tregs) have also been implicated in vitiligo through genetic studies [66] and the fact that patients with immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome, who lack Tregs, have an increased incidence of vitiligo [67]. However evidence is conflicted regarding whether their numbers are normal or fewer, whether they have difficulty homing to the skin, and whether or not they are functionally impaired [68–72].

Hair depigmentation is not commonly observed in vitiligo, most likely due to hair follicle immune privilege. Immune privilege is defined as tissue that is not readily inflamed, possibly due to limited access by the immune system and/or the expression of immunoregulatory proteins

or cell populations [73–75]. Melanocyte stem cells reside in hair follicles, which migrate out into the epidermis to repigment the skin [76]. When repigmentation occurs in patients, it usually begins around pigmented hair follicles and spreads outward, indicating that the stem cell populations in the hair follicles may be maintained through immune privilege. Little is known about the mechanisms of repigmentation, though this is critical for effective therapy (see also section "Future Perspectives into Vitiligo Treatment Strategies"). A summary of vitiligo pathogenesis is presented in Fig. 28.2.

Genetic Contributions

Genetic contributions to vitiligo are well-established, based on familial associations of the disease, as well as the increased concordance in identical twins [16, 66]. It is likely that inherited factors affect all pathways involved in vitiligo pathogenesis, including melanocyte stress, innate inflammation, and adaptive autoimmunity. The earliest genetic associations with vitiligo were with the HLA-A haplotypes, required for T cell recognition of their target cells [77]. The first non-HLA gene found to confer risk for vitiligo was NACHT, LRR and PYD domains-containing protein 1 (NLRP1). NLRP1 is a component of the innate immune response, confirming a causative role of innate immunity in vitiligo [78]. A recent study using functional genomics demonstrated that NLRP1 haplotypes associated with vitiligo cause increased IL-1β secretion by monocytes, potentially predisposing patients to innate immune activation and lowering the tolerance threshold [79].

Genome-wide association studies (GWAS) have revealed a large number of additional genes, the majority of which also play roles in immune responses (PTPN22, TSLP, HLA class I/II/III, CCR6, IL2RA, UBASH3A, and FOXP3), including specifically cytotoxic T cell responses

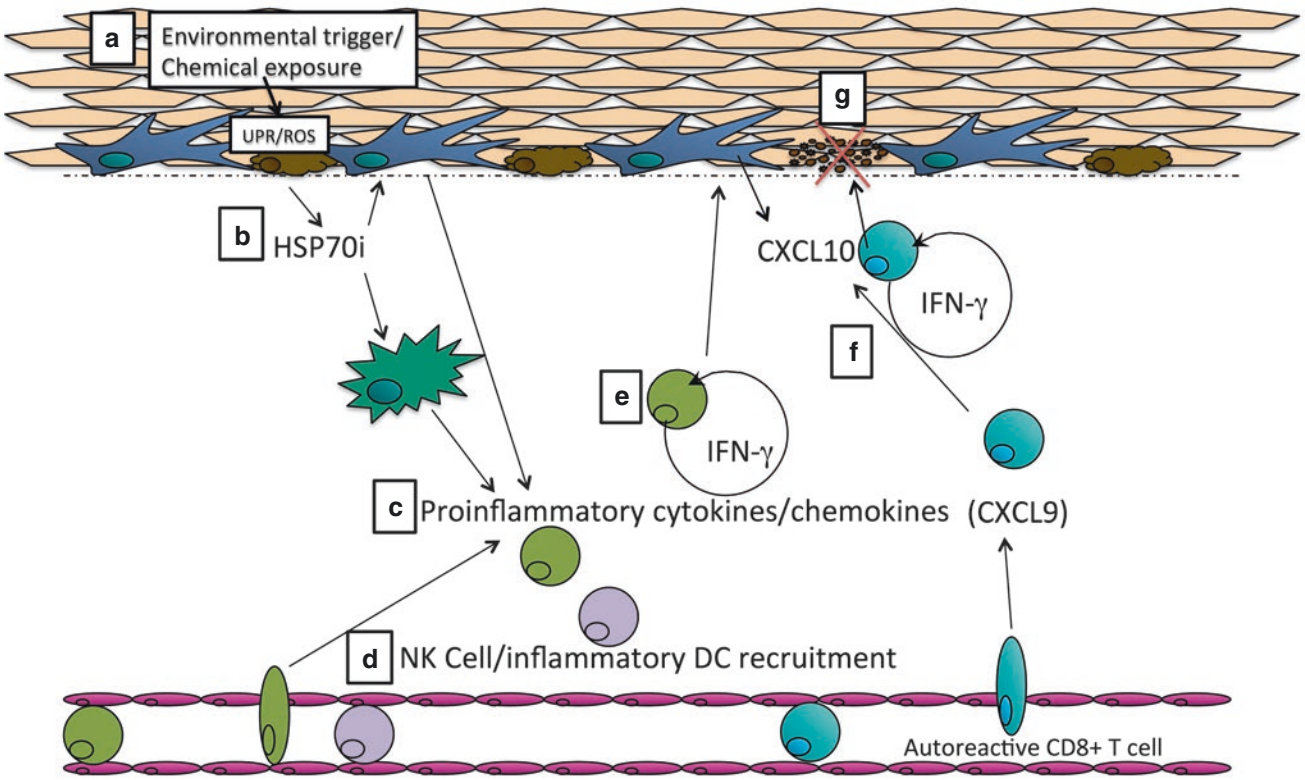

Fig. 28.2 Vitiligo pathogenesis: linking melanocyte stress, innate immune triggering and adaptive immune responses. (*A*) Exposure to an environmental insult, such as a phenol, may induce stress responses in melanocytes through activation of the unfolded protein response (*UPR*) or production of reactive oxygen species (*ROS*). (*B*) This results in release of stress/danger signals such as HSP70i, which are detected by other skin resident immune cells such as Langerhans cells or dermal dendritic cells. (*C*) These cells secrete proinflammatory cytokines and chemokines to recruit more immune cells to the skin, including natural killer (*NK*) cells or inflammatory dendritic cells (*DCs*). (*D*) NK cells produce IFN-γ, which stimulates CXCL10 production by skin resident cells (*E*). (*F*) Established gradients of CXCL9 and CXCL10 from the dermis to the epidermis direct autoreactive $CD8^+$ T cell recruitment to the skin and up to the epidermis, where they kill melanocytes either through cell contact dependent mechanisms or through IFN-γ production, which induces additional chemokine production to promote the cycle (*G*)

(GZMB), supporting earlier histologic and *in vitro* mechanistic data [18]. An allele of tyrosinase, the enzyme responsible for creating melanin, is protective against melanoma but causes an increased risk of vitiligo [80, 81]. X-box binding protein 1 (XBP1) variants were also identified as risk factors for vitiligo [82]. XBP1 promotes class II HLA expression (important for antigen presentation to CD4 T cells) [83], IL-6 and IL-8 production [84], and it is also a member of the UPR that protects the cell from stress-induced apoptosis [85].

In addition to a proinflammatory role in vitiligo, XBP1 may also participate in the stress response, as decreased expression or impaired activity of the protein may increase stress levels of the melanocyte [85], indirectly leading to activation of autoimmunity. Consistent with this hypothesis, studies in inflammatory bowel disease have identified two hypomorphic variants of XBP1 that contribute to a proinflammatory state of the intestine as a result of increased cellular stress [86]. To date, only a hypermorphic variant has been described in vitiligo [87], which may induce an exaggerated inflammatory response to cellular stress [41]. Another stress-related genetic factor that has been identified is tyrosinase-related protein 1 (TRP1). Mutations in TRP1 have been identified in vitiligo patients, and have been shown to activate cellular stress responses in melanocytes via chaperone proteins such as calnexin [32]. Further studies will be required to determine the functional role of other identified genetic factors in disease pathogenesis (Fig. 28.3).

Basic and Translational Approaches for Studying Vitiligo

Basic Science Approaches: Animal Models of Disease

One of the earliest models of vitiligo identified was a spontaneous mouse model caused by a point mutation in the microphthalmia-associated transcription factor (MITF) gene, which caused melanocyte degeneration as the mouse aged. However due to its monogenic nature and lack of autoimmunity [88, 89], this strain (mi^{vit}) does not appear to accurately model human vitiligo pathogenesis. Several other mouse models have been developed to study vitiligo, including models induced through exposure of the skin to melanocyte antigens plus adjuvant to induce depigmentation [90], or through adoptive transfer of transgenic T cells engineered to react against melanocyte antigens. Early models were developed from animal models of melanoma immunotherapy [91–95] in which TCR transgenic mice responding to gp100 (called PMEL for pre-melanosome protein were used to mediate tumor clearance. The mice spontaneously developed white hair, and several studies have used these PMEL host mice to study spontaneous hair depigmentation [43]. We developed a model through the adoptive transfer of PMEL T cells into hosts that have epidermal melanocytes [95], which resulted in progressive epidermal depigmentation with sparing of the hair (Fig. 28.4a). Studies using this

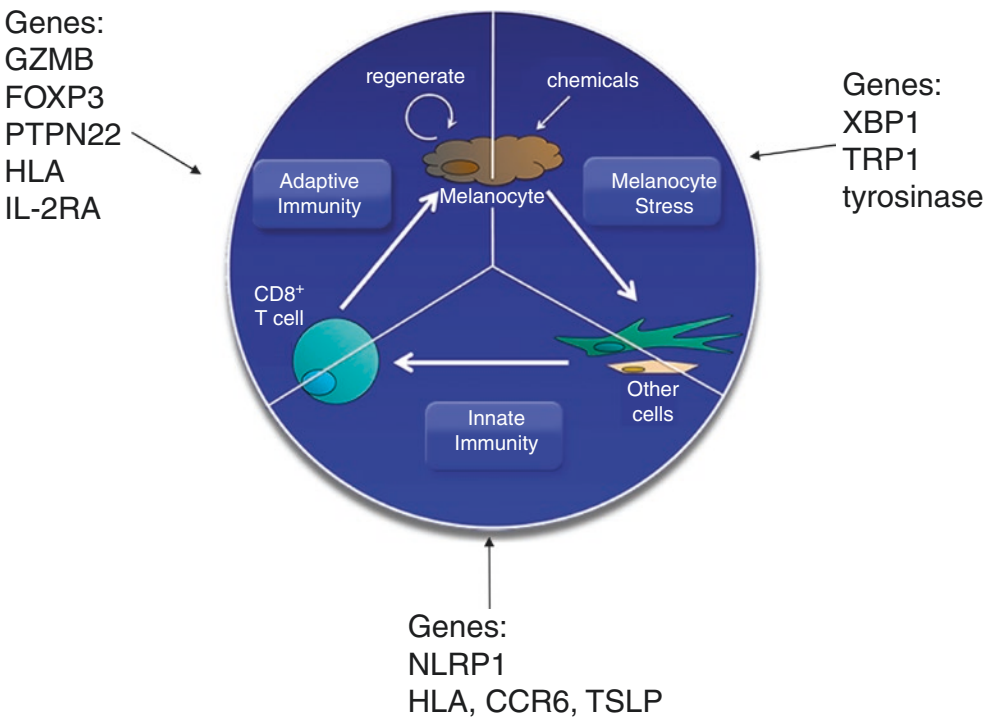

Fig. 28.3 Multiple pathways contribute to vitiligo pathogenesis. Melanocyte stress can be induced by chemicals and other environmental insults, resulting in release of stress signals that are detected by other cells in the skin. This can trigger activation of the innate immune system, resulting in production of chemokines required for autoreactive $CD8^+$ T cell recruitment to the skin. These $CD8^+$ T cells target and kill melanocytes, resulting in release of more proinflammatory signals in the skin, thereby re-activating the cycle. As melanocytes try to regenerate, they may continue to be targeted by CD8+ T cells in the skin, resulting in maintenance of clinical depigmentation. Examples of genes that may directly affect these three major pathways in disease are highlighted

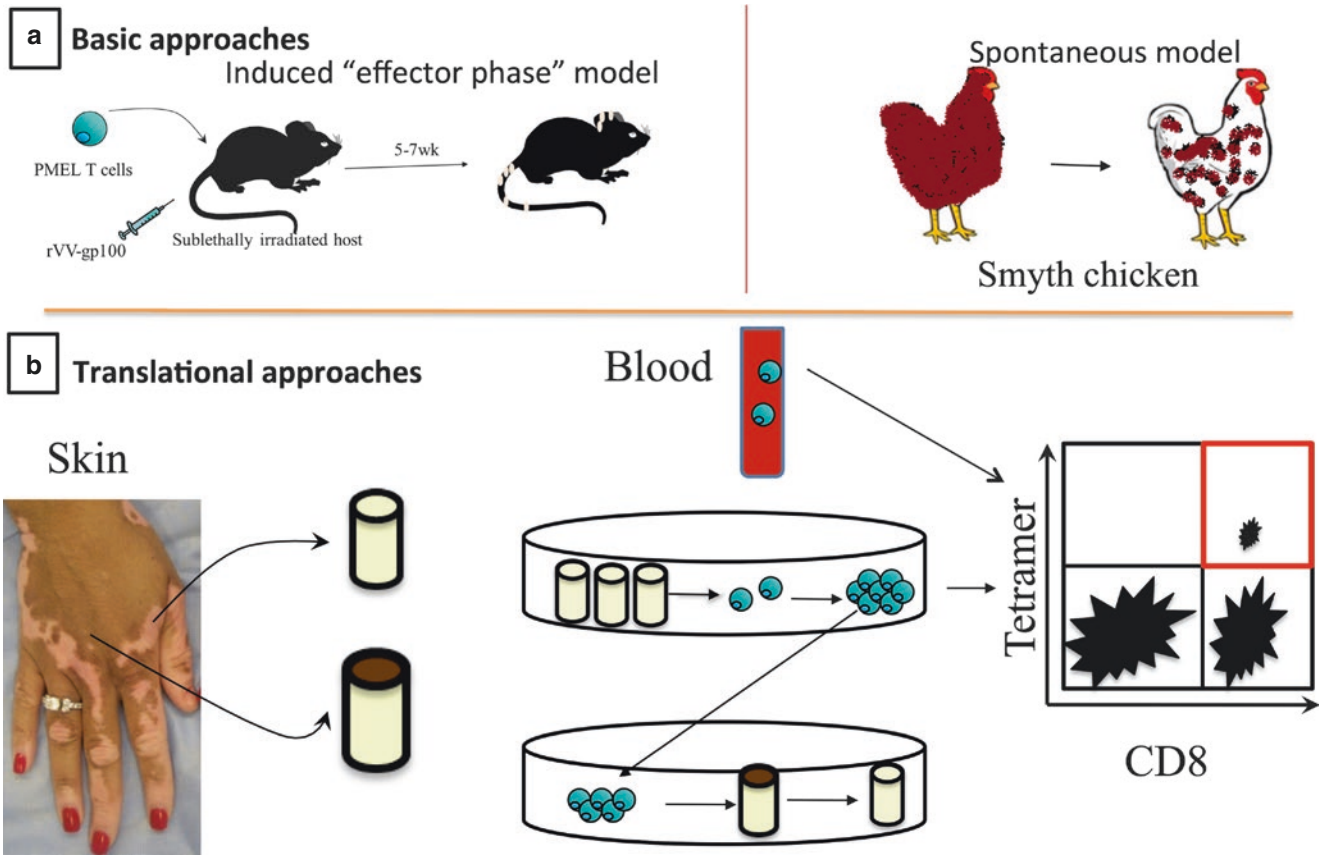

Fig. 28.4 Experimental approaches for studying vitiligo. (**a**) Basic science approaches for studying vitiligo: animal models. Adoptive T cell transfer model for studying the effector phase of vitiligo in mice. Melanocyte antigen-specific T cells (*PMEL*) are adoptively transferred into hosts and activated *in vivo* with an attenuated recombinant vaccinia virus expressing their cognate antigen gp100 (*rVV-gp100*). After 5–7 weeks, mice develop patchy depigmentation. This model is most useful for studying the "effector phase" of vitiligo. Alternatively, the Smyth Line Chicken spontaneously develops vitiligo, and is a more appropriate model for studying induction of vitiligo. (**b**) Translational approaches for studying vitiligo. Human samples can be analyzed *ex vivo* to examine elements involved in disease pathogenesis. Using skin biopsies, antigen-specific T cells can be isolated from lesions, cultured, expanded, and used to examine killing ability in normally pigmented skin cultures [46]. Antigen specificities of these skin T cells as well as T cells isolated from peripheral blood of vitiligo patients can be analyzed with tetramers, which are fluorescently labeled reagents comprised of MHC:peptide complexes. Most of the melanocyte tetramer reagents available are for CD8+ T cells, though it is possible that reagents for CD4+ T cells will become available in the future

model implicated IFN-γ and the IFN-γ-induced chemokine CXCL10 as required for both disease progression and maintenance [54]. This model is most useful for studying the effector phase of vitiligo, as loss of tolerance through innate immune activation is largely bypassed.

Perhaps the most representative animal model of vitiligo is the Smyth Line (SL) chicken, which develops spontaneous depigmentation of its feathers over time (Fig. 28.4a). Affected brown SL chickens develop white feathers with age and produce anti-melanocyte antibodies. Depigmentation is due to melanocyte loss by apoptosis induced by cytotoxic T cells. Interestingly, the SL chicken also has melanocyte defects, including autophagocytosis of melanosomes and increased generation of ROS. Consistent with the theory that vitiligo pathogenesis is multifaceted, these melanocyte defects are not sufficient for depigmentation in the absence of a functional immune system [96]. While the SL chicken appears to model vitiligo in a way that is most similar to human disease, the paucity of tools and expertise for investigation of chicken cells limits its use. Recent studies determined that IFNγ, IL-21 and IL-10 are expressed in evolving lesions [97], and microarray analysis has been conducted to begin to address gene signatures in the model [98].

Translational Approaches Using Patient Samples

Histology and immunohistochemistry were the earliest methods used to study inflammatory infiltrates in vitiligo. Histologic analysis of vitiligo patient biopsies revealed a lack of epidermal melanocytes with accompanying lymphomonocytic infiltrates. Immunohistochemistry revealed infiltrates of CD8 and CD4 T cells that are most often detected perilesionally [3, 99, 100]. CD8 T cells have been

observed infiltrating the epidermis and may be found proximal to dying melanocytes. Innate immune populations including CD11b+ CD11c+inflammatory DCs [43] and NK cells [44] have also been identified in vitiligo lesions.

Van den Boorn et al. used human skin organ culture and autologous T cells to demonstrate that $CD8^+$ T cells are necessary and sufficient to induce melanocyte apoptosis in human skin, consistent with a causative role for adaptive immunity in vitiligo [46]. This approach models processes that occur within the skin, and provides the normal 3D architecture and maintains most of the microenvironment. Punch biopsies of lesional skin from vitiligo patients were cultured *ex vivo*, T cells migrated out of the explant, and were expanded *in vitro*. Expanded populations can then be analyzed, manipulated, and re-exposed to punch biopsies of normally pigmented skin from the same patient to determine their effects on melanocytes *in situ* (Fig. 28.4b). Disadvantages of this approach include the inability to model recruitment of T cells from the blood to the tissue, and the fact that over time culture of skin results in the loss of resident immune cell populations through emigration [101].

Immune responses can be categorized by the cytokines that are expressed during the response. For example, in general T_H1 responses target tumors and intracellular pathogens and are characterized by the expression of interferons, including IFN-α, IFN-β, and IFN-γ. T_H17 responses target extracellular pathogens and are characterized by IL-17, IL-23, and TNF-α. T_H2 responses are involved in protection against parasites, and utilize IL-4 and IL-13. Autoimmune responses like vitiligo are mediated by inappropriate immune responses to self-tissues, and are often characterized by the cytokines that drive them as well. Enzyme-linked immunosorbent assays (ELISAs) have been used to measure cytokines in peripheral blood, serum, and/or plasma from vitiligo patients in an effort to characterize immune responses in vitiligo and to serve as potential biomarkers. We found that CXCL10 is elevated in the serum of vitiligo patients compared to controls [54], and others have been reported as well, although with variable results [102–106]. One caveat of studying cytokines present in serum in vitiligo patients is that the autoimmune process occurrs in the skin rather than the blood. Presumably serum chemokines have diffused from lesional skin, and so will likely be dilute, and detection will vary according to their magnitude and location of expression in tissue, solubility, and binding to other proteins in the serum.

Studies to detect the expression of cytokines directly in human lesional skin include quantitative reverse-transcriptase PCR (qRT-PCR), gene expression microarray, histology, and flow cytometry on T cells isolated from lesional skin. We used microarray to determine that the IFN-γ-induced chemokines CXCL9, CXCL10, and CXCL11 are significantly elevated in human skin [54], consistent with a previous report that IFN-γ is induced using qRT-PCR [107]. Other studies have reported other cytokines as well, including small amounts of IL-17, TNF-α, and IL-1β [104, 107–109]. Immunofluorescence on fixed vitiligo lesions revealed a number of infiltrating $IL\text{-}17^+$ cells, although other cytokines were not analyzed, and therefore it is difficult to say whether the number detected is the predominant population [108]. Flow cytometry on T cells isolated from lesional skin reveal some heterogeneity among individual patients, but included IFN-γ, TNF-α, IL-17, and IL-4 [46]. While cytokine detection directly in lesional skin appears to be more relevant to disease than serum cytokines, their functional roles are difficult to determine without the use of animal models.

Tetramers, which are comprised of fluorescently tagged, peptide-bound HLA molecules, can be used to detect melanocyte antigen-specific $CD8^+$ T cells in blood using flow cytometry (Fig. 28.4b). This approach has revealed that melanocyte-specific $CD8^+$ T cells are elevated in vitiligo patients compared with controls, and the numbers in the blood correlate with disease activity [47]. We found that they express high levels of CXCR3, and therefore have the capacity to respond to the high levels of CXLC9, CXCL10, and CXCL11 expressed within the skin [54]. Healthy donors also have melanocyte-specific T cells in the blood, although at frequencies much lower than that of vitiligo patients [47, 54]. It is likely that these melanocyte-reactive T cells also play a role in protection against melanoma. One study demonstrated that a large proportion of T cells isolated directly out of lesional vitiligo skin are positive for tetramer staining, thereby demonstrating melanocyte-specific T cells directly within lesions [46].

The combination of human correlative studies with mechanistic studies in representative preclinical animal models is a powerful approach to study disease pathogenesis and develop new treatments, and it is likely that the complimentary use of both basic and translational strategies to understand vitiligo pathogenesis will be necessary to develop badly needed new treatments for the disease.

Future Perspectives into Vitiligo Treatment Strategies

In general, new vitiligo treatments may address any key aspect of vitiligo pathogenesis and repigmentation, which includes melanocyte stress, autoimmune destruction, and melanocyte regeneration (Fig. 28.5). Approaches to normalize melanocyte stress have included the administration of antioxidants orally (Vitamin E, etc) to counteract ROS, as well as the topical administration of pseudocatalase to help break down ROS. To date, these approaches have not demonstrated consistent efficacy in clinical trials, and their use as

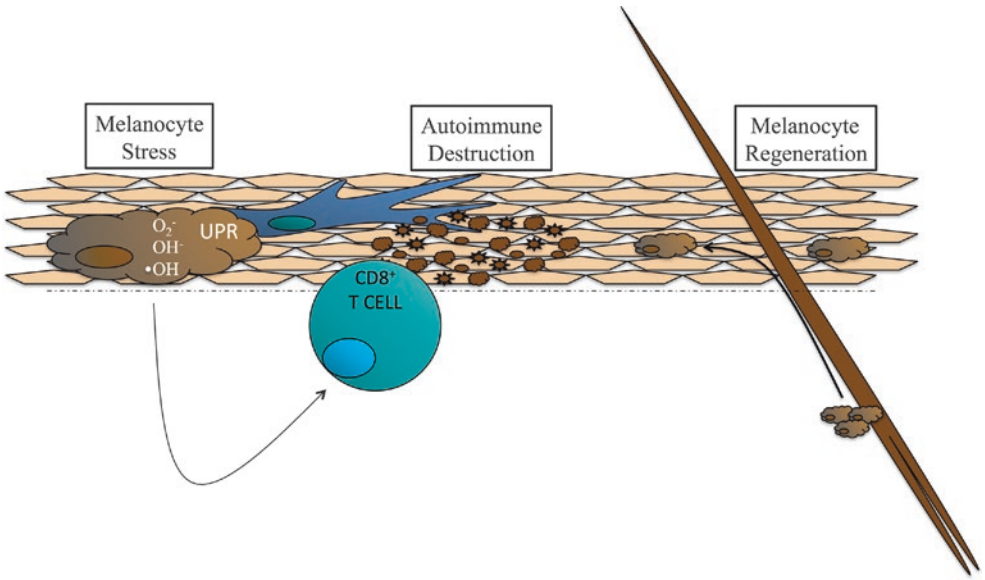

Fig. 28.5 Treatment goals for vitiligo: Target all three aspects of disease. Ideally, treatments for vitiligo will reduce melanocyte stress, suppress autoimmune destruction, and promote melanocyte regeneration

treatments remains controversial [39, 110]. Further study into these approaches is warranted however, and a holistic approach to treatment, including stabilizing melanocyte stress, is an attractive strategy.

Current first-line treatments for vitiligo include nbUVB, topical steroids and topical calcineurin inhibitors. While their precise mechanisms of action are not known, general immunosuppression is likely, and therefore they primarily address the autoimmune component of disease. Future treatments may be developed that provide more targeted immunosuppression, such as interfering with key cytokines and chemokines, or their signaling pathways. For example, based on translational studies with human tissues as well as functional studies in our mouse model, we suspect that interfering with the IFN-γ-CXCL10-CXCR3 axis could be a highly effective strategy for the development of new treatments [54, 95]. CXCL10 and its receptor CXCR3 are particularly attractive candidates, and a number of pharmaceutical companies have developed antibodies and/or small molecules that target these proteins. Janus kinase (JAK) inhibitors, which are currently being developed as well, block signaling through the IFN-γ receptor and may also prove to be effective treatments for disease. It was recently reported that a mutant form of the chaperone protein HSP70, called HSP70i, could prevent depigmentation in a mouse model of vitiligo [43]. This may also be an effective future therapy, and likely helps to uncouple chronic melanocyte stress from innate immune triggering.

Melanocyte regeneration is critical for reversing vitiligo through repigmentation, which often occurs spontaneously when autoimmunity is addressed. Melanocytes typically regenerate and migrate out of pigmented hair follicles, presumably supplied from a protected pool of stem cells within the follicle [76]. Therefore, promoting melanocyte regeneration may improve treatments that target autoimmunity. In addition to the immunosuppressive effects of phototherapy, melanocytes are stimulated as well, promoting repigmentation. Additional approaches to repopulate epidermal melanocytes include the melanocyte-keratinocyte transplant procedure (MKTP), which directly transplants melanocytes and keratinocytes from uninvolved areas of skin to affected areas. However it is only effective in stable conditions, where the autoimmune response has presumably stabilized, as in the segmental variant [111]. Afamelanotide, an analog of α-MSH, is a novel treatment that has been tested in open-label clinical studies. The hypothesis is that stimulating melanocytes through the MSH receptor will promote their regeneration and growth, improving repigmentation. Because it does not address ongoing autoimmunity, clinical studies paired its use with nbUVB with promising results. Repigmentation indeed appeared to occur faster in subjects treated with afamelanotide, although placebo-treated controls were not included in initial pilot studies, and whether the overall quantity of repigmentation was increased is not clear [111]. Additional studies will be required to determine how afamelanotide, and other strategies to support melanocyte regeneration, will contribute to overall clinical treatment strategies of the future.

Conclusion

Vitiligo is a common autoimmune disease in which melanocytes are targeted for destruction. Disease pathogenesis is dependent on genetic susceptibility and environmental triggers related to both the melanocytes and the immune system. Current treatment options focus on general immunosuppression, though investigational procedures and

new research supports targeted treatments combined with therapies that promote melanocyte regeneration. In addition to developing a deeper understanding of the contributions of melanocyte stress, innate immunity, and adaptive immunity in vitiligo pathogenesis, future studies should seek to understand the interactions among them, as well as functional genomics to understand the role of specific genes that affect these pathways. New treatments are on the horizon, and will be exciting to watch as they enter clinical trials in vitiligo patients.

Questions

1. Briefly describe the clinical presentation of vitiligo. What is required for a definitive diagnosis?

   Vitiligo presents as patches of depigmentation, most commonly on the hands, feet, face, and genitals, followed by the trunk/back. Depigmentation may be bilateral or unilateral, and is thus classified as either non-segmental or segmental, respectively. Signs for highly active vitiligo include confetti or trichrome depigmentation, as well as the koebner phenomenon. A definitive diagnosis of vitiligo requires biopsy demonstrating a lack of melanocytes by immunohistochemical staining, although this is not commonly required.

2. What are current treatment options and how do they work? How would future treatment options improve upon these?

   Current treatment options for vitiligo include general immunosuppressants, including topical steroids and calcineurin inhibitors like tacrolimus, and narrow band UVB, which is thought to both suppress autoimmune inflammation and promote melanocyte differentiation/melanin production in the skin. Melanocyte transplantation surgery, or MKTP, often works for patients with stable disease such as segmental vitiligo patients. Future treatment options would improve upon these by more specifically targeting the immune cells responsible for vitiligo maintenance and progression, and through direct stimulation of melanocytes to better promote repigmentation.

3. Briefly describe the pathophysiology of vitiligo. Be sure to discuss cellular stress, innate immunity, adaptive immunity?

   Vitiligo is caused by a combination of genetic susceptibility and environmental exposures that lead to immune activation. Melanocyte stress is thought to be the first step in vitiligo pathogenesis. Genetic factors may cause increased basal melanocyte stress, and/or exposure to phenolic chemicals would cause increased melanocyte stress through interaction with tyrosinase. This stress is likely sensed by innate immune cells that reside in tissues, such as dendritic cells and macrophages, who in turn present melanocyte antigens to T cells in skin draining lymph nodes. These T cells then infiltrate the skin to target and kill melanocytes.

4. What are some genetic contributions to vitiligo? Please describe the functions of these genes in relation to disease pathogenesis.

   Some genetic contributions to vitiligo include HLA-A2, which is important for presentation of melanocyte antigens to T cells; innate immune genes such as NLRP1, which may play a role in sensing melanocyte stress; XBP-1, a chaperone that may contribute to melanocyte stress; genes affecting Treg function including PTPN22, IL2RA, FOXP3; and genes affecting effector T cell function such as granzyme B.

5. Name two research tools currently being used to better understand mechanisms of vitiligo. How does each tool help to identify new potential treatments?

   Two research tools currently used to better understand vitiligo are animal models and translational studies using human skin biopsies. Animal models help identify disease mechanisms and potential treatments, as T cell responses may be assessed in skin and lymphoid tissues following experimental treatments at specific time points, which cannot be assessed in humans. Skin biopsies, however, provide insight into human disease and may be assessed for inflammatory markers and cellular phenotypes.

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Alopecia Areata

29

Ali Jabbari, Lynn Petukhova, and Angela M. Christiano

Abstract

Alopecia areata (AA) is an autoimmune disease characterized by targeting of the hair follicle. Clinically, patients exhibit nonscarring hair loss with varying presentations across all age groups and follow an unpredictable course. Our understanding of the pathogenic mechanisms underlying AA has been greatly enhanced by recent large scale studies of genetic associations of disease. Descriptive studies in humans in tandem with mechanistic experiments in mice have helped define cellular and soluble disease drivers at the level of the end-organ target, the hair follicle, as well as the immune system. In AA, those mechanisms that protect the hair follicle from immune attack and maintain the immune privileged status of this site become disrupted and allow autoreactive cytotoxic immune cells to recognize and respond to self-antigens associated with the hair follicle. Our enhanced understanding of the disease has led to the identification of new therapeutic targets for AA. In particular, targeting of JAK molecules, proximal intermediates that transduce ligand binding signals for a wide variety of cytokine receptors, have shown promise in the treatment of this disease in animals models and humans.

Keywords

Alopecia areata • Hair • Immune privilege • Autoimmune • Immunogenetics • Immunotherapy

Introduction

Alopecia areata (AA) is an autoimmune disease characterized by targeting of the hair follicle. Clinically, patients exhibit nonscarring hair loss with varying presentations across all age groups. The most common form of the disease, patchy AA, is characterized by well-defined, coin-shaped or ovoid patches of alopecia that typically involve the scalp or beard area (Fig. 29.1), although any part of the body can be affected. These areas may resolve spontaneously or with treatment, or may be persistent. In approximately 1 in 20 cases of patchy AA, the alopecic areas will grow and coalesce, resulting in a more severe form of AA [1]. AA that involves the entire scalp (Fig. 29.2) is called alopecia totalis (AT), and AA that involves the entire body is called alopecia universalis (AU). The course of disease in patients with AT and AU can vary, although a much higher percentage of these patients exhibit persistent disease when compared with patients with patchy or localized AA. These more severe forms of the disease are also largely recalcitrant to long-term treatment. In addition, specific patterns of hair loss can be observed in patients with AA. In the ophiasis pattern, first described by Aulus Cornelius Celsus circa 30 AD, a band-like area of hair loss is observed extending from the occipital scalp bilaterally to the temporal scalp [2]. The inverse of this pattern has been described; in the sisaipho pattern, the vertex

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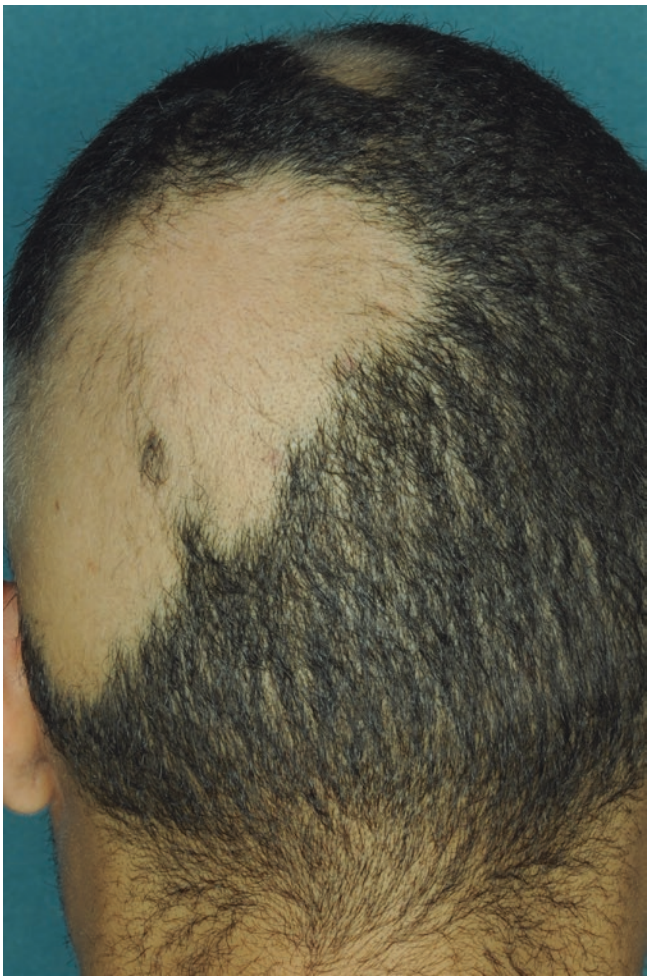

Fig. 29.1 Alopecia areata, patchy type. Nonscarring patches of alopecia on the posterior scalp of an AA patient

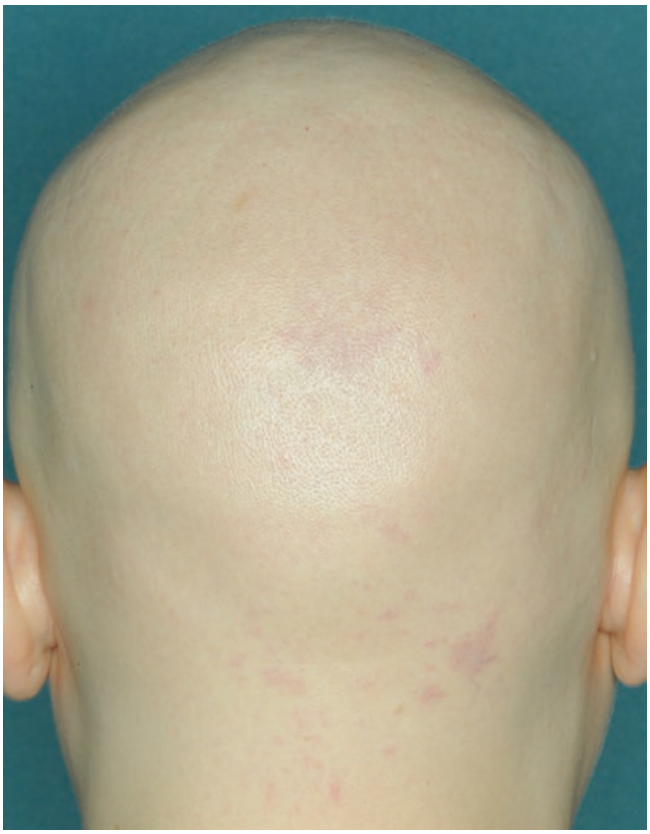

Fig. 29.2 Alopecia areata totalis. Alopecia involving the entire scalp in a patient with AT

and frontal scalp are affected with relative sparing of the band of hair between the occiput and bilateral temporal scalp. Like the severe forms of AA, both of these variants are usually recalcitrant to currently available therapies.

Genetic Architecture of AA

Human genetic studies in AA have played a pivotal role in understanding its etiology. Initial evidence for a genetic basis to the disease arose from family studies that demonstrated increased risk of disease in first degree relatives [3, 4], twin studies [5, 6], and studies in animal models [7]. The earliest genetic studies, limited by the availability of high throughput cost-efficient technologies for genotyping or sequencing, utilized a candidate gene approach testing only a few variants in pre-selected genes, utilizing small cohorts. These initial genetic studies demonstrated associations to several immune related genes, including genes within the human leukocyte antigen (HLA) complex such as HLA-DQB1, HLA-DRB1, HLA-A, HLA-B, HLA-C, NOTCH4, MICA, as well as PTPN22 and AIRE, which are located outside the HLA. While these studies provided consistent support to the theory of an autoimmune basis for the disease, they were limited in generating new etiological insight. With the advent of commercial single nucleotide polymorphism (SNP) genotyping arrays, it became feasible to query the entire genome in large cohorts of patients and controls, enabling unbiased testing in well-powered studies. The first genome-wide association study (GWAS) conducted for AA identified eight regions in the genome with statistically significant evidence for association [8]. Of these, only the HLA had previously been implicated in AA. A subsequent replication study utilizing this GWAS data and genetic data in an independent cohort identified two additional loci in the genome [9]. Most recently, the first meta-analysis GWAS was performed in AA in a cohort of 10,000 samples. The substantial increase in sample size increased the power to detect association, and four additional loci were at or near genome-wide statistical significance [10].

GWAS loci typically contain multiple genes, but the technique does not allow individual genes within loci to be prioritized for etiological significance. In order to gain mechanistic insight from GWAS evidence, pathway analysis

Table 29.1 GWAS loci in AA implicate biological processes perturbed in disease

Pathway, GWAS locus (pathway gene)
T cell activation, co-stimulation, and differentiation 1p13.2 (PTPN22), 2q33.2 (CD28, CTLA4), 4q27 (IL2, IL21), 5q31.1 (IL13, IL4, IL5, IRF1), 6p21.32 (HLA-DMA, HLA-DOA), 10p15.1 (IL2RA, PRKCQ), 11q13.1 (BAD), 12q13 (IL23A, IKZF4 (Eos)), 16p13.13 (CIITA)
JAK-STAT signaling 4q27 (IL2, IL21), 5q31.1 (IL13, IL4, IL5), 10p15.1 (IL2RA, IL15RA), 12q13 (IL23A, STAT2), 16p13.13 (SOCS1)
Viral defense response 5q31.1 (IRF1, IL4), 6q25.1 (ULPB3, ULBP6 (RAET1L)), 10p15.1 (IL2RA), 11q13.1 (BAD)
Steroid Hormone Signaling 6p21.32 (RXRB), 9q31.1 (NR4A3), 11q13.1 (ESRRA)
Oxidation Reduction 2q13 (ACOXL), 5q31.1 (P4HA2, UQCRQ), 6p21.32 (HSD17B8), 6q25.1 (IYD), 11q13.1 (PRDX5), 12q13 (SUOX, RDH5, GLS2), 12q24.12 (ALDH2, ACAD10)
Apoptosis 10p15.1 (IL2RA), 11q13.1 (MEN1, PRDX5, BAD), 12q13 (ERBB3, GLS2), 16p13.13 (LITAF), 1p13.2 (HIPK1, MAGI3, BCL2L15), 2q13 (BCL2L11), 2q33.2 (CD28), 4q27 (IL2, FGF2), 5q31.1 (IL4)

There are 14 regions in the genome that demonstrate significant association with AA by GWAS, which range in size from 30 Kb up to 500 Kb and can contain many genes, in addition to regulatory elements that influence genes proximal to GWAS loci [10]. Pathway analysis of the 228 genes within 1 Mb of a GWAS loci implicates several molecular pathways. Genes contributing to pathway evidence are listed by region

is used to identify sets of functionally related genes. In AA, the 14 GWAS loci contain 226 protein coding genes and pathway analysis demonstrates enrichment of genes involved with a small number of biological processes (Table 29.1). For instance, pathways that implicate specific immune perturbances include T-cell activation/differentiation, JAK-STAT signaling, and viral defense response. There are additional pathways implicated by GWAS that suggest disease mechanisms that could be operating in either cells of the immune system, or alternatively, within the hair follicle itself, including oxidation-reduction, apoptosis, and steroid hormone signaling.

Of clinical relevance, some of the etiological pathways that came to light as a result of these genetic studies are targeted by therapeutics already in use to treat other autoimmune diseases, but had not previously been considered within the context of AA treatment. For example, the costimulatory pathway is targeted by abatacept, and JAK-STAT signaling is therapeutically targeted by ruxolitinib and tofacitinib, discussed further below. Trials to test these interventions in the treatment of AA have yielded promising early results [30]. Thus GWAS in AA have rapidly realized the initial promise of the human genome project, to improve the care of patients.

Hair Follicle Immune Privilege

An 'immune privileged' (IP) site was initially defined as a body site to which foreign grafts could be placed without being rejected [11]. This concept was first described by Peter Medawar, after conducting experiments in rabbits demonstrating relative resistance to rejection of allogeneic skin grafted into the anterior chamber of the eye [12]. IP was extended to describe not only sites or organs but also tissues, which were so defined by their relative resistance to rejection if transplanted onto conventional, non-immune privileged sites [13, 14]. Immune privileged sites include the eye, brain, gametes, pregnant uterus, as well as the hair follicle. Indeed, early experiments in which black skin from guinea pigs was grafted onto immunologically nonreceptive white skin of a different guinea pig strain showed rejection of donor skin but prolonged retention of the donor hair follicles [13, 15, 16]. More recently, hair follicle cells from a human male donor were implanted into the skin of an unrelated female, without evidence of rejection [17].

Many factors contribute to IP in the hair follicle. HLA expression is minimal in the hair follicle [18, 19] below the level of the isthmus, preventing recognition of cell-associated antigens by T cells. The hair follicle also employs various methods to avoid or minimize recognition and attack by natural killer (NK) cells, which may be expected due to low HLA expression, including the expression of macrophage migratory inhibitory factor and relative absence of ligands of NK cell stimulatory receptors [20, 21]. Evidence exists that the local environment is comprised of other immunoregulatory proteins, such as TGF-β1 [22], α-MSH [23, 24], and cell-associated PDL1 [25] that may be serving to inhibit immune responses to hair follicle-associated antigens [26]. Lastly, there is an alteration and overall diminution in immune cells that may be contributing to inflammation. Langerhans cells, an immune component of the skin epidermis, are relatively deceased in numbers below the level of the isthmus [19, 27], as are T cells [19]. The contribution of these cells to pathologic states where IP is altered is currently an active area of research.

AA is associated with a collapse of the immune privileged status of the hair follicle [26]. Hair follicles within and adjacent to alopecic patches in AA patients exhibit marked expression of HLA molecules [28], and infiltration of the hair follicle with CD4 and CD8 T cells is observed in AA

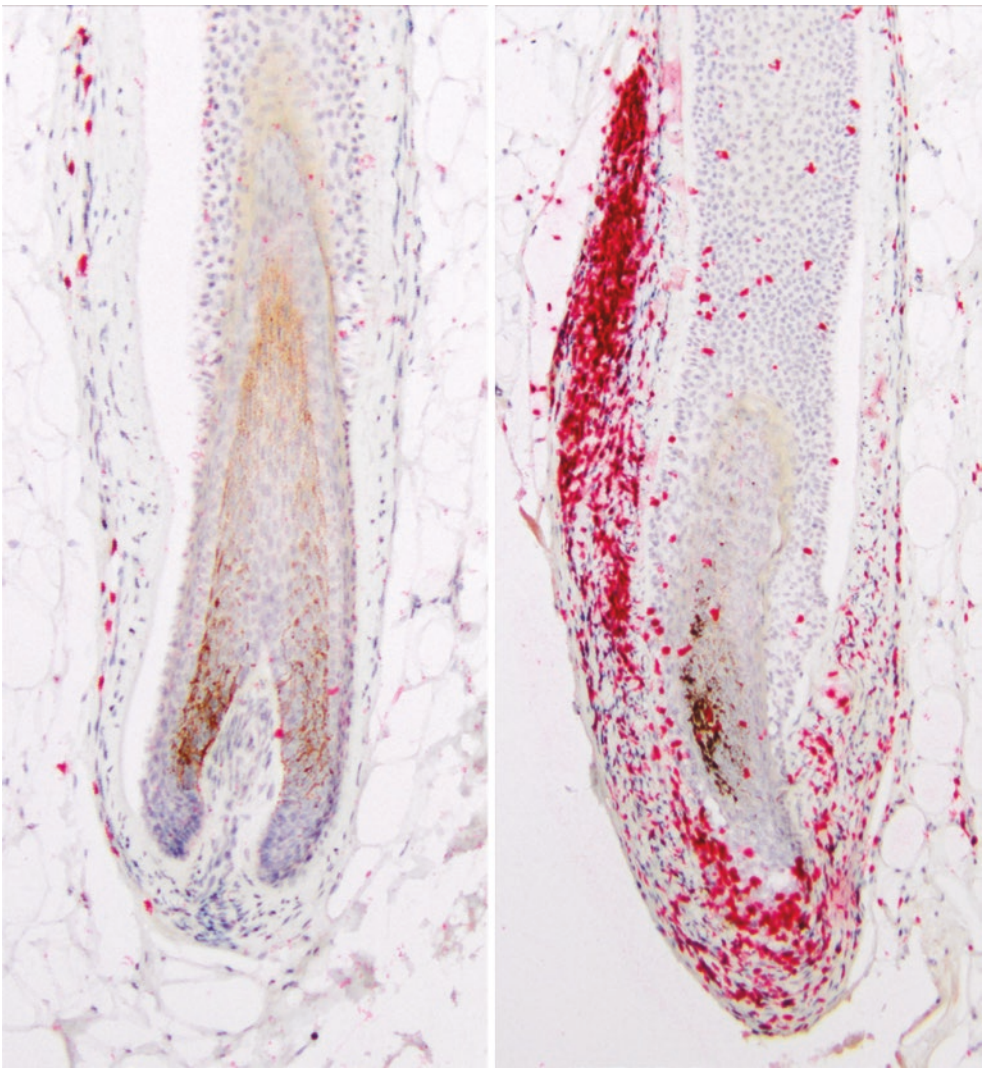

Fig. 29.3 Peribulbar CD3 infiltrate in AA. CD3 immunohistochemical staining of hair follicles in a normal patient (*left panel*) and AA patient (*right panel*). The peribulbar infiltrate has been likened to a "swarm of bees"

lesions (Fig. 29.3), in a pattern termed the "swarm of bees" [1, 29]. Furthermore, the hair follicle begins expressing ligands for NK cell stimulatory receptors, including MICA [20] and several ULBP molecules [8]. The cytokine milieu is also skewed toward a proinflammatory profile, exemplified by the marked increase in the expression of interferon (IFN)-response genes as well as common γ-chain (γ_c) receptor cytokines in AA lesions [30]. This loss of IP in the hair follicle is likely a necessary step for antigenic responses by the immune system and clinical disease in AA.

Cellular Immune Effectors in AA

Much of the work to identify the cellular pathogenic mediators of AA has been conducted in animal models. The C3H/HeJ mouse is a murine model of spontaneous AA development, wherein approximately 10–20 % of mice develop nonscarring alopecia that replicates many features of the human disease [31]. Subcutaneous injection of unmanipulated lymph nodes cells from alopecic C3H/HeJ mice to unaffected mice resulted in hair loss after 5 weeks, implicating the presence of an immune effector mediating the disease [32]. Another model of alopecia, in which human punch biopsy samples from normal patients are grafted onto immunodeficient recipient mice, requires the subcutaneous injection of peripheral blood mononuclear cells after culture in IL-2, corroborating the necessity of a cellular immune effector [33]. More recently, spurred by multiple lines of evidence including (1) the recognition of increased expression of NKG2D ligands in AA skin biopsies [8, 20], (2) our identification of a genetic association between AA and NKG2D ligands [8], and (3) the presence of $CD8^+NKG2D^+$ cells surrounding the hair follicle in patients with AA, adoptive transfer studies were performed demonstrating that $CD8^+NKG2D^+$ cells from skin draining lymph nodes of alopecic C3H/HeJ mice were necessary and sufficient to induce systemic disease in previously unaffected

C3H/HeJ mice [30]. Several lines of evidence support the NKG2D-NKG2D ligand interaction as a critical component of disease, including the upregulation of NKG2D ligands in the hair follicle of AA patients [8] and affected C3H/HeJ mice [30] as well as the known capacity of NKG2D to potentiate antigen-induced T cell responses [34, 35]. These identify $CD8^+NKG2D^+$ T cells as critical players in AA pathogenesis.

Other T cell populations are also likely influencing the development of AA. CD4 T cells comprise part of the peribulbar infiltrate seen in lesions from AA patients [20], and the strongest AA genetic associations have been with HLA Class II molecules [8], which present peptides to CD4 T cells. Adoptive transfer studies in the C3H/HeJ model indicate that CD4 T cells from the lymph nodes of affected mice were sufficient to induce the disease in unaffected, AA-prone mice [36]. Experiments in which the CD4 T cell population have been segregated based on CD25 expression, putatively separating regulatory T cells and conventional CD4 T cells, indicate that the $CD4^+CD25^-$, or conventional T cell, population is the more potent subgroup of this population, whereas $CD4^+CD25^+$, or Treg, cells have a protective role and act to prevent the development of AA [36]. In humans, analyses of serum cytokines in AA patients show reduced levels of circulating TGF-β1 and elevated levels of IL-2, IFN-γ, IL-13 and IL-17, supporting a reduction in the ratio of Treg/conventional T cell activity [37].

The role of NK cells is currently less clear. Although the expectation may be that NK cells would be playing a pro-inflammatory role in AA pathogenesis, data exists supporting an immunoregulatory role for NK cells [38]. The mechanisms of this immunoregulation have not yet been elucidated. It has become challenging to interpret prior studies interrogating the role of NK cells in AA, and the recent discovery of a CD8 T cell population that co-expresses NK markers in AA invites a re-evaluation of prior studies examining NK cells.

Mast cells have also been described in increased numbers around the hair follicle and in the perifollicular dermis in patients with AA [39]. However, studies of mast cells in AA have been limited, although mast cells are able to act as professional antigen presenting cells [40, 41] and elaborate T cell chemotactic molecules [42]. While the mast cells in AA lesions exhibit a pro-inflammatory profile skewing [39], their contribution to AA pathogenesis is unclear.

Macrophages also make up part of the peribulbar infiltrate in AA. In the normal murine hair cycle, the transition from telogen to anagen is marked by a decrease in the number of macrophages, and experimental depletion of macrophages hastened the transition from telogen to anagen [43]. The heightened presence of these cells in AA may indicate that macrophages may be playing in a role in regulating the hair cycle in AA, although this has yet to be substantiated.

Cytokines in AA

IFNs have been implicated in the induction of AA in human disease and in mouse models. Scalp lesions in AA exhibit a strong IFN signature [30], with both type I and II IFNs likely contributing. In particular, IFN-γ, the sole member of the type II IFN family, appears to strongly influence the immune status of hair follicles. Injection of IFN-γ into C3H/HeJ mice reverses the IP status of the hair follicle, demonstrated by increased MHC Class I and II expression, T cell infiltration, and hair loss [44], although some studies of this effect have shown contrasting results [45]. Genetic ablation of IFN-γ prevented the development of spontaneous AA in C3H/HeJ mice [46]. Furthermore, in a modified murine AA model in which alopecic C3H/HeJ skin has been grafted onto previously unaffected C3H/HeJ recipients, which induces hair loss in 90–100 % of graft recipients, neutralizing antibodies to IFN-γ prevented the development of AA [30].

Evidence that type I IFNs, including IFN-α and IFN-β, contribute to the development of AA can be inferred from studies in which patients were treated with IFN for other medical conditions. IFN-α is used as a treatment for chronic hepatitis C, and case reports exist exhibiting the development of AA with treatment and subsequent resolution after the treatment course is completed [47, 48]. Examinations of lesional biopsies from patients with AA demonstrate expression of MxA [49], an IFN-stimulated gene. However, more extensive studies examining the necessity and sufficiency of type I IFNs in AA pathogenesis, for example in mouse models, are lacking.

The participation of certain γ_c receptor cytokines, especially IL-2 and IL-15, has also been implicated in the development of AA. IL-2 and IL-15 function in many ways in the immune system, including providing survival and proliferative signals to T cells. IL-15 can also induce the expression of NKG2D on T cells and can prime NKG2D signaling [50]. IL-2 haploinsufficent C3H/HeJ graft-recipient mice were relatively protected from developing AA, compared to IL-2 sufficient control graft recipients [51]. Antibody treatment targeting IL-2 or the shared β subunit for the IL-2/IL-15 receptor prevented the development of AA in a mouse model of the disease [30]. In AA patients, IL- 15 is expressed at high levels on the hair follicle [30] and has been implicated in another autoimmune disorder, celiac disease [52–54]. Both IL-2 and IL-15 are likely crucial to the development of AA.

Putative Antigens in AA

It is currently unknown whether a shared specific antigen or set of antigens is the target of autoimmune attack for patients with AA. Several lines of evidence support the notion that an

antigen-specific response is involved, including the genetic association between AA and HLA alleles [8], the congregation of T cells around hair follicles in skin samples of affected patients [1], and the ability to induce disease from one mouse to another by adoptive transfer of T cells [30, 36]. Attempts at identifying target antigens in AA have mostly focused on autoantibodies in patients with AA, and the role of antibodies in AA has not been defined. Autoantibodies against a hair follicle-associated antigen have been detected at higher levels in AA patients compared with normal controls, although the identity of this antigen is not known. Trichohyalin has been identified as an autoantibody target in patients with AA [55], although whether autoantibody formation to this or other putative antigens is critical to the pathogenesis of AA or a marker of hair follicle damage remains an open question.

Immunointerventions in AA

There are currently no US FDA approved drugs for the treatment of AA. A Cochrane systematic review conducted in 2008 concluded that there is no convincing trial evidence that any treatments provide long-term benefit to patients with any form of AA [56]. Interventions for AA have, for the most part, consisted of local or systemic immunosuppressive regimens that are relatively nonspecific in their effects and have included topical or intralesional glucocorticoids or steroid-sparing agents.

Glucocorticoids are likely the most commonly used treatment for AA. Glucorticoids inhibit the production of pro-inflammatory cytokines and chemokines and induce the apoptosis of dendritic cells and lymphocytes. Localized treatments are often performed using topical or intralesional delivery methods, and systemic treatment is usually performed only in the context of severe disease. No double blind, randomized controlled trials exist of these treatments, and it is unknown whether these treatments alter the long-term disease course.

Prolonged treatment with systemic steroids has the potential to cause many adverse effects. Steroid-sparing immunosuppressive agents are usually used in the context of severe disease and in cases where the need for a prolonged treatment course are suspected. These treatments include methotrexate, cyclosporine, azathioprine, and sulfasalazine. Only case reports exist of the use of these treatments for AA [57].

Topical immunomodulation is another treatment strategy used for AA. The rationale for these treatments is that the end-organ inflammatory milieu may be redirected to a different, non-hair follicle associated target or can be changed in such a way that it is no longer permissive for continued attack of the hair follicle by the immune system. Anthralin has been cited as a topical immunomodulator, likely due to its proinflammatory effects causing a dermatitis to applied areas. The time that anthralin is allowed to remain on the skin is often titrated to a maximum of 1 h or until a dermatitis is achieved. The mechanism by which AA is inhibited by this drug, however, is unknown.

Topical sensitization to either diphenylcyclopropenone or squaric acid dibutylester is another form of topical immunomodulation that is used for severe cases of AA. These treatments consist of a sensitization phase, in which an allergic response is generated to the drug, and a treatment phase, in which the drug is applied to the affected area with the intent of causing a low-level dermatitis and, secondarily, allowing hair regrowth. Studies in which half the head had been treated with the topical immunomodulator while the contralateral side was left untreated demonstrated some efficacy for these treatments in severe forms of AA, although the hair regrowth is often not sustained long-term [58]. The proposed mechanisms by which these drugs effectively treat AA include antigenic competition, in which an immune response to a separate antigen is elicited with accompanying regulatory/suppressor cells that quell responses to competing antigens [59]. Other studies have shown that DPCP also increases the number of infiltrating CD8 T cells in perifollicular and epidermal areas [60] and decreases the CD4:CD8 ratio [61], but it is unclear how this contributes to ameliorating hair loss in AA since CD8 T cells are thought to be the primary cellular effectors of disease.

Targeted treatments, specifically identified for AA or for immune pathways active in AA, have been lacking. The identification of candidate therapeutic targets for drug development have been recently spurred by results from a GWAS for AA in 2010 as well as gene expression profiling of affected skin from human AA patients and the C3H/HeJ mouse model [62]. Many of these treatments have been tested in the C3H/HeJ model, with a few beginning to be assessed in human AA patients. Antibodies to IFN-γ, IL-2, and the IL-2/15R β chain have shown their utility in the prevention of AA onset in the C3H/HeJ murine model. The efficacy of blocking these cytokine pathways in the murine model led to investigation into whether inhibiting the signaling pathways activated by these cytokines is also an effective treatment strategy.

Notably, γ_c receptors and IFN receptors signal through JAK molecules, with the γ_c cytokines signaling predominantly through JAK1 and JAK3 and the IFN-γ receptor signaling through JAK1 and JAK2. Ruxolitinib and tofacitinib are tyrosine kinase inhibitors with selectivity for JAK molecules that have been approved for use by the US FDA in myelofibrosis and rheumatoid arthritis, respectively. Both of these drugs were able to prevent the development of AA and effectively treat established disease in the C3H/HeJ graft model [30]. Furthermore, topical formulations of these inhibitors reversed established disease in the C3H graft model [30].

Experience with these drugs in the treatment of AA has been limited to a very small number of patients. A single patient with psoriasis and AA universalis treated with tofacitinib experienced partial scalp hair regrowth at 2 months when treated with 5 mg twice daily. Complete scalp hair regrowth with regrowth of eyelashes, eyebrows, axillary hair and pubic hair was observed after an additional 3 months of taking 10 mg in the morning and 5 mg at night. No adverse symptoms or laboratory abnormalities were reported for the patient.

The use of ruxolitinib has also been reported in a series of three patients with moderate to severe AA [30]. All three patients experienced striking hair regrowth after 12 weeks of treatment. Adjunctive studies performed on these patients demonstrated diminished skin infiltration by CD4 and CD8 T cells, and resolution of the observed IFN and cytotoxic T lymphocyte signatures, which were elevated at baseline prior to treatment. Open label pilot studies of ruxolitinib and tofacitinib in patients with AA are currently underway. Larger placebo-controlled studies are needed to determine whether JAK inhibitors are a safe and efficacious treatment for AA, to validate the striking results reported to date.

Conclusions

AA is a common autoimmune disease in which the hair follicle is the target of immune attack. Genetic studies support the immune-related etiology of AA and have been critical in the identification of cellular effectors in AA and pathways that underlie the pathogenesis of the disease. $CD8^+NKG2D^+$ cells, which were first implicated by the genetic association between AA and NKG2D ligands, have since been found to be necessary and sufficient for the development of AA in mouse models. The IFN and γ_c cytokine circuits, identified in the AA GWAS and/or gene expression analyses in humans and mice, play critical roles in the collapse of immune privilege and the development of AA in mouse models and warrant further investigation as targets of biologics in humans for treatment development.

JAK inhibition, resulting in ablation of these cytokine signaling pathways, is a strategy showing promise in effectively treating AA but will likely require further studies.

Questions

1. A class of medications that has recently shown promise in AA:
   A. NSAIDs
   B. TNF inhibitors
   C. Interferons
   D. ***JAK inhibitors***
   E. ACE inhibitors
2. These cells are not commonly found in the peribulbar infiltrate in skin biopsies sections of AA lesions:
   A. ***Plasma cells***
   B. CD4 T cells
   C. CD8 T cells
   D. NK cells
   E. Macrophages
3. Activation of this pathway is associated with enhanced immune responses to the hair follicle:
   A. Interferon-γ
   B. IL-2
   C. TGF-β
   D. all of the above
   E. ***A and B only***

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Cutaneous Lupus Erythematosus

30

Christopher B. Hansen, David F. Fiorentino, and Richard D. Sontheimer

Abstract

Cutaneous lupus erythematosus is a heterogeneous skin disease that is identified based on histopathological and clinical features. It can exist in the presence or absence of systemic manifestations of lupus erythematosus. Classification of cutaneous lupus into subtypes is clinically useful in predicting clinical course and potential association with systemic disease. Pathogenesis involves a genetic susceptibility and environmental triggers. An increasing number of therapeutic options are available.

Keywords

Lupus • Cutaneous • Epidemiology • Therapy • Photosensitivity

Lupus erythematosus (LE) is a broad term referring to disease phenotypes that are characterized by a particular form of aberrant immune activation. Disease presentation is heterogeneous, ranging from a single organ (i.e., skin) disorder to a multi-systemic disease. As such, the term LE should be used with certain modifiers to specify more precisely the type of clinical illness that is being discussed. Systemic LE (SLE) refers to a unique medical diagnosis that is characterized by involvement of multiple organ systems and, although heterogeneous, is bound by certain commonalities of immune dysfunction. The term cutaneous LE (CLE) describes the spectrum of skin disease that has a pathophysiology that is related to LE and is bound by common histopathological and/or molecular criteria. After accepting this definition of CLE, it is paramount to understand that the same lesions can also be found as isolated cutaneous findings in an otherwise healthy individual.

When considering the patient with SLE, one must remember that CLE is not the only form of skin condition related to the underlying disease. It is useful to consider the classification scheme developed by Gilliam and Sontheimer [1] in which the skin findings in a patient with SLE are further characterized as either "LE specific" or "LE non-specific". LE-specific skin disease refers to CLE. LE-specific skin disease generally has one or more of the following histologic features: lichenoid tissue reaction with or without basal keratinocyte vacuolar change; hyperkeratosis; thickening of the epidermal basement membrane; pervivascular and/or perifollicular mononuclear cell infiltrate; and dermal mucin deposition.

In contrast, LE non-specific disease refers to those cutaneous findings that, although driven by the underlying SLE, do not possess the typical histologic features of CLE and can also seen in other disorders. LE non-specific disease includes vascular abnormalities (i.e., vasculitis, vasculopathy, Raynaud's), mucosal ulceration, alopecia, and photosensitivity [2]. This chapter will only concern itself with LE-specific skin disease (i.e., CLE). However, it should be noted that LE non-specific skin disease is of paramount importance to the clinician, as its presence can be an indicator of systemic disease and can reflect SLE disease activity.

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Classification

LE-specific skin disease can be classified in various ways—clinically, serologically, or histopathologically. We will employ the scheme of Gilliam and Sontheimer [1], which relies heavily on clinical manifestations and divides lesions into acute (ACLE), subacute (SCLE), and chronic (CCLE). It should be noted that these terms refer both to the morphologies and pace of the cutaneous lesions themselves. The fact that some forms of LE specific skin disease such as ACLE are strongly associated with SLE disease activity is one reason why it is so vital to categorize carefully LE-specific disease, as this can give a clue if the findings are isolated or are occurring in the context of SLE. As with any classification system, there are cases that do not fit neatly into a group or are considered indeterminate. In addition, these categories are not mutually exclusive; that is, patients may have more than one type of LE-specific skin lesion, either at the same time or during the course of their disease.

Epidemiology

There are few population-based data concerning the epidemiology of CLE, particularly isolated forms of CLE such as SCLE and classical discoid LE (DLE) that typically are not associated with clinically significant SLE. A retrospective population based study over 40 years suggested that the incidence of CLE is comparable to the published incidence of SLE [1].

In the context of SLE, the skin appears to be second only to the joints as the most frequently affected organ [5–7]. For ACLE, the association is so strong with SLE that the epidemiology of ACLE would be expected to be similar to that of patients with SLE [3]. Assuming that ACLE is synonymous with "malar rash" as well as "maculopapular rash", facial ACLE appears to occur in 20–60 % of large LE patient cohorts, while the "maculopapular rash" of SLE occurs in 35 % of SLE patients [6]. Malar rash/ACLE is more common in whites than blacks, women, and younger patients [6, 8, 9].

SCLE occurs predominately in white females of all ages. The original cohort of Sontheimer et al. showed that 70 % were female and 85 % were white, with a mean age of 43.3 years [10]. These patients have been shown to comprise 7–27 % of the total LE cohort in several studies [3]. A study using an anti-Ro/SSA registry followed by patient reported photosensitive skin disease estimates an incidence and prevalence of 4.8 (per year) and 6.2–14 per 100,000 persons, respectively, for SCLE [11].

Chronic cutaneous LE (CCLE) can occur with or without systemic disease. There are no reliable population-based data for CCLE, as these patients are underrepresented in studies from rheumatologists and internists and over represented by dermatologists. It appears that DLE lesions, the most common form of CCLE, can be found in 15–30 % of SLE patients at some point in time during their disease course [12]. Approximately 5–10 % of SLE patients will present with DLE skin lesions [13]. Female:male ratio of DLE is between 3:2 and 3:1 (much lower than SLE); males and females are equally affected, and typical age of onset is between 20 and 40 years [2, 14].

Cutaneous Manifestations

Acute Cutaneous Lupus Erythematosus

ACLE can either be localized to the face or generalized. The classic "butterfly rash" consists of confluent or patchy macular erythema with or without papules, edema and induration, scattered across the malar eminences and bridge of the nose. The forehead, chin, and V-area of the neck can be involved (Fig. 30.1a). The nasolabial folds are typically spared. Although usually symmetrical, this is not always the case. There can be some mild degree of hyperkeratosis.

Generalized ACLE is less common and presents as a widespread morbiliform eruption, often accentuated in a photodistributed pattern over the extensor aspects of the arms, forearms, and dorsal hands and fingers. Over the dorsal fingers during the early phase of the disease, the hair-bearing interphalyngeal areas are especially targeted while the knuckles are spared. Some patients experience an extreme form of ACLE that simulates toxic epidermal necrolysis (TEN), due to the intense lichenoid inflammation [15]. Of note this is one mechanism for the development of vesicular lesions in CLE.

LE patients can experience several types of vesiculobullous skin disease. In "bullous SLE," patients typically with active SLE can present with vesicles (which appear similar to dermatitis herpetiformis) or bullae on the face, arms, and trunk [3]. Histopathological examination of the skin often reveals papillary dermal neutrophilic microabcesses as well as deposition of multiple immunoreactants at the dermal-epidermal junction. In some cases, these antibodies have been shown to bind type VII collagen, while in others the multiple immunoreactants are more consistent with nonspecific immune complex deposition. Thus, these lesions clinically and histologically simulate dermatitis herpetiformis or epidermolysis bullosa acquisita, but the clinical distribution and multiplicity of immunoreactants and the presence of other features of LE help to distinguish these cases.

As "bullous SLE" does not share the histopathologic findings that are typical of CLE, it can be considered as a form of LE-nonspecific vesiculobullous skin disease. Vesiculobullous annular SCLE is an example of vesiculobullous LE-specific

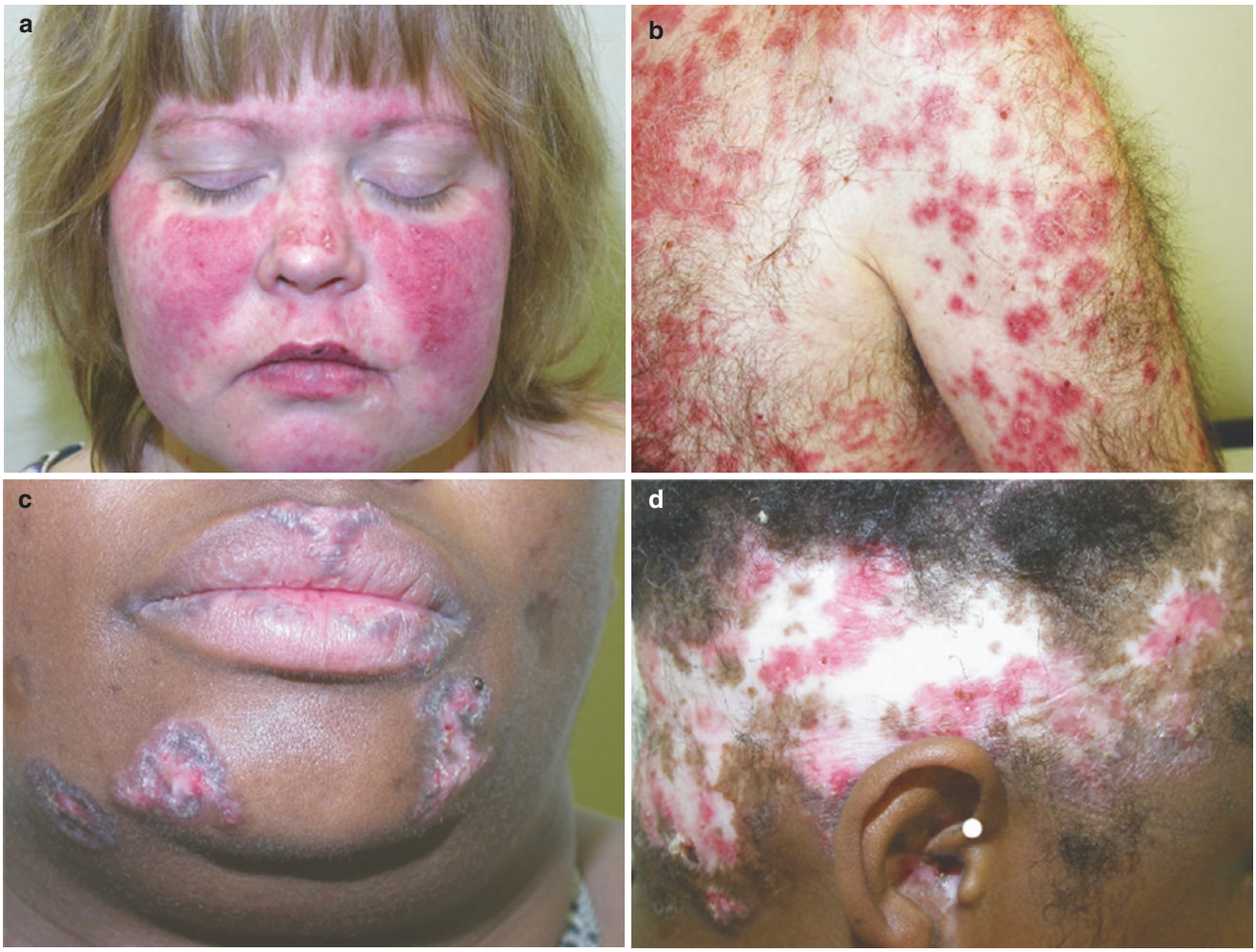

Fig. 30.1 Lupus-specific skin disease. (**a**) Acute cutaneous LE. (**b**) Drug-induced subacute cutaneous LE secondary to rabeprazole (Aciphex) (personal unpublished observation, R. D. Sontheimer). (**c**) Classical discoid LE affecting the chin and lips. (**d**) Classical discoid LE affecting the scalp and external ears with scarring alopecia and postinflammatory hypopigmentation

skin disease. Of note, vesiculation can also result from other blistering disorders, such as bullous pemphigoid, dermatitis herpetiformis, porphyria cutanea tarda, and pemphigus vulgaris that have rarely been reported to occur concordantly with LE [79–82].

Superficial ulceration of the oral or nasal mucosa can also occur in ACLE. These lesions are often asymptomatic, transient, and tend to occur on the hard palate (although virtually any area of the oral mucosa can be involved) [2, 16].

The lesions of ACLE are typically photosensitive and transient, usually lasting several days or weeks. Patients can concurrently develop SCLE, or, less commonly, DLE lesions. ACLE lesions do not scar but can result in predominate post-inflammatory pigmentary alteration, especially in dark-skinned patients.

The differential diagnosis for ACLE includes any dermatosis that can produce a red face, with common diagnoses being acne rosacea, dermatomyositis, seborrheic dermatitis, contact dermatitis, polymorphous light eruption (PMLE), and drug eruptions. Rosacea deserves special mention as a mimic of ACLE. In a study of patients referred to a dermatology clinic with a presumptive diagnosis of cutaneous LE, there were 21 that in fact had a different dermatological condition. Sixteen of these 21 patients had rosacea based on biopsy or diagnostic clinic features [2].

Subacute Cutaneous Lupus Erythematosus

First described as a distinct entity by Gilliam in 1977, SCLE is the prototype of a LE-specific skin disease that is defined by clinical, serological, and genetic features [10]. Clinically, these lesions present as either scaling papules or small plaques (“psoriasiform type”) (Fig. 30.1b) or scaling, annular and/or polycyclic plaques (“annular type”)—these forms are equally prevalent [2]. In general, one

individual presents with one or the other type, though both forms can occur in a patient.

Lesions are characteristically photodistributed on the chest, back, extensor arms and V-area of the neck—it is the experience of the authors and others [92] that these lesions occur less commonly on the face compared to ACLE and DLE [2, 17]. Eighty-five percent of all SCLE patients report photosensitivity [18]. The inactive central portion of the radially spreading annular lesions is often hypopigmented. As in ACLE (see above), intense basovacuolar degeneration of epidermal keratinocytes can result in vesiculation and/or crusting, which usually occurs at the active edge of annular lesions. This can resemble TEN in its extreme form [19]. Lesions of SCLE typically heal without scarring, but permanent hypopigmentation and/or telangiectasias can occur. SCLE patients can also develop the lesions of ACLE or classic DLE. Localized facial ACLE has been reported to occur in 20 % of patients [3], while various reports document DLE lesions in 0–30 % of SCLE patients [11, 18]. In contrast to ACLE, SCLE lesions tend to be less transient, more scaly, less edematous, associated with more pigmentary change, and, as previously mentioned, less commonly affect the face. The absence of induration in SCLE lesions can serve as a clinical distinguisher from DLE and LE tumidus.

The differential diagnosis for SCLE includes psoriasis, dermatophyte infections, pityriasis rubra pilaris, polymorphous-light eruption (PMLE), nummular eczema, dermatomyositis, and mycosis fungoides. Annular lesions can be confused with granuloma annulare, erythema multiforme, and gyrate erythemas. However, the inactive centers of SCLE lesions are typically hypopigmented while those of other annular disorders are typically pigmented normally or hyperpigmentated.

Chronic Cutaneous Lupus Erythematosus

Classic DLE is the most common form of CCLE. These lesions begin as erythematous papules, which then develop scale and evolve into larger plaques covered by adherent scale that are usually associated with follicular plugging and peripheral hyperpigmentation. When the adherent scale is peeled back, follicle-sized keratotic spikes can be seen to project from the underside (the so-called "carpet tack sign"). The lesions expand slowly leaving central atrophy, scarring, telangiectasia, and depigmentation (Fig. 30.1c). Hyperpigmentation is often seen at the active borders of lesions. The combination of peripheral hyperpigmentation and central depigmentation is especially prominent in African American DLE patients. Some lesions of DLE can present only as macular hyperpigmentation, especially in Asian Indians [20].

DLE lesions occur most often on the face, ears (especially the conchae), scalp, V-area of the neck, and extensor aspect of the arms. Any facial structure can be involved, including eyebrows, eyelids, nose and lips. Periocular lesions are often misdiagnosed and can present as blepharitis, conjunctivitis, or periorbital edema [2]. Lesions can occur in the malar distribution, but their chronicity, epidermal change and scarring should distinguish them from the classic malar rash of ACLE. An acneiform pattern (often in the perioral area or the chin) that resolves with pitted scarring is rarely seen [2, 21]. Compared with ACLE or SCLE, DLE is less commonly reported to be associated with ultraviolet (UV) exposure. Patients are often unaware of the time lag (up to 4 weeks) following sun exposure, and many lesions do not occur in sun-exposed areas (e.g., hair-bearing scalp, conchal bowl of ears) [22, 23].

Scalp involvement occurs in 60 % of patients with DLE, with persistent activity resulting in permanent scarring (Fig. 30.1d). However, alopecia that is associated with DLE can be reversible when it is secondary to early inflammation of DLE; the telogen effluvium that represents an increase in underlying SLE activity; and alopecia areata that has been shown to be commonly associated with DLE [24].

DLE lesions are termed "localized" if they occur only on the head and neck, while lesions above and below the neck are referred to as "generalized". Lesions can also occur on the palmar or plantar surfaces [25, 26], the nail unit [2, 27], in areas of trauma (the Koebner or isomorphic response) [28]. Follicular DLE lesions have been described, often around the elbow, and may be more common in African-American and Asian patients [28, 29].

Hypertrophic DLE is a rare variant in which hyperkeratotic lesions occur (often on the extensor extremities, upper back, and face). Histopathology can reveal features of squamous-cell carcinoma which can lead to confusion regarding diagnosis [115]. Even if patients have classic DLE lesions elsewhere, the clinician should still be aware that squamous-cell carcinoma can develop in long-standing, scarring DLE lesions [30, 31].

Well-developed lesions of DLE do not usually present a problem with differential diagnosis, although early lesions can be confused with PMLE, granuloma faciale, sarcoidosis, cutaneous lymphoid hyperplasia, lupus vulgaris, angiolymphoid hyperplasia with eosinophilia, and tertiary syphilis.

Mucosal DLE occurs in approximately 25 % of CCLE patients [16]. Oral lesions tend to occur on the buccal mucosa, and less commonly on the palate, gums, and tongue. Lesions have a sharply marginated, scalloped white border with central erythema. Central areas can erode, although lesions are typically painless. The surfaces of well-developed plaques on the palate can have a meshwork of raised hyperkeratotic strands giving a "honeycomb" appearance [16]. Fixed mucosal DLE lesions can be distinguished clinically

from the transient superficial mucosal ulcerations that are often seen in active SLE patients. The lips can be involved with well-defined plaques or a diffuse cheilitis [3]. Such lesions can degenerate into squamous cell carcinoma [32]. Involvement of the nasal, conjunctival, and genital mucosa can occur [33].

Chilblain LE is characterized by red-purple patches or papules on the toes, fingers, and/or face that are precipitated by cold or damp climates. At the onset these lesions are clinically indistinguishable from simple chilblains (or, pernio) lesions that occur in healthy individuals [34, 35]. However, chilblains LE lesions tend to evolve into more classic acral DLE lesions, and it is postulated that this may be the result of a Koebner phenomenon in otherwise typical lesions of perniosis [3]. Differential diagnosis includes other cold-induced vasculopathies, such as cold agglutinin disease or cryoglobulinemia.

LE profundus (syn. LE panniculitis) is a form of CCLE characterized by inflammation in the lower dermis or subcutis. This lesion occurs more commonly in women and is seen in 1–3 % of SLE patients [3]. Approximately 70 % of patients will have overlying DLE lesions [36, 37]. Some have used the term "LE profundus" to specify those lesions that have concurrent overlying DLE activity and the term "LE panniculitis" to refer to lesions displaying only subcutaneous inflammation. However, this is not a universally accepted convention.

LE profundus/panniculitis lesions are characterized by firm, deep nodules with initially normal appearing overlying skin. With time, the nodules resolve and draw the surface of the skin inward, leaving deep, saucerized depressions. Lesions tend to occur in the head, upper arms, buttocks and thighs. Rarely, this entity can present as periorbital edema. Dystrophic calcification can occur in older lesions. Breast lesions ("lupus mastitis") can be confused with carcinoma [2]. Persistent, extensive LE profundus/panniculitis lesions of the breast can necessitate mastectomy. Early lesions can be confused with morphea, while other forms of panniculitis (subcutaneous panniculitic T cell lymphoma, sarcoidosis. factitial or traumatic panniculitis, subcutaneous granuloma annulare) and lipoatrophy (partial lipodystrophy associated with autoimmune disease, drug-induced lipoatrophy, HIV-associated lipoatrophy) must be ruled out.

Typical lesions of lupus erythematosus tumidus (LET) are succulent, edematous papules and plaques that arise due to accumulation of dermal mucin. These lesions are found with decreasing frequency on the face, back, arms, and chest [38, 39]. In some individuals, large annular edematous plaques can be seen. The largest series reported resolution with no or mild topical treatment in nearly half of cases [39], although it has since been questioned that this series may have included many patients with PMLE [38]. It is possible that this might account for the extreme photosensitivity that was reported in these patients [39]. Other authors report these lesions to be chronic and difficult to treat and such patients are typically ANA-negative. Most affected patients do not have SLE [38–41].

Lesions are characterized by perivascular and periappendageal lymphocytic inflammation with dermal mucin deposition. Unlike other forms of LE-specific skin disease, there is absence of basal vacuolar changes in 80–100 % of cases, with positive cases showing only focal and sparse keratinocyte necrosis [38, 42]. This fact has led to discussions about how to best categorize LET. Some authors have characterized LET as a separate category of CLE called "intermittent CLE" due to histological differences and lack of scarring potential seen in other forms of CCLE [3]. LET lesions must be differentiated from PMLE, Jessner's lymphocytic infiltrate, atypical lymphoid infiltrates, myocosis fungoides, reticular erythematous mucinosis, DLE, SCLE, and figurate erythemas. Some have argued that LE tumidus and Jessner's lymphocytic infiltrate in reality cannot be clearly be distinguished [39].

Laboratory Abnormalities

Little data are available concerning laboratory assessment of patients with ACLE. It is assumed that this would closely parallel the data that are available for patients with SLE.

Approximately 60–80 % of SCLE patients have detectable antinuclear antibodies (ANAs) with a speckled/particulate ANA pattern being most common [43]. This disease is characterized by positive anti-Ro/SSA antibodies, present in 40–100 % of patients, depending on the assays used [44, 45]. Anticardiolipin antibodies are present in 10–16 % [46]. Rheumatoid factor is present in one third of SCLE patients [44], and some patients initially present with rheumatoid arthritis long before a diagnosis of SCLE is made. Sm, dsDNA, and U1RNP antibodies are present in 10 % of SCLE patients [44]. Anti-thyroid antibodies were reported in 18 % [47] and 44 % [48] of SCLE patients. Depending on the presence of SLE disease activity, cytopenias, hypergammaglobulinemia, proteinuria, hematuria, and depressed complement levels can also be seen.

In DLE, low ANA titers/levels (e.g., ≤1:40) are present 30–40 % of the time in assays that employ human tumor cells as substrates. However, higher titers that are typically seen in SLE (≥1:160) are rarely encountered in patients having isolated forms of DLE [2]. Anti-Ro/SSA antibodies are occasionally found, but the presence of anti-Sm, dsDNA, and La/SS-B antibodies is uncommon [49]. Fewer than 10 % of patients have IgG anticardiolipin antibodies [50]. A small percentage of DLE patients will have positive rheumatoid factor, slight depression in complement, and leucopenia (see below). Antinuclear antibodies are present in approximately

75 % of patients with lupus profundus/panniculitis [2]. The frequency of ANAs in patients with chilblains lupus was reported as 9 out of 14 patients, with anti-dsDNA antibodies in 4 of 14 patients—these numbers might be an overestimate [35]. Anti-Ro/SS-A antibodies have been variably found in these patients, and some authors have suggested that this is a marker for this disorder [35, 51].

Relationship to Systemic Disease

For ACLE, as it is generally presumed that this is a fundamental component of SLE, there are not many data regarding the relationship of ACLE and SLE disease activity. One study suggests that the course of rash severity parallels SLE activity [12]. Interestingly, authors have failed to support an association with renal or CNS disease, although this has not been studied adequately [2]. One small study indicates that SCLE and DLE patients with normal lymphocyte counts are unlikely to have SLE [52].

Approximately 50 % of patients with SCLE meet American College of Rheumatology (ACR) criteria for SLE [44, 53]. However, severe systemic disease (i.e., nephritis, CNS disease) develops in only 10 % of SCLE patients [44]. Some data support that the papulosquamous form of SCLE is more associated with renal involvement [2]. As stated above, rheumatoid arthritis has been reported to precede as well as follow a diagnosis of SCLE. In addition, 3–12 % of SCLE patients will later develop Sjögren's syndrome [2]. Finally, there are some reports that suggest that SCLE might be a paraneoplastic syndrome [2]—due to the paucity of cases, a causal relationship has not been proven.

Historically it has been felt that 5–10 % of patients presenting only with DLE lesions will eventually develop SLE [2]. Two more recent prospective studies suggest the risk may be in the 10–20 % range [1, 4] However, a minority of those that eventually develop SLE by criteria experience moderate or severe disease [4]. Risk factors for progression include lesions above and below the neck, unexplained anemia, leucopenia, persistently positive high-titer ANA, hypergammaglobulinemia, and positive lupus band test of nonlesional skin [54]. Patients with evidence of nephropathy or arthralgias are also at increased risk of having SLE [55]. Similar to SCLE, patients with lupus panniculitis have a 50 % chance of having SLE, although this is usually mild with only 10 % meeting strict ACR criteria for SLE [2].

In patients with known SLE, the presence of CCLE lesions (namely DLE, lupus panniculitis, chronic mucosal plaques), appears to be associated with less severe systemic disease [2]. A more recent study suggested SLE patients with DLE had a decreased risk of arthritis and pleuritis but no effect on risk of nephritis [5].

Histopathology

The histopathology of CLE will be mentioned only briefly as it has been described in detail elsewhere [2]. In general, ACLE, SCLE and DLE have similar features that do not allow for distinction between the subsets of CLE. Characteristic findings include liquefactive degeneration of the epidermal basal-cell layer, variable hyperkeratosis, dermal edema and mucin deposition, and mononuclear cell infiltration around the dermal-epidermal junction and dermis. This infiltrate consists mainly of CD3+ (both CD4+ and CD8+) cells, with other cell types including histiocytes and plasmacytoid dendritic cells (see below). In DLE, the dermal infiltrate is generally denser and can extend more deeply into the reticular dermis. In addition, DLE lesions can demonstrate follicular plugging and more pronounced basement membrane thickening.

Variable deposition of immunoglobulin (IgM, IgG, IgA in decreasing frequency) and complement components can also be detected at the basement membrane zone of lesional skin. The frequency and intensity with which this is detected varies between studies, anatomic location of skin biopsies, and type of CLE [2].

Hypertrophic forms of DLE are characterized by a greater degree of epidermal acanthosis and hyperkeratosis. Notably, some areas can have features of squamous cell carcinoma or keratoacanthoma. Lupus panniculitis/profundus generally spares the dermal-epidermal junction (if overlying DLE is not present), and is characterized by a lobular panniculitis and perivascular mononuclear cell infiltrate [2]. The infiltrate in the fat is composed of histiocytes and lymphocytes (sometimes forming nodules) and can show variable hyaline-fat necrosis or calcification. LE tumidus shows a perivascular and periadnexal lymphocytic infiltrate with dermal mucin deposition. Studies show focal spotty keratinocyte necrosis in 0–20 % of cases [38, 42]. Chilblain LE shows basal vacuolar degeneration, superficial dermal edema, and a perivascular lymphocytic infiltrate. Some authors conclude that these entities can be distinguished by histopathology, with idiopathic chilblains being characterized by perieccrine inflammation and spongiosis [56, 57].

Pathogenesis

Most of the work pertaining to pathogenesis of cutaneous lupus relates to those forms of CLE that are characterized by interface dermatitis (i.e., ACLE, SCLE, DLE). Thus, this section will focus on these manifestations of CLE. In addition, much of the discussion will not distinguish between the different types of CLE, except for when specifically noted in the text.

Before considering the etiology of LE-specific skin disease, it is interesting to consider its relationship with disease mechanisms that are associated with SLE. Evidence for a pathogenic relationship between cutaneous and systemic disease includes the association of LE-specific skin disease with SLE as well as the fact that, even in "skin-limited" disease, certain characteristic T and B lymphocyte abnormalities can be found systemically that mirror those seen in SLE [58, 59]. The general concept that genetic susceptibility (i.e., HLA haplotypes) and environmental triggers (infection, medication, ultraviolet light) result in a loss of immunologic self-tolerance which then is manifested by generation of autoantibodies and antigen-specific T lymphocytes that mediate tissue injury is likely operative in both cutaneous and systemic disease. However, it should be noted that there is no definitive evidence at present that demonstrates the cutaneous inflammation of CLE is due to an autoimmune response to antigen(s) in the skin. However, studies showing an oligoclonal expansion of T cells in the CCLE lesions are suggestive of an antigen driven reaction either in the skin or periphery [60, 61]. Interestingly, there is no evidence for this in infiltrates of lupus panniculitis [62].

Various genetic abnormalities are associated with different forms of LE-specific skin disease. Several HLA haplotypes have been associated with ACLE, SCLE, and DLE [3]. This implicates a role of T lymphocytes, and may relate specifically to their role in providing help for antigen-specific B cell responses, as a particular HLA B8 DR2 (DRB1*1501) DR3 (DRB1*0301) extended haplotype correlates with the anti-Ro/SS-A response [2]. A polymorphic variant in the TNF-α promoter that is associated with increased TNF-α production is highly associated with SCLE and neonatal LE [63, 64]. Genetic deficiencies in complement components, such as C2, C3, C4 and C5, have been associated with SCLE and/or DLE [2]. The role of C1q seems important, as complete congenital genetic deficiencies of this protein are a strong risk factor for photosensitive SLE [2]. In addition, a polymorphism in the C1QA gene is associated with SCLE [65].

Environmental factors play a role in the pathogenesis of CLE. The paramount role of UV irradiation is discussed below. Assuming that ACLE is triggered by the same mechanisms as for SLE, chemicals such as L-canavanine present in alfalfa sprouts (which induce SLE) may be important [2]. Infections, especially those caused by viruses, are also triggers for SLE. Multiple medications have been associated with the clinical induction of SCLE and less so with DLE [2, 116, 117].

It has been proposed that these may do so via inducing photosensitivity, which might result in disease activity via UV-specific mechanisms or simply via the Köebner phenomenon that results from photodamage. Although numerous drugs can induce SLE (e.g., procainamide, hydralazine, isoniazid), drug-induced SLE is typically not associated with cutaneous findings. Similarly, trauma appears to induce a Köebner phenomenon, especially in DLE patients. Smoking has been implicated as a risk factor for the development of SCLE and DLE [2]. It is unclear if this reflects a primary role for smoking in the disease process or simply results from its known association with antimalarial resistance.

Any consideration of the molecular pathogenesis of CLE must involve consideration of the role of ultraviolet (UV) light. Evidence for the role of UV light in CLE is strong: first, most CLE lesions are in photo-exposed regions of the body; second, 50 % of patients with lupus report photosensitivity; third, 54 % of patients with CLE demonstrate UV photoprovocation of their lesions in the lab [66]. Finally, an immune response against UV-altered DNA has been shown to occur in both mouse models of lupus and patients with SLE. However, the relative importance of UV light in the pathogenesis of likely multiple genetic and phenotypic forms of CLE is not currently known. It is likely that other environmental triggers (i.e., infection, cellular injury, medications) can lead to CLE as well, although the mechanisms for these are not well worked out. Thus, although most of the data presented related to how UV light induces CLE, other forms of keratinocyte damage and/or activation of the cutaneous immune system can be applied in the final model.

Although it is clear that UV light (UVA or UVB) can induce CLE lesions in susceptible patients, the mechanistic link between UV exposure and the cutaneous inflammation that is observed is still not clear—many of the proposed mechanisms may be operating simultaneously. Ultraviolet light can generate neoantigens, such as UV-modified DNA; when injected into mice, this altered DNA can cause lupus like disease [66]. Another mechanism might be the ability of UV light to induce apoptosis of keratinocytes by multiple mechanisms, including oxidative damage to mitochondrial membranes, damage to DNA, induction of p53, activation of membrane death receptors (Fas), and sensitization to TNF-α (and TNF-related apoptosis inducing ligand, TRAIL). Normally, the immunologic clearance of apoptotic cells is a non-inflammatory event. An increased rate of formation and/or decreased ability to "clear" these apoptotic cells, however, can lead to early necrosis of cells and result in their capacity to stimulate the immune system. This occurs via multiple mechanisms, including the ability of necrotic elements to induce maturation and activation of local antigen presenting cells [67]. Necrotic cells can release pro-inflammatory mediators such as high mobility group 1 (HMG1) protein, which is found in high levels in the skin of CLE patients [68]. The C1QA and other complement deficiencies that are associated with CLE suggest that these patients might have a defect in removal of apoptotic cells. Is there evidence of defective

clearance of apoptotic keratinocytes in CLE? Data are conflicting in this regard [69, 70]. Whether or not CLE patients have an increased number of apoptotic cells, it is still possible that these cells could somehow lead to inflammatory sequelae in CLE patients. Indeed, a recent observation suggests that detectable inflammation correlates with the presence of apoptotic cells in the near vicinity in CLE patients [70].

If one accepts that in CLE patients, UV-damages keratinocytes with an inflammatory response, then how might such a response be propagated? One theory is that the binding of circulating autoantibodies against cellular constituents of dying keratinocytes results in a local inflammatory response (via FcR-dependent or complement-dependent mechanisms) [66]. Since the seminal observation by Casciola-Rosen and colleagues that UV light induces translocation of intracellular keratinocyte antigens to the cell surface (in structures called blebs) [71], and that these antigens include the SSA/Ro and SSB/La antigens that are the targets of commonly found autoantibodies in CLE (especially SCLE), the ability to definitively link this process with the clinical findings of CLE has eluded investigators. In fact, the frequency and/or titers of SSA/Ro antibodies do not always correlate with skin activity in CLE patients [66]. However, it has recently been suggested that SSA/Ro autoantibodies are capable of interfering with the clearance of apoptotic cells [119]. Still, it might be that the critical antibodies are not being measured, and that apoptotic-modified forms of these antigens are the critical targets [66]. In general, LE patients have antibodies that appear to be directed against antigens involved in the cellular-stress response and heat shock response. Whatever the answer, it seems unlikely that such autoantibodies play a role in initiating CLE disease, as the deposition of antibodies in CLE tends to follow, not precede, the cellular inflammation [72].

UV light can also promote inflammation by inducing the secretion of cytokines and chemokines, as well as upregulating the expression of adhesion molecules [73]. UVB induces IL1-α and TNF-α in the epidermis. These cytokines induce release of IL-6, PGE2, IL-8 and granulocyte-monocyte colony-stimulating factor (GM-CSF) by keratinocytes [66]. The end result is activation of Langerhans cells, chemotaxis of lymphocytes, and up-regulation of adhesion molecules on keratinocytes (ICAM-1) and endothelial cells (ICAM-1, VCAM-1, E-selectin). UVB irradiation also induces expression of chemokines, such as CCL5, CCL27 and CXCL8 [74]; these have all been found at high levels in CLE skin, and function to recruit memory T cells into the vicinity. Via production of oxygen-free radicals, UVA upregulates ICAM-1 in keratinocytes, but, because it can penetrate deeper into the skin, is also able to upregulate vascular endothelial ICAM-1 and E selectin, which allows leukocyte extravasation into the skin [66]. In addition, UVA is able to induce secretion of IL-12, a potent immunostimulant [66].

T lymphocytes are likely to play a major pathological role in CLE. Skin infiltrates consist primarily of CD3+ T cells, both CD4+ and CD8+ [66]. CD4+ cells appear early in the skin, with CD8+ appearing later. Recent data suggest that skin homing (CLA+) CD8+ cytotoxic cells might be responsible for the scarring that is seen in CCLE [75]. These cells are seen predominantly in the skin of DLE patients (as opposed to other CLE subsets), and an expanded population of circulating, CCR4+, CLA+ CD8+ cells is associated exclusively with generalized DLE [76]. These cells secrete granzyme B, a serine protease that causes tissue death and could conceivably account for injury (and resulting scar) to adnexal and epidermal structures. IFN−α may also play a role in this process, as local IFN-α activity was found to be correlated with CD8+ cell infiltration. Further evidence for the role of activated T cells in CLE comes from the increased expression of HLA-DR and CD25 (both activation markers) on circulating CD4+ and CD8+ cells in patients with DLE and SCLE—furthermore, these levels correlated with cutaneous disease activity [58, 59].

The cytokine expression pattern found in DLE lesions is representative of a mixed Th1 and Th2 profile. Lesions are characterized by high levels of IL-1, IL-2, IFN-γ, TNF-α, IFN-α, IL-5 and IL-10 [66, 67]. TNF-α is found in increased levels in the skin of DLE and SCLE patients and serum levels correlate with disease [77, 78]. TNF-α can promote many of the findings seen in CLE: translocation of SSA/Ro to the cell surface of keratinocytes; apoptosis of keratinocytes; hyperkeratosis; and increased expression of adhesion molecules that favors cutaneous leukocyte infiltration [79]. In addition to the effects mentioned above, IFN-γ has been shown to cause keratinocyte apoptosis [80]; a mouse strain that overexpresses IFN- γ in keratinocytes results in clinical features of SLE and cutaneous inflammation [66]. IL-1 is generated by keratinocytes in response to UV light, and transgenic expression of IL-1 in mice results in hair loss, scaling, and focal inflammatory lesions [66]. Keratinocytes also express an antagonist of the IL-1 receptor, and null alleles of this protein have been reported in SLE patients with photosensitivity as well as in CCLE patients [66].

The role of the innate immune system in CLE is beginning to be explored. It has recently been discovered that IFN-α plays an important role in the pathogenesis of SLE [81]—evidence shows that the source of this IFN-α is the plasmacytoid dendritic cell (pDC). High numbers of pDC have been detected in the lesions of CLE [82], with accompanying high levels of local IFN-α activity [76, 82–84]. IFN-α is known to induce the chemokines CXCL9, CXCL10, and CXCL11, which are also at high levels in CLE skin [74]. The ligand for these chemokines, CXCR3, is found on infiltrating T cells in CLE. Thus, local emigration of activated pDC to the skin of CLE patients might represent the mechanism whereby T cells initially migrate into cutaneous lupus lesions. At present it is unclear what the signals are that cause

pDC migration to the skin; however, it is tempting to speculate that locally deposited immune complexes (i.e., the lupus band) might play a role in their ongoing local activation, since it is known that immune complexes containing nucleic acid (from apoptotic cells) activate pDC via toll-like receptors (TLRs), resulting in the production of IFN-α [85].

Treatment

Due to the critical role of UV light in CLE pathogenesis, the mainstay of any treatment regimen is sun protection. Protection against UVB and UVA are critical, as both can induce CLE in the laboratory [23, 86]. Patients should be counseled on the importance of avoiding direct sun exposure, as well as the benefits of protective clothing including hats, and tightly woven clothing. Broad-spectrum sunscreens are important, and there is evidence showing that the use of these agents inhibits experimentally-induced CLE lesions in patients [87] as well as potentially decreasing the burden of systemic disease in SLE patients [88].

Local therapy with corticosteroids and/or calcineurin inhibitors (tacrolimus or pimecrolimus) can be used in CLE [2]. The current strengths and formulations of calcineurin inhibitors available in the United States have limited efficacy for skin sites other than the face. However, higher concentrations of calcineurin inhibitors have been reported anecdotally to be of value in classical DLE [89]. Calcineurin inhibitors are presumed to act by inhibiting activation of infiltrating T cells that are known to be present in CLE.

Antimalarials (hydroxychloroquine, chloroquine, and quinacrine) are used as first-line systemic agents for SCLE or DLE lesions, with approximately 75 % of patients responding favorably [3]. Efficacy data is based mostly upon anecdotal reports, although one controlled trial with hydroxychloroquine suggested 50 % efficacy for DLE or SCLE [90] while another with chloroquine showed 82 % efficacy for ACLE, SCLE, or DLE [91]. More common side effects include gastrointestinal disturbance, and blue-black pigmentation of skin or mucosa. Rarer side effects include retina, muscle, nerve, and hepatic toxicity. Potential mechanisms of action include inhibition of antigen presentation (via their known ability to disrupt lysosomal acidification), inhibition of cytokine secretion, interference with activation of extracellular signal-regulated kinses (ERKs), inhibition of prostaglandins, stabilization of membranes, and photoprotection [92]. Recent evidence suggests that they might operate via interference with intracellular TLR signaling [93], which is tantalizing given the mounting evidence for a role of the innate immune system (i.e., pDCs) in LE and its dependence on TLR-signaling.

The value of systemic photo-protective agents in cutaneous lupus is not well studied. One case report suggested a benefit of adding the supplement polypodium leucotomos, which has photoprotective effects on human keratinocytes, to hydroxychloroquine in a patient with SCLE [6].

Diaminodiphenylsulfone (Dapsone) is also used for CLE, especially when treating the patient with LE-nonspecific vesiculobullous lesions related to SLE [2]. Others have reported efficacy in SCLE [94]. Hematologic, renal, and hepatic toxicity can occur with this drug. Dapsone is known to affect neutrophil function at many levels (chemotaxis, CD11b-mediated epidermal adherence, enzyme production, generation of reactive oxygen intermediates) [95] and this may be relevant in light of the variable cutaneous infiltrate of neutrophils that is seen in CLE.

Retinoids (acitretin and isotretinoin) are advocated for SCLE and hypertrophic DLE lesions [2], although disease usually returns following removal of the drug. The mechanism of action is unclear, although inhibition of cutaneous T lymphocyte infiltration has been demonstrated in humans and animal models [96, 97].

Thalidomide can be especially effective for SCLE and DLE [99, 118], with a response seen in 75 % of antimalarial-resistant patients [98]. Efficacy is seen as early as two weeks and peaks at three months, although relapses following cessation of therapy are common. The most common side effect is sedation. Besides its teratogenicity, it can produce a sensory neuropathy in 50–70 % of patients, with no correlation between cumulative dose and duration of treatment [98]. In some cases the neuropathy can be persistent despite withdrawl of the medication. Reports of thromboembolic events occurring in thalidomide-treated patients should prompt caution with its use in high-risk patients, including those with antiphospholipid antibodies. A thalidomide anolog, lenolidomide, has more recently been reported to be effective in refractory cutaneous lupus and may carry less risk of neuropathy and thrombosis [7, 8].

Traditional immunosuppressive agents are sometimes used for CLE. These include methotrexate, mycophenolate mofetil, azathioprine, and cyclophosphamide [2]. Methotrexate has perhaps the most data to support its use, with a retrospective study of 43 CLE patients (including ACLE, SCLE, and DLE) showing improvement in 98 % of patients [100]. Mycophenolate mofetil has been used effectively in CLE patients that have adequately responded to antimalarials. Doses of 2–3 g a day appear most effective.

Intravenous immunoglobulin (IVIG) has been used in some cases of CLE, the largest being an open label prospective study of 12 patients with SCLE or DLE in which 5/12 had virtual clearing of disease [101]. Some investigators, however, have not observed a beneficial effect [102]. Possible mechanisms of action have been reviewed elsewhere [103].

Belimumab is a human monoclonal antibody that in 2011 became the first drug to receive FDA approval for the treatment of active systemic lupus in over 50 years. The

drug's mechanism of action is to inhibit B-lymphocyte stimulator which affects B-cell survival and reduces the differentiation of B cells into immunoglobulin producing plasma cells [10]. Studies assessing the organ specific response of cutaneous lesions to belimumab in LE patients have not yet been performed. Rituximab is a chimeric monoclonal antibody that destroys B cells. While it is thought to be a promising treatment for LE, two randomized trials in SLE have failed to meet efficacy endpoints and experience in CLE is limited [11].

One intriguing and somewhat counterintuitive therapy for LE is that of UV light, namely low dose UVA1 (340–400 nm). This may not be so unexpected, as the wavelengths responsible for inducing SLE flares are thought to be within the UVB (280–320 nm) and UVA2 (320–340 nm) wavebands [23, 104], and "pure" (not contaminated with UVC or longer wavelengths) of UVA1 and UVB do not induce (and may improve) CLE [105]. Several small trials using low dose UVA1 (6–12 J/cm^2) have demonstrated benefit in systemic symptoms of SLE (including a decrease in autoantibodies). As for the skin, one of the trials noted "improvement of rash" [106], and this was also reported separately in two patients with DLE [107]. Caution should be noted, as exacerbation of skin disease has been noted in some patients [108, 109]. The mechanism of action is unclear, but might involve immunodeviation to Th1 patterns, apoptosis of resident T cells, inhibition of antigen presentation, or inhibition of TNF-α production [110].

Future Directions

An increased understanding of the immune dysregulation seen in CLE will lead to more effective therapies for this disease. Many of these therapies will parallel development of treatments for SLE [111]. Novel treatments might include agents targeted at T lymphocytes, such as anti-CD4 antibodies or CTLA4-Ig (which blocks T cell costimulation). Although clearly important in SLE, the role of B lymphocytes is unclear in CLE, and it will be interesting to evaluate the current application of B cell directed therapies (such as Rituximab) for efficacy in LE-related skin disease. There is anecdotal evidence for efficacy of TNF-α inhibitors such as etanercept in some forms of CLE [112–114]. However, the tendency for this class of biologics to induced lupus serological changes (ANA, double-stranded DNA antibodies) and, at times, clinical changes of cutaneous and SLE limits their value in this regard. The increasing role of innate immunity, pDCs, and TLR signaling suggests that agents specifically designed to inhibit TLR signaling or pDC infiltration/maturation might be very effective for CLE. The compelling role of IFN-α, along with studies showing interference with type-I interferon receptor signaling improves lupus-like disease in mice, make this an attractive target.

Questions

1. What is the association between different types of cutaneous lupus and systemic lupus erythematosus?
   A. All forms of cutaneous LE are strongly associated with SLE
   B. Certain forms of cutaneous LE such as acute cutaneous LE are strongly associated with SLE
   C. All patients with chronic cutaneous LE meet ARA criteria for SLE
   D. Subacute cutaneous LE is never associated with SLE

Correct Answer: (B) Some forms of cutaneous LE are strongly associated with SLE

2. What is the difference between lupus-specific and lupus non-specific skin disease?
   A. All skin manifestations of LE are lupus-specific
   B. The non-Lupus specific cutaneous manifestations of lupus are not relevant to the clinical course of the disease
   C. The non-lupus specific cutaneous manifestations can be observed in other disorders (for example, alopecia)
   D. Chronic cutaneous LE is one of the few lupus-specific manifestations
   E. A + B only
   F. C + D only

Correct answer: (F)

3. What genetic HLA types, polymorphisms and deficiencies have been associated with cutaneous lupus?
   A. Certain HLA types
   B. Cytokine promoter polymorphisms
   C. Polymorphisms of the C1qA genes
   D. Genetic deficiencies of complement
   E. All of the above
   F. None of the above

Correct answer: (E) All of these genetic factors have been associated with different presentations of cutaneous LE

4. How might ultraviolet light exposure induce cutaneous lupus in susceptible individuals?
   A. Increased immune response to altered DNA
   B. Increase in Th2 lymphocyte activity
   C. Loss of Tregulatory cells
   D. Acceleration of DNA repair

Correct answer: (A) Increased immune response to altered DNA

5. What are treatment options for patients with cutaneous lupus that cannot tolerate or do not respond to antimalarials?
   A. Dapsone
   B. Isotretinoin
   C. Cytotoxic drugs

D. Thalidomide
E. All of the above
F. None of the above

Correct answer: (E) All of these drugs represent treatment options for the patient who fails antimalarial therapy

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Lichen Planus

31

Aaron R. Mangold and Mark R. Pittelkow

Abstract

Lichen planus (LP) is the prototypical lichenoid tissue reaction (LTR) that affects approximately 1 % of the population worldwide. Classic LP is characterized by violaceous, flat-topped, polygonal papules, which favor the flexor aspects of the body. LP can affect any ectodermally derived tissues (skin, hair, nail, mucous membranes, esophagus) and therefore a complete physical examination and multidisciplinary approach is often needed. Histologically, LP is characterized by basal keratinocyte damage with a brisk LTR. While there is a clear genetic association with LP, the pathogenesis remains unknown. Antigen presenting cells and T-lymphocytes play a key role in antigen recognition, lymphocyte activation, keratinocyte apoptosis, and disease resolution. Numerous therapeutic modalities have been utilitzed in LP. We recommend the usage of anti-inflammatory agents, which specifically target T-lymphocytes. Future, novel therapeutic targeting of cytotoxic T-lymphocytes make mechanistic sense; although, future studies are needed for both clinical efficacy and safety.

Keywords

Lichen Planus • LP • Tree moss • Morphology • Skin • Hair • Nail • Mucous membranes • Esophagus • Skin disease • Pathogenesis • Cutaneous disease • Hepatitis C

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Key Points

- Prevalence: Approximately 1 % worldwide
- Morphology: Classic disease is characterized by violaceous, flat- topped, polygonal papules
- Distribution: Classic disease commonly affects the flexor aspects of the body. Any ectodermally derived tissues (skin, hair, nail, mucous membranes, esophagus) may be involved
- Histology: Basal keratinocyte damage with a lichenoid tissue reaction
- Pathogenesis: Idiopathic, T-cell mediated process without a clear auto antigen

Introduction

Lichen planus (LP) is derived from the Greek word, *leichen*, meaning "tree moss" and the Latin world, *planus*, meaning "flat." LP is a often a clinical diagnosis typified by: purple, polygonal, pruritic, papules and plaques. LP has pathognomonic histological findings and represents the prototype of a broader category of lichenoid tissue reactions (LTRs).

History

Dr, Erasmus Wilson was the first to describe lichen ruber in 1869 [1]. He delineated the inflammatory nature of the lesions and its focus upon the epidermis. In all likelihood, Wilson was referring to the same entity previously described by Hebra. Similarly, Weyl originally described the white striae overlying lichenoid papules in 1885 and Wickham later elaborated upon them [2, 3]. Gougerot and Burnier later described the multimucosal involvement of LP [4].

Epidemiology

The exact incidence and prevalence of LP is unknown. The largest studies on LP focus on oral disease or cutaneous disease but few include both the cutaneous and mucosal spectrum.

In adult oral lichen planus (OLP), the data is heterogeneous within different geographic regions with reported rates ranging from 0.1 to 4 %, the best estimate is approximately 1.3 % (1 % men and 1.6 % women) [5]. Because the most common form of OLP is asymptomatic, oral disease is under-represented and, additionally, most of the studies did not differentiate LP from LTRs. The incidence of OLP is even less well studied and is essentially indeterminate. The best population studies found a peak age of onset between 55 and 74 in OLP [6]. There is no clear sexual predilection of disease and women tend to have an earlier age of onset than men.

Initial estimates found that 1–5 % of LP occurs in children. However, it is now believed that childhood LP may be much more common than previously thought with the largest childhood cohort representing 19 % of the total LP population [7]. The largest series of childhood LP are from India. Therefore, the generalizability of data as well as the possibility of genetic predisposition has come into question. The largest US study showed childhood LP to be more common in African Americans [8]. There is no clear sex predilection and disease onset is between 8 and 12 years of age [7–10].

True familial LP is rare; however, a strong family history has been reported in 1.5 % in adults and 3.8 % of pediatric cases [7, 11]. Small studies in adults suggest that familial LP may make up 5–10 % of cases [12, 13]. Familial LP is characterized by: earlier onset, widespread disease, mucosal involvement, and frequent relapses [14, 15]. Familial cases of bullous LP have also been reported [16].

Clinical Features

LP may affect any ectodermally derived tissue including: skin, mucous membranes (most commonly oral), hair, nails, genitalia and, rarely, esophagus. We will emphasize the findings in adult disease. However, LP in children has classic cutaneous findings in 42–60 % of cases, oral involvement in 17–30 % of cases, hair involvement in 2–6 % of cases, and nail involvement in 0–19 % of cases [7–10].

Skin lesions develop over the course of weeks. LP has many clinical subtypes based upon the configuration of lesions, morphological appearance, and site of involvement. The nuances of these subtypes are beyond the scope of this chapter and emphasis will be placed upon the classic findings and a few special forms of disease. Table 31.1 shows the variants of cutaneous, oral, and special forms of LP.

Cutaneous Disease

LP is characterized by four to six descriptive, repetitive "P" terms (pruritic, purple, polygonal, planar, papules and plaques). The individual lesions vary in size from a few millimeters to large, coalescent plaques (Fig. 31.1). The violaceous color of longstanding LP plaques results from the combination of red blood in the capillaries, interface lymphocytes, and the bluish-gray hue of dermal melanin

Table 31.1 Clinical subtypes of lichen planus

Subtypes	Most common sites of involvement
Cutaneous lichen planus	
Actinic	Sun-exposed areas
Annular	Male genitalia, intertriginous
Atrophic	Predominately on lower extremities, may occur on other areas of the body
Erosive	Soles of the feet
Guttate	Trunk
Hypertrophic	Anterior legs, ankles, interphalangeal joints
Linear	Lower extremities
Follicular	Trunk and proximal extremities
Papular	Flexural surfaces
Bullous	Feet
Pigmentosus	Sun-exposed areas
Pigmentosus-inversus	Intertriginous and flexural areas
Nail	Fingernails > toenails
Palmoplantar	(1) Malleoli (2) Soles
Lichen Planopilaris	(1) Classic- vertex scalp (2) Frontal fibrosing alopecia (3) Graham-Little-Piccardi-Lasseur Syndrome
Mucosal lichen planus	
Oral	
Reticular	(1) Buccal mucosa and mucobuccal folds
Atrophic	Attached gingiva
Hypertrophic	Buccal mucosa
Erosive	(1) Lateral and ventral tongue (2) Buccal mucosa
Bullous	Posterior and inferior areas of the buccal mucosa
Plaque-like	Dorsum of tongue and buccal mucosa
Vulvovaginal	Vaginal introitus, clitoral hood, labia minora, labia majora, vagina
Esophageal	(1) Proximal esophagus (2) Proximal and distal esophagus (3) Distal esophagus (80 % have mucosal involvement)
Special forms	
Drug-induced	Sun-exposed areas
Lichen planus-lupus erythematosus overlap	Distal extremities, sun-exposed
Lichen planus pemphigoides	Extremities

Adapted from: Gorouhi et al. [32]

[17]. Fine white lines, which represent areas of focal epidermal thickening, are termed Wickham's Striae (Fig. 31.2). The lesions are more clearly visualized using contact dermoscopy. LP is often localized to the flexor aspects of the extremities as well as the oral and genital mucosa. The face and interfollicular scalp are typically spared. Isomorphic phenomenon (Koebnerization) (Fig. 31.3) and Wolf's isotopic response are both seen in LP. The former represents disease at the site of prior trauma and the later represents secondary disease occurring in previously diseased skin, most commonly varicella zoster virus (VZV) [18–20].

Nail Disease

Nail disease occurs in up to 10 % of patients with LP and the fingernails are more commonly affected than the toenails [21, 22]. LP can affect the nail folds, bed, and matrix. The most common findings are: thinning, ridging (trachyonychia), and distal splitting of the nail plate (onychoschizia) (Fig. 31.4) [22]. One study found that nail matrix involvement was associated with trachyonychia (40 %), pitting (34 %), and dorsal pteryigium (21 %) [23]. Nail bed involvement was associated with chromonychia (56 %) and nail fragmentation (51 %). Paronychia was seen in 32 % of cases and disease of all

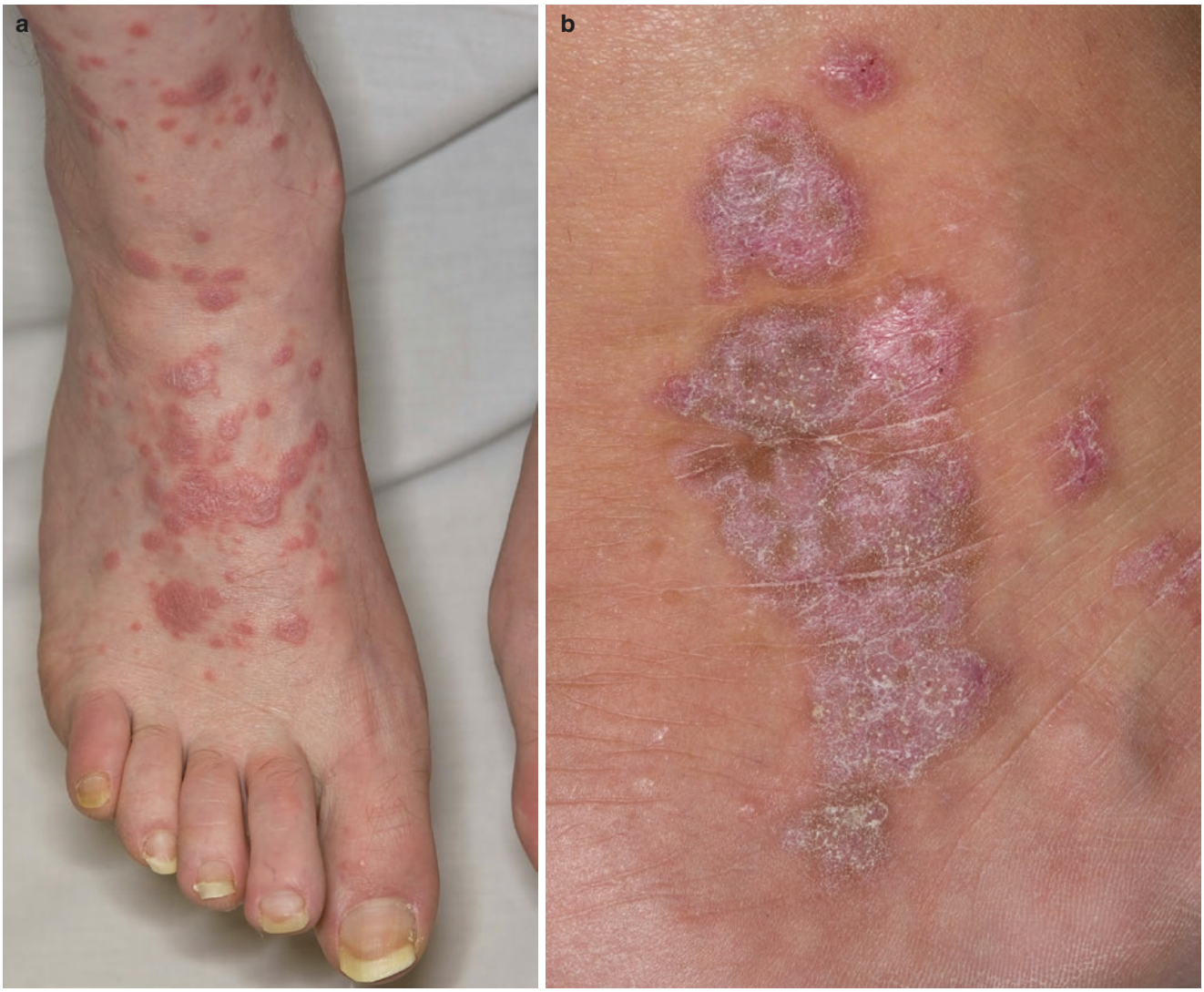

Fig. 31.1 Classic cutaneous lichen planus (**a**) Left-Violaceous papules and plaques on the dorsal foot (**b**) Right-Violaceous papules and plaques with overlying reticulate, white striae (Wickham's striae)

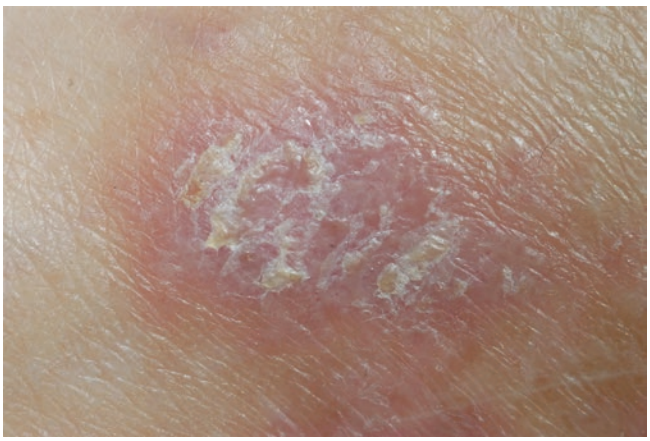

Fig. 31.2 Wickham striae: Violaceous plaque with overlying lace-like, reticulated white striae

three-nail components was associated with longitudinal streaks (82 %). Similar nail patterns may be seen in alopecia areata, psoriasis, eczema, and pemphigus vulgaris.

Mucosal Disease

LP commonly involves the oral mucosa but the penis, vulva, vagina, anus, nose, larynx, esophagus and conjunctiva can also be affected. OLP is more common in women than men (2:1). Oral involvement occurs in approximately 60–70 % of individuals with LP and may be the only site of involvement in 20–30 % [11, 24–27].

Multiple subtypes of OLP have been described: reticular, erosive, atrophic, papular, plaque, and bullous. Ulcerative OLP is the most common subtype reported in studies, likely

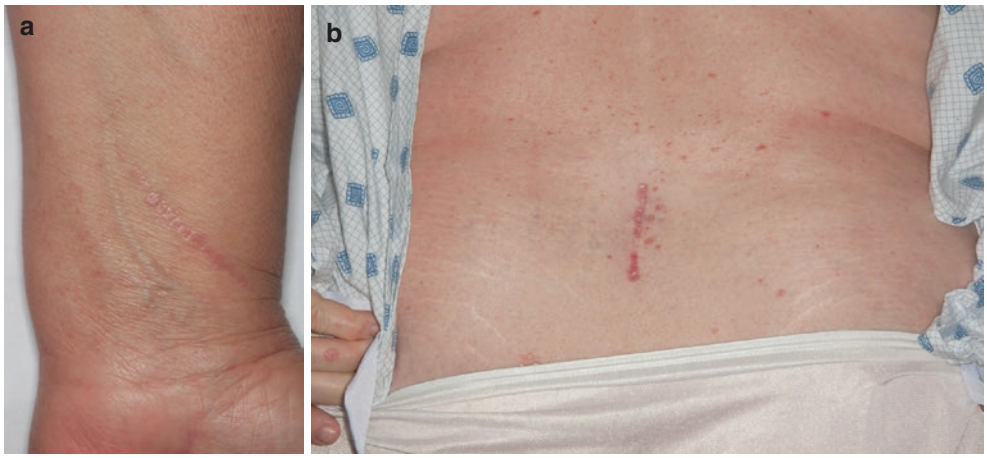

Fig. 31.3 Koebnerization (**a**) Left-Linear cluster of lichenoid papules corresponding to the site of prior trauma (**b**) Right-Clustered papules at site of prior surgery

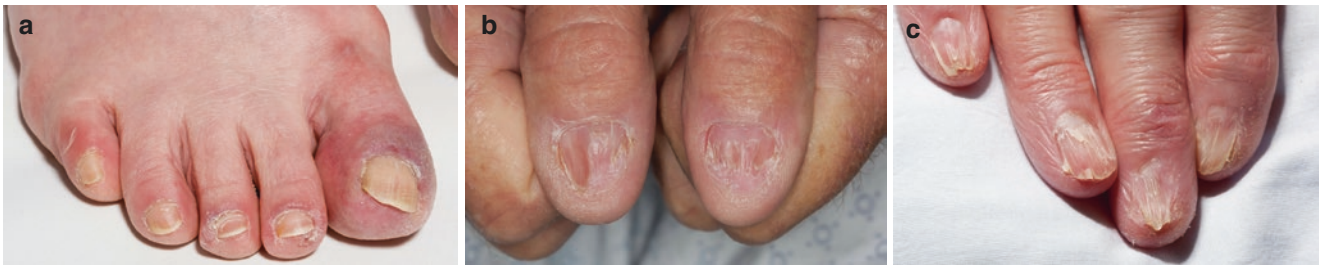

Fig. 31.4 Nail lichen planus (**a**) *Left*- Purple, red plaques surrounding the proximal and lateral nail folds (paronychia) (**b**) *Middle*- Longitudinal ridging with forward growth of the eponychium and adherence to the proximal nail plate (dorsal pterygium) (**c**) *Right*- Longitudinal ridging of all nails (trachyonychia) with distal splitting (onychoschizia)

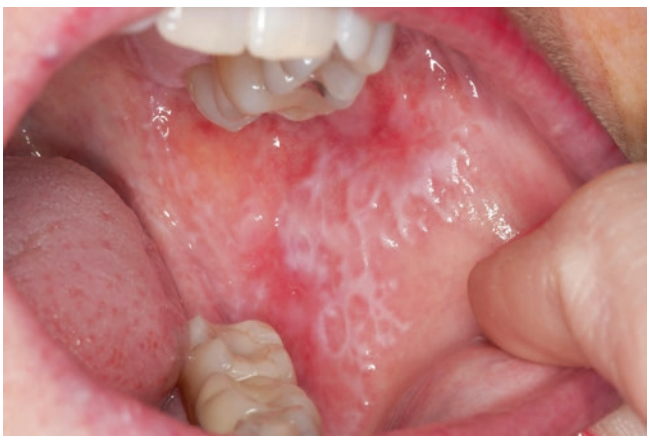

Fig. 31.5 Oral lichen Planus: Typical lace-like whitish, reticulated pattern (Wickham striae) on the buccal mucosa

due to symptomatology and severity of disease [26, 28]. However, clinical experience indicates that the reticular subtype is more common but patients are less likely to seek medical care due to its indolent and often asymptomatic nature. The lesions are often symmetrical and the buccal mucosa is the most common site of involvement (80–90 %) (Fig. 31.5) [25, 29–32]. Gingival involvement is uncommon, being the sole presentation in 8 % of cases, but remains the most common cause of desquamative gingivitis (75 % of cases) [33]. The classic lacy streaks (Wickham striae) are most commonly seen on the buccal mucosa. Koebnerogenic factors: smoking, dental caries and amalgams may exacerbate disease.

Oral lichenoid contact stomatitis can be clinically and histologically indistinguishable from OLP. In patients with OLP, one study identified a contact hypersensitivity in 40 % of the cases [34]. The most common culprits are amalgams in fillings as well as food flavorings (cinnamon, cinnamaldehyde, and spearmint) [34–36]. Some individuals may benefit in removal of damaged and corroded fillings despite negative patch testing [37]. This may be due to false negative patch testing, which would have been picked up with other methods (repeat open application testing (ROAT)) or due to the removal of a mucosal irritant.

Genital lesions (Fig. 31.6), including vulvar, vaginal, and penile, have been reported in 19–57 % of individuals with OLP and 15–50 % of cutaneous LP [25, 38–40]. Of patients with vaginal involvement, 44–100 % have oral disease. Therefore, when examining a patient with vulvar or vaginal inflammation, an oral and cutaneous examination is essential. Vulvo-vaginal gingival (VVG) syndrome is a specific subtype of vulvar and vaginal LP with oral involvement. Genital lesions, specifically penile, often have an annular appearance.

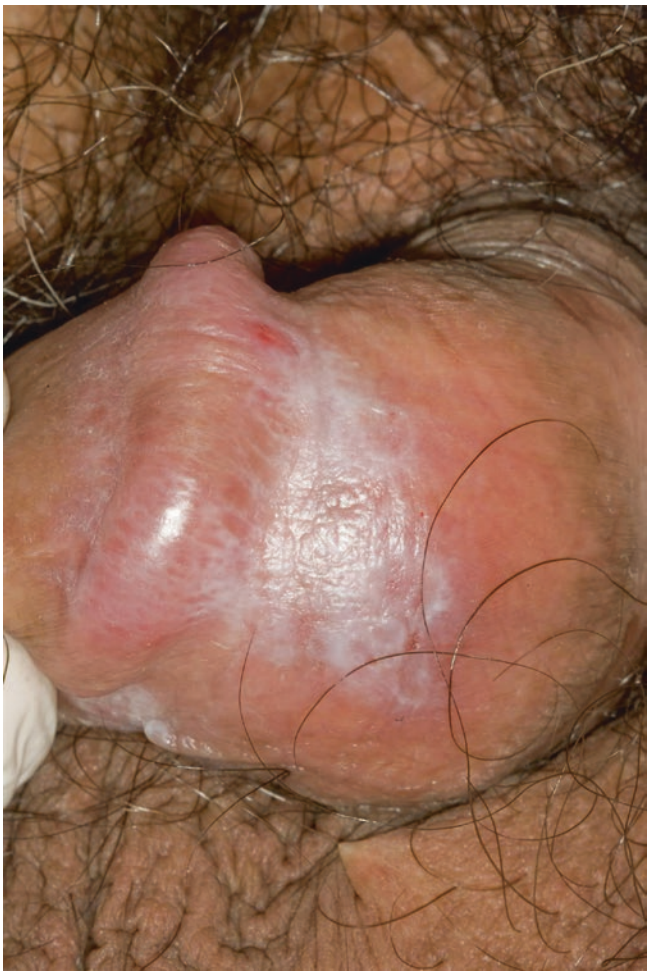

Fig. 31.6 Genital lichen planus: Shiny white, reticulated plaque with a violet hue and a small superficial erosion on the corona

Ocular disease is likely under-reported and up to one-third of patients with LP report blepharitis [41]. There are varying degrees of ocular involvement from the minor symptoms of dryness to cicatricial conjunctivitis [42].

Hair Disease

Hair disease has been reported in up to 40 % of LP [43–45]. Follicular involvement on the body often occurs; however, the classic cicatrical scalp alopecias of LP are the focus. Three clinical subtypes are: classic lichen-planopilaris (LPP), frontal fibrosing alopecia(FFA), and Gram-Little-Piccardi-Lasseur Syndrome(GLPLS) [44, 46]. LP of the scalp occurs more frequently in women (5 to 31:1) with FFA dominating the most [44]. Classic LPP affects the vertex scalp and consists of diffuse erythema with perifollicular hyperkeratosis and livid erythema (Fig. 31.7) [44]. Dermoscopy can aid in the diagnosis of early scarring LPP. Dermoscopic features of LPP include: absence of follicular opening, cicatricial white patches, peripilar casts and perifollicular scale, blue-gray dots, perifollicular erythema, and polytrichia (2–3 hairs) [47]. Late stage disease and progressive disease often lead to nondescript scarring (Pseudopelade of Brocq).

FFA most commonly affects postmenopausal women and is characterized clinically by a band-like alopecia of the frontal hairline [48]. Up to 75 % of women with FFA report concomitant loss of the eyebrows, which tends to be non-inflammatory [49]. Thyroid dysfunction is more common in individuals with FFA versus LPP (31 % versus 10 %) [44]. The difference seen may be partially age related. However, a more recent study found an elevated rate of thyroid dysfunction in individuals with LPP versus controls (34 % versus 11 %) [50]. The role of thyroid hormone receptors on keratinocytes, follicular function, and hair organ homeostasis remains unknown. GLPLS is a rare subtype characterized by Cicatricial alopecia of the scalp, non-scarring alopecia of the axilla and groin, and follicular papules on the trunk and extremities [51].

Special Forms

Drug-Induced Lichen Planus

Lichenoid drug eruptions (LDE) can be virtually indistinguishable from LP. LDE have been described with: oral ingestion or subcutaneous injection (angiogtensin converting enzyme inhibitors (ACEi), antimalarials, calcium channel blockers (CCB), gold, non-steroidal anti-inflammatory drugs (NSAIDs), tumor necrosis factor-alpha (TNF-α) inhibitors and contact factors (mercury, copper, gold, phenylenediamine (PPD) derivatives) [52]. Classically, LDE involve a photodistributed area, lack prominent epidermal changes, and lack mucosal involvement (with oral ingestion). LDE are typically delayed a few months from initial exposure but onset can vary from days to years [52]. LDE typically clear within a few weeks to few months after drug discontinuation. However, reactions post-gold exposure can persist for years.

Overlap Syndromes

LP pemphigoides has distinct features of both LP and bullous pemphigoid (BP) and occurs secondary to BP antigen 2 (BPAG-2) antibodies to the Medical College of Wisconsin-4 (MCW-4) epitope on the non-collagenous 16A (NC16A) domain [53]. The bullous and lichenoid lesions tend to occur on the extremities [54]. LP/Lupus erythematosus (LE) overlap has clinical, histological, and immunofluorescent features which overlap between these

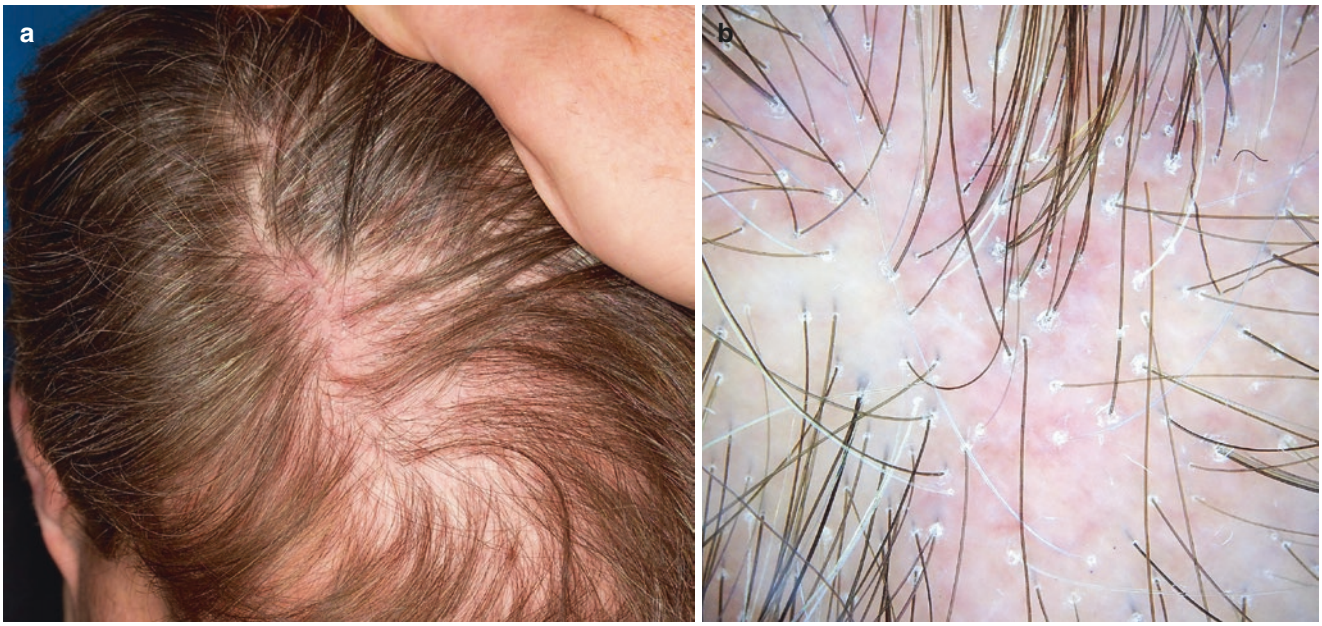

Fig. 31.7 Lichen planopilaris (**a**) *Left*- Early disease with widening of the hair line and erythema of the scalp (**b**) *Right*- Dermoscopy (10× magnification) provides much higher resolution and shows classic features of lichen planopilaris: loss of follicular ostia, perifollicular scale and erythema

two entities and can have a positive anti-nuclear antibody (ANA). The extremities are commonly involved and the clinical features are atypical for LP with atrophic plaques and minimal epidermal disruption [55].

Associations with Systemic Diseases and Infections

Systemic Diseases

Patients with LP are at a higher risk of metabolic syndrome and tend to have more cardiac risk factors than healthy individuals [56, 57]. Thyroid dysfunction is found in up to 34 % of patients with LPP [50]. Lichen sclerosus et atrophicus (LS et A) is seen in up to 16 % of patients with OLP [38, 58]. OLP is associated with chronic liver disease.

Psychological and Neurogenic Factors

Increased stress, anxiety, sleep disturbance, and depression are reported in LP [59–61]. Up to 60 % of patients had chronic nervous distress or a stressful event near the time of a flare [11]. Recent studies examined the role of neural pathways and neurogenic factors in LP and showed co-localization of mast cells and nerve fibers as well as increased peripheral nerve innervation in OLP [62, 63]. The pattern of innervation appears to be different between lichenoid reactions and OLP [64]. The differences in nociceptive features are partially explained by the nature of the lesions (reticular versus erosive) rather than the innervation. Neuro-modulating drugs have been reportedly successful in OLP although larger studies verifying these findings are needed [65].

Infections

Patients with OLP and liver disease have significantly higher rates of hepatitis C (Hep C) in selected populations (78 % versus 3 %) [66]. There may be specific unidentified genetic factors contributing to this co-occurrence, as large studies on Hep C have not found LP to be a common finding [67]. Hep C and LP are associated in certain endemic regions (East and Southeast Asia, South America, the Middle East, and Southern Europe) but not in others (North America, South Asia, and Africa) [68]. In general, patients with LP have a six-fold risk of having Hep C relative to the control population [32]. The heterogeneity of Hep C and OLP may be related to the human leukocyte antigen-DR6 (HLA-DR6) haplotype, which is found in endemic regions with Hep C and LP [69, 70]. There is no strong link between hepatitis B (Hep B) and LP [71].

Other infections have been implicated in LP. Human papilloma virus (HPV) is more common in OLP with overall odds ratio (OR) of 5.12 and HPV-16 of 5.61 [72]. In

addition, the higher rates of oncogenic subtypes may explain part of the increased malignancy risk. Individuals with OLP are more likely to be colonized with Candida species than controls [73]. Non-Candida albicans species were isolated in patients with OLP, particularly those with OLP and diabetes mellitus (DM) [73]. In zosteriform LP, the lesions have expressed VZV antigens. In addition to being a potential trigger of LP, VZV antigen expression may differentiate zosteriform LP from linear presentations of LP [74].

Malignant Transformation

The malignant potential of LP is dependent upon the site of involvement, clinical subtype, duration of disease, and patient population. The reported rates of squamous cell carcinoma (SCC) development have varied: 0.8 % of OLP in the United States, 1.9 % in the United Kingdom, 0.6 % in China, and 1 % in the Swedish population [75–78]. Longstanding erosive and atrophic disease appears to have the highest risk of malignant transformation. The tongue is the most common site of involvement. Other risk factors for oral SCC, including tobacco and alcohol, have not been observed at greater frequency in OLP. Candida as well as HPV have been observed in higher frequency in OLP and may be contributory [72, 73].

No overall increased risk of malignancy has been observed in cutaneous LP [78]. There are rare case reports of cutaneous SCC arising in LP. Risk factors include: hypertrophic or verrucous LP, location on the lower extremity, a history of arsenic or x-ray exposure, and longstanding disease (average of 12 years) [78].

Laboratory Tests

No specific laboratory test abnormalities are seen in LP. Hep C testing should be considered in those with risk factors as well as those in endemic areas having a prevalence of greater than 7 % [79]. Testing for dyslipemia and thyroid dysfunction (thyroid stimulating hormone (TSH) and thyroid peroxidase (TPO) antibody) is part of routine health maintenance and determining the psychosocial impact of disease is essential [50, 56, 57, 60, 61]. Individuals with a normal TSH and TPO antibodies will require follow up monitoring for the development of clinical hypothyroidism. In oral disease, allergic contact dermatitis (ACD) should be ruled out, as oral contact stomatitis can appear identical to LP [34–36, 80].

In most cases, LP is a clinical diagnosis and should be confirmed with a biopsy when atypical or overlap features are present. Dermoscopic driven biopsies showing the key features led to definitive diagnosis in 95 % of LPP cases [81]. In cases of vesiculobullous disease or erosive disease, direct immunofluorescence (DIF), indirect immunofluorescence (IIF), as well as enzyme linked immunosorbent assay (ELISA), may be needed to differentiate from other immunobullous diseases.

Pathology

Pinkus described the initial, modern understanding of the LTR which focused on the spectrum of epidermal damage to the basement membrane and its resulting cascade of events [82]. Two major pathologic findings were described, the lymphocytic LTR and consequent damage to basal, epidermal keratinocytes [82].

The earliest changes can be seen in uninvolved and non-inflamed skin and are characterized with colloid body formation and pigmented macrophages in the dermis [83]. Colloid bodies (also known as Civatte bodies) are degenerated keratinocytes and electron microscopy has shown fibrillar degeneration of basal keratinocytes specifically [84, 85].

Classic changes are seen in established lesions with: ortho-hyperkeratosis, wedge-shaped hypergranulosis, saw-toothed rete ridges, epidermal hyperplasia, lymphocytes at the dermal-epidermal junction, Max Joseph spaces (focal, sub-epidermal clefts seen in 20 % of cases), squamatization (loss of maturation and flattening of the basal layer), and colloid bodies [86] (Fig. 31.8). LP is characterized by a dense, continuous, and band-like lympho-histiocytic infiltrate at the dermal-epidermal junction (DEJ). The heavy infiltrate can result in effacement of the DEJ. Parakeratosis and eosinophils are absent.

Late disease is characterized by: atrophic epidermis, effacement of the rete ridges, occasional colloid bodies, dermal fibrosis, and melanophages. When few colloid bodies are present, distinguishing from poikiloderma may be very difficult.

Hypertrophic LP is characterized by: hyperkeratosis, acanthosis, papillomatosis, and thickened collagen bundles in the dermis. Hypertrophic LP can be mistaken for squamous cell carcinoma; therefore, good clinical pathological correlation is needed to avoid inappropriate treatment [87]. Eosinophils are more commonly seen in hypertrophic lesions [88]. Mucosal lesions tend to have less specific changes and genital disease can often be inconclusive. Parakeratosis and an absent granular layer are common at mucosal sites.

LPP is characterized by a perifollicular, lymphohistiocytic inflammatory reaction with perifollicular fibrosis, scarring, and follicular atrophy. Initial inflammation is at the level of the isthmus and infundibulum and spares the

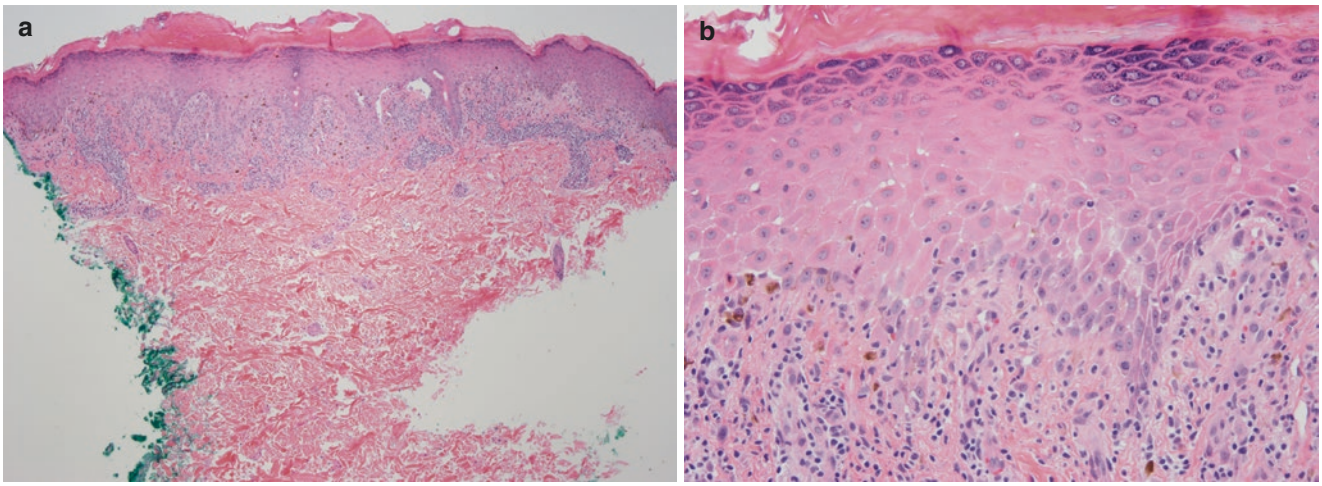

Fig. 31.8 Histology of classic cutaneous lichen planus (**a**) *Left*- Hematoxylin and Eosin (H&E) (40×) The characteristic findings of compact ortho-hyperkeratosis, wedge shaped hypergranulosis, saw-toothed rete ridges, and a lichenoid infiltrate. Small epidermal clefts (Max-Joseph space) can be seen centrally. (**b**) *Right*- (H&E 200×) Dense, lichenoid lymphocytic infiltrate with scattered apoptotic keratinocytes and pigment incontinence (Courtesy of David J DiCaudo MD)

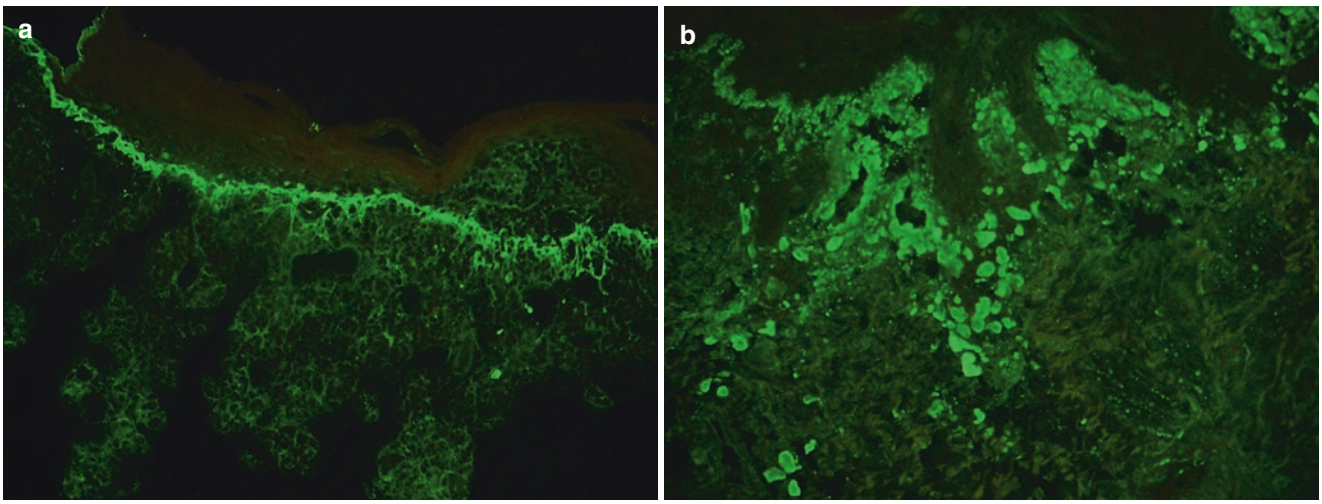

Fig. 31.9 Immunofluorescence of lichen planus (**a**) Shaggy basement membrane zone for fibrinogen (**b**) Scattered and clumped cytoid bodies with Immunoglobulin M (IgM) (Courtesy of Michael J Camilleri MD)

lower segment [45]. Permanent hair loss likely results from damage to the stem cells in the bulge and subsequent fibrosis [89].

LDEs may be indistinguishable from LP. However, if present, atypical features like parakeratosis, eosinophils (seen in up to two-thirds of cases), plasma cells, large numbers of apoptotic keratinocytes, and perivascular lymphocytes extending into the reticular dermis may aid in the diagnosis [90, 91]. In one study, focal parakeratosis, focal clusters of colloid bodies in the granular layer and stratum corneum, and focal epidermal disruption were seen in 50 % of LDEs and no cases of LP [91]. Cluster of differentiation-8 (CD8) positive Granzyme B positive lymphocytes appear to predominate in LDE versus classic LP [90].

Immunofluorescence

In cases of LP with positive immunofluorescence, fibrin and IgM (bound to colloid bodies) are present in 100 % and 93–100 % of cases, respectively [83, 92] (Fig. 31.9). Shaggy fibrin is the most common finding and best predictor of LP and can be seen in early disease without a prominent inflammatory infiltrate. The criterion for LP requires basement membrane zone fibrinogen and colloid bodies with one or more conjugates (Mayo Clinic Criteria) [93]. Colloid bodies have a better predictive value in oral lesions. DIF of OLP (sensitivity of 61 % and specificity of 96 %) is inferior to both hematoxylin and eosin (H&E) (sensitivity of 84 % and specificity of 93 %) and clinical impression (sensitivity of

Table 31.2 Differential diagnosis of lichen planus

Morphology and age:
Classic: Psoriasis, Lichenoid drug, Lichen simplex chronicus, Chronic cutaneous lupus, Graft versus host disease, Secondary syphilis, Pityriasis rosea, Mycosis fungoides
Annular: Granuloma annulare, Tinea corporis
Linear: Lichen striatus, Linear epidermal nevus, Psoriasis, Darier's disease
Hypertrophic: Lichen simplex chronicus, Prurigo nodularis, Lichen amyloidosis, Kaposi sarcoma, Squamous cell carcinoma, Psoriasis
Atrophic: Lichen sclerosus, Cutaneous lupus erythematosus, Poikiloderma
Vesiculobullous: Lichen planus pemphigoides, Epidermolysis bullosa pruriginosa, Pemphigus vulgaris, Bullous pemphigoid, Bullous amyloidosis
Follicular: Lichen nitidus, Lichen spinulosa
Childhood: Lichen nitidus, Lichen striatus, Pityriasis lichenoides, Papular acrodermatitis of childhood
Special site:
Nail: Psoriasis, Onychomycosis, Alopecia areata
Genital: Psoriasis, Seborrheic dermatitis, Fixed drug eruption
Palms and soles: Secondary syphilis, Erythema multiforme
Scalp: Cicatricial alopecia, Lupus erythematosus, Inflammatory folliculitis, Alopecia areata, Cicatricial pemphigoid, Keratosis follicularis spinulosa decalvans
Mucosal: Paraneoplastic autoimmune multi-organ syndrome/Paraneoplastic pemphigus, Candidiasis, Lupus erythematosus, Leukokeratosis, Secondary syphilis, Traumatic patches, Chronic ulcerative stomatitis, Erythema multiforme
Histological:
Lichenoid keratosis, Poikiloderma, Chronic cutaneous lupus erythematosus, Fixed drug eruption, Lichen nitidus, Lichen striatus, Lichenoid drug, Graft versus host disease, Mycosis fungoides, Contact dermatitis, Keratosis lichenoides chronica, Erythema dyschromicum perstans, Lichenoid and granulomatous dermatitis

74 % and specificity of 87 %) [93]. These findings emphasize the importance of clinical examination and diagnosis in classic LP. DIF has a role in atypical disease as well as ulcerative and vesiculobullous variants.

Location of disease governs sensitivity of detection by DIF (in decreasing order): mouth floor, the ventral side of the tongue, superior labial mucosa, hard palate and buccal mucosa. The gingiva is the least sensitive location, although other studies refute this, and suggest the dorsal tongue [93, 94]. The optimal location for biopsy of cutaneous LP is on the proximal trunk with avoidance of the distal extremities [92]. A biopsy of lesional tissues for DIF can yield false negatives since immune deposits are degraded by intense inflammation or damage in the basal membrane zone.

Traditionally, biopsies have been taken from perilesional skin; however, more recent data, in OLP, suggests the same diagnostic yield of more distant biopsies (greater than 1 cm from the lesion) [94, 95]. Splitting of biopsy samples is common in oral disease; however, the sensitivity is significantly less on split samples (40 % versus 65 %) [94]. Subsequent studies of mucosal and glaborous skin showed no difference in sensitivity and specificity of DIF [92].

On DIF, there can be considerable overlap between LP and LE with the majority of cases of LE showing immune deposition on colloid bodies and fibrin at the DEJ. The clustering of colloid bodies is much more common in LP. Multiple immunoglobulin (IgG, A, and M) conjugates and granular, basilar deposition of Igs are more common in LE, and the diagnosis should be suspect when present in LP [96].

Differential Diagnosis

The differential diagnosis of LP is quite broad. A more practical approach is to look at the age of the individual, morphology of the primary lesion, and site of involvement (Table 31.2).

Prognosis

Most cutaneous LP resolves within 1–2 years. Disease duration, in ascending order, is: generalized cutaneous, non-generalized cutaneous, cutaneous and mucosal, mucosal, hypertrophic, and LPP [97]. Recurrence is seen in up to 20 % of cases but is more common in generalized cutaneous disease [11, 98]. In higher Fitzpatrick skin types, post-inflammatory changes manifest as significant, persistent pigmentary abnormalities.

Pathogenesis

The pathogenesis of LP is unknown. Many contributing factors are implicated and include, infectious, autoimmune, metabolic, psychosomatic, and genetic causes. The initial theories of LP, described by Sabouraud in 1910, emphasized a dermally based process with secondary involvement of the basal cells of the epidermis [99]. Thyresson and Moberger challenged this concept and proposed that LP was a process

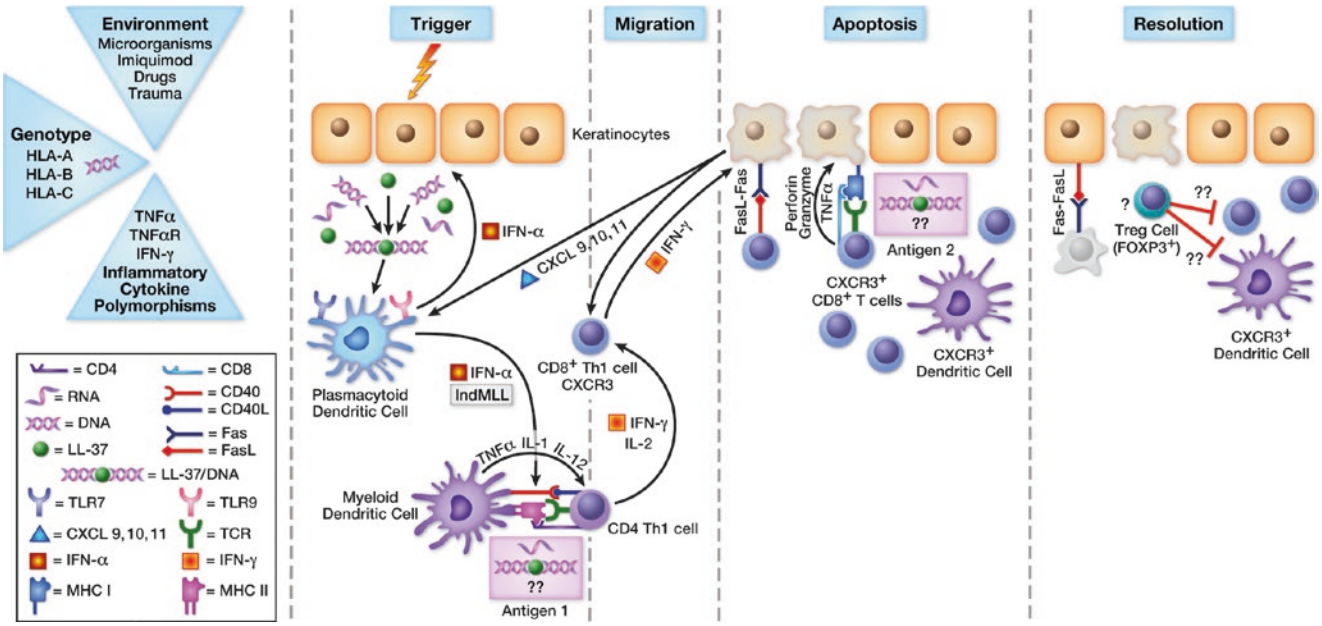

Fig. 31.10 Immunopathogenesis of lichen planus. The occurrence of triggering factors in a genetically predisposed individual carrying LP-associated genes, results in disease development. During the initiation phase, damage to keratinocytes results in the release of deoxyribonucleic acid (*DNA*), ribonucleic acid (*RNA*), and cathelcidin (*LL37*). These proteins stimulate plasmacytoid dendritic cells (*PDC*) via toll like receptors (*TLR*)-7,-9 which result in the release of interferon alpha (*IFN-α*). IFN-α can have both local and distant affects upon myeloid dendritic cells (*MDC*) as well as keratinocytes. Stimulated MDC will interact with CD4-Th cells with the correct antigen. The antigen remains unknown but may represent a viral derived peptide. MDC will stimulate CD4-Th cells via the release of TNF-α, Interleukin (IL) -1, and −12. In addition, CD40 and CD40 ligand (*CD40L*) will result in the co-stimulation of the CD4-Th/MDC interaction. Stimulated CD4-Th cells then release IFN-γ and IL-2. These cytokines stimulate CD8-Tc cells. The stimulated CD8-Tc cells expressing Chemokine receptor-3 (*CXCR-3*) will migrate to the DEJ following the release of chemokine ligands (*CXCL*) −9, −10,−11. These chemokines are released by stressed and stimulated keratinocytes. The stimulated CD8-Tc cells will interact with the stressed keratinocytes and can induce apoptosis with the proper signaling receptors. This antigen also remains unknown but may be a self-antigen released by local stress. The major kill signals are TNF-α, granzyme, and perforin. Fas and Fas ligand (*Fas-L*) are expressed on both keratinocytes and lymphocytes. Therefore, the Fas -Fas-L interaction is likely involved in both apoptosis as well as disease resolution. Local CXCR-3+ DC and T-regulatory (*T-reg*) cells may also modulate the LTR

focused at the DEJ [84]. Black provided evidence of decreased respiratory enzyme activity in the epidermal cells as a possible early driver of LP [100]. However, the changes seen may have been a secondary phenomenon to the basal damage. The basal keratinocyte damage was thought to be secondary to targeted cell mediated immune (CMI) mechanisms, but the exact pathogenesis remained largely illusive [101].

Shiohara et al. further described the T-cell mediated attack upon the epidermis and re-focused attention to CMI and away from secondary humoral mediated processes [102]. Immune complexes have been found to down regulate the ability of macrophages to present antigen as well as stimulate a cytotoxic response [103, 104]. CD4-positive T-helper (CD4-Th) cells persistent in the dermis despite disease chronicity, and CD8-positive T-cytotoxic (CD8-Tc) cells have been found in close proximity to damaged basal keratinocytes in conjunction with exocytosis [105].

Modern theories encompass three major stages: antigen recognition, lymphocyte activation, and keratinocyte apoptosis. A fourth stage, resolution, is a new and emerging topic and will be discussed briefly. Research on LP often separates oral and cutaneous disease. However, the major mechanisms underlying disease subtypes are likely the same, although mechanisms targeting specific epithelial sites are enigmatic. A unified theory of LP does not exist; however, we present the modern view of the topic (Fig. 31.10).

The Cellular Immune Response

Antigen Recognition

The predominate cell mediating LP is the CD8-Tc cell. The targeted antigen(s) and or trigger(s) for LP remain unknown. However, in other diseases like lichenoid graft versus host disease (GVHD), the target antigens are the alloantigens. In oral disease, a LP-specific antigen associated with major histocompatibility complex (MHC) class I on keratinocytes has been reported [106]. It remains to be established if this antigen is an auto-reactive peptide or an exogeneous antigen. Circulating antibodies have been identified in multiple

studies without a clear target antigen [83, 107]. The adaptive immune response is triggered by a "foreign" stimulus, and CD 56-positive CD 16-negative natural killer (NK) cells are recruited [108]. These cells are found in early lesions, express chemokine receptor-3 (CXCR-3), chemokine (c-c) motif ligand (CCL) -5, −6, and release IFN-γ and TNF-α [108]. NK cells may play a role in early propagation of LP.

The role of CD4-Th cells has come into focus in recent years. The CD4-Th population tends to be localized to the dermis with scattered cells in the epidermis. The level of CD4-Th cells correlates with the number of Langerhans cells (LC). LCs, one of the principal antigen-presenting cells of skin, upregulate MHC class II receptors in lichenoid disease [109]. In particular, the CD4-positive LC is seen in close approximation with the HLA-DR positive keratinocytes [110]. In addition, the CD4-Th cells have restricted V-beta gene expression, which suggests antigen specific oligoclonal T-cell expansion [111, 112]. Upon costimulation, these cells release large amounts of inflammatory cytokines including IFN-γ [113]. Taken together, these findings suggest an integral role of Langerhans cells, keratinocytes, and CD4 T helper cells in antigen presentation as well as propagation of the Th1 response via the production of IFN-γ.

The exact roles of exogenous and endogenous antigens in development of LP remain unknown. However, many drugs (see section "Drug-Induced Lichen Planus"), infections (see section "Infections"), contact allergens, and ultraviolet radiation (seen in LE) have been implicated [114–117]. With oral contactants, it has been debated if there is a bonafide hapten reaction or simply chronic inflammation, which manifests as a LTR. With the expanding use of biologics and inevitable adverse reactions, particularly TNF-α inhibitors, specifically LDE, imbalance and upregulation of type I IFN have been implicated in disease pathogenesis [118, 119]. This further substantiates previous reports of development of LP with therapeutic use of IFNs [120].

Lymphocyte Activation

Following antigen recognition, CD8-Tc cells are activated and undergo oligo-clonal expansion (outlined above). A cascade of both pro- and anti-inflammatory cytokines is released including: IL-2, −4, −10, IFN-γ, TNF-α, and transforming growth factor beta 1 (TGF-β1) [121, 122]. In LP, the balance between lymphocyte activation, down regulation, and the cytokine milieu may well determine the disease phenotype.

IFN-γ plays a central role in LP [123]. IFN-γ induces the expression of inflammatory chemokines such as chemokine ligand (CXCL)-9, −10, and −11 [123–125]. CXCR-3, their matching receptor, is predominantly expressed on the surface of IFN-γ-producing CD4-Th cells [124–127]. Peroxisome proliferated-activated receptor (PPAR) gamma inhibits CXCL-10 and −11 and its loss can result in scarring alopecia [128, 129]. IFN-γ increases peripheral blood mononuclear cell (PBMC) binding to HLA-DR positive keratinocytes [124]. However, blockade of the HLA-DR does not inhibit the interaction and lymphocyte function associated antigen-1 (LFA-1) is implicated in the interaction as it is reversed by neutralizing antibody [125]. LFA-1 is expressed on lymphocytes and interacts with intercellular adhesion molecule-1 (ICAM1) [130]. ICAM1 and vascular cell adhesion molecule (VCAM) expression is also enhanced by IFN-γ [126]. Mice pretreated with IFN-γ prior to transfer of T-helper cells exhibited a more brisk LTR but it was unchanged by pretreatment with TNF-α [127]. Therefore, IFN-γ is fundamentally involved in the upregulation of cellular adhesion molecules and the migration of lymphocytes to the DEJ [131, 132].

Keratinocyte Apoptosis

CD8-Tc cells are likely the terminal effector cells in LP. They have been found to co-localize with apoptotic keratinocytes and to have in-vitro cytotoxic activity against autologous keratinocytes [106, 133]. The cytotoxic effects of the CD8 positive cells can be inhibited by blockade of the MHC class I domain [106]. In addition to cytotoxic effects, lymphocytes in LP may be resistant to apoptosis suggested by increased levels of B-cell lymphoma-2 (Bcl-2) [134]. The markers of apoptosis include Caspase-3 and Bcl-associated X (BAX) expression. These proteins are elevated in LP in the basal and suprabasilar epidermis, respectively [134].

The exact mechanism of apoptosis in LP remains unknown. The possible mechanisms include: granzyme-B release, TNF-α-TNF-α R1 receptor interaction, and Fas-Fas-L interaction. Granzyme-B and granulysin are expressed at 100–200 fold higher levels in LP relative to normal skin [135]. Granzyme B, excreted by CD8-Tc cells, activates caspase-3 and likely promotes apoptosis in LP [136]. TNF-α upregulates the expression of matrix metalloproteinase-9 (MMP-9) in lesional T lymphocytes of OLP and has little effect upon the tissue inhibitor of metalloproteases (TIMPs) [137]. This likely leads to disruption of the basement membrane and damage to basilar keratinocytes. MMP-9 levels correlate with the phenotype, with higher lesional levels of messenger ribonucleic acid (mRNA) expression being associated with oral ulcerative disease [138]. MMP-9 is also involved in terminal differentiation and apoptosis of keratinocytes [139]. Taken together, MMP-9 likely disrupts the basement membrane homeostasis, blocking normal cell survival signaling and leads to apoptosis and cell death. Fas-Fas-L expression is elevated in OLP, correlates with disease progression, and likely contributes to apoptosis of keratinocytes [140–142].

Resolution

LP tends to be a self-resolving disease; however, there is a paucity of research into the resolution phase of disease. T-Regulatory (T-reg) cells are seen in OLP and correlates with disease subtype and activity [143, 144]. In vivo research in acute GVHD has shown the key role that T-reg cells play in the disruption of DC and allogeneic T-cell interactions [145]. DCs in LP have also been found to play a key role in T-cell migration [146]. However, DCs role is likely more complex as higher levels inversely correlate with the inflammatory infiltrate [147]. Fas-L, granzyme-B, and perforin can be expressed by keratinocytes allowing for apoptosis of lymphocytes [148, 149].

Genes and Gene Expression

Teleologically, immune system targeting of various naturally occurring but potentially deleterious antigens i.e., viruses and bacteria (see section "Toll-Like Receptors"), malignant cells, and exogenous contactants has potential benefit to the organism [150]. However, the generation of an immune response to exogenous antigens poses risk in the development of cross-reactivity to self-antigens or antigen mimicry and potential for cross-presentation [151, 152].

Genetic polymorphisms have been implicated in the risk of development of LP including: HLA, immune signaling molecules and receptors, and other polymorphisms, but their exact roles remain unknown. Copeman found HLA-B7 in 80% of individuals with familial LP; however, further studies found HLA-B7 to be in 0–50% of cases [15, 153–155]. Additional HLA types associated with familial and non-familial LP include: HLA-A3, −Aw19, −B18, −Cw8, −DR1, −DRB1*0101, −DQ1, −DQB1*0201 [32, 97]. The mode of inheritance appears to be autosomal dominant with variable penetrance. A study of OLP, in a Chinese family, found chromosome 3p14-3q13 as the candidate gene region for OLP [156].

Polymorphisms have been reported in immune related genes: IFN-γ, TNF-α, TNF-α R2, IL-4, −6, and −18 [32]. Polymorphisms in other genes include those involved in oxidative stress, prostaglandin-E2 (PGE2) synthesis, formation of transglutaminase, thyroid hormone synthesis, prothrombin, nuclear factor kappa-light-chain-enhancer of activated B cell (NFkB) as well as epigenetic regulation of genes by micro-RNA (miRNA)-146a and −155 [32] These polymorphisms may regulate increased activity of pro-inflammatory mediators as well as dysfunctional proteins and aberrant signaling. Gene expression profiling of LP, compared to atopic dermatitis and psoriasis, identified expression of the CXCR-3 ligand, CXCL-9, as the most specific marker for LP [157]. In addition, keratinocytes were confirmed as the source of type I IFNs (−α & -β) [157]. Future research using large, case-control genome-wide array studies, as was done with psoriasis, may clarify the current risk alleles and identify additional risk alleles for LP [158].

Cellular Immune Response

Dendritic Cells

Dendritic cells (DCs) play a key role in antigenic stimulation of naïve T-cells. Three major dendritic cell populations are involved in development of LP, including LCs (CD1a +, Langerin +), dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin positive DCs (DC-SIGN DCs), and plasmacytoid DCs (PDC) (CD-123+, BDCA+) [159]. DCs are the primary antigen presenting cells and are involved in antigen processing as well as presentation to T-cells [160]. When DCs encounter an antigen, they undergo maturation, resulting in specific acquisition of phenotypic properties, including CCR7 expression, adhesion, and co-stimulatory marker expression, which induce nodal homing and T-cell stimulation [161, 162].

Langerhans Cells

LCs express: CD1a, Langerin, and E-cadherin, and are usually located in the suprabasal layer of the epidermis [163]. In LP, LCs are critical in the epidermal migration of T-cells. LCs present autoantigens or foreign antigens to T-cells, which can activate T-cells. Upon activation, LCs downregulate Langerin and E-cadherin as well as upregulate CD80 and 86, which enhances T-cell stimulation [164]. Over time, the LCs migrate towards the basal epidermis [165].

The exact role of LCs remains unknown, though they likely play integral roles in both promoting and regulating the inflammatory response. Mouse models show that the migration of T-cells can be impaired with tape-stripping of the skin (95% of LCs removed) [146]. In OLP the number of LCs inversely correlates with the number of inflammatory cells [147]. The paradoxical findings related to DCs may eventually be reconciled by the intricate balance of deletion of autoreactive populations versus potential propagation of autoimmunity, which may not be mutually exclusive [166].

DC-SIGN Dendritic Cells

The role of DC-SIGN DCs is unknown in LP. Interstitial DCs do not express langerin but do express DC-SIGN, which binds to ICAM2 and −3 and allows for extravasation as well as interaction of DCs and naïve T-cells [167–170]. DC-SIGN DCs have pluripotent differentiation in that one population

stimulates T-cells and another is macrophage-like, which lack T-cell stimulatory function and can stimulate B cells [171].

Plasmacytoid Dendritic Cells

PDCs express CD68, cutaneous lymphocyte-associated antigen (CLA), IL-3Rα (CD-123) and blood dendritic cell antigen-2 (BDCA2), but lack expression of CD11c [172]. PDCs are found in the dermis and are likely responsible for the maturation of DCs and play a central role in the amplification of cytotoxicity in lymphoid cells. Upon stimulation, PDCs upregulate CD40-L, express IL-12, and type I IFN, which stimulate a Th1 response [172]. With maturation, PDCs downregulate CXCR-3 and L-selectin, which allows for migration from the epidermis into the stroma, lymphatics, and eventually the lymph nodes [172, 173].

PDCs have the unique capacity to rapidly produce large amounts of interferon- α (IFN-α) upon recognition of viral RNA and deoxyribonucleic acid (DNA) through TLR7 and −9, respectively [174]. Type I IFN producing PDCs recruit CD8-Tc cells, carrying CXCR-3 ligands in their granules, via CXCR-3–CXCL-10 interaction, which represents an important amplification step in LP [150, 175]. In addition, PDCs express CXCR-3, which allows for migration, amplification, and modulation of the local LTR [175].

PDCs are normally unable to respond to self-nucleic acids; however, in cutaneous LE PDCs become activated to produce type I IFNs by self-nucleic acids in complex with antibodies to DNA or nucleoproteins [176–178]. Type I IFN results in the maturation of MDCs, DC-SIGN DCs and LCs, and increases MDCs' ability to stimulate T-cells, which can result in auto-reactive immune events [179]. It remains speculative whether these triggers exist in LP.

T-Cells

Both cytotoxic and helper T-lymphocytes play central roles in LP as described earlier (see section, "Antigen Recognition," "Lymphocyte Activation," "Keratinocyte Apoptosis," and "Resolution"). The role of T-Reg cells also remains speculative but their general mechanisms of activity are discussed below.

T-Regulatory Cells

Foxp3-positive CD4-positive CD25-positive regulatory T cells (T-Regs) are critical for the regulation of host tolerance and suppression of pathological immune responses. T-Reg cells can be readily identified in the skin; however, no significant differences were found between normal skin and many inflammatory dermatoses, including LP [180]. Recently, T-reg cells and the inducers and signaling molecules, TGF-β and IL-10, were found to be upregulated in OLP and were diminished after effective treatment [181]. This suggests a role for T-Regs, induced by IL-2 and TGFβ, as well as synthesis of IL-10 and TGF-β in the resolution of LP [182, 183]. Human anti-inflammatory macrophages (m2 type) induce T-Reg cells and may also play a role in disease resolution [184].

Polymorphisms in specific cytokines, and receptors, as well as pathways in the generation of suppressive immune cells may lead to a prolonged or inappropriate inflammatory response. Genetically altered mice with autoreactive CD8+ T cells do not develop toxic epidermal necrolysis, a severe, necrotic form of interface dermatitis, until they are depleted of T-Reg cells [185]. Taken together, T-Reg cells are involved in attenuating the immune response and are diminished in cutaneous LE and other LTR, but their role in propagation and clearance of LP has yet to be clearly defined [186].

Cytokines/Chemokines

Chemokines are a subset of cytokines that mediate migration and homing of leukocytes [122, 187]. Chemokines possess homeostatic as well as inflammatory properties.

CXCR-3 Ligands

CXCR-3 ligands, CXCL9, −10, −11, are required in the recruitment of CD8-Tc cells as well as PDCs in LTR [188]. CD8-Tc cells harbor CCL5 and CXCR10 in their granules, which suggest auto-inductive and propagating pathways of activation [188]. CXCL-12 is homeostatic in nature, has an unclear role in LP, but is upregulated in oral disease [189]. The upregulation of inflammatory and homeostatic chemokines results in the recruitment of memory T cells [114, 190]. IFN-γ and TNF-α stimulate keratinocytes to release CXCR-3 ligands [123, 191]. These ligands are upregulated in LP relative to other inflammatory skin diseases [157]. CXCR-3 positive CD8-Tc cells as well as PDCs have a high affinity for CXCR-3 ligands that facilitate migration and recruitment at the DEJ with subsequent basement membrane disruption [175, 190, 192]. Similar mechanisms, although mediated by different stimuli, have been described in cutaneous LE, and lichenoid GVHD [114, 192].

Interferon-α

IFN-α is produced primarily by PDCs and is a critical mediator in DC maturation (see section "Dendritic Cells"),

as well as the amplification and the recruitment of CD8-Tc cells [150] IFN-α is a potent mediator of the expression of CXCR-3 ligands. IFN-α rapidly induces CXCL-9, −10, −11, and −12 expression in primary keratinocytes, dermal fibroblasts, and dermal endothelial cells. The upregulation of CXCL-12 in conjuction with the CXCR-3 ligands suggests that IFN-α plays a key role in PDC recruitment as well as CD8-Tc cells and memory T cells. T-lymphocyte recruitment produces IFN-γ, which results in an amplification loop.

Interferon-γ

IFN-γ plays a central role in CMI and is over-expressed in lesional LP (see section "Lymphocyte Activation") [189, 193]. Upregulation of IFN-γ has been identified in keratinocytes as well as cytotoxic T cells of LP [113]. IFN-γ induces expression of Fas on keratinocytes contributing to apoptosis [194].

Toll-Like Receptors

TLRs are part of the innate immune response, which recognize specific molecular components conserved among microorganisms [195]. TLRs induce an innate inflammatory response and mediate antigen-specific adaptive immunity [195].

PDC are likely activated by TLR7 and −9 (see section "Dendritic Cells"). While specific triggers of LP are unknown, human herpes virus type 7 (HHV7) in LP has been found to co-localize with PDCs [115–117]. Though not conclusively confirmed, this suggests that viral DNA in conjunction with TLR9 may activate PDCs in early lesions of LP [115–117]. Increased expression of TLR9 has been demonstrated in both oral and cutaneous LP [196, 197]. Similarly, imiquimod, an inducer of TLR7, is reported to cause LP [198–200].

Koebnerization, disease triggered by local skin injury, may be induced by cathelcidin (LL37), an endogenous antimicrobial peptide, which can bind to self-DNA. While this has not been observed in LP, activation of PDCs by LL37 bound to self-DNA has been shown in psoriasis [201]. Meller has suggested that a similar mechanism may occur in an individual with a different genetic predisposition [122]. TLR2, which drives a humoral-mediated immune response, is downregulated in OLP while TLR4, which drives a CMI response, is upregulated in OLP [202]. TLR3 polymorphism has been associated with an increased risk in developing OLP as well as being a risk factor for poor survival in oral cancer [203, 204]. Taken together, there is an integral role between the host-microbial response in OLP and likely in cutaneous LP as well.

Treatment

The treatment of LP is challenging for both physicians as well as patients. Due to its ability to affect multiple ectodermally derived tissues, LP often requires a multidisciplinary approach with dermatologists, dentists, and gynecologists. The goal of therapy is to minimize morbidity and improve the patient's quality of life.

The basic concepts in the treatment of LP have remained largely unchanged over the last decade. The therapies are mainly divided into skin directed and systemic agents. The therapies for various ectodermal tissues are also similar. Nearly all of these agents act in a manner to depress the immune response. To date, there are not disease specific medications for LP. However, Janus Kinase (JAK) inhibitors target CD8-Tc cells and represent a potential, disease-specific treatment of lichenoid diseases, including LP [205]. This section will provide a brief overview of the general approach to therapy for LP; however, emphasis will be placed upon unique treatments for variants of LP and their potential pharmacological targets.

Cutaneous Lichen Planus

Topical Corticosteroids

Despite few clinical trials, high potency topical corticosteroids are considered first-line therapy for limited cutaneous LP. Occlusion may be necessary to increase penetration in cases of hypertrophic LP. The sole randomized controlled trial comparing calcipotriene to betamethasone valerate (twice daily for 12 weeks) found no difference between treatments [206]. If no response is observed to twice daily application for 2–4 weeks, changing to a higher potency corticosteroid or intralesional injections should be considered [207].

Topical Calcineurin Inhibitors

There are no trials using topical calcineurin inhibitors (TCIs) in cutaneous LP. However, clinical data in OLP suggests that topical TCIs are likely the most effective topical therapy for LP. Application of tacrolimus 0.1 % ointment is as effective as 0.05 % clobetasol [208].

Intralesional and Systemic Corticosteroids

Intralesional corticosteroids (5–10 mg/mL injected on a monthly basis) can be highly effective in resistant and hypertrophic LP. However, one should use caution to prevent

excessive trauma to avoid Koebnerization. Systemic corticosteroids for cutaneous LP have only been reported in one study with a 90 % response rate and 32 % relapse rate at 6 months [209]. In our clinical experience, systemic corticosteroids are highly effective but are associated with high rates of relapse upon discontinuation. Therefore, oral and intralesional therapy should always be combined with topical therapy.

Phototherapy

Phototherapy has been used successfully in many inflammatory diseases of the skin. Ultraviolet-B (UVB) exposure of DCs results in impaired DC-CD4-Th cell interaction that culminates with T-cell apoptosis [210]. In addition, phototherapy alters cytokine expression, which suppresses CD8-Tc cells [211]. UVB (three times weekly until remission with taper after remission over 3–6 weeks) has a 70 % remission rate and 85 % of those patients remained in remission at 34 months [212]. Narrowband UVB (311 nm) is as effective in LP and has largely supplanted Ultraviolet-A (UVA), psoralen plus UVA (PUVA), and UVB phototherapy [213].

Miscellaneous

The mechanism of action of metronidazole in LP is unknown but may be mediated by its antimicrobial activity as well as immunosuppressive effects on human blood lymphocytes [214]. An open labeled study of oral metronidazole (250 mg three times daily for 12 weeks) showed a 74 % response rate at 3 months of follow up [215]. Alternate dosing (500 mg twice daily for 20–60 days) has also been reported to be successful [216]. Due to its side effect profile, metronidazole is often considered first line; however, one should caution patients as well as monitor for possible sensory peripheral neuropathy [217].

Sulfasalazine (initial dose 1 g daily with an increase every 3 days by 0.5 g to a max of 2.5 g daily) has the highest level of evidence of efficacy for LP with an 83 % improvement in skin lesions and a 91 % improvement in itch at 6 weeks in the therapy group [218, 219]. Sulfasalazine likely downregulates the expression of ICAM1, decreases leukotriene synthesis, and decreases the number of lesional T lymphocytes [220].

A double blind, placebo control trial of acetretin (30 mg daily for 8 weeks) showed marked improvement in 64 % of individuals [221]. Mucocutaneous side effects and hyperlipidemia were common. Retinoids may have activity by downregulating VCAM1 on endothelial cells and modulation of cyclooxygenase-2 (COX2) and TNF-α preventing the homing of lymphocytes to the skin and stimulation of the inflammatory cascade [222, 223].

Methotrexate has been shown to be of benefit for more recalcitrant disease as well as in specific forms of disease, including hypertrophic LP and LPP. Methotrexate preferentially targets lymphocytes 1000-fold relative to keratinocytes and inhibits proliferation and has cytotoxic activity [224]. Recent, non-randomized, prospective data has shown methotrexate (15–20 mg weekly for 4–24 weeks) to be highly efficacious with complete responses in 58–91 % of cases [225–227].

Other therapies including: mycophenolate mofetil, cyclosporine, TNF-α inhibitors, trimethoprim-sulfamethoxazole, griseofulvin, itraconazole, terbinafine, antimalarials, tetracyclines, laser, IFN, allitretinoin, thalidomide, and low-dose heparin have also been reported.

Oral Lichen Planus

The cornerstone of treatment in OLP is good oral hygiene with regular professional dental cleanings [228]. Minimizing other exacerbating factors such as: contact allergens, drug reactions, reducing oral microbes, and minimizing trauma can reduce disease severity as well as frequency of flares. Replacement of dental amalgams and gold dental restorations can be beneficial, even in patients with negative patch testing [229, 230]. However, removal and restoration should be individualized based upon the severity of disease as well as the index of suspicion of the level of involvement of the metal or prosthesis.

Topical Corticosteroids

Topical steroids are first-line therapy in OLP with overall clinical responses on the order of 70–80 %. Although few direct comparisons between topical corticosteroids in LP exist, the most beneficial are likely: triamcinolone acetonide, clobetasol-17-propionate and fluocinonide [231]. However, only clobetasol and fluocinonide have clear benefit over placebo. In general, application of topical corticosteroids two to six times daily is indicated. The major complications of topical corticosteroids are fungal infections and, in general, higher rates of fungal infections are seen with more potent topical corticosteroids. Therefore, concomitant therapy with oral chlorohexidine gluconate mouthwash or other topical anti-candidal medications is recommended [232, 233].

Triamcinolone acetonide 0.1 % mouthwash and paste (four times daily for four weeks) are equally effective in the treatment of OLP [234]. Clobetasol 0.05 % in Orabase®

(twice times daily for 3 weeks, daily for 3 weeks, and every other day for 3 weeks) was found to be superior to triamcinolone 0.1 % in Orabase® at 3 weeks with equal efficacy at 6 and 9 weeks [235]. Another study found clobetasol to be superior to fluocinonide in OLP with faster resolution of lesions [232]. Studies comparing various preparations of clobetasol 0.05 % showed that there was faster clearing time in those using twice daily applications of clobetasol 0.05 % in adhesive denture paste [236]. There is no clinically significant difference in efficacy between clobetasol 0.05 % gel (twice daily for 2 months) versus clobetasol 0.025 % gel [237]. However, at 2-month follow up, the patients in the high concentration group had more stable disease. Fluocinonide 0.025 % (six times daily) in adhesive base was found to be superior to placebo [238]. Fluocinolone 0.1 % (four times daily for two to four weeks) in various preparations was found to be equally effective [239].

Topical Calcineurin Inhibitors

Pimecrolimus 1 % cream is effective in curing the erosive lesions of OLP but is ineffective at symptomatic control [231, 240]. Meta-analysis has found tacrolimus 0.1 % ointment (used one to four times daily) has been found to be more effective than clobetasol propionate ointment [231]. Unlike pimecrolimus, tacrolimus is effective at controlling the symptoms of OLP as well. However, the number of patients in the three RCTs is much lower than those using corticosteroids and; therefore, tacrolimus is reserved as a second-line topical therapy. Cyclosporine in various preparations has been found to be less effective than both clobetasol and triamcinolone [231, 241]. In lieu of alternative commercially available calcineurin inhibitors (CIs) and increasing restrictions on compounding, we would recommend this therapy third-line. Transient burning with usage of topical CIs is common and overlap therapy with topical corticosteroids may alleviate the burning sensation.

Intralesional and Oral Corticosteroids

Systemic steroids are the most effective treatment for OLP and can provide rapid improvement in acute exacerbations. However, there are no RCTs for systemic corticosteroids. Both clinical experience as well as prospective studies indicate that oral corticosteroids (1.5–2 mg/kg) are highly effective [242, 243]. Intralesional triamcinolone (0.5 mL of 40 mg/mL on a weekly basis for four weeks) is as effective as 0.1 % triamcinolone (three times daily for 6 weeks) mouthwash [244]. However, due to the discomfort of injection and few well-controlled studies, intralesional therapies are reserved after exhausting topical therapies. Additionally, studies examining pulsed betamethasone (5 mg on 2 consecutive days weekly for 3 months) versus topical 0.1 % triamcinolone paste (three times daily for 3 months) showed a more rapid response but no clear long-term advantage [245]. Intralesional and oral therapy should be done concomitantly with topical therapy, as patients will often flare upon discontinuation of oral therapy.

Miscellaneous

Many other therapies including: topical retinoids, mesalazine, antibiotics, hydroxychloroquine, griseofulvin, thalidomide, etretinate, acetretin, isotretinoin, cyclosporine, methotrexate, mycophenolate mofetil, azathioprine, as well as parenteral alefacept, extracorporeal photophoresis, laser therapy, and PUVA therapy. However, these therapies often have attendant side effects or do not show clear efficacy. They are reserved as third-line therapies, and the risks and benefits of therapy should be considered prior to institution.

Lichen Planopilaris-Frontal Fibrosing Alopecia

There are no randomized controlled trials in LPP and only one controlled study. Therefore, many of the therapies in LPP are based upon expert opinion [246]. LPP has three major subtypes; however, for therapeutic purposes, they will be divided into LPP and FFA. These clinically distinct entities also appear to be responsive to slightly different therapies [247]. When considering therapeutic modalities, the psychosocial effects of scarring alopecia should be taken into account. While the pathogenesis of LPP is unknown, there is destruction of the bulge region, which leads to scarring. Recent studies have indicated a deficiency of peroxisome proliferator-activated receptor PPARγ, which may lead to loss of immune privilege and scarring alopecia [129].

Lichen Planopilaris

First-line therapies for LPP are mid to high potency topical corticosteroids with an average 53 % response rate [43, 45, 247]. There is only limited data to determine the efficacy of topical CIs, but the side effect profile makes it common second-line topical therapy. Hydroxycholorquine (6.5 mg/kg for 6–12 months) is the second most commonly used drug

and showed a good response in 23 % of cases [247, 248]. There have been several negative studies using hydroxychloroquine, and there is good evidence that while clinical evidence of inflammation is decreased, progressive scarring of follicles continues [246]. Oral corticosteroids (1 mg/kg/day for 15 days with taper over 4 months) and cyclosporine (300 mg/day for 3–5 months) are the third most commonly used drugs with a good response in 60 % of cases [43, 45, 247, 249]. Relapse is common upon discontinuation of these medicines; therefore, topicals or intralesional therapy should be used concomitantly. Approximately one third of patients receiving oral tetracyclines (doxycycline 100 mg twice daily for 3–6 months), mycophenolate mofetil (2–3 g daily for 3–12 months), or intralesional steroids (10 mg/mL injected on a monthly basis) had marked improvement [43, 250–252].

One approach is superpotent topical corticosteroids, clobetasol, twice daily for 1 month, daily for 3 months, and every other day for 3 months [249]. Intralesional corticosteroids as well as oral hydroxychloroquine can be added if there is no effect at 3 months, recognizing that hydroxychloroquine alone is likely ineffective. Other systemic agents above should be reserved for severe cases.

Frontal Fibrosing Alopecia

High potency topical corticosteroids have not shown to be effective in FFA, for 93 % of cases, while intralesional corticosteroids (10–20 mg/mL injected every 3–6 months) led to partial improvement in 60 % of cases [247, 249, 253, 254]. Oral 5 alpha-reductase inhibitors, such as finasteride (2 or 5 mg daily dose for 12–18 months) or dutasteride (0.5 mg daily for 12 months), are the most commonly used and most effective drugs, and result in a good response in 45–47 % of cases [247, 253, 255–258]. The largest study of FFA to date found that androgenetic alopecia (AGA) was concurrently present in 40 % of the women and 67 % of the men [254]. Therefore, the benefit seen with anti-androgens may be due to the component of AGA. Hydroxychloroquine has been reported effective in approximately 30 % of cases; however, many of the studies had concomitant topical therapy with immunomodulators and minoxidil [49, 247]. Other reported therapies, e.g., systemic prednisone, hormone replacement, topical calcineurin inhibitors, topical minoxidil, cyclosporine, and mycophenolate mofetil have only been reported in small series and have highly variable response rates.

Based upon the above evidence, many hair experts will use as first-line therapy: oral 5-alpha reductase inhibitor, intralesional corticosteroids, and topical minoxidil.

Nail Lichen Planus

Nail LP can be extremely difficult to treat. The goal of therapy should be to reduce the number of inflammatory cells within the nail and to prevent irreversible pterygium. A therapeutic approach should be very similar to cutaneous LP. Ultra-potent topical and intralesional corticosteroids (5–10 mg/mL injected on a weekly basis) are first line therapies. Systemic therapies should be reserved for cases manifesting significant compromise of function and causing debilitating pain.

Conclusions

The exact mechanism of LP remains unknown. However, insight from other lichenoid diseases, specifically lichenoid GVHD and cutaneous LE, as well as psoriasis, have provided insight into the basic inflammatory cascade. Further insight into disease pathogenesis will likely come from better characterization of genetic, epigenetic, and microbiomic aberrations and their interactions [259–262].

Questions

1. What HLA type is common in the setting of hepatitis C and oral lichen planus (LP)?
   (A) HLA-B51
   (B) HLA-DR6
   (C) HLA-Cw6
   (D) HLA-DQ2
   (E) HLA-B*1502

Answer:

(A) HLA-B51- HLA-B51 is associated with Behçet' disease and is seen in approximately 80 % of Asian individuals but is much less common in Caucasians

(B) **HLA-DR6- HLA-DR6 is seen in patients with OLP and Hepatitis C (over 50 % of cases) and may explain geographic heterogeneity of the association of OLP and Hepatitis C** [1, 2]

(C) HLA-Cw6- HLA-Cw6 is associated with psoriasis (included in the PSORS1 locus) and is a risk factor for early onset of disease

(D) HLA-DQ2- HLA-DQ2 recognizes the gliadin peptide and is associated with dermatitis herpetiformis (DH). Nearly 90 % patients with DH have HLA-DQ2

(E) HLA-B*1502- HLA-B*1502 is associated with carbamazepine induced SJS/TEN in Asians in East India but not Europeans

2. What systemic disease is most strongly associated with lichen planopilaris (LPP)?
   (A) Hyperlipidemia
   (B) Diabetes
   (C) Hepatitis C
   (D) Hyperthyroidism
   (E) Hypothyroidism

Answer:

(A) Hyperlipidemia- Hyperlipidemia is not strongly associated with LPP and may even be less prevalent in LPP versus controls

(B) Diabetes- Diabetes is not strongly associated with LPP and may be less prevalent in LPP versus controls

(C) Hepatitis C- The association with Hepatitis C is strongest in OLP and may be related to HLA-DR6

(D) Hyperthyroidism- There is no clear association with hyperthyroidism

(E) **Hypothyroidism- There is a strong association with hypothyroidism and LPP with 29% of patients with LPP having hypothyroidism compared to 9% of controls** [3]

3. What is true in regards to the risk of malignant transformation in LP?
   (A) The risk of malignant transformation is highest in longstanding oral erosive disease
   (B) The risk of malignant transformation is highest in generalized cutaneous lichen planus
   (C) Smoking and alcohol consumption are clear risk factors for malignant degeneration
   (D) HPV16 is less common in those with oral lichen planus (OLP)
   (E) Hypertrophic LP and squamous cell carcinoma (SCC) can clearly be distinguished histologically

Answer:

(A) **The risk of malignant transformation is highest in longstanding oral erosive disease** [4, 5].

(B) The risk of malignant transformation is highest in generalized cutaneous lichen planus. Risk factors for malignant transformation in cutaneous LP include: hypertrophic or verrucous LP, location on the lower extremity, a history of arsenic or x-ray exposure, and longstanding disease (average of 12 years) [5]

(C) Smoking and alcohol consumption are clear risk factors for malignant degeneration- Interestingly, smoking and alcohol have not shown a clear association with malignant transformation of OLP

(D) HPV16 is less common in those with oral lichen planus (OLP)- HPV16 is nearly five times more common in OLP compared to controls and may account, in part, for the increased risk of malignancy [6]

(E) Hypertrophic LP and squamous cell carcinoma (SCC) can clearly be distinguished histologically.- Hypertrophic LP is often confused histologically with SCC; therefore, good clinical pathological correlation is require [7]

4. What is the key effector cell in LP?
   (A) T-cytotoxic cell
   (B) T-helper cell
   (C) NK cell
   (D) Langerhans cell
   (E) Plasmacytoid dendritic cell

Answer:

(A) **T-cytotoxic cell- The T-cytotoxic cells are likely the terminal effector cell in LP. These cells localize to the dermal-epidermal junction and in close proximity to damaged basal keratinocytes** [8]

(B) T-helper cell- T-helper cells tend to localize to the dermis and are involved in antigen recognition and are often found in close proximity to Langerhans cells [9]

(C) NK cell- NK cells may be involved in the early propagation of LP [10]

(D) Langerhans cell- Langerhans cells are involved in antigen presentation and T-cell stimulation [11]

(E) Plasmacytoid dendritic cell- Plasmacytoid dendritic cells produce large amounts of type I interferon and likely act as an early amplifying loop in LP [12]

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Cutaneous Fibrosis and Normal Wound Healing

32

Emily Hamburg-Shields, Peggy Myung, and Shawn E. Cowper

Abstract

Normal cutaneous wound healing is a highly orchestrated process requiring the participation of numerous cell types, each performing highly individualized functions at specific times under the guidance of dozens of circulating proteins that are themselves tightly controlled by transcriptional and translational mechanisms. It should come as no surprise that genetic and environmental perturbations could lead to the disruption of the successful completion of this process, or that the mechanisms themselves could be hijacked and diverted into the production of unnecessary scar.

The first portion of this chapter introduces the cellular players in this drama, their normal roles in the maintenance of the extracellular matrix, and the overarching and coordinated production that is known as "normal" wound healing. The chapter culminates with the detailed examination of several cutaneous fibrosing processes that result when genetic and/or environmental influences steer the mechanisms of wound healing and injury repair into "pathological" fibrosing disorders that can be notoriously challenging to correct.

Keywords

Cutaneous • Fibrosis • Scar • Pathology • Mechanism • Pathway • TGF-β • Wound healing • Fibroblast • Myofibroblast • Scleroderma • Systemic sclerosis • Lichen sclerosus • Keloid • Nephrogenic systemic fibrosis

Important Cellular Contributors to Cutaneous Fibrosis

The cells that promote cutaneous fibrosis include fibroblasts, fibroblast progenitors, T lymphocytes, and macrophages. Additionally, in autoimmune fibrosing disorders such as systemic sclerosis (SSc), B lymphocytes play an important role, reflecting the diverse and unique pathogenesis of different fibrotic disorders. Each of these cell types, with additional focus on particular subsets of fibroblast progenitors, will be discussed in detail in this section.

Activated Fibroblasts and Myofibroblasts

Fibroblasts are a heterogeneous group of cells that are responsible for the production of the extracellular matrix (ECM)—composed broadly of collagen, elastin, and myxoid ground substance. Cutaneous fibrosis occurs when abnormal fibroblast activity leads to ECM with excess collagen. The dermis contains resident fibroblasts which produce type I collagen (the predominant type of collagen in adult skin) and other matrix proteins including type III collagen, fibronectin, fibrillin, and elastin [1]. Many different stimuli, both signaling factors and mechanical forces, can regulate the activity

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of fibroblasts. These stimuli vary among the different fibrosing conditions.

Some conditions have a characteristically elevated fibroblast proliferation rate. These include normal healing wounds, keloidal and hypertrophic scars, and radiation-induced skin fibrosis [2–4]. In other fibrotic conditions, increased fibroblast proliferation is not a typical feature (i.e. SSc, morphea, and stiff skin syndrome).

Myofibroblasts, a fibroblast subtype, are present in many fibrosing conditions and in normal dermal wound healing [5, 6]. These contractile cells are defined by the presence of stress fibers and prominent endoplasmic reticulum and are critical for tissue contraction during wound healing. Expression of α-smooth muscle actin is widely accepted as a marker for myofibroblasts, although it is not specific; it is also expressed in endothelial cells, pericytes, and smooth muscle cells [7]. In addition, α-smooth muscle actin identifiable by confocal microscopy may not be readily seen in typical immunohistochemical preparations [8, 9]. Myofibroblasts are typically present in healing or fibrosing conditions, although fibrosis can occur in their absence. For example, early stage SSc skin does not always have myofibroblasts [10].

Potential Activated Fibroblast/Myofibroblast Sources

Determining the cellular sources of the fibroblasts that contribute to fibrosis is an active area of research and debate (Fig. 32.1). This question is relatively understudied in skin fibrosing processes as compared to renal, pulmonary, and hepatic fibrosis. Resident dermal fibroblasts are ideally situated to respond to injury and profibrotic stimuli, allowing for their local activation and contribution to fibrogenesis. However, several other cell types have been hypothesized to contribute to the population of ECM-producing fibroblasts in fibrosis [11]. For example, pericytes are perivascular mesenchymal progenitor cells that have been shown to contribute to murine fibrosis in multiple organs including skin [12–14] and to human fibrosis in lesional SSc skin, where they express markers common to myofibroblasts [8]. Circulating fibrocytes (CFs) are a population of monocyte-lineage, bone marrow-derived mesenchymal progenitor cells [15] that home to injured tissues and contribute to fibrosis in wounds, scars (including keloidal scars), and nephrogenic systemic fibrosis (Section Nephrogenic Systemic Fibrosis) [16, 17]. They have also been demonstrated in a bleomycin-induced murine model of skin fibrosis [18].

Furthermore, growing evidence suggests that subcutaneous adipocyte progenitors may also contribute to fibrosis, especially in SSc, in which adipose tissue is replaced by fibrotic tissue [19, 20]. Finally, both epithelial cells and endothelial cells have been postulated to trans-differentiate into fibroblasts in fibrosing skin and other tissues. Until further studies dissect the relative contributions of these diverse lineages to different fibrotic conditions, each of these cell types can be considered a potential source of matrix-producing fibroblasts in addition to the resident dermal fibroblasts.

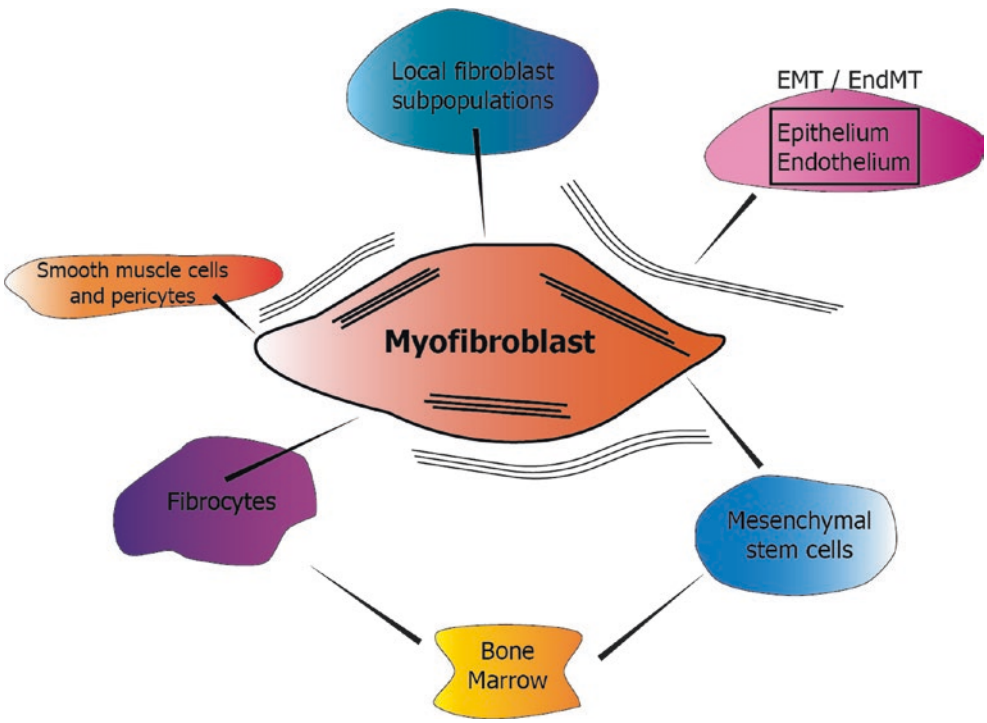

Fig. 32.1 Potential sources of myofibroblasts. Myofibroblasts, besides resulting from the differentiation of normal cutaneous resident fibroblasts, may also derive from several cellular sources, including pericytes, epithelial cells, endothelial cells, circulating fibrocytes, and mesenchymal stem cells. *EMT* epithelial to mesenchymal transition, *EndMT* endothelial to mesenchymal transition (Figure adapted per Creative Commons license from Micallef et al. [253])

Endothelial Cells

Endothelial cells are crucial mediators of the wound healing process (see Section Cutaneous Wound Healing). In fibrotic skin disorders, endothelial or microvascular dysfunction may play an important role in early stages of injury response leading to fibrogenesis [21].

The role of endothelial cells in fibrosis is of particular interest in SSc (see Section Scleroderma/Systemic Sclerosis), which is accompanied by skin microvessel vasculopathy and endothelial apoptosis and is often preceded by the appearance of Raynaud's phenomenon [22]. Tight-skin mice, which have an in-frame duplication of one allele of the gene encoding fibrillin-1, recapitulate several features of SSc, including excess collagen, thickened skin, and endothelial dysfunction [23]. In addition to contributing to tissue hypoxia, endothelial cell dysregulation may also directly contribute to fibrosis via endothelial-to-mesenchymal transition (EndMT), a phenomenon that produces activated fibroblasts in injured tissue [24, 25].

How endothelial cells might contribute to fibrotic scar formation is unclear. In contrast to SSc, keloidal and hypertrophic scars do not exhibit endothelial cell apoptosis [26]. Instead, dermal microvessels are more abundant in these scar tissues than in normal skin and tend to exhibit occlusion that may contribute to local tissue hypoxia [27, 28].

Epithelial Cells

Epithelial cells, including basal keratinocytes and hair follicle-associated stem cells, contribute to re-epithelialization during wound healing [2]. Therefore, in wound healing and scar formation, epithelial proliferation and migration are key steps that regulate fibrotic processes (see Section Cutaneous Wound Healing). As discussed in Section Activated Fibroblasts and Myofibroblasts, epithelial-to-mesenchymal transition (EMT) may serve as a source of matrix producing cells. EMT, a phenomenon in which epithelial cells transdifferentiate into mesenchymal cells, has important roles in embryonic development and tumorigenesis. It has been suggested that EMT contributes to the activated fibroblast population in fibrosis of the kidney, lung, and liver [29]. In contrast, the role of EMT in skin fibrosis has not been carefully studied [30]. In a transgenic mouse model of fibrosis, overexpression of profibrotic CTGF in mouse fibroblasts *in vivo* was sufficient for an EMT gene expression signature to become detectable in skin and lung epithelium [31]. However, dedicated lineage-tracing studies are still required in order to demonstrate a role for EMT in the bleomycin-induced and tight-skin murine models of skin fibrosis.

T Lymphocytes

T cells are present in the inflammatory phase of normal wound healing as well as in keloidal scars and lesional tissue affected by SSc and acute graft versus host disease (GVHD) [2, 32–34]. T lymphocytes have several potential roles in fibrosis. First, they can stimulate fibroblasts to produce excess collagen [35]. Second, they can induce local macrophages to produce profibrotic cytokines [21]. Finally, they can promote vascular dysfunction by inducing apoptosis of vascular endothelial cells, as observed in SSc [36].

Studies in animal models have not demonstrated a consistent role for T cells in skin fibrosis. CD4-deficient tight-skin mice (Tsk/+) have diminished skin fibrosis suggesting that CD4+ T lymphocytes are important for the development of skin fibrosis in this model [37]. Similarly, donor T cells appear to contribute to fibrosis in the murine model of sclerodermoid GVHD [38]. Conversely, subcutaneous injection of bleomycin in severe combined immunodeficiency mice, which lack functional T cells and B cells, does not appear to affect the fibrotic response [39]. These results indicate that the role for T cells in fibrogenesis may vary between animal models and human diseases. The balance between distinct regulatory and other CD4-lineage T cells is likely important and deserves further investigation [40].

B Lymphocytes

B cell activation is of mechanistic and therapeutic interest in SSc and morphea. B lymphocytes are a component of the inflammatory infiltrate in lesional SSc skin. They are detectable upon immunohistochemical staining using CD20 surface marker, and SSc skin has elevated expression of signature genes that are consistent with the presence of B cells [41–43]. Peripheral blood B cells from SSc patients have an activated phenotype, with elevated expression of surface marker CD19 and B cell activating factor receptor (BAFFR) [44, 45]. This observation suggests that B cells in SSc may be activated by B cell activating factor (BAFF). Similar to SSc, patients with morphea also have elevated serum levels of BAFF that correlate with disease severity [45–47].

The profibrotic effects of B cell activation are likely mediated by their production of specific autoantibodies and cytokines. In SSc, B cells produce multiple autoantibodies including anti-DNA topoisomerase I, anti-centromere, anti-fibrillin-1, anti-PDGFR, anti-fibroblast, anti-endothelial cell, anti-nuclear antibodies, and others [43]. Some of these autoantibodies may have direct pathogenic roles by inducing an inflammatory response against their antigenic targets [48] (Section Scleroderma/Systemic Sclerosis). In addition, B lymphocytes produce IL-6, a cytokine that stimulates fibroblast growth and collagen production [49, 50].

Two mouse models of skin fibrosis that possess B cell abnormalities have illuminated the precise pathogenic roles of B cells in autoimmune fibrosis. Both the tight-skin mouse (Tsk/+), a genetic model of skin fibrosis, and mice subcutaneously injected with bleomycin, an injury-induced model of skin fibrosis, have activated B cell phenotypes and autoantibody production [51, 52]. Depletion of CD19+ B cells in these mouse models, as well as inhibition of BAFF in tight-skin mice, inhibit the development of skin fibrosis [52–54]. These studies suggest that inhibition of B cell activation may have therapeutic benefit for fibrotic skin diseases.

Ongoing studies in SSc patients are investigating the therapeutic benefit of B cell depletion using rituximab, a chimeric anti-CD20 antibody. Recent studies have shown a significant reduction in clinical indicators of SSc severity, including the modified Rodnan skin score, with rituximab treatment [55–57]. In addition to being potential therapeutic targets, B cell-related factors such as BAFF may also serve as biomarkers of disease progression in SSc [58].

Macrophages

Macrophages are involved in wound healing and in skin fibrosis. They are present during the inflammatory phase of wound repair [2] and in skin lesions of SSc [59, 60], acute GVHD [61], and keloidal scars [32]. Although resident macrophages are present in normal dermis, recruited monocyte-derived macrophages also contribute to wound healing [62] and SSc [63].

Macrophages are activated by profibrotic signaling factors including TGF-β, IL-13, and platelet-derived growth factor (PDGF). Functional studies in mouse models have suggested that macrophage activation is a response to factors secreted by Th2 cells, a subset of T helper cells [21]. In turn, macrophages contribute to fibrosis by secreting profibrotic cytokines including angiotensin II, CCL3, and TGF-β1 [21, 64, 65].

In mice, macrophages are present in the dermis of the bleomycin and cutaneous GVHD mouse models of fibrosis [38, 39] and are required for bleomycin-induced skin fibrosis [66] as well as for normal wound healing [62, 67]. Specifically, CCL3 produced by macrophages is critical for fibrosis in the bleomycin-induced pulmonary fibrosis mouse model [64], suggesting that this cytokine may be a key mediator of fibrogenesis in these conditions.

Signaling

Fibrosis involves altered signaling activity between all of the cellular players. In particular, inflammatory cells and fibroblasts actively secrete signaling molecules that promote the development and maintenance of pathologic fibrosis, while many of these molecules are also important in physiologic wound healing.

Signaling molecules discussed here include interleukins, chemokines, and growth factors. Recipient cells detect these secreted molecules via specific membrane-bound receptors. Generally, these signals serve to alter the target cell's behavior (inducing migration, cell division, or another activity), identity (for example, differentiation of a mesenchymal stem cell to a fibroblast), or secretory profile (of additional signaling factors, extracellular matrix proteins, etc.). Often these changes are executed by downstream regulation of nuclear gene expression by the recipient cell. Dissecting the specific signaling pathways that result in fibrosis will provide important insight into potential targets for therapeutic intervention. As effective therapies for fibrotic diseases are sorely limited, identifying novel fibrotic signaling mediators and testing their importance in animal models is an active area of research [68].

The goal of this section is to focus on some of the prominent profibrotic signaling molecules that have been directly tested in murine models of fibrosis and wound healing. Their relevance to specific fibrosing disorders will be discussed in Section Cutaneous Fibrosing Disorders. The influence of mechanical stresses and the extracellular matrix on the signaling environment will also be discussed.

Inflammatory and Immunomodulatory Cytokines

IL-4/IL-13

IL-4 and IL-13 are cytokines produced by Th2 cells (a subset of T lymphocytes). They bind to a common receptor, IL-13Rα1 [69, 70] and both have profibrotic activity in skin.

The profibrotic effects of IL-4 include stimulating collagen production by fibroblasts, inducing expression of TGF-β, and enhancing mononuclear cell infiltration [43]. Similarly, IL-13 stimulates activation of TGF-β by cleavage from its latent binding complex, induces expression of TGF-β by macrophages, and induces expression of collagen and fibronectin by human dermal fibroblasts [21, 43, 71]. IL-13 can also induce expression of profibrotic CC-chemokines including CCL3 and CCL2 [21, 72].

Both IL-4 and IL-13 show elevated expression in lesional skin of SSc patients [71, 73], and IL-4 is expressed during normal wound healing [74]. In addition, a genetic signature consistent with IL-13 activation has been identified in the murine sclerodermatous GVHD model and in a subset of SSc patients with inflammatory disease. In these patients, IL-13 activation correlates with the severity of skin fibrosis [72]. Inhibition of IL-4 prevents dermal fibrosis in the

tight-skin mouse model of SSc, and results in delayed wound healing in mice [35, 74]. Similarly, deficiency of IL-13 or its receptor IL-4Rα1 is protective against fibrosis in the sclerodermatous GVHD murine model [72].

IL-17

IL-17 is an inflammatory cytokine characteristically produced by Th17 cells, a subset of T lymphocytes. The profibrotic effects of IL-17 in skin are largely indirect. IL-17 stimulates expression of inflammatory cytokines and adhesion factors by other cell types [70]. Specifically relevant to fibrosis, IL-17 stimulates fibroblast proliferation and endothelial cell production of IL-1 and adhesion molecules. Expression of IL-17 is increased in T cells in SSc peripheral blood and skin lesions [75]. Conversely, mice lacking IL-17A have a delayed wound closure phenotype [76]. In mouse models of bleomycin-induced skin and lung fibrosis, IL-17 and other Th17 cytokines showed elevated expression, suggesting a role for Th17 mediation of the early fibrotic response [77].

IL-1

IL-1 comprises a family of inflammatory cytokines with an established profibrotic role in fibrosis of the kidney and other organs. In renal fibrosis, IL-1β, via TGF-β1, regulates epithelial-mesenchymal transition and myofibroblast conversion from fibroblasts [78]. Its role in skin fibrosis and wound healing has not been as extensively studied as in fibrosis of other organs. Both IL-1α and IL-1β are upregulated during the inflammatory phase of wound healing [79], predominantly by polymorphonuclear leukocytes and macrophages [80]. IL-1β, possibly secreted by fibroblasts, is increased in SSc. It is thought to mediate inflammation in SSc and may drive myofibroblast conversion and collagen production [81]. There is also a potential role for IL-1α from keratinocytes in stimulating fibroblast activation and contractility in fibrosis and wound healing [82]. Functional studies in animal models are needed in order to more precisely determine the sources and roles of IL-1α and IL-1β in fibrosis *in vivo*.

Chemokines

Chemokines (chemotactic cytokines) include CXC- and CC-molecules. These are secreted factors that bind to specific cell surface G-protein coupled receptors (GPCRs). Their potential roles in fibrosis and wound healing include inflammatory cell recruitment to the wound site, re-epithelialization, tissue remodeling, and angiogenesis [83]. Here, we will focus on two CC-type chemokines, CCL2 and CCL3. Both CCL2 and CCL3 have established profibrotic effects in murine studies of pulmonary fibrosis, possibly mediated by induction of IL-13 [21].

CCL2

CCL2 (formerly known as MCP1) acts upon monocytes/macrophages, T cells, and mast cells [83]. It is a monocyte chemoattractant and may mediate profibrotic behaviors of T cells. During wound healing, CCL2 is expressed in monocytes, macrophages, and keratinocytes [83–85]. It is also elevated in the serum in early-stage SSc, perhaps corresponding with the inflammatory phase of the disease, and is elevated in an inflammatory subset of SSc patients [72, 86]. CCL2 stimulates fibroblast expression of genes encoding TGF-β1 and type I collagen, which may be an indirect effect mediated by IL-4 produced by T cells [87, 88].

CCL2 knockout mice have delayed wound re-epithelialization and reduced angiogenesis and collagen production, without altered macrophage recruitment to the wound bed [89]. Therefore, the role of CCL2 during wound healing may be predominated by its direct or indirect effects on fibroblasts, endothelial cells, and keratinocytes, rather than by monocyte chemoattraction. CCL2 knockout mice are also resistant to bleomycin-induced skin fibrosis [66]. Furthermore, treatment with an anti-CCL2 antibody prevents the development of fibrosis in the sclerodermatous GVHD mouse [72].

CCL3

CCL3 (formerly known as MIP1α) is expressed early during wound healing in mice, corresponding with macrophage infiltration [83]. CCL3 is present at elevated levels in SSc patient serum; however, unlike CCL2, the amount of CCL3 does not correspond to the degree of skin fibrosis [90]. CCL3 knockout mice have normal wound healing [89], and CCL3 mRNA is not detected in acute adult human wounds [85]. Although this chemokine may not have a compelling role in wound healing in mice or humans, its elevated expression in SSc tissues and the possible profibrotic role of its receptor CCR5 [91] warrants its further investigation as a profibrotic mediator in human disease.

Growth Factors/Morphogens

TGF-β (Transforming Growth Factor-β)

TGF-β is the prototypic profibrotic signaling factor, and has been extensively studied in human disease conditions and animal models (Fig. 32.2). The TGF-β ligand family comprises three isoforms; TGF-β1 is the most abundant isoform in tissues. The ligand is secreted in a complex with a latent binding protein, which must be proteolytically cleaved to yield active extracellular ligand [92]. The active ligand binds to cell surface receptors, which are heterotrimers of TGF-β receptor I and TGF-β receptor II (TGFBRI and TGFBRII), stimulating their serine-threonine kinase activity [83]. Downstream intracellular transducers of TGF-β signaling

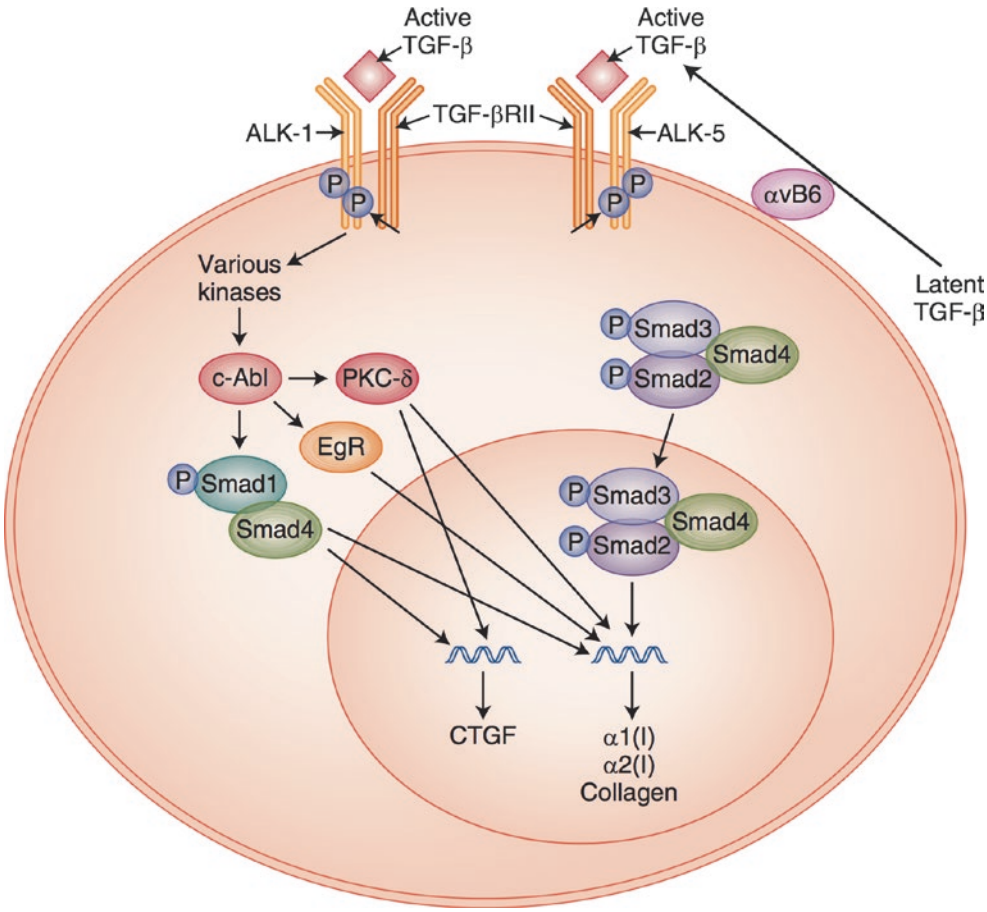

Fig. 32.2 TGF-β signaling in fibroblasts. Extracellular latent TGF-β is activated by αvβ6 integrin to its active form. Active TGF-β can signal through the classical pathway utilizing ALK-5 to phosphorylate Smad2 and -3, which then complex with Smad4. The complex is transported to the nucleus where, through additional factor interactions, critical genes can be transcribed. Non-classical signaling through ALK-1 can effect similar results through alternate pathways

activity include the canonical Smad2/Smad3-mediated response as well as non-canonical pathways [93].

The TGF-β ligand is expressed and secreted by platelets [83], activated T lymphocytes [94], monocytes and macrophages, fibroblasts, neutrophils, and epithelial cells [92]. Cells that respond to TGF-β ligand include neutrophils, macrophages, and fibroblasts [83]. The functions of TGF-β are pleiotropic and sometimes even contradictory. Depending on the cellular context, its effects may be both pro- and anti-fibrotic [21] and pro- and anti-inflammatory [92]. In the settings of fibrosis and wound healing, the prominent functions of TGF-β include promotion of monocyte activation, chemotaxis, cytokine production [92], fibroblast proliferation, expression of ECM proteins and integrins, and myofibroblast conversion [83, 95].

Elevated expression of TGF-β ligand and/or its receptors is broadly evident in fibrotic tissues including SSc lesional skin, hypertrophic scars, and keloidal scar fibroblasts [96–98]. In SSc primary fibroblasts, a TGF-β-responsive gene signature is correlated with more severe disease, providing a rationale for this signaling pathway as a therapeutic target [99]. In a transgenic mouse model, expression of constitutively active TGF-β type I receptor in fibroblasts is sufficient to cause dermal fibrosis [100]. Conversely, inhibition of TGF-β signaling results in reduced matrix deposition and scar formation in a rat model of wound healing [101] and prevents skin and lung fibrosis in the mouse model of sclerodermatous GVHD [102]. Deletion of *TGFBR2* expression in fibroblasts of transgenic mice results in altered wound healing, including reduced collagen deposition with increased macrophage infiltration, and accelerated re-epithelialization and wound closure [103]. This complex phenotype highlights the importance of TGF-β signaling in regulating a variety of cellular behaviors *in vivo*.

Due to its many profibrotic effects on both fibroblasts and macrophages, TGF-β signaling has been under intense investigation for the treatment of fibrosis in skin and other organs. Nevertheless, neutralizing antibodies against TGF-β have not been successful in clinical trials [93]. Alternative strategies for inhibiting the profibrotic effects of TGF-β include precisely manipulating other mediators of this pathway in order to avoid off-target effects of ligand inhibition [104] and combination therapies involving inhibition of both TGF-β and other profibrotic signaling pathways [105]. A currently promising strategy involves blockade of αV integrin-mediated activation of TGF-β. This membrane-associated integrin liberates the TGF-β ligand from its inactive complex with latent binding protein (Fig. 32.2). The

newly activated TGF-β then mediates myofibroblast conversion and *COL1A2* expression in SSc fibroblasts [106]. Integrin blockade is anti-fibrotic in pulmonary fibrosis [107], and genetic deletion of the αV subunit in mice is protective against lung, liver, and renal fibrosis [108]. Therefore, integrin blockade and other novel manipulations of TGF-β ligand activity are of continued interest for the treatment of skin fibrosis.

Embryonic Developmental Pathways: Wnt, Notch, and Hedgehog Signaling

Classic embryonic developmental pathways are often less utilized in healthy adult tissues, but are re-activated in the contexts of tissue regeneration, wound healing, and fibrosis [19, 109]. Notch, Hedgehog, and canonical Wnt signaling are three pathways that have important gene-regulatory roles in skin development, wound healing, and fibrosis [105, 110].

The canonical Wnt/β-catenin signaling pathway is active in the presence of extracellular Wnt ligands. Activation of cell surface receptors by ligand binding results in signal transduction by intracellular β-catenin and regulates target gene expression by the Tcf/Lef family transcription factors. Wnt signaling is important for dermal reconstitution during wound healing and contributing to fibroblast proliferation, migration, motility, and matrix production [111]. Wnt signaling is activated in hypertrophic scar and keloidal scar fibroblasts and the lesional dermis of SSc and morphea patients [112–114], perhaps due to elevated expression of Wnt ligands in the skin [115, 116]. Importantly, inhibition of Wnt signaling is therapeutic in mouse models of skin fibrosis [117].

Notch signaling depends upon the interaction between transmembrane Delta-like/Jagged ligand on one cell and transmembrane Notch receptor on an adjacent cell. Mediation of this interaction by the intracellular domain of the Notch receptor results in regulation of target gene expression by the recipient cell [110]. The Notch-1 receptor is required for normal wound healing in mice, and appears to promote macrophage recruitment as well as migration of fibroblasts and vascular endothelial cells [118, 119]. This signaling pathway has elevated activity in SSc skin, and inhibition of Notch signaling is therapeutic in mouse models of skin fibrosis [120].

Hedgehog signaling involves binding of an extracellular ligand such as Sonic hedgehog (Shh) to its transmembrane receptor Patched (PTCH). This signal is transduced intracellularly to regulate target gene expression [110]. In wound healing, Shh signaling is required for normal wound closure, granulation tissue formation, and vasculogenesis [121]. This signaling pathway has elevated activity in SSc skin, and inhibition of its transducer Smoothened (Smo) is therapeutic in mouse models of skin fibrosis [122].

CTGF

Connective tissue growth factor (CTGF/CCN2) is a matricellular protein that is typically secreted by mesenchymal cells, including fibroblasts and endothelial cells [123]. Its expression is induced by TGF-β signaling (Fig. 32.2) as well as other profibrotic signaling pathways [123]. In the extracellular space, CTGF can bind to integrins as well as other secreted proteins, including TGF-β1 [124]. Based upon these properties, it follows that CTGF can mediate fibroblast and endothelial cell adhesion and potentially TGF-β1 ligand bioavailability [124, 125]. Consistent with this, CTGF is sufficient to cause increased collagen and fibronectin synthesis in human fibroblasts *in vitro* [126].

CTGF expression is elevated in lesional fibroblasts and plasma from SSc patients. CTGF levels correlate with the severity of fibrosis, suggesting its possible utility as a biomarker of fibrotic disease [123, 124, 126]. The role of CTGF in fibrosis and wound healing has been tested using multiple animal models. In murine models of skin fibrosis, *CTGF* over-expression in fibroblasts is sufficient to cause dermal, renal, and pulmonary fibrosis; and deletion of *CTGF* in fibroblasts protects against bleomycin-induced dermal fibrosis [127, 128]. CTGF expression is also elevated in healing wounds in rats during granulation and vasculogenesis [129]. Finally, inhibition of CTGF mRNA in a rabbit model of scar formation resulted in reduced hypertrophic scarring with lower numbers of myofibroblasts, but with maintenance of normal wound healing with respect to inflammation and angiogenesis [130].

PDGF

Platelet-derived growth factors (PDGFs) are secreted growth factors that bind to cell surface receptors that are homo- or heterodimers of PDGFRα and PDGFRβ. The PDGFRs are receptor tyrosine kinases that, when activated, trigger downstream signaling cascades including the Ras/MAP kinase (ERK) pathway. PDGF ligands are produced by fibroblasts, endothelial cells, macrophages, and platelets [131]. Fibroblasts express the PDGF receptor and respond to this signal with increased proliferation, migration, expression of extracellular matrix genes (including collagen-encoding genes), and conversion to a myofibroblast phenotype [132, 133]. Murine dermal fibrosis is associated with activation of PDGF receptors, presumably due to increased production or availability of PDGF ligands [134]. In SSc, activation of PDGF receptors can occur in response to stimulatory autoantibodies against the receptor. These autoantibodies are able to stimulate tyrosine kinase activity, type I collagen gene expression, and myofibroblast conversion in human fibroblasts in vitro [135] and therefore may be an important factor in the pathogenesis of SSc (see Section Scleroderma/Systemic Sclerosis).

A study in mice found that transgenic expression of activated PDGFRA is sufficient to cause fibrosis in skin

and other organs [136]. Many more studies have evaluated readily available tyrosine kinase inhibitors (TKIs), which block PDGF signaling and other related pathways, to test for a possible therapeutic benefit of PDGF inhibition [137]. Imatinib mesylate, a TKI, inhibits PDGF signaling and reduces fibroblast proliferation, migration, collagen gene expression, and myofibroblast conversion in mouse wound healing and fibrosis models [132, 138]. One caveat is that some of the *in vivo* effects of imatinib and other TKIs may be due to inhibition of c-Abl (Fig. 32.2) or other protein tyrosine kinases [139, 140]. The therapeutic effects of imatinib against pre-existing dermal fibrosis in mouse models [141] provided a strong rationale for studies of its efficacy in SSc skin fibrosis. However, a phase II clinical trial did not demonstrate clinical efficacy over 6 months of treatment [142]. Additional studies addressing the profibrotic functions of specific tyrosine kinase receptors and the specificities of particular TKIs against these receptors will yield additional therapeutic options in the future.

Extracellular Matrix, Adhesion, and Mechanical Stresses

Recently, there has been a growing appreciation of the importance of tissue stiffness on cell behavior and gene expression, especially in the context of fibrosis and wound healing [143]. The fibrotic extracellular matrix has different mechanical properties from normal tissue, and this results in altered forces on fibroblasts and other cells. Elevated matrix stiffness *in vitro* results in increased conversion of dermal fibroblasts to a myofibroblast phenotype, and elevated expression of fibrosis-related genes such as *COL1A1* and *CTGF* [144]. The mechanoresponsiveness of fibroblasts may be via signaling pathways that have already been established to be profibrotic, such as TGF-β/Smad2/3 (Fig. 32.2) and Wnt/β-catenin pathways [145].

Adhesion molecules, namely integrins, also mediate effects of the extracellular environment on fibroblast biology. Focal adhesion kinase (FAK), the downstream signaling transducer of integrins, is required in dermal fibroblasts for mechanosensation during scar formation; mice lacking FAK in dermal fibroblasts have reduced CCL2-mediated recruitment of inflammatory cells after wounding. This results in reduced fibrosis in a hypertrophic scar model [146]. In addition, FAK is required for dermal fibroblast expression of fibrosis-related genes including *ACTA2*, *CTGF*, and *COL1A1* [147]. These findings suggest that modulating the extracellular matrix and mechanical environment during scar formation may be of therapeutic benefit. They also illustrate how physical factors can be integrated with molecular and cellular events during wound healing.

Genetic Mutations and Epigenetic Factors

Many of the classic profibrotic signaling factors exert their effects by altering expression of particular target genes. Consequently, cells and tissues in fibrosing diseases and related animal models have characteristic gene signatures that have been defined by microarray-based studies. These gene signatures are useful for identifying up- or downregulated genes that encode novel mediators of fibrosis, both in human disease [99, 148] and animal models of fibrosis [149]. In SSc, these genetic profiles can be used to divide patients into stable subtypes that predict the clinical response to treatment [150–152]. Gene expression studies can also yield insight into the pathogenesis of fibrotic disease, for example by the discovery of a B lymphocyte expression signature in SSc patient skin [42]. Next-generation sequencing technology has recently enabled the discovery of elevated fungal (*Rhodotorula*) gene transcripts in SSc patient skin, suggesting that this pathogen may play a provocative role in susceptibility to or in the pathogenesis of SSc [153].

In addition to altered regulation of protein-coding genes, fibrotic conditions can be associated with genetic mutations. Namely, a mutation in the protein-coding region of *FBN1*, which encodes fibrillin-1, causes stiff-skin syndrome [154]. This mutation is an in-frame duplication, in contrast to the single point mutation in *FBN1* that causes Marfan syndrome. In addition, several single-nucleotide polymorphisms (SNPs) were found to be associated with fibrotic disease, including a SNP in the promoter region of *IL1A* in SSc patients [155, 156].

The presence of broad changes in gene expression, as well as the stability of the fibrosis phenotype in lesional fibroblasts cultured *in vitro*, has provided strong evidence for the role of epigenetic changes in fibrosis [157]. Epigenetic regulation of gene expression is often repressive in nature and can be mediated by micro-RNAs (miRNAs), methylation of DNA, and chemical modifications of DNA-associated histone complexes [158]. Down-regulation of micro-RNA (miR)-29 has been found in the skin of SSc patients and the bleomycin-induced mouse model of skin fibrosis, and miR-196a is downregulated in keloidal fibroblasts. Both of these miRs appear to inhibit expression of type I and type III collagen genes in dermal fibroblasts; therefore, decreased expression of each miR could enable elevated expression of collagen in the dermis [159, 160].

A role for DNA methylation in fibrosis has been found in dermal fibroblasts from SSc patients, where the promoter region of *FLI1*, which encodes a repressor of *COL1A1*, is heavily methylated. This methylation results in repressed expression of *FLI1*, which enables increased expression of type I collagen [161]. A role for decreased histone modifications in fibrosis has been identified in mice, where inhibition of methylation of lysine 27 on histone H3 (H3K27me3) is sufficient to cause dermal fibrosis, possibly by enabling expression of the profibrotic transcription factor Fra2 [162].

The increasing feasibility of genomic studies in human patients and mouse models will certainly yield additional novel pro-fibrotic mediators that may serve as therapeutic targets or biomarkers of disease progression, in addition to providing insight into how epigenetic factors might contribute to the pathogenesis of fibrosis.

Cutaneous Wound Healing

Wound healing is a physiologic process that involves many of the same cellular players and molecular pathways that promote pathologic fibrosis. Cutaneous wound healing serves to prevent infection and to repair the wound, thus preventing dehydration and restoring the protective characteristics of intact skin. In adult human skin, this process is not completely restorative, since the resulting scar tissue is mechanically weaker than native dermis and lacks functional structures, such as hair follicles and sweat glands [163]. Consequently, several studies of wound healing in humans and animal models have focused on elucidating the factors that drive fibrotic repair as opposed to faithful regeneration of skin to its original form and function.

Principles of Wound Healing

In adult mammalian tissue, partial- and full-thickness cutaneous wounds are resolved in a series of phases. Each phase involves particular cellular constituents and signaling pathways. The initial injury results in vascular disruption at the wound site, with activation of the clotting cascade producing a fibrin clot (Fig. 32.3). In the inflammatory phase of wound healing, which begins within hours of the injury, platelets in

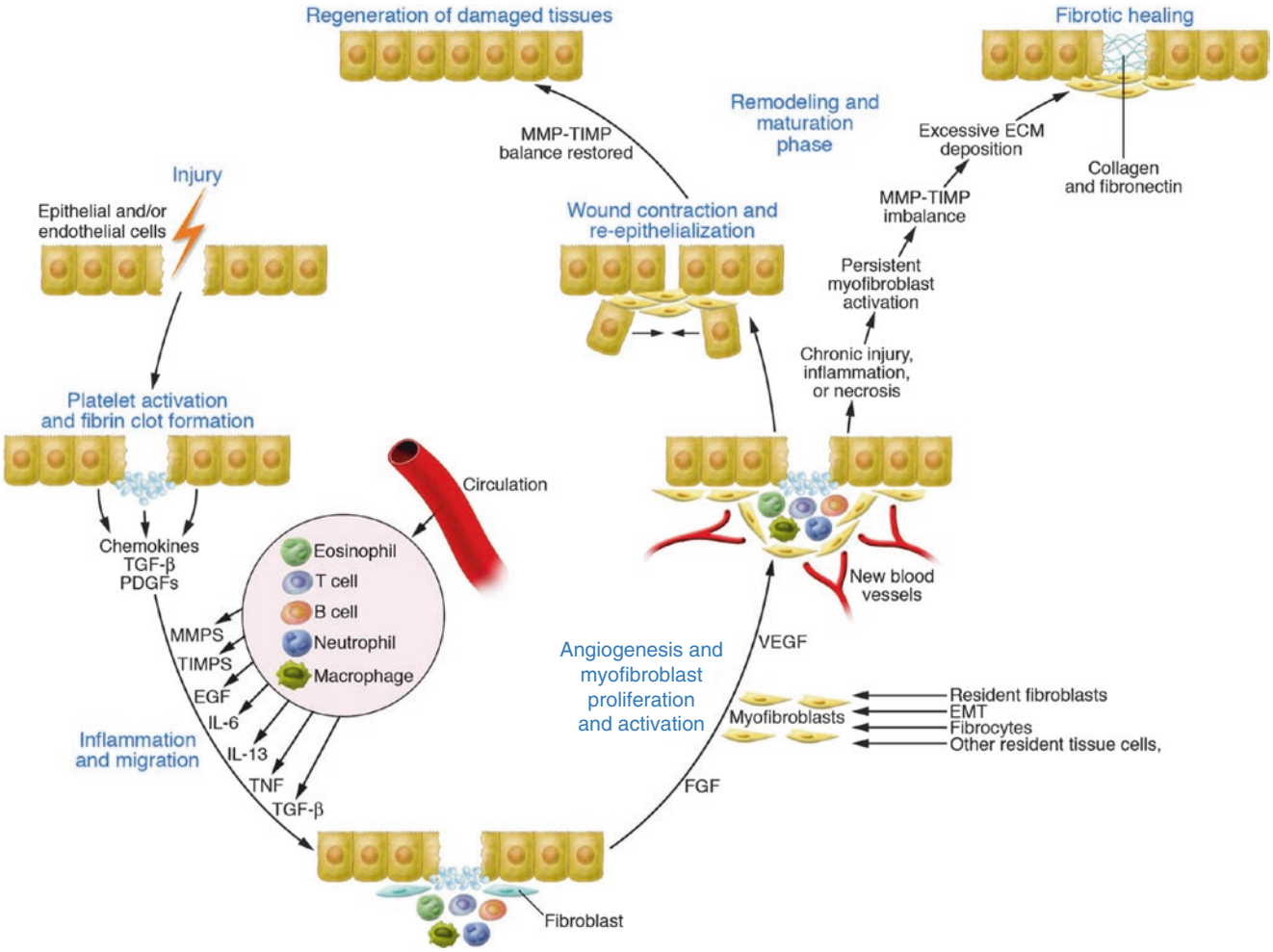

Fig. 32.3 Wound healing: regeneration and fibrosis. Following injury, signaling molecules influence the migration of fibroblasts, macrophages, and other inflammatory cells to the wound. Myofibroblasts, derived from a variety of potential sources, initiate the deposition of extracellular matrix elements and contract the wound. Provided re-injury and/or chronic inflammation do not ensue, tissue regeneration should occur. Persistent inflammation and injury signals may result in excessive matrix deposition, leading to a fibrotic scar. Many fibrosing conditions, in lieu of an actual wounding event, can elaborate similar inflammatory signals that also result in fibrosis and potential organ dysfunction (Figure utilized with permission from Wynn [68])

the clot release chemotactic factors that recruit neutrophils and monocytes to the site [2]. Neutrophils predominate during the early inflammatory phase to protect against infection [164]. Monocyte-derived tissue macrophages predominate later in the inflammatory phase (within days following the wound) and release growth factors that are important for new tissue formation [62].

The new tissue that forms at the wound site during the days and weeks following the initial injury is called granulation tissue. Cells in the local inflammatory infiltrate produce growth factors that promote fibroproliferation and migration of fibroblasts, including myofibroblasts, into the wound bed [5, 6]. Angiogenesis and vasculogenesis, which involve proliferation and migration of endothelial cells, also occur during this phase. Wound repair also requires re-epithelialization over the granulation tissue and is dependent upon growth and chemotactic factors released by activated keratinocytes and underlying fibroblasts [2]. Particular populations of epidermal stem cells, which have been carefully defined in murine studies (see Section Principles of Wound Healing), contribute to the newly forming epidermis.

In the weeks to months following injury, the granulation tissue is replaced by mature scar tissue. Inflammation resolves and myofibroblasts undergo apoptosis [6]. Mature scar tissue is more collagenous than granulation tissue, better approximating the dermal extracellular matrix [2]. However, mechanical and functional properties of the skin are not wholly restored to their original state [165].

Repair and Regeneration in Animal Models of Wound Healing

Studies of wound healing in transgenic mice have defined specific populations of cells that contribute to tissue repair and regeneration. Extensive lineage-tracing experiments have labeled epidermal stem cell and progenitor populations and followed their contribution to re-epithelialization following wounding [166]. This work has shown that the healed epithelium is derived from epidermal stem/progenitor populations peripheral to the wound [167, 168].

Similar lineage-tracing studies have recently defined the mesenchymal populations that contribute to the dermal component of wound healing. PDGFRα-expressing fibroblasts residing in the upper (papillary) and lower (reticular) dermis adjacent to the wound bed contribute substantially to the healing dermis, with earlier and more robust invasion by the deeper dermal fibroblasts [169]. These fibroblasts may be recruited or supported by intradermal (subcutaneous) adipocytes, which are required for normal wound healing [170].

Mammalian fetal skin and lower vertebrates, such as zebrafish and amphibians, are champions of scarless wound healing and perfect tissue regeneration in the absence of fibrotic repair [110, 163]. One of the key differences between scarring and scar-free wound healing in these studies is that animals that can heal wounds without scars are relatively immunodeficient, suggesting that inflammation is directly related to fibrotic wound healing. Determining the specific cell types and molecular pathways engaged in tissue regeneration may point toward therapeutic strategies to help regenerate functional skin (or other organs) after injury without scarring and loss of organ function. Other than fetal wound healing, there are few examples of true regeneration in mammalian tissues. However, one such example is adult murine wound-induced hair neogenesis, in which a subpopulation of γδ T cells has been shown to be important for the formation of neogenic hair follicles in healing full-thickness wounds by regulating dermal fibroblasts to acquire more inductive characteristics [171, 172]. Continued studies of this and similar regenerative phenomena may provide provocative keys to promoting regeneration instead of scar formation during cutaneous wound healing and fibrosing skin disorders.

Cutaneous Fibrosing Disorders

Cutaneous fibrosis is a final common pathway for many disorders characterized by chronic inflammation and/or tissue injury. As noted in the preceding sections, a complex interplay of cells, growth factors, chemokines, cytokines and signaling pathways are involved in the processes of wound healing, injury recovery, and matrix homeostasis. Imbalance of any of these factors has the potential to create self-sustaining feedback loops of signaling that eventuate in fibroblast/myofibroblast activation and excess tissue matrix deposition. While a detailed mechanistic discussion of all such conditions (Table 32.1) is beyond the scope of this chapter, an in depth discussion of several common and/or well-characterized conditions is provided in the following sections to illustrate how pathological fibrosis can be triggered and sustained.

Scleroderma/Systemic Sclerosis

Clinical

Scleroderma (also known as systemic sclerosis, SSc) is a chronic progressive systemic disease of unknown etiology, characterized by autoimmunity, inflammation and multi-organ tissue fibrosis [173, 174]. Various lines of evidence suggest SSc is caused by interactions between the host genetic background and certain environmental factors [175]. The pathophysiology of SSc is characterized by (1) immune system activation/autoimmunity, (2) proliferative and obliterative

Table 32.1 Disorders commonly associated with cutaneous fibrosis

Dermatofibroma
Diabetic digital sclerosis
Eosinophilic fasciitis (Shulman syndrome)
Hypertrophic scar
Keloidal scar
Lichen sclerosus
Lipodermatosclerosis
Morphea
Nephrogenic systemic fibrosis
Porphyria cutanea tarda (sclerodermoid)
Radiation induced fibrosis
Scleredema diabeticorum
Sclerodermoid GVHD
Scleromyxedema
Stiff skin syndrome/congenital fascial dystrophy
Systemic sclerosis (scleroderma)
Toxin-associated (Spanish toxic oil syndrome, vinyl chloride)

vasculopathy, and (3) tissue fibrosis [176]. The clinical presentation of SSc is heterogeneous and can be subdivided into two entities based upon the level of skin involvement: limited (with fibrosis distal to the elbow and knee including the face), and diffuse (including proximal fibrosis) [177]. A subset of patients with limited cutaneous SSc present with "CREST" syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia). While both limited and diffuse SSc can exhibit internal organ involvement, life-threatening damage to the lungs, heart, gut, and kidneys is more common in diffuse SSc. Localized scleroderma (morphea, linear scleroderma) does not have internal organ involvement [176].

Histopathology

Skin biopsies from patients with SSc are essentially indistinguishable from those with localized scleroderma (morphea). In both entities there is thickening of collagen with loss of the clefts between the bundles. Over time, a relatively acellular hyalinized matrix replaces the fibrillar collagen. There is a variably dense superficial and mostly deep perivascular and interstitial lymphoplasmacytic infiltrate with occasional eosinophils that typically extends into the underlying subcutis by widening of the fatty septa. Loss of peri-eccrine fat is typical [178].

Autoimmunity

Oligoclonal expansion of T cells, induced by presumed environmental antigens, have been observed in SSc [179]. A variety of autoantibodies may be found in SSc [180, 181], although there is disagreement regarding their roles. Some investigators believe the autoantibodies are directly pathogenic while others suggest they may be epiphenomena, arising only after tissue damage has already occurred [182]. It is surmised that some, including antinuclear antibodies (ANA) and anti-endothelial cell antibodies, lead to the microvascular endothelial apoptosis that has been identified as the initiating pathogenic event in SSc [26, 183]. Damage to the microvascular endothelium leads to the release of proinflammatory mediators, growth factors, and chemokines.

Vasculopathy

Raynaud's phenomenon, usually the earliest clinical sign of the disease [184], signals the presence of vasomotor dysregulation and vasculopathy. Endothelial damage triggers the activation of the coagulation and fibrinolytic cascades and ultimately the vascular occlusion, capillary necrosis, and capillary dropout seen in SSc [175, 185]. Loss of capillaries leads to tissue hypoxia, which, in turn, induces pro-angiogenic signals [186]. Other investigators have shown that exposure of endothelial cells to IFN-γ stimulates the profibrotic mediators ET-1, CTGF and TGF-β2, and that these induce the transition of endothelial cells to myofibroblasts [187].

Fibrosis and Matrix Alterations

The inappropriate leukocyte accumulation triggered by antibody-driven vascular damage leads to the elaboration of TNF (tumor necrosis factor), the release of degradative

matrix metalloproteinases (MMPs), and the production of reactive oxygen species (ROS), which perpetuate additional tissue injury. With tissue hypoxia as a driving force, multiple genes for chemokines, cytokines, growth factors and their receptors are upregulated [175]. Fibroblasts respond to hypoxia by producing proteins involved in the remodeling of the ECM, such as TGF-β, thrombospondin, and CTGF [188] (see Section Growth Factors/Morphogens). In SSc, serum levels of tissue inhibitors of metalloproteinases (TIMPs) are significantly elevated, leading to an imbalance in the turnover of the ECM that favors matrix deposition [182]. In skin biopsies from SSc patients, TIMP-1 is markedly elevated while MMP-1 is not detectable [189].

The net result of these processes is the production of "damage-associated membrane proteins" (DAMPs). DAMPs, which result from cell damage, oxidative stress, and ECM remodeling, and are recognized by Toll-like receptors (TL4) on the surface of fibroblasts. One particular DAMP, a variant of fibronectin (FnEDA), seems to fulfill the functions of both structural ECM scaffold and signaling molecule [173]. FnEDA is present in significantly elevated quantities in the serum and tissue of patients with SSc. Increased FnEDA increases collagen cross-linking, promotes myofibroblast differentiation, and increases collagen gene expression. FnEDA expression, normally absent in adult tissue except during tissue injury, can also be expressed by normal fibroblasts when stimulated by TGF-β. In turn, activated fibroblasts can produce additional FnEDA, which then perpetuates a self-sustaining feedback loop that promotes the ongoing profibrotic milieu and the differentiation of a steady supply of matrix producing myofibroblasts [173].

Myofibroblasts are the primary effector cells in SSc [174], although their precise origin is debated (see Section Activated Fibroblasts and Myofibroblasts). Myofibroblasts may potentially derive from several sources (Fig. 32.1) including circulating fibrocytes (CFs), resident fibroblasts, pericytes, and epithelial or endothelial cells (via epithelial- and/or endothelial- to mesenchymal transition) [182].

The expression of genes in SSc has offered a few clues. In one gene microarray study comparing cultured fibroblasts from patients with diffuse SSc to those from normal control patients, only down-regulation of the gene MMP9 was significant after quantitative PCR analysis [190]. Another study comparing lesional and non-lesional fibroblasts in patients with diffuse SSc by gene microarray analysis showed that 26 markers performed well as predictors of fibrosis [191]. The findings of several such studies suggest that SSc is characterized by a proinflammatory gene profile that includes TGF-β and Wnt expression [192]. Activation of the Wnt pathway also stimulated an increase in fibroblast migration and proliferation, activities known to be critical in SSc [113] (Section Growth Factors/Morphogens).

Patients with SSc have also been shown to have elevation of sonic hedgehog protein (Shh) in skin fibroblasts, endothelial cells, and keratinocytes. Shh can induce myofibroblast differentiation, and its inhibition has resulted in anti-fibrotic effects in a model of SSc [122] (Section Growth Factors/Morphogens).

In human SSc, cutaneous CFs (CD45+/Collagen+) are present at high levels in the dermis, and are attracted along a chemical gradient of CCR5 (which is overexpressed by SSc monocytes). CSD (Caveolin-1 scaffolding domain peptide) has been shown in a mouse model of SSc to inhibit the accumulation of CFs, CCR5, and the CCR5 ligands CCL3 and CCL4 in the dermis. These results parallel the findings in a similar study examining lung fibrosis triggered by bleomycin injury, suggesting blockade of this CCR5 axis may have general applicability in many fibrotic conditions [91].

Nephrogenic Systemic Fibrosis

Clinical

Nephrogenic systemic fibrosis (NSF), first described in 2000, is an acquired fibrotic dermatosis associated with the use of gadolinium-containing magnetic resonance contrast agents in the setting of renal insufficiency [193]. While there is lingering controversy over the precise triggering events, evidence supports the hypothesis that certain chemical formulations of these agents are capable of promoting fibrosis in patients with transient or chronic renal disease [194, 195].

The clinical lesions of NSF typically present on the extremities (legs more commonly than arms), and occasionally involve the trunk, buttocks, and in very rare instances, the face. They consist of papules, plaques and nodules that coalesce into erythematous to brawny skin thickening, often with a peau d'orange appearance. Encompassing dermal fibrosis near joints often leads to restriction of range of motion and contractures. Pruritus and burning pain are common clinical complaints, and sometimes there is palpable warmth. It is not uncommon to observe an evolution of lesions that appears first as dependent edema, gradually becoming more erythematous, and then evolving into woody induration [193, 194].

Microscopic

Biopsies of early NSF are remarkably bland, with splaying of dermal collagen by edema and increasing numbers of banal, spindle cells in the dermis, that frequently extend into the subcutaneous tissue along fibroconnective septa [193]. There is no inflammation, although small collections of

mononuclear cells may be noted adjacent to blood vessels. Fully involved biopsies show markedly thickened dermal collagen bundles associated with a complex network of CD34+ dermal spindle cells. Besides the membranous staining observed with CD34, the cytoplasm of these cells stains with procollagen I [16, 194]. Procollagen I can also be found in the dermis between these fibrocytes, along with increased amounts of dermal elastin and mucin. Collagen bundles are typically separated by clefts from one another, and a mix of thick and thin bundles is typical. These findings extend into the subcutaneous tissue along septa, and will frequently infiltrate the fascia and underlying skeletal muscle. CD68+ histiocytes are also present in variable numbers, sometimes forming multinucleated giant cells. In a small number of cases pale pink osteoid forms around protruding elastin fibers creating the so-called "lollipop" body, which is considered virtually pathognomonic for NSF. On occasion these bodies will mineralize [194]. Rarely, widespread mineralization of dermal collagen can occur. Initial tissue staining studies showed markedly increased TGF-β1 mRNA by in situ hybridization throughout the dermis of all patients examined [196].

Triggers

Epidemiological evidence has convincingly established that NSF is a disease of the renally impaired. Patients at risk for NSF have acute kidney injury (AKI) or an estimated glomerular filtration rate (eGFR) of less 30 ml/min/1.73 m^2. Investigators have concluded that NSF is triggered by the administration of gadolinium based contrast agents (GBCAs) to patients with the requisite degree of renal impairment. Gadolinium (Gd) is a paramagnetic element of the lanthanide series, making it an ideal contrast agent in magnetic resonance imaging (MRI) studies. As ionized Gd^{3+} is considered toxic because of its potent calcium channel blocking abilities, the ion is chelated with a proprietary organic ligand that may be linear or macrocyclic in structure. This reduces the toxicity of Gd while simultaneously facilitating the excretion of the chelated complex, which in a patient with normal renal function has a half-life of about 90 min. Over the years since the discovery of NSF, there have been several brands of GBCA available on the market in the United States and abroad. Epidemiological evidence has associated the vast majority of cases of NSF with three brands of GBCA, these being agents with a linear organic ligand. The use of these agents in the renally-impaired has been specifically contraindicated by regulatory agencies, leading to a very rapid decline in new cases of NSF since 2010 [195].

While these agents were still being used in the at-risk population, it became clear that only 2–6 % of those at risk actually contracted NSF upon exposure to one or more doses of the agent. The reasons for this variability in the at-risk population are unknown, although some have surmised there might be a genetic propensity or some other as yet unrecognized risk factor for NSF [197].

Cell Culture Studies

Epidemiological and clinical aspects of NSF have implicated excess extravasated GBCA as the initial triggering event in NSF. Electron microscopy with energy dispersive spectroscopy has identified Gd within the macrophages seen in abundance in early lesions of NSF [198]. The dominant cells in the histopathological specimens from NSF, macrophages and fibrocytes, have been extensively studied by cell culture techniques.

Fibroblasts/Fibrocytes

In 2003, a compelling case was made for the involvement of bone marrow-derived circulating fibrocytes in NSF [16]. Circulating fibrocytes, defined by their dual marker positivity for CD34 and procollagen I, participate in normal wound healing and have been shown to be active in several fibrotic processes. In their fully mature form they histologically resemble and likely supplement the activities of resident dermal fibroblasts.

When added to the serum of normal cultured fibroblasts, Omniscan™, the GBCA associated with the majority of NSF cases, increases the fibroblast proliferation by more than two-fold. It also increases MMP-1 (1.5-fold) and TIMP-1 (12-fold). While individual cell procollagen production was not directly stimulated, the cumulative effects of increased fibroblast proliferation and altered collagen turnover were judged to contribute to the fibrosis of NSF [199]. While both MMP-1 and TIMP-1 are increased, the vast excess production of TIMP-1 effectively outweighs the collagenolytic activity provided by MMP-1 [200]. The increased TIMP-1 and absent MMP-1 pattern has been directly visualized in immunohistochemical studies from NSF biopsy specimens, corroborating the culture observations [189].

Dermal fibroblasts obtained from patients with NSF display a marked increase in the production of type I (2 to 3-fold) and type III (3.5 to 6-fold) collagen, fibronectin (3 to 7-fold), and hyaluronic acid (4 to 5-fold). This effect was maintained after 11 culture passages, suggesting the effect might be a stable epigenetic change. Collagen I production was increased at both the translational and transcriptional levels. The increased transcriptional activity of the COL1A1 promoter was due to increased cREL, NF1, and CBF transcription factor binding [201]. In NSF, cREL and NF1 appeared to show the most binding

activity, in contrast to CBF and Sp1, which are the most active transcription factors in SSc [202]. The elevation of TGF-β combined with the markedly increased cREL component of the NF-kB family of transcription factors suggests that there may be an important interaction between these pathways in NSF [201].

A culture study conducted on normal human fibroblasts concluded that Gd^{3+} directly or indirectly modulates signaling through the PDGF receptor. The investigators postulated that Gd^{3+} attaches to an available protein or some other "carrier molecule" that allows it to remain soluble long enough to interact with the PDGF receptor. They also noted that fibrotic changes similar to those seen in NSF have been produced in rats exposed to other chelated lanthanides, suggesting that the observed effects are not Gd specific. This observation offers the intriguing possibility that metallic elements other than Gd, in the correct clinical setting, may be capable of interacting with the PDGF receptor to produce similar fibrotic outcomes [203].

Another study examining normal human fibroblasts concluded that the PDGF stimulatory effects of Omniscan™ could be abrogated by exogenously administered CCN3 (also known as nephroblastoma overexpressed gene). CCN3 is a matricellular protein that is able to suppress CTGF/CCN2, a molecule critical to the initiation and progression of renal fibrosis [204]. The authors noted that Omniscan™ suppressed the endogenous production of CTGF/CCN2, leading to a permissive environment for both PDGF and TGF-β driven fibrogenic processes [205].

Monocyte/Macrophages

In human peripheral blood monocyte studies, Gd compounds caused significant upregulation of multiple cytokines and growth factors, including VEGF (253-fold), IL-13 (68-fold), IL-4 (28-fold) and IL-6. TGF-β was increased two to three fold (depending on the agent used) and IFN-γ was elevated up to 50-fold. The timing and magnitude of the effects varied with the agents used and the subjects, although intrasubject response was stable [206]. Normal fibroblasts cultured with media conditioned by peripheral blood monocytes that had been exposed to Omniscan™ displayed a dose-dependent increase in production of type I procollagen [206].

In a gene microarray evaluation of normal human macrophages incubated with 50 mM Omniscan™, 551 differentially expressed genes were noted. There was potent stimulation of CCL8 (669 ± 108-fold), CXCL10 (401 ± 72-fold), CCL2 (245 ± 36-fold) and CXCL11 (551 ± 48-fold). Pathway analysis proved that exposure to Omniscan™ elicits chemokine gene expression in macrophages that is dependent on NFkB activation [207]. As it is widely accepted that circulating fibrocytes home to the skin following a specific chemokine gradient, the investigators concluded that macrophages stimulated by this mechanism produce the chemokines needed for fibrocyte chemotaxis to skin.

In addition, the effects of Omniscan™ and gadodiamide in macrophages are dependent on TLR4 and TLR7. It is hypothesized that these agents possess a unique, specific molecular shape or pattern that renders them capable of this interaction. Initial engagement of TLR4 by Omniscan™ at the macrophage surface could induce and increase the rates of phagocytosis, which could amplify its TLR signaling capacity through the engagement of TLR7 within the endosome [208]. As fibroblasts also express surface TLR4, it is possible that some of the direct effects of Gd compounds on fibroblasts could be due to engagement of these receptors (Fig. 32.4) [208].

These investigators conclude that all Gd compounds examined were capable of inducing expression of genes associated with TLR signaling and that the shape of individual GBCAs, in combination with direct effects of free Gd^{3+} likely both contribute to NSF pathogenesis [209].

Animal Modeling

Utilizing a rat model of NSF exposed to gadodiamide (the active ingredient in Omniscan™) investigators have induced high levels of serum cytokines, the most significantly elevated being CCL2 (6.5-fold) and CCL7 (6.8-fold), macrophage inflammatory proteins CCL4 (2.8-fold) and CXCL2 (2.5-fold), TIMP-1 (3.6-fold), TNF-α (3.4-fold), osteopontin (6.1-fold), and VEGF (1.6-fold) [210]. The role of osteopontin is particularly intriguing to these investigators as it is known as a mediator in lung disease, interacting with receptors (CXCR4, CCR7) of circulating fibrocytes [211].

These same investigators also noted a drop in serum albumin that correlated with marked vascular permeability within 6 h of gadodiamide exposure [212], a finding that parallels the early edema and erythema seen in NSF patients clinically. This tissue vascular leakage facilitates the extravasation of GBCA into the extravascular space, where it is subsequently phagocytosed by macrophages.

Fascinating work completed using mouse modeling has shown that Gd^{3+} and GBCA activate the NLRP3 inflammasome, primarily in undifferentiated and LPS-primed M2 macrophages [213]. The NLRP3 inflammasome is involved in the rapid recruitment of inflammatory monocytes and granulocytes to the site of Gd deposition in vivo, and can be stimulated by extracellular calcium and Gd^{3+} through a calcium-sensing receptor (CASR) [214]. GBCAs also induce the production of mature IL-1β by macrophages in vitro through NLRP3-dependent mechanisms. IL-1β can promote fibrosis by stimulating fibroblasts and by inducing TGF-β1 [213].

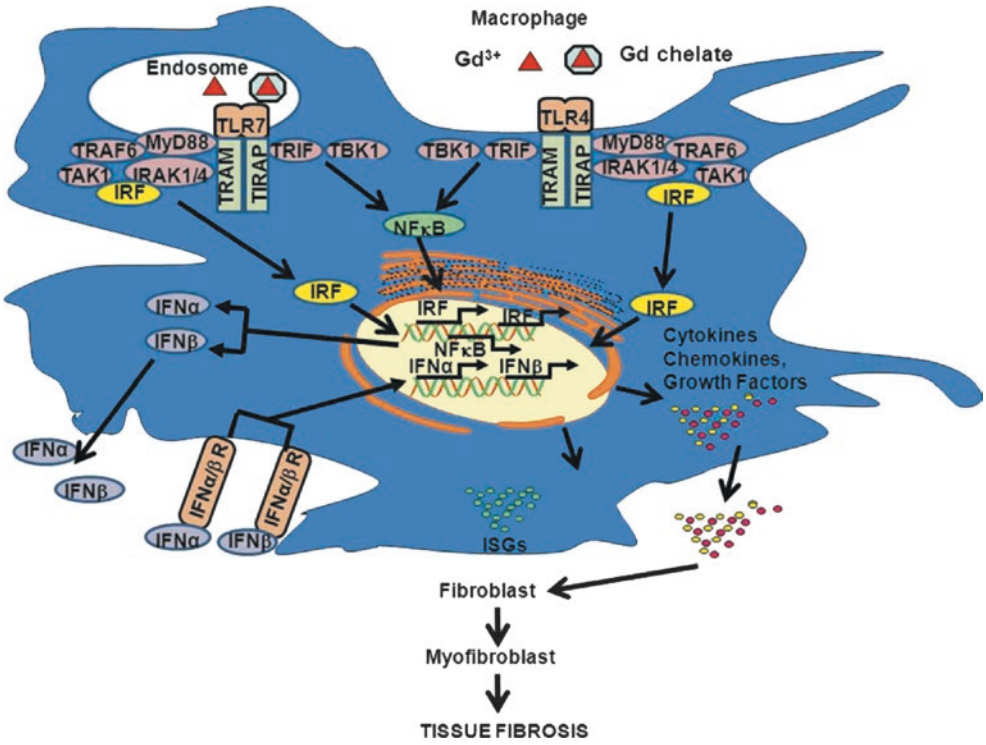

Fig. 32.4 Gadolinium based contrast agent (GBCA) induction of a proinflammatory/profibrotic phenotype in normal human macrophages. Gd^{3+} or GBCA is phagocytosed. Within the acidic environment of the endosome, dechelation is facilitated. Either Gd^{3+} or GBCA interact through TLR 4 and 7 to upregulate the NFkB protein complex, leading to the production of proinflammatory and profibrotic factors that are subsequently secreted by these "activated" macrophages. These secreted factors facilitate the development and migration of myofibroblasts from a variety of tissue sources, which then result in sustained and dysregulated matrix production (Figure utilized with permission from Wermuth and Jimenez [209])

Keloids

Clinical

Keloids are defined as exuberant scar tissue formation extending beyond the area of original tissue injury [215]. Clinically, keloids are firm broad nodules with a shiny surface that often have a claw-like appearance beyond the original site of injury [215]. Keloids may show a delayed onset, sometimes forming months after the original injury. They can be painful or pruritic [215]. Persons of African or Asian heritage are reportedly ten times more susceptible to keloids than are whites [216].

Microscopic

Histologically, keloids show thickened, hyalinized collagen fibers that frequently form whorls and nodules. The collagen fibers can be large and irregular, and tend to be organized somewhat randomly. Blood vessels and ground substance are more abundant than in typical scars, and the epidermis can be effaced [217]. Partial occlusion of blood vessels by exuberant endothelial cell growth has led some investigators to hypothesize that keloids arise in a hypoxic milieu [218]. Some believe that keloidal fibroblasts may derive from pericytes that accompany the attendant hypoxia-driven proliferation of capillary endothelial cells [28].

Culture Studies

Cultured keloid derived fibroblasts (KDFs) have been shown to have several unusual growth characteristics. KDFs overproduce type I procollagen while keeping type III procollagen levels unaltered. This results in a markedly increased type I/III procollagen mRNA ratio [219]. The rate of collagen synthesis in KDFs is twice that of normal scars at six months, and continues to be higher for two to three years after wounding [217]. Collagenase activity is also increased in KDFs, although the abundance of chondroitin-4-sulfate may make collagen fibers resistant to degradation. Keloidal collagen has a low proportion of cross-linking despite having normal lysyl-oxidase activity [217].

KDFs have a reduced growth factor requirement for proliferation and have been reported to grow to higher cell densities in low-serum medium than normal dermal fibroblasts [220]. In a three-dimensional culture system of KDFs, type I and III procollagen levels remain high, despite the tendency for both procollagen types to gradually diminish over time in cultures of normal fibroblasts [221]. KDFs overproduce fibronectin and maintain its high expression over time as well [222].

Three dimensionally cultured KDFs have a high level of plasminogen activator inhibitor-1 (PAI-1) expression and a low level of urokinase plasminogen activator expression [223]. The elevated PAI-1 expression seems to be induced by hypoxia, and is supported by the observations of increased levels of the hypoxia marker HIF-1a (hypoxia induced

factor-1a) [224] and increased vascular endothelial growth factor (VEGF) expression, also triggered by hypoxia [225].

TGF-β1 induced collagen synthesis is more marked in KDFs than in those from the normal dermis [226]. This effect can be further increased after co-stimulation with insulin-like growth factor 1 (IGF-1), an effect correlated with increased phosphorylation of p38 MAP kinase and ATF-2 [227]. KDFs exposed to TGF-β1 showed increased production of reactive oxygen species (ROS) via the action of NAPDH oxidase 2 (NOX2). NOX2 has been shown to be essential for the transformation of KDFs to myofibroblasts in response to TGF-β1. NADPH oxidase-derived ROS also stimulate MAPK phosphorylation and Smad2/3 activation [228].

Ladin et al. [229] demonstrated that the apoptosis rate of KDFs was relatively low, an observation corroborated by others [230, 231]. KDFs are significantly more resistant than normal fibroblasts to Fas-mediated apoptosis [232]. In addition, their over-expression of IGF-1 receptor protects KDFs from ceramide-induced apoptosis [233]. Co-culture of KDFs with keloidal keratinocytes induced the expression of anti-apoptotic bcl-2 and the phosphorylation of ERK and JNK in KDFs, also known to be anti-apoptotic [215].

Normal keratinocytes have been shown to express IL-1, which is known to stimulate the expression of keratinocyte growth factor (KGF) in dermal fibroblasts. Co-culture experiments using keloidal and normal keratinocytes and fibroblasts have yielded interesting results. In these studies, keloid-derived keratinocytes promoted the proliferation of KDFs to a greater extent than keratinocytes derived from normal skin, lending credibility to the hypothesis that keloid pathogenesis involves interactions between abnormal fibroblasts and abnormal keratinocytes [215].

An enhanced response of KDFs to PDGF has also been reported [234].

Gene Studies

To date, no single gene mutation sufficient for keloids has been reported. The multiple growth and behavior anomalies noted in cultured KDFs suggest that a single gene could not account for all of the findings [215].

In DNA microarray studies, KDFs show an upregulation of approximately 15% of genes. Among the most pertinent of these are interleukin (IL)-1α, IL-1β, IL-6 and TNF-α [235, 236].

The NF-kB signaling pathway is activated in KDFs. NF-kB is involved in signal transduction pathways essential for the transcription of a variety of pro-inflammatory genes, and it is presumed that its activation contributes to the persistence of inflammation in keloids and also the inhibition of apoptosis in keloidal fibroblasts [236]. The observed clinical inhibition of keloid formation by glucocorticoids may be mediated through inhibition of this signaling pathway [237].

Proteomic analysis of normal and keloidal tissue has revealed 11 key proteins with altered expression in keloids. Of these, annexin A2 was prominently downregulated in keloidal tissue [238]. Annexin A2 is a profibrinolytic receptor that assists in localizing fibrinolytic activity to endothelial and monocyte cell surfaces [239]. In annexin A2 deficient animals, the primary histological feature is the accumulation of fibrin within microvessels in all tissues examined [240]. Additionally, keloid derived fibroblast proliferation, ordinarily inducible by recombinant human epidermal growth factor, is blocked by blocking the action of annexin A2. These findings suggest that the observed annexin A2 downregulation would result in fibrin accumulation within vessels and diminished fibroblast proliferation. In scars, a decrease in annexin A2 inhibits wound healing at the remodeling phase. It may be that keloids result from an inability to properly remodel collagen laid down following injury [238].

Lichen Sclerosus

Clinical

Lichen sclerosus (LS) is an acquired, chronic, inflammatory dermatosis with a predilection for genital skin. Symptoms include pruritus and scarring of skin and squamous mucosa. LS can lead to physical morbidity and serious sexual dysfunction [241]. Longstanding cases may be complicated by squamous cell carcinoma [241, 242]. Women are reportedly affected up to ten times more frequently than men, and several studies have identified an increased rate of coincident autoimmunity [242].

The underlying cause of LS is unknown although genetic, infectious and environmental triggers have been proposed [241, 242]. Some investigators offer strong circumstantial evidence that genital LS is due to chronic, intermittent exposure to urine, and that in certain individuals this may unmask site-specific skin epitopes that promote the development of autoantibodies [241].

The clinical lesions of LS consist of indurated papules and plaques with ivory-white, scar-like atrophy. The lesions commonly involve the vulva and perianal areas in women and the distal penis in men. Extragenital involvement of the scalp, neck, palms and soles, oral cavity, and peristomal sites has also been reported [242].

Microscopic

Early biopsies of LS reveal a band-like infiltrate of lymphocytes at the dermal-epidermal junction. As the process continues, this infiltrate retreats into the deeper dermis, leaving a homogenized and hyalinized (sclerotic) matrix in its wake [242]. Within the

hyalinized zone elastic fibers are typically lost [243]. The inflammatory component consists chiefly of T lymphocytes, and these are joined in later stages by macrophages and mast cells. Over time, initially normal or thickened epidermis becomes markedly atrophic.

Lipoid proteinosis, a rare genodermatosis associated with pathogenic mutations in the extracellular matrix protein 1 (ECM1) [244], shares some histopathological features with LS. This observation prompted investigation of the potential role of ECM1 in the pathogenesis of LS. In 2003, investigators found circulating IgG class antibodies to ECM1 in 74 % of female patients with LS [242] and subsequent work has confirmed similar results in male patients with LS [241]. There are mixed opinions regarding whether the circulating autoantibodies are responsible for triggering LS or are a consequence of the unmasking of antigens after inflammatory damage has already occurred. The finding of a variety of other autoantibody populations in LS has led many investigators to favor the latter interpretation [245, 246].

Matrix Analysis

The profound histopathological changes seen in the zone of dermal sclerosis in LS has focused attention on the constituents of the ECM. Investigators have determined that the distribution of collagens I and III, elastin, and fibrillin are abnormal in LS. Type I and III collagens provide tensile strength to the dermis. Type I collagen (80 % of dermal collagen) is normally distributed throughout the dermis, whereas type III collagen tends to localize to the papillary dermis. In the dermis of patients with LS, the proportions of these collagen types are variably and unpredictably increased or reduced relative to normal controls. In addition, the fibrillar nature of the proteins becomes indistinct and homogeneous [243].

Elastic fibers, which provide elasticity to dermal tissues, are reduced throughout the sclerotic zone of most patients with LS [243]. Fibrillin staining is also reduced throughout the sclerotic zone [243]. The mechanisms contributing to the reduction of elastin and fibrillin are unknown.

Compared to normal controls, tenascin staining is increased within the areas of sclerosis in LS [247, 248]. The normal role of tensacin in the dermis is not fully understood, although some lines of evidence indicate it is involved in cell adhesion and/or mobility. Tenascin is also increased in morphea and SSc, two other fibrosing disorders with histopathological features that overlap with LS [247]. Tenascin has also been reported to upregulate the synthesis of MMPs. Tenascin production by human fibroblasts is stimulated by IL-4 [249]. Investigators have reported variable responses to tenascin production by TGF-β [248, 249]. Tenascin mRNA signals in LS have been identified in fibroblasts, endothelial cells and basal keratinocytes [248].

Fibrinogen and fibronectin are ECM components that appear early in the process of wound repair and scar formation. Because of their many attachment points for extracellular macromolecules and cell surface proteins, they are ideally suited to creating a provisional matrix optimized for cell migration. In LS, fibronectin is reduced in areas of dermal sclerosis but increased in the zone of active inflammation immediately subjacent to the sclerotic areas [247]. Like tenascin, fibronectin has been reported to upregulate the synthesis of MMPs. Compared to normal controls, fibrinogen shows increased staining in both the zones of sclerosis and the underlying inflammatory zone.

Since tenascin and fibronectin both increase the levels of dermal MMPs, it is important to understand the role of MMPs in the normal dermis. MMPs are endopeptidases that are capable of degrading several different macromolecules in the ECM. As such, they are critical in tissue remodeling, wound healing, and cancer invasion. MMP activity is modulated by specific inhibitors known as tissue inhibitors of metalloproteinases (TIMPs). The balance between MMP and TIMP activity determines whether ECM components are being actively synthesized or degraded [250]. MMPs and TIMPs are activated in the extracellular environment and are found predominantly in the active form in the ECM [251].

In the dermis of patients with LS, MMP-2 and MMP-9 density is increased over that of normal skin if stromal cells, inflammatory cells, and vessels are all included in the analysis. It is hypothesized that the increased MMP-2 in the sclerotic zone of LS leads to MMP-2 driven degradation of decorin [250]. Decorin is a peri-cellular matrix proteoglycan that binds to type I collagen and modulates the effects of TGF-β during matrix assembly. Since MMP-2 and MMP-9 are also increased in the keratinocytes of LS, investigators have speculated that the basement membrane changes seen in LS are the result of MMP-driven degradation of laminin and type IV collagen, important components of the basement membrane zone at the dermal-epidermal junction [250]. Unsurprisingly, TIMP-1 and TIMP-2 activity is also greater in LS than in normal skin, a pattern verified through cell culture studies.

Growth Factors

In LS fibroblasts, similar to the pattern seen in scleroderma fibroblasts, maintenance of the profibrotic phenotype can be independent of Smad-dependent TGF-β signaling [252]. While there is significant upregulation of CTGF/CCN2 with concurrent elevations of mRNA levels of several ECM components (biglycan, versican, fibronectin), observations indicate that TGF-β1 and Smad-3 expression is not significantly altered in chronic lesions of LS [252].

Clonal T-Lymphocytes

Approximately 30–50 % of biopsies of genital LS contain T cells with monoclonally rearranged T-cell receptor γ-chain genes. These cases show predominantly CD4-positive T-cells that form an irregular meshwork with B lymphocytes and antigen presenting dendritic cells. The target antigen triggering this clonal growth is not known; however, the persistence of this clonal population may be further evidence of the systemic immune dysfunction inferred by epidemiological associations between LS and other autoimmune disorders [246].

Conclusion

In many ways, the study and understanding of cutaneous fibrosis is in its earliest stages. Conceptually, fibrosis represents a perturbation of a normal process, namely, coordinated wound healing. In an extended analogy, it is fair to say that a successfully healed and remodeled wound is analogous to a classical Shakespearian drama—the actors (cells) are clearly defined, the script is historically (evolutionarily) refined and unchanging, and the storyline and plot (pathways) lead to established and expected consequences and a satisfying resolution. The addition or subtraction of actors and plot elements from Hamlet will almost certainly create confusion—and so, too, will the addition of unexpected elements to the orderly repair of tissue damage (i.e. continuing infectious insult, re-injury, environmental factors, autoimmunity). It is this perturbation of a complex and harmonious process that leads to undesired fibrosis, and it will be the re-establishment of the natural storyline that will eventually result in its eradication.

Questions

1. Cellular origins of fibrosis: which of the following cell types is NOT considered to be a potential source (cellular progenitor) of extracellular matrix-producing fibroblasts in fibrosis?
   A. Pericyte
   B. Fibrocyte
   C. Epithelial cell
   D. Lymphocyte
   E. Adipocyte progenitor cell

(Answer = D)

2. Name three specific effects of elevated TGF-beta signaling activity on fibroblasts or other cell types.

   (Potential answers include fibroblast proliferation; fibroblast expression of extracellular matrix genes and deposition of ECM; conversion of fibroblasts to myofibroblasts; monocyte activation; cytokine production.)

3. True or false: Microarray-based gene expression profiles of fibrotic tissue versus healthy tissue are essentially identical.

   (False; many studies have identified differential expression of specific genes in fibrotic tissue.)

4. Identify and elaborate on the phases of cutaneous wound healing.

   (Hemostasis (formation of fibrin clot), inflammatory phase, proliferative phase (formation of granulation tissue), resolution (remodeling to mature scar tissue).)

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Pemphigus Family of Disease

33

Jun Yamagami and Masayuki Amagai

Abstract

Pemphigus is a group of autoimmune blistering diseases of the skin and mucous membranes, which are mediated by IgG autoantibodies against desmogleins (Dsgs), cadherin type cell-cell adhesion molecules in desmosomes. Oral and cutaneous blisters and erosions with acantholysis, which is defined as intraepidermal blisters due to the loss of cell-cell adhesion of keratinocytes, are typically observed. For the diagnosis of pemphigus, immunopathological findings of *in vivo*-bound and circulating IgG autoantibodies directed against the cell surface of keratinocytes are essential. Pemphigus has three major forms: pemphigus vulgaris (PV), pemphigus foliaceus (PF), and paraneoplastic pemphigus (PNP). Patients with PV and PF have IgG autoantibodies against Dsg3 and Dsg1, respectively, while patients with PNP are characterized by autoantibodies against plakin molecules in addition to Dsgs and interface dermatitis suggesting the involvement of cell-mediated cytotoxicity. The relationship between clinical features and antibody profiles in pemphigus are logically explained by the Dsg compensation theory: The intraepithelial expression pattern of Dsg1 and Dsg3 is different between the skin and mucous membranes, and Dsg1 and Dsg3 compensate for each other when they are coexpressed in the same cell. Systemic corticosteroids are the mainstay of therapy for pemphigus, and adjuvant therapies, including immunosuppressive agents, plasmapheresis, high-dose intravenous immunoglobulin, and anti-CD20 monoclonal antibody are used for severe cases.

Keywords

Pemphigus • Skin disease • Desmosomes • Desmogleins • Mucous membrane • Acantholysis • Pemphigus vulgaris • Pemphigus foliaceus • Paraneoplastic pemphigus • Blisters • Autoimmune • Intraepithelial blister • Autoimmunity • Autoantibody

Key Features

- Pemphigus is a group of autoimmune blistering diseases of the skin and mucous membranes, which are mediated by IgG autoantibodies against cadherin type cell-cell adhesion molecules in desmosomes, desmogleins.
- Pemphigus is histologically characterized by acantholysis, i.e. intraepidermal blisters due to the loss of cell–cell adhesion of keratinocytes, and immunopathologically by the finding of *in vivo*-bound and circulating IgG autoantibodies directed against the cell surface of keratinocytes.
- Pemphigus has three major forms: pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic pemphigus. Pemphigus vegetans is a variant of pemphigus vulgaris. Pemphigus erythematosus is a localized variant of pemphigus foliaceus and fogo

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selvagem is an endemic variant of pemphigus foliaceus.
- Patients with pemphigus vulgaris and pemphigus foliaceus have IgG autoantibodies against desmoglein 3 and desmoglein 1, respectively, while patients with paraneoplastic pemphigus have IgG autoantibodies against plakin molecules in addition to desmogleins.
- Systemic corticosteroids are the mainstay of therapy for pemphigus, and adjuvant therapies, including immunosuppressive agents, plasmapheresis, high-dose intravenous immunoglobulin, and anti-CD20 monoclonal antibody are used for severe cases.

Historical Background

The term pemphigus stems from the Greek *pemphix* meaning blister or bubble and it describes a group of chronic blistering skin diseases in which autoantibodies are directed against the cell surface of keratinocytes, resulting in the loss of cell–cell adhesion of keratinocytes through a process called acantholysis (Table 33.1). The modern history of pemphigus began with the discovery by Beutner and Jordon in 1964 of circulating antibodies directed against the cell surface of keratinocytes in the sera of pemphigus vulgaris patients [1]. In the late 1970s to early 1980s, pemphigus autoantibodies were shown to have a pathogenic activity in induction of blister formation in skin organ-culture systems as well as by passive transfer of patients' IgG to neonatal mice [2, 3]. In the mid and late 1980s, the target antigens of pemphigus were characterized by immunochemical methods, such as immunoprecipitation and immunoblotting [4, 5]. In the early 1990s, the isolation of cDNA for pemphigus antigens demonstrated that the target antigens in pemphigus are desmogleins [6, 7].

Table 33.1 Classification of pemphigus

Pemphigus vulgaris
Pemphigus vegetans
Pemphigus foliaceus
Pemphigus erythematosus: localized
Fogo selvagem: endemic
Paraneoplastic pemphigus
Drug-induced pemphigus
IgA pemphigus

Pathogenesis

Pemphigus Target Antigens are Desmogleins

The hallmark of pemphigus is the finding of IgG autoantibodies against the cell surface of keratinocytes. The pemphigus autoantibodies found in patients' sera play a primary pathogenic role in inducing blisters. When IgG fraction from patients is passively transferred to neonatal mice, the mice develop blisters with typical histologic findings [3]. Even monovalent Fab' fragments of IgG or single chain fragment variables (monoclonal antibodies isolated using phage display technique, consisting of the variable regions of light chain and heavy chain of immunoglobulin) from patients are sufficient to cause blisters in neonatal mice, indicating complement activation and surface cross-linking may not be relevant in keratinocyte detachment [8–10].

Immunoelectron microscopy localized both pemphigus vulgaris and pemphigus foliaceus antigens to the desmosomes, the most prominent cell–cell adhesion junctions in stratified squamous epithelia [11]. Immunochemical characterization of pemphigus antigens by immunoprecipitation or immunoblotting with extracts from cultured keratinocytes or epidermis demonstrated that the pemphigus vulgaris and foliaceus antigens were 130 kD and 160 kD transmembrane glycoproteins, respectively [4, 5, 12]. The 160 kD protein recognized by pemphigus foliaceus sera was subsequently shown to be identical with desmoglein 1 (Dsg1) by comparative immunochemical studies [13].

Molecular cloning of cDNA encoding Dsg1 and pemphigus vulgaris antigens indicated that both molecules were desmogleins, which are the members of the cadherin supergene family [6, 7] (Fig. 33.1). Thus, pemphigus was discovered to be an anti-desmoglein autoimmune disease. The pemphigus vulgaris antigen was termed desmoglein 3 (Dsg3).

Desmosomes are intercellular adhesive junctions in the epidermis and mucous membranes and contain two major transmembrane components, desmogleins and desmocollins, both of which are cadherin type cell adhesion molecules. Desmogleins have four isoforms (Dsg1 to Dsg4). Expression of Dsg1 and Dsg3 is basically restricted to stratified squamous epithelia, where blisters are formed in pemphigus, while Dsg2 is expressed in all desmosome-possessing tissues, including simple epithelia and myocardium [14]. Dsg4 plays an important adhesive role mainly in hair follicles because mutations in *DSG4* gene cause abnormal hair development [15]. The molecular structure of desmogleins is unique and they have four cadherin repeats in their extracellular domain as do classic cadherins and have an extra carboxyl-terminal domain containing repeats of a 29 ± 1 residues (Fig. 33.1).

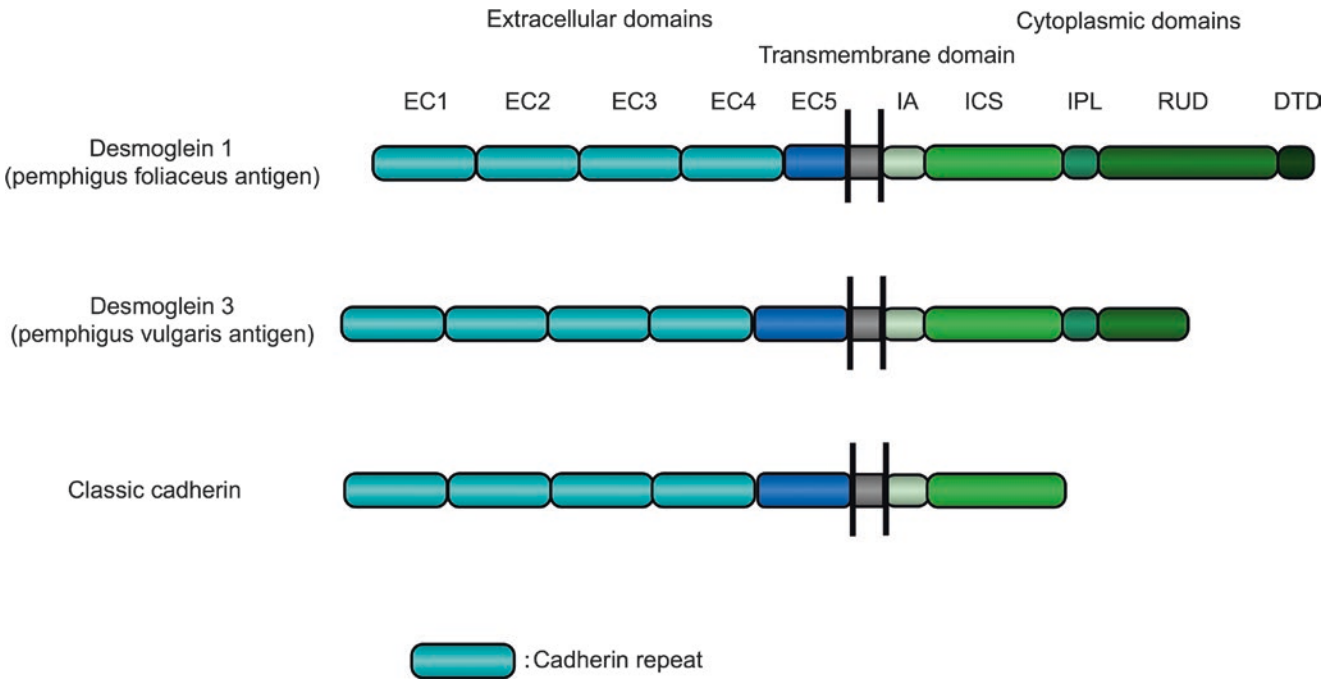

Fig. 33.1 Molecular structure of the pemphigus antigens. Cadherin is a single span transmembrane protein with a unique structure. The extracellular (EC) region of each cadherin member has four cadherin repeats of about 110 amino acid residues with calcium-binding motifs. *Boxes* with the *same color* have similarities in their amino acid sequences. Desmogleins have their own unique sequences of 29 ± 1 residues (repeating unit domain or RUD). *ICS* intracellular cadherin-specific domain, *IA* intracellular anchor domain, *IPL* intracellular proline-rich linker, *DTD* desmoglein-specific terminal domain

Compelling evidence has accumulated that IgG autoantibodies against Dsg1 and Dsg3 are pathogenic and play a primary role in inducing the blister formation in pemphigus. Essentially, all patients with pemphigus have IgG autoantibodies against Dsg1 and/or Dsg3, depending on the subtype of pemphigus [16, 17]. When anti-desmoglein IgG autoantibodies are removed from patients' sera of pemphigus vulgaris, pemphigus foliaceus or paraneoplastic pemphigus by immunoadsorption with recombinant desmoglein proteins, the sera are no longer pathogenic in blister formation [18, 19]. Furthermore, anti-desmoglein IgG autoantibodies that were affinity-purified from pemphigus sera on the desmoglein recombinant proteins can cause blisters when injected in neonatal mice [19, 20]. Some pemphigus sera react with Dsg4 due to cross-reactivity of a subset of anti-Dsg1 IgG, although Dsg4/Dsg1-cross-reacting IgG has no demonstrable pathogenic effect [21]. IgG autoantibodies against acetylcholine receptors or annexin-like molecules are reported, but their pathogenic relevance in pemphigus remains to be determined [22–24].

Thus, the basic pathophysiology of pemphigus is that IgG autoantibodies raised against Dsg1 and/or Dsg3 inhibit their adhesive function and lead to the loss of the cell–cell adhesion of keratinocytes, resulting in blister formation. Recent studies, in which pathogenic and non-pathogenic monoclonal antibodies were isolated from pemphigus patients, suggest that patients would have polyclonal IgG with diverse pathogenic activities [10, 25, 26]. Although the mechanism of blister formation in pemphigus is not fully understood, it is considered that the loss of cell-cell adhesion is triggered by the combination of direct inhibition of Dsg interactions (steric hindrance), the activation of cellular signal pathways, and endocytosis of cell surface Dsg [27–29].

Desmoglein Compensation Theory as Explanation for Localization of Blisters

Although the disruption of desmoglein-dependent cell adhesion by autoantibodies is the basic pathophysiology underlying blister formation in pemphigus, the clinical spectrum is more complex. The complex clinical features of pemphigus are explained logically by the desmoglein compensation theory: Dsg1 and Dsg3 compensate for each other when they are coexpressed in the same cell [30–32] (Fig. 33.2).

The intraepithelial expression pattern of Dsg1 and Dsg3 is different between the skin and mucous membranes. In the skin, Dsg1 is expressed throughout the epidermis, but more

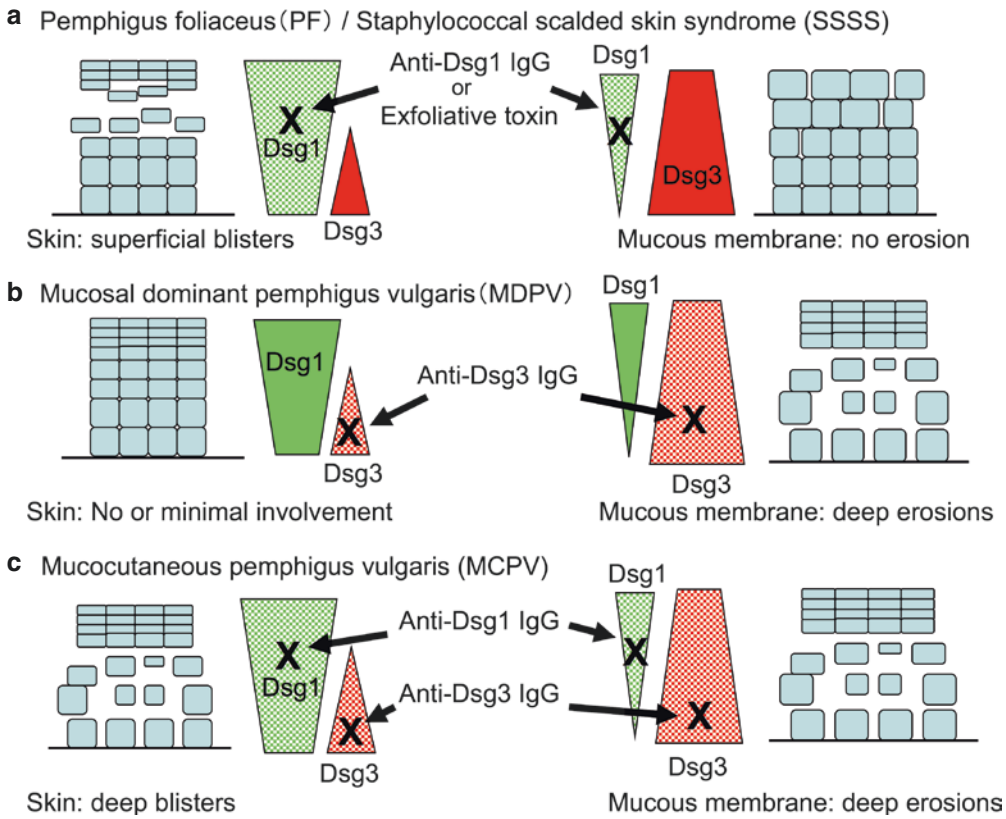

Fig. 33.2 Desmoglein compensation theory. Anti-Dsg1 IgG autoantibodies in serum from patients with pemphigus foliaceus or exfoliative toxin in staphylococcal scalded-skin syndrome (SSSS) cause superficial blisters in the skin; no blisters form in the lower epidermis or mucous membrane, because Dsg3 maintains cell-cell adhesion in those areas (**a**). Serum from patients with mucosal-dominant pemphigus vulgaris contains only anti-Dsg3 IgG, which causes mucosal blisters and erosions where there is no significant compensation by Dsg1, but no or minimal involvement in the skin where Dsg1 maintains cell-cell adhesion (**b**). Serum from patients with mucocutaneous pemphigus vulgaris containing anti-Dsg1 and Dsg3 causes blisters and erosions in the epidermis as well as in the mucous membranes because of no compensation (**c**)

intensely in the superficial layers, while Dsg3 is expressed in the lower portion of the epidermis, primarily in the basal and parabasal layers. In contrast, Dsg1 and Dsg3 are expressed throughout the squamous layer of mucosa, but Dsg1 is expressed at a much lower level than Dsg3.

Patients with pemphigus foliaceus have only anti-Dsg1 IgG autoantibodies (Table 33.2). Patients with the mucosal dominant type of pemphigus vulgaris have only anti-Dsg3 IgG autoantibodies, whereas those with the mucocutaneous type of pemphigus vulgaris have both anti-Dsg3 and anti-Dsg1 IgG autoantibodies [33, 34].

For example, when sera contain only anti-Dsg1 IgG, which interferes with the function of Dsg1, the presence of Dsg3 compensates for the loss of function of Dsg1 in the lower epidermis. In contrast, in upper epidermis, there is no compensation by Dsg3. Therefore, blisters only appear in the superficial epidermis of the skin because that is the only area in which Dsg1 is present without coexpression of Dsg3. Although the anti-Dsg1 IgG binds to mucosa, no blisters are formed because of the coexpression of Dsg 3. Thus, sera containing only anti-Dsg1 IgG cause superficial blisters in the skin without mucosal involvement, as is seen in patients with pemphigus foliaceus.

The desmoglein compensation theory also explains the clinical and histological phenotype of bullous impetigo and staphylococcal scalded skin syndrome (SSSS) [32]. The blisters of bullous impetigo and SSSS are caused by exfoliative toxin (ET), which is produced by *Staphylococcus aureus*. ET was recently discovered to bind specifically to Dsg1 and cleave only the site after glutamic acid residue 381 between extracellular domains 3 and 4 [35–37]. When ET reaches the skin and digests Dsg1 in the lower layers of the epidermis, Dsg3 compensates for the loss of function of Dsg1 and manages to maintain cell–cell adhesion, while no compensation by Dsg3 occurs in the superficial layers of the epidermis. Therefore, ET induces superficial blisters on the skin. In mucous membranes, the Dsg3 expressed throughout the squamous layers of the mucosa compensates for the impaired Dsg1 and maintains cell–cell adhesion with no mucosal involvement.

Table 33.2 Target antigens in pemphigus

Diseases	Autoantibodies	Antigens
Pemphigus vulgaris		
Mucosal dominant type	IgG	Desmoglein 3
Mucocutaneous type	IgG	Desmoglein 3
		Desmoglein 1
Pemphigus foliaceus	IgG	Desmoglein 1
Paraneoplastic pemphigus	IgG	Desmoglein 3
		Desmoglein 1
		Plectin/HD1 (500 kD)
		Desmoplakin I (250 kD)
		Desmoplakin II (210 kD)
		BPAG1 (230 kD)
		Envoplakin (210 kD)
		Periplakin (190 kD)
		alpha-2-macroglobulin-like-1 (170 kD)
Drug-induced pemphigus	IgG	Desmoglein 3
		Desmoglein 1
IgA pemphigus		
SPD type	IgA	Desmocollin 1
IEN type	IgA	?

Paraneoplastic Pemphigus Has a More Complex Autoimmune Reaction Than Classic Pemphigus

In addition to IgG autoantibodies against Dsg3 and/or Dsg1, patients with paraneoplastic pemphigus (PNP) develop characteristic IgG autoantibodies against multiple antigens with molecular weights of 500, 250, 230, 210, 190 and 170 kDa [19, 38] (Table 33.2). By immunochemical studies and cDNA cloning, most of these antigens were identified. The 500 kD antigen is plectin. The 250 kD and 210 kD antigens are desmoplakins I and II, respectively. The 230 kDa antigen is bullous pemphigoid antigen 1, the major plaque protein of the epidermal hemidesmosome and also a target antigen in bullous pemphigoid. The 210 kDa band also contains envoplakin. The 190 kDa antigen is periplakin, and 170 kDa antigen was recently identified as alpha-2-macroglobulin-like-1, a broad-range protease inhibitor [39, 40].

Anti-desmoglein antibodies play a role in inducing the loss of cellular adhesion of keratinocytes and initiate blister formation, while the pathophysiological relevance of the anti-plakin autoantibodies is unclear. The intracellular location of plakin proteins makes it unlikely that anti-plakin autoantibodies initiate pathology in paraneoplastic pemphigus because IgG cannot penetrate cell membranes. It is also important to bear in mind that not only humoral immunity but also cell-mediated cytotoxicity is involved in the pathogenesis of paraneoplastic pemphigus in a form of interface dermatitis. Patients with PNP show more severe and refractory oral erosions and stomatitis as well as more polymorphic skin eruptions when compared with classic forms of pemphigus. Recent studies using the pemphigus mouse model indicated that $CD4^+$ T cells recognizing Dsg3 are able to directly infiltrate to dermal-epidermal junctions and induce interface dermatitis, suggesting the involvement of the cellular autoimmune reaction to epidermal antigens in PNP [41].

Clinical Features

Pemphigus Vulgaris

Pemphigus vulgaris has two clinical subtypes: mucosal dominant type and mucocutaneous type. Patients with mucosal dominant type show mucosal erosions mainly in the oral cavity with minimal or limited skin involvement. Patients with mucocutaneous type show extensive flaccid blisters and erosions on the skin in addition to the mucosal erosions. Any stratified squamous epithelia where Dsg1 and/or Dsg3 are expressed can be involved in pemphigus vulgaris.

Mucous membrane lesions are usually seen as painful erosions (Fig. 33.3). Intact blisters are rare, probably because they are fragile and break easily. Scattered and often extensive erosions may be seen on any part of the oral cavity. Extensive erosions and painful lesions in the mouth may

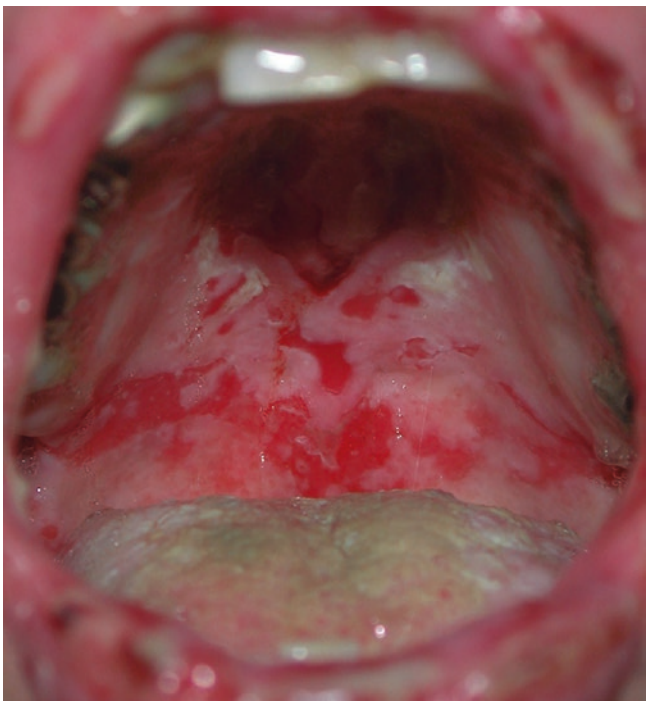

Fig. 33.3 Pemphigus vulgaris. Essentially all patients develop painful oral mucous membrane erosions

result in decreased food and drink intake. Involvement of throat may produce hoarseness and difficulty in swallowing. The esophagus, conjunctiva, nasal mucosa, vagina, penis, anus and labia may also be involved. The diagnosis of pemphigus vulgaris tends to be delayed in patients presenting with only oral involvement, as compared to patients with skin lesions.

The primary skin lesions of pemphigus vulgaris are flaccid, thin-walled, easily ruptured blisters that appear anywhere on the skin surface (Fig. 33.4). The blisters arise on normal-appearing skin or erythematous bases. The blisters are fragile and soon rupture to form painful erosions that ooze and bleed easily. The erosions soon become partially covered with crusts that have little or no tendency to heal. Without appropriate treatment, pemphigus vulgaris can be fatal because large area of the skin lose epidermal barrier function, leading to loss of body fluids or secondary bacterial infection. Because of absence of cohesion in the epidermis, the upper layers are easily made to slip laterally by slight pressure or rubbing in active patients with pemphigus (Nikolsky sign).

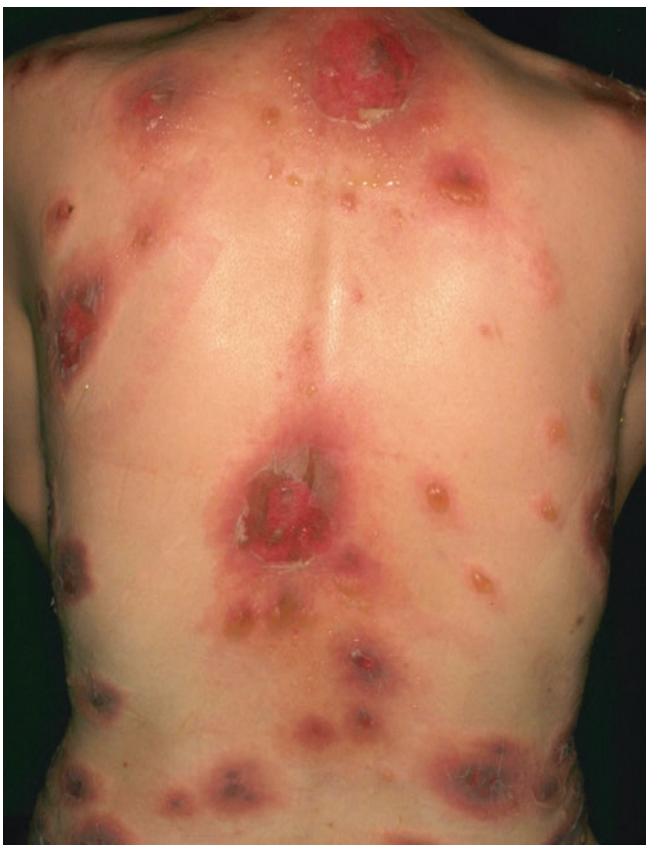

Fig. 33.4 Pemphigus vulgaris. Skin lesions are flaccid blisters which are fragile and soon rupture to form painful erosions that ooze and bleed easily

Pemphigus Foliaceus

Patients with pemphigus foliaceus develop scaly, crusted erosions, often on an erythematous base in the skin, but do not have apparent mucous membrane involvement even with widespread disease (Fig. 33.5). The absence of oral involvement may be a clue to clinically differentiate pemphigus foliaceus from pemphigus vulgaris.

The onset of disease is often subtle with a few scattered crusted lesions which come and go, and are frequently mistaken for impetigo. These lesions are usually well demarcated and scattered in a seborrheic distribution, including face, scalp, and upper trunk. Because the vesicle is so superficial and fragile, often only the crust and scale that result from a ruptured vesicle are seen. Disease may stay localized for years or may rapidly progress, in some cases, to generalized involvement resulting in an erythrodermic exfoliative dermatitis. Nikolsky sign is present. Generally patients with pemphigus foliaceus are not severely ill.

Paraneoplastic Pemphigus

Paraneoplastic pemphigus is a recently described form of pemphigus that occurs in association with underlying neoplasms [38, 39]. PNP is unique and distinct from the classic forms of pemphigus vulgaris and foliaceus by clinical, histologic, and immunopathologic criteria. The associated neoplasms are non-Hodgkin's lymphoma (42 %), chronic lymphocytic leukemia (29 %), Castleman's tumor (10 %), malignant and benign thymoma (6 %), spindle cell neoplasms (reticulum cell sarcoma) (6 %), and Waldenstrom's macroglobulinemia (6 %). The combination of non-Hodgkin's lymphoma and chronic lymphocytic leukemia represents almost two thirds of cases. Castleman's disease,

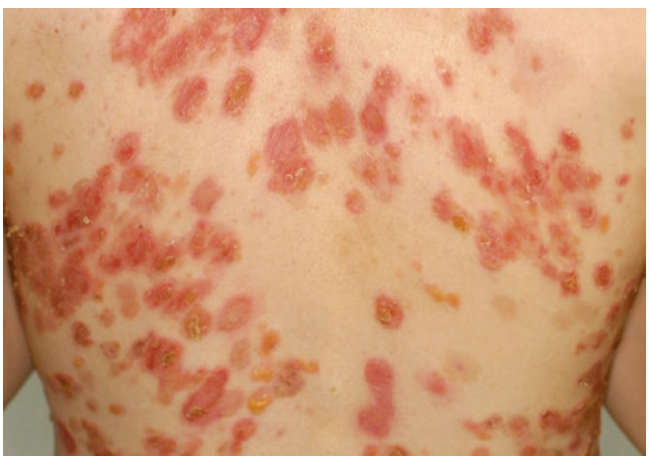

Fig. 33.5 Pemphigus foliaceus. Skin lesions are scaly crusted erosions and vesicles that are fragile and easily ruptured

which is a very rare lymphoproliferative lesion, is the third most commonly associated neoplasm. The absence of common tumors, such as adenocarcinoma of breast or bowel and squamous cell carcinomas, is notable.

The most constant clinical feature of paraneoplastic pemphigus is the presence of intractable stomatitis (Fig. 33.6). The severe stomatitis is usually the earliest presenting sign and after treatment it is the one that persists and is extremely resistant to therapy. This stomatitis consists of erosions and ulcerations that affect all surfaces of the oropharynx and characteristically extend onto the vermillion of the lip. Most patients also have a severe pseudomembranous conjunctivitis with scarring. Esophageal, nasopharyngeal, vaginal, labial, and penile mucosal lesions may also be affected.

The cutaneous lesions are quite polymorphic and may appear as erythematous macules, flaccid blisters and erosions resembling pemphigus vulgaris, tense blisters resembling bullous pemphigoid, erythema multiforme-like lesions, and lichenoid eruptions. Extensive cases show clinical resemblance with toxic epidermal necrolysis (TEN). The occurrence of blisters and erythema multiforme-like lesions on the palms and soles can be used to clinically differentiate paraneoplastic pemphigus from pemphigus vulgaris. Cutaneous lichenoid eruptions are very common together with severe stomatitis. In the chronic form of the disease lichenoid eruption may predominate over blistering lesions.

Paraneoplastic pemphigus is the only form of pemphigus that has involvement of non-stratified squamous epithelia. Approximately, 30–40 % of patients develop pulmonary symptoms [42, 43]. The earliest symptoms are progressive dyspnea, and pulmonary function studies show airflow obstruction, involving large and small airways, as seen in bronchiolitis obliterans, which can be fatal through respiratory failure. Recently, it has been demonstrated that the ectopic expression of Dsg3 or other epidermal antigens in the lung in the form of squamous metaplasia, which is often found in the lungs of PNP patients, is able to render the lung a target organ in PNP [44].

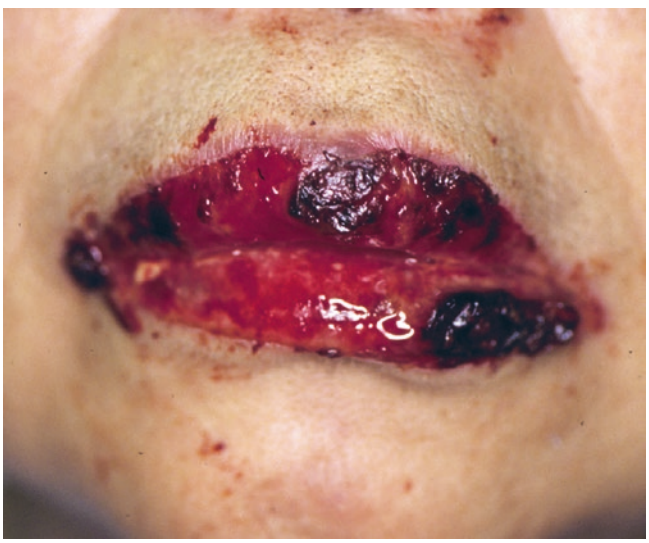

Fig. 33.6 Paraneoplastic pemphigus. The characteristic clinical feature is severe intractable stomatitis that extends onto the vermillion of the lip

Other Forms of Pemphigus

Pemphigus Vegetans

Pemphigus vegetans is a rare vegetative variant of pemphigus vulgaris and considered to be one reactive pattern of the skin to autoimmune insult of pemphigus vulgaris. Pemphigus vegetans is characterized by flaccid blisters that become erosions and form fungoid vegetations or papillomatous proliferations, especially in intertriginous area and in the scalp or on the face. Pustules rather than vesicles characterize early lesions but these soon progress to vegetative plaques.

Pemphigus Erythematosus (Senear-Usher Syndrome)

Pemphigus erythematosus is simply a localized variant of pemphigus foliaceus. Typical scaly and crusted lesions of pemphigus foliaceus occur across the malar area of the face and in other seborrheic areas. Originally, pemphigus erythematosus was introduced for patients with immunological features of both lupus erythematosus and pemphigus, i.e. *in vivo* IgG and C3 deposition on keratinocyte cell surfaces as well as basement membrane zone and circulating antinuclear antibodies [45]. However, only few patients have been reported to actually have the two diseases concurrently [46].

Drug-Induced Pemphigus

There are sporadic cases of pemphigus in association with the use of drugs, such as penicillamine and captopril [47]. Pemphigus foliaceus is more common than pemphigus vulgaris in penicillamine-treated patients. Although most of patients with drug-induced pemphigus are shown to have autoantibodies against Dsg1 or Dsg3 [48], evidence suggests that some drugs may induce acantholysis without production of antibodies. Both penicillamine and captopril contain sulfhydryl groups that are speculated to interact with the sulfhydryl groups in desmoglein 1 and 3. Most, but not all, patients with drug-induced pemphigus go into remission after the offending drug is stopped.

IgA Pemphigus

IgA pemphigus is a newly characterized group of autoimmune intraepidermal blistering diseases presenting with a vesiculopustular eruption, neutrophilic infiltration, and in vivo-bound and circulating IgA autoantibodies against the keratinocyte cell surface, but no IgG autoantibodies [49–51]. IgA deposition

on cell surfaces of the epidermis is present in all cases by direct immunofluorescence, and many patients have detectable circulating IgA autoantibodies by indirect immunofluorescence. There have been two distinct types of IgA pemphigus, subcorneal pustular dermatosis (SPD) type and intraepidermal neutrophilic (IEN) type. IgA autoantibodies in the SPD type react with desmocollin 1, which is expressed on COS7 cells [50], while autoimmune targets of the IEN type remain to be identified (Table 33.2). Subsets of IgA pemphigus patients have IgA autoantibodies against Dsg1 or Dsg3, making the autoimmune target of IgA pemphigus more heterogeneous. The exact pathogenic role of IgA autoantibodies in pustular formation in IgA pemphigus remains to be elucidated.

The patients with both types of IgA pemphigus clinically present with flaccid vesicles or pustules both on erythematous or normal skin. In both types the pustules tend to coalesce to form an annular or circinate pattern with crusts in the central area, although sunflower-like configuration of pustules is a characteristic sign of the IEN type. The predilection sites are the axillary and groin areas, but the trunk, proximal extremities, and lower aspect of the abdomen are commonly involved. Mucous membrane involvement is rare. Pruritus is often a significant symptom. Because the SPD type of IgA pemphigus is clinically and histologically indistinguishable from classic subcorneal pustular dermatosis (Sneddon-Wilkinson disease), immunological characterization is essential to differentiate the two diseases.

Histology

Pemphigus Vulgaris

The characteristic histological finding of the classic form of pemphigus is intraepidermal blister formation due to loss of cell-to-cell adhesion (acantholysis) of keratinocytes without keratinocyte necrosis. In pemphigus vulgaris, acantholysis usually occurs just above the basal cell layer (suprabasilar acantholysis) (Fig. 33.7). A few rounded up (acantholytic) keratinocytes as well as clusters of epidermal cells are often seen in the blister cavity. Although the basal cells lose contact with their neighbors, they maintain their attachment to the basement membrane, thus giving the appearance of a "row of tombstones". Eosinophilic spongiosis can be also seen in very early lesions of pemphigus.

Pemphigus Foliaceus

In pemphigus foliaceus, acantholysis is found in the upper epidermis, within or adjacent to the granular layer (Fig. 33.8). As the blisters are superficial, it is often very difficult to obtain an intact lesion for diagnosis. These histologic findings of superficial blisters are indistinguishable from those seen in staphylococcal scalded skin syndrome or bullous impetigo, because desmoglein 1 is targeted in both diseases. Sometimes the blisters contain numerous acute inflammatory cells, particularly neutrophils.

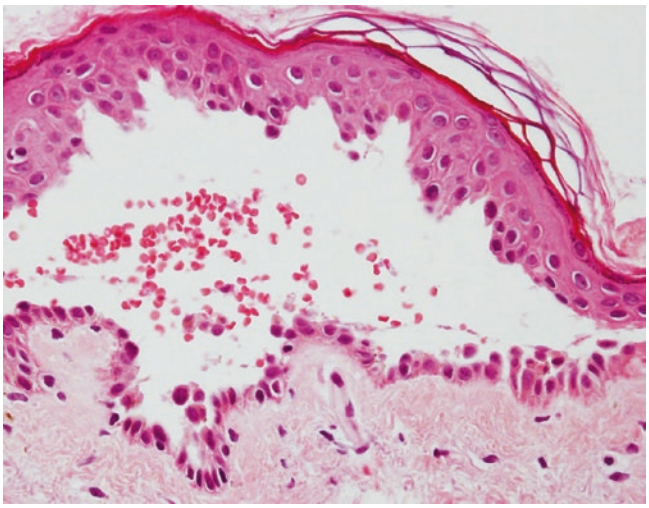

Fig. 33.7 Histology of pemphigus vulgaris. The characteristic histological finding of pemphigus is intraepidermal blister formation due to loss of cell-to-cell adhesion of keratinocytes without keratinocyte necrosis. In pemphigus vulgaris, blisters usually occur just above the basal cell layer (suprabasilar acantholysis)

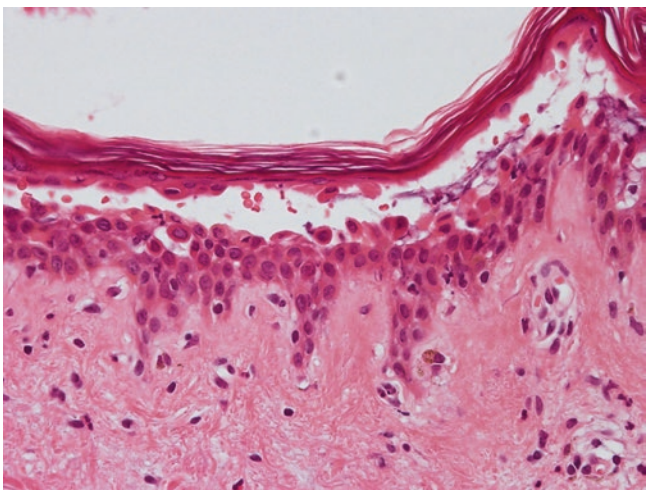

Fig. 33.8 Histology of pemphigus foliaceus. In pemphigus foliaceus, blisters usually occur around the granular layer of the epidermis (subcorneal acantholysis)

Paraneoplastic Pemphigus

In paraneoplastic pemphigus, the histologic findings of lesions show considerable variability, reflecting the polymorphism of the clinical lesions. The lesions show a unique

combination of pemphigus vulgaris-like histology and erythema multiforme-like or lichen planus-like histology. Intact cutaneous blisters may show suprabasilar acantholysis and individual keratinocyte necrosis with lymphocytic infiltration in the epidermis. In addition, basal cell liquefactive degeneration or band-like dense lymphocytic infiltration in the upper dermis can be seen.

Diagnosis

Once the diagnosis of pemphigus is suspected from clinical findings, it is important to take a biopsy for histology and direct immunofluorescence as well as perform serum tests to look for IgG autoantibodies against cell surfaces of keratinocytes or desmogleins. The definitive diagnosis of pemphigus requires the demonstration of the IgG autoantibodies. Methods to demonstrate pemphigus autoantibodies include direct immunofluorescence, indirect immunofluorescence, immunoprecipitation, immunoblot, and enzyme-linked immunosorbent assay (ELISA).

Direct immunofluorescence examines patients' skin or mucous membranes to demonstrate *in vivo* bound IgG deposition on the keratinocyte cell surfaces (Fig. 33.9). Direct immunofluorescence is the most reliable and sensitive diagnostic test for all forms of pemphigus. If the direct immunofluorescence is negative, the diagnosis of pemphigus should be seriously questioned. The biopsy specimen should be taken from perilesional normal skin or mucous membrane, because blister sites may give a false negative. IgM deposition is not seen, but occasionally IgA deposition may be seen in addition. Complement (C3) deposition is not necessarily demonstrated, probably because the dominant subclass of IgG is IgG4, which does not fix complement. In IgA pemphigus IgA deposition, but not IgG deposition, is detected on keratinocyte cell surfaces.

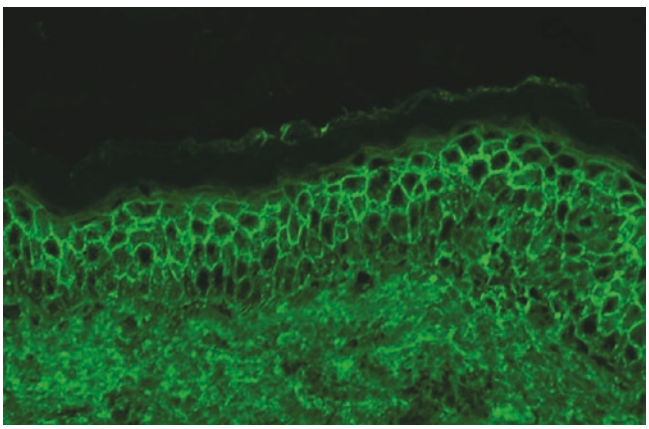

Fig. 33.9 Direct immunofluorescence of pemphigus foliaceus. Direct immunofluorescence using patients' skin as a substrate shows *in vivo* bound IgG deposition on the keratinocyte cell surfaces, indicating IgG autoantibodies bind to the native target antigen, desmoglein1 and/or desmoglein3, as an initial step of blister formation in pemphigus

Indirect immunofluorescence examines patients' sera to demonstrate circulating IgG autoantibodies that react with epithelial cell surfaces. Expression level of Dsg1 and Dsg3 varies among different epithelial cells. As a substrate of indirect immunofluorescence staining, monkey esophagus is more suitable for detecting anti-Dsg3 IgG autoantibodies, and normal human skin or guinea pig esophagus is better for anti-Dsg1 IgG autoantibodies. Rat bladder is used for paraneoplastic pemphigus or anti-plakin autoantibodies. Despite the different antigens involved in pemphigus vulgaris and foliaceus, the staining pattern using direct or indirect immunofluorescence is similar, which makes it difficult to serologically distinguish the two diseases.

ELISA provides a specific, sensitive, and quantitative assay to detect and measure circulating IgG autoantibodies in the diagnosis of pemphigus [16, 17]. The patient's serum is tested on ELISA plates pre-coated with recombinant proteins of Dsg1 or Dsg3. ELISA enables us to serologically distinguish subtypes of pemphigus vulgaris and foliaceus. In general, if a serum is positive against desmoglein 1 but negative against desmoglein 3, it suggests a diagnosis of pemphigus foliaceus. If negative against desmoglein 1 but positive against desmoglein 3, it suggests a diagnosis of mucosal dominant type of pemphigus vulgaris. If positive against both desmoglein 1 and desmoglein 3, it suggests a diagnosis of mucocutaneous type of pemphigus vulgaris. Furthermore, ELISA scores show parallel fluctuation with the disease activity. Thus ELISA is also useful to monitor the disease activity to plan tapering schedules of corticosteroids and to predict flares or relapses before clinical evidence of disease flares are noticed.

Treatment

The introduction of systemic glucocorticoids and immunosuppressive agents has greatly improved the prognosis of pemphigus, however, the morbidity and mortality is still significant because death sometimes occurs from complications of therapy. Systemic glucocorticoids are the mainstay of therapy for pemphigus and immunosuppressive agents are often used for a steroid-sparing effect to reduce the side effects of steroids. The goal of therapy is to control the disease at the lowest possible dose of glucocorticoids. Complete remission on therapy is defined as the absence of new or established lesions while the patient is receiving minimal therapy (less than or equal to 10 mg/day of prednisone or the equivalent and /or minimal adjuvant therapy) [52].

Prednisone at 1.0 mg/kg/day (usually 60 mg/day) is a typical initial treatment. During initial treatment, disease activity is assessed primarily through clinical symptoms,

typically based on PDAI (pemphigus disease area index), a recently established scoring system that can reliably capture all ranges of cutaneous and mucosal disease extent, and prednisone is planned to be gradually tapered [53]. However, once clinical remission is obtained, changes in the titers of circulating autoantibodies are helpful in gauging the dose of prednisone [17, 54–56].

Immunosuppressive agents, such as azathioprine (2–4 mg/kg/day) and cyclophosphamide (1–3 mg/kg/day), when combined with corticosteroids, are beneficial to gain early control of the disease and increase numbers of remissions [57–59]. If complete clinical remission is achieved with the combined therapy, the dosage of the immunosuppressive drug is maintained while the prednisone is gradually tapered to 5 mg/day. In young patients the potential increase in malignancies that might be associated with the use of these drugs must be taken into account. Mycophenolate mofetil (2–3 g/day) is another choice for an effective immunosuppressive agent of the combination therapy with glucocorticoids [60].

Plasmapheresis is useful to quickly reduce the titers of circulating autoantibodies and should be considered for severe pemphigus if the disease is unresponsive to a combination of prednisone and immunosuppressives [61]. Concomitant immunosuppression with glucocorticoids and cyclophosphamide prevents a rebound increase in the production of autoantibody.

High dose intravenous immunoglobulin is another option for resistant disease [62]. Intravenous immunoglobulin has immunomodulatory effects when used in a high dose although their exact mechanisms remain to be elucidated. The efficacy of IVIG (400 mg/kg/day for 5 consecutive days) has been verified by a multicenter, randomized, placebo-controlled, double-blind clinical trial [63]. Recently, anti-CD20 monoclonal antibody (rituximab) was reported to be effective in patients with refractory pemphigus by a single cycle or multiple cycles of the treatment [64–66].

Conclusion

Pemphigus is a group of autoimmune blistering diseases caused by IgG autoantibodies against desmogleins. Understanding the pathomechanism leads to the correct diagnosis and appropriate treatment in pemphigus.

Questions

1. How do you explain the sites of blisters in pemphigus with anti-desmoglein antibody profiles in a logical way?
2. What are essential findings to confirm the diagnosis of pemphigus vulgaris or foliaceus?
3. What is the key difference between paraneoplastic pemphigus and pemphigus vulgaris?
4. How do you set the goal of therapy for patients with pemphigus?

Answers

1. The relationship between phenotypes of pemphigus and anti-desmoglein antibody profile is explained by desmoglein compensation theory. Dsg1 and Dsg3 compensate for each other when they are coexpressed in the same cell. The intraepithelial expression pattern of Dsg1 and Dsg3 is different between the skin and the mucous membranes
2. Clinical findings, histological findings (intraepidermal blister formation with acantholysis), and detection of in vivo bound IgG deposition on the keratinocyte cell surface in direct immunofluorescence of patient's skin or mucous membranes
3. Not only humoral immunity but also cell-mediated cytotoxicity is involved in the pathogeniesis of paraneoplastic pemphigus
4. The goal of therapy is to control the disease at the lowest possible dose of glucocorticoids

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Immunoglobulin A Dermatoses

34

Julia A. Curtis and John J. Zone

Abstract

Immunoglobulin A (IgA) dermatoses represent a distinct but diverse category of immunologic skin diseases, including linear IgA bullous dermatosis, dermatitis herpetiformis, IgA subcorneal pemphigus, IgA intraepidermal pemphigus and IgA vasculitis (Henoch-Schönlein purpura).

The common findings that tie all of these disorders together are the cutaneous deposition of IgA at the histopathologic site of inflammation on direct immunofluorescence microscopy and a neutrophilic inflammatory infiltrate on hematoxylin and eosin staining of involved skin.

IgA is found in respiratory, gastrointestinal and genitourinary mucosal secretions, as well as human serum. The IgA dermatoses share activation of the IgA receptor, FcαR1, on neutrophils, which is also present on eosinophils and monocytes. This receptor activation starts an inflammatory cascade believed to be essential in these disorders that include: a neutrophilic response, endocytosis, antibody-dependent cell-mediated cytotoxicity, the respiratory burst, degranulation in the skin and subsequent tissue damage.

The main therapy for these disorders remains dapsone, a sulfone antibiotic, and its related medications, which are effective agents against neutrophil activity that include inhibition of adhesion, chemotaxis and myeloperoxidase production for the respiratory burst. Topical therapy consists mainly of high potency corticosteroids.

Keywords

Immunoglobulin A • Bullous dermatoses • Vesiculobullous • Bullae • Linear IgA • LABD • Mucosal involvement • Subcorneal pemphigus • Intraepidermal pemphigus • Pustules • Drug-induced • Dermatitis herpetiformis • Vesicles • Papules • Papulovesicular • Duhring's • IgA vasculitis • Henoch-Schönlein purpura • Neutrophilic dermatoses • IgA FcαR1 • Dapsone

Key Points

- Immunoglobulin A (IgA) dermatoses encompass a number of diseases, including linear IgA bullous dermatosis, dermatitis herpetiformis, IgA subcorneal pemphigus, IgA intraepidermal pemphigus and Henoch-Schonlein purpura. The common finding is that all of the disorders are characterized by deposition of IgA on direct immunofluorescence at the histopathologic site of inflammation in the skin.

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- Additional shared findings include a neutrophilic inflammatory infiltrate and a response to dapsone therapy.
- Neutrophils have an IgA receptor, FcaR1, which is not present on all immune response cells. Activation of this receptor starts the cellular cascade that is believed to be operative in these disorders: neutrophilic response, degranulation, respiratory burst, endocytosis and subsequent tissue damage.

The Immunoglobulin A (IgA) dermatoses represent a classification of immune cutaneous disorders sharing the finding of IgA deposition on direct immunofluorescence at the site of histopathologic inflammation. Neutrophils are the predominant inflammatory infiltrate in the skin in this group of diseases and dapsone is generally an effective pharmacologic agent.

Immunoglobulin A

IgA is abundantly found in the mucosal secretions of the respiratory, gastrointestinal and genitourinary tracts where it neutralizes pathogens; however, it is also present in human serum at a concentration second to that of IgG. This presence implies an immunologic function in the serum; however, this has yet to be elucidated [1].

There are two subclasses of IgA: IgA1 and IgA2. IgA1 is produced by the bone marrow and is found in higher quantities than IgA2 in the serum. IgA2 is found in high quantities in the secretions of the lungs, gastrointestinal and genitourinary tracts and accounts for 60–70 % of the total output of antibodies in the body [2]. Secretory IgA (S-IgA) is a dimer held together by a J chain with a polypeptide that is produced in mucosal lymphoid tissue by antigen-stimulated B cells and is transported to the apical epithelial border by a polymeric immunoglobulin receptor (pIgR). Once at the border of the mucosal cell, S-IgA is proteolytically cleaved and IgA is secreted into the lumen. This pIgR also transports secretory IgM into intestinal secretions [2]. Secretory IgA is the key defense mechanism preventing microbes from gaining access to the mucosae by coating them and preventing adherence. Additionally, the FcaR1 receptor, uniquely present on neutrophils, eosinophils, and monocytes activates antigen presentation, endocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC), the respiratory burst and degranulation in the skin leading to the clinical findings of these diseases [2].

General Features of Immunoglobulin A Dermatoses

Microscopic examination of cutaneous histology reveals a neutrophil-predominant inflammatory infiltrate consistent with the IgA immune response. Because eosinophils and macrophages also have FcaR1, these immune cells can also be found scattered throughout the tissue. The main therapeutic agent for these dermatoses remains dapsone, an effective weapon against neutrophil activity, including inhibiting adhesion, chemotaxis and production of myeloperoxidase for the respiratory burst [3].

Dermatitis Herpetiformis

Dermatitis herpetiformis (DH) is unique among autoimmune diseases because there is a known human antigen – epidermal transglutaminase or transglutaminase 3 (TG3), a known human antibody – IgA and a known cause dietary gluten and celiac disease.

Dr. Louis Duhring first described DH in 1884. Patients develop an eruption of intensely pruritic, symmetrical inflammatory papules and papulo-vesicles on the extensor surfaces of the bilateral upper and lower extremities, as well as the scalp and buttocks and in areas of pressure or trauma such as the belt line. The vast majority of patients have associated celiac disease (CD), also referred to as gluten-sensitive enteropathy. Only 15–20 % of cases have gastrointestinal symptoms, but virtually all patients respond over time to dietary gluten restriction with healing of both the cutaneous and intestinal pathology. DH responds well to dapsone alone; however, symptoms recur in 48–72 h if dapsone is discontinued. Dietary gluten restriction produces a long-term remission but DH usually recurs in weeks to months if a gluten containing diet is resumed.

Epidemiology

DH occurs more frequently in patients of northern European descent. Incidence rates in northern Europe are between 0.4 and 3.5 per 100,000 people per year. Prevalence rates are between 1.2 and 75.3 per 100,000 people. One Finnish study found that the incidence of DH decreased over three successive decades beginning in 1970 possibly due to citizens having increased awareness of the association between gluten consumption and subclinical celiac disease [4].

A population-based study in Utah found incidence and prevalence rates similar to those reported in Europe, likely related to the high proportion of residents of northern European descent. In 1987 the incidence of DH was 0.98 per 100,000 people per year and the prevalence was 11.2 per 100,000 people [5].

Most epidemiologic studies report an increased frequency in males compared to females, ranging from 1.1:1 to 1.9:1. The cause of this male predominance has not been further illuminated [4].

Individuals of all ages may be affected; however, DH is uncommon in children and most patients develop it in the fourth or fifth decade of life [4].

Like CD, DH is associated with specific human leukocyte antigen (HLA) genes, HLA-DQ2 or HLA-DQ8 haplotypes [6]. One comparative study of 50 subjects with DH and 290 healthy controls found that 86 % of the patients with DH carried the HLA-DQ2 gene compared with 35 % of the healthy controls. Additionally, six of the seven DH patients without the HLA-DQ2 gene were positive for the HLA-DQ8 gene [7]. These HLA genes allow antigen presenting cells to recognize the alpha-gliadin antigen in wheat and other prolamins in rye and barley with the subsequent T cell response.

Additional support for the genetic contribution to DH is revealed in familial studies demonstrating that first-degree relatives of patients with DH have an increased risk for DH and celiac disease [8]. In a Finnish population of over 1000 patients with DH and celiac disease, 4–6 % of patients had a first-degree relative with DH and celiac, respectively [9].

Pathogenesis

Granular IgA deposition in dermal papillae is the hallmark of DH. DH has a complex pathogenesis, stemming from both intrinsic and extrinsic factors. DH virtually always occurs in genetically susceptible individuals as described above, and usually remits with a gluten-free diet.

Greater than 90 % of DH patients have small bowel biopsy findings consistent with a gluten-sensitive enteropathy, including varying degrees of atrophy of the jejunal villi, crypt hyperplasia and a lymphocytic and plasma cell infiltrate in the lamina propria and within the mucosal epithelium. These findings are indistinguishable from the findings in CD and span the entire gamut of CD histopathologic severity (Marsh grades 0–3). DH is best regarded as a cutaneous manifestation of CD. Up to 80 % of CD and DH patients have the histopathologic abnormality of gluten sensitive enteropathy but do not have classical gastrointestinal complaints of abdominal cramping, gas, bloating and diarrhea.

Gluten is a group of proteins in wheat, rye and barley that is not water-soluble. Prolamins are the alcohol-soluble portion of gluten and include gliadin in wheat, secalin in rye and hordein in barley. These peptides fit in the HLA determined antigen groove of antigen presenting cells. The protein is then deamidated by tissue transglutaminase (tTG or TG2), a calcium-dependent enzyme that also catalyzes crosslinking between glutamine and lysine protein residues and forms covalent bonds with gliadin. This deamidated gliadin and the bound tTG are key antigens in the pathogenesis of the disease and are used in diagnostic testing [10]. The deamidated peptide is then recognized by T helper (Th) cells. These activated Th cells produce proinflammatory cytokines and matrix metalloproteinases (MMP) that damage the intestinal mucosa and produce antibodies from B cells against tTG. Cutaneous manifestations of this inflammatory state are possibly mediated through epitope spreading. Epitope spreading occurs when the immune response from an endogenous antigen or multiple antigens spreads to self-antigens through the release of self peptides from tissue damage, mediated by self-reactive T helper 1 cells reacting against a chronic inflammatory state from endogenous antigens [11]. The antibodies that are produced from this process may bind and form an antigenic complex to epidermal transglutaminase (TG3). The IgA TG3 antibodies reach the dermis through the bloodstream and bind to TG3 produced by overlying epidermal cells. The TG3 enzyme continues to be enzymatically active and crosslinks IgA to surrounding dermal connective tissue. This deposition of immune complexes then stimulates an inflammatory state leading to cutaneous blistering from the recruitment of neutrophils, neutrophil chemotaxis and proteolytic cleavage in the lamina lucida of the basement membrane zone [12–14].

Clinical Features

The hallmark feature is the development of multiple intensely pruritic vesicles and papules, grouped in herpetiform arrangement, on the elbows, dorsal forearms, knees, scalp, back and buttocks; however, erosions, papulo-vesicles and excoriations are more readily found on exam and urticarial plaques may occur (Fig. 34.1a, b). Fully developed vesicles are rare and bullae are extremely uncommon.

Patients with mild disease usually have involvement of the knees, elbows and dorsal forearms. Those patients with severe disease usually present with additional involvement of the trunk and extremities. Lesions also frequently occur in areas of pressure such as the belt line. Although the skin generally heals without scarring after resolution of the symptoms, post-inflammatory hyperpigmentation may occur [10].

Other uncommon cutaneous findings are petechiae on the fingers or palms, mainly occurring in children, but also found in adults [15, 16]. Occasionally, these palmar lesions are the primary manifestation of the disease [17].

DH may also occur in the oral mucosa; however, this has not been confirmed through direct immunofluorescence microscopy, and in some cases may have been aphthous stomatitis that is known to be an oral manifestation of CD [10]. Mucosal and tongue involvement may occur as vesicles, erosions or erythematous macules with or without discomfort.

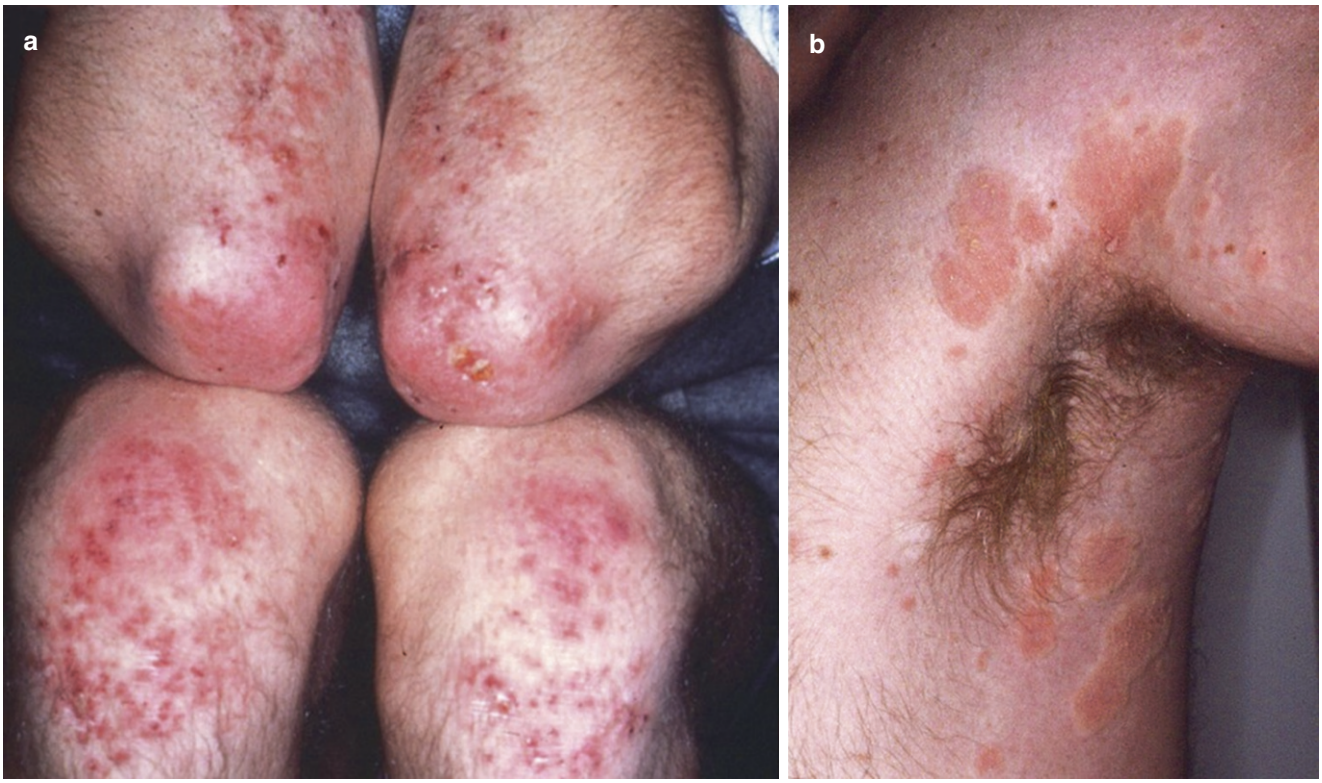

Fig. 34.1 Dermatitis herpetiformis. (**a**) Excoriated papules and papulo-vesicles on elbows and knees of a DH patient. (**b**) Urticarial plaques of DH

An additional association with celiac disease reveals itself in tooth enamel defects, such as horizontal grooves, pits or discoloration [18]. One study supporting this reported that in 30 adults with DH, 53 % had enamel defects compared with 2 % of 66 healthy controls [19]. One small study of ten children compared to healthy children controls showed eight had enamel defects while only 13 % of healthy children had similar defects [20].

Associated Diseases

The most common associated condition is autoimmune thyroid disease, with hypothyroidism being more likely [21–23]. One retrospective study of 264 adults with DH revealed that 11 % had thyroid disease [22].

Type 1 diabetes mellitus is also associated with DH with estimates between 2 and 5 % [10]. Pernicious anemia has a slightly higher frequency in patients with DH, ranging between 1 and 3 % [21].

An increased risk for non-Hodgkin lymphoma has reportedly been associated with celiac disease and this may also occur in DH [24]. One Swedish population-based study with 1354 patients with DH revealed a slight increased overall risk for malignancy (standard incidence ratio [SIR] 1.2, 95 % CI 1.0–1.4), due to increased cases of lymphoma and leukemia [25]. The effect of adherence to a gluten-free diet on the reduced risk for lymphoma was reported in one retrospective study in the United Kingdom; however, more studies are necessary to demonstrate the link between gluten-free diets and reduced risk of malignancy in DH. There is an increased risk of malignancy in CD, so it is likely that with a big enough sample size, a similar risk would be documented in CD's cutaneous manifestation, DH [25].

Additional autoimmune diseases with a possible increased frequency are alopecia areata, Addison's disease and vitiligo [21, 26].

Diagnosis

Confirming the diagnosis of DH through laboratory studies is essential, through histopathology of lesional skin, direct immunofluorescence microscopy (DIF) of perilesional skin and serologic studies. DIF is the gold standard test for diagnosis.

A 4 mm punch biopsy of a small intact vesicle is best for hematoxylin and eosin (H&E) staining. If there are no intact vesicles, involved erythematous skin is better than excoriated lesions, as these lesions may yield nonspecific results.

Findings on H&E staining vary with the age of the sampled lesion. Early lesions may show only a neutrophilic

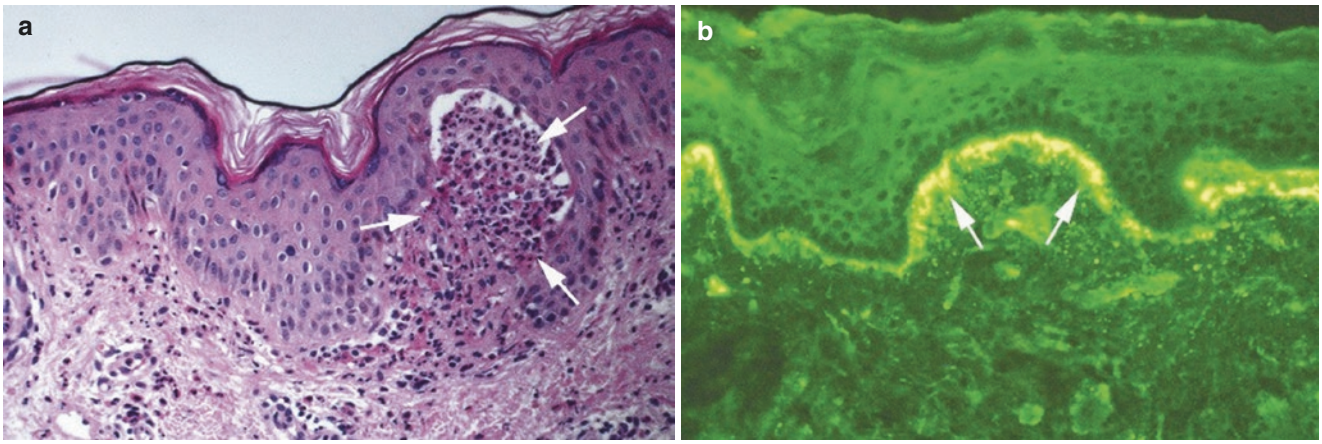

Fig. 34.2 Dermatitis herpetiformis. (**a**) Neutrophils in dermal papillary tips (*arrows*) on hematoxylin and eosin staining of biopsy from involved skin. (**b**) Granular IgA in the papillary dermis (*arrows*) with focusing of grains in dermal papillae from perilesional, clinically normal-appearing skin

infiltrate, with or without eosinophils, and papillary microabscesses, which are neutrophils in the tips of the dermal papillae (Fig. 34.2a) [27]. After 48 h, lesions on H&E show subepidermal vesiculation at the dermal tips which over time bridge to form larger subepidermal vesicles, containing neutrophils, eosinophils and fibrin. Additionally, the dermis shows a perivascular lymphocytic infiltrate with neutrophils and eosinophils [27].

The characteristics of DH on H&E histopathology are similar to other subepidermal bullous disorders. DH can be particularly difficult to distinguish from linear IgA bullous dermatosis, bullous systemic lupus erythematosus, and even bullous pemphigoid; however, numerous eosinophils are usually present in pemphigoid [27].

A punch biopsy for DIF must come from perilesional, clinically normal-appearing skin immediately adjacent to a lesion as biopsies taken from lesional skin are more likely to yield false negative findings [28]. The characteristic finding is granular deposits of IgA in the dermal papillae (Fig. 34.2b). DH may also show deposits of IgM, fibrinogen and C3 in a similar pattern [27]. A fibrillar pattern of IgA deposition is found occasionally rather than a granular one [29]. A retrospective study of 264 patients with DH found the DIF was positive in 92 % of patients (244) [21]. Of note, the DIF may be negative in patients on a strict gluten-free diet as this reduces IgA deposits in the skin. One retrospective series found that 10 of 41 patients with DH on a strict gluten-free diet after 13 years did not have detectable IgA deposition in the skin [30]. Treatment of DH with dapsone alone does not alter the findings of IgA deposition on DIF [10].

At times, granular deposits of IgA along the basement membrane appear on DIF, which may confound the diagnosis with linear IgA bullous dermatosis; however, the sharp linear IgA deposition usually separates the two disorders [6, 31]. In these instances, indirect immunofluorescence testing for basement membrane antibodies of linear IgA disease and serologic testing for IgA antibodies to TG2 and TG3 in DH may aid in clarification of the diagnosis. The same testing is useful in the extremely rare occasions when the DIF findings are equivocal or negative [32]. Additionally, these levels fall when a strict gluten-free diet is followed serving as a useful tool for monitoring response and adherence to this diet. Lastly, another useful serology in the evaluation of patients with DH is a serum total IgA level for detecting partial IgA deficiency, which occurs at an increased frequency in celiac disease. Such patients may not show IgA autoantibodies; therefore, IgG tissue transglutaminase and endomysium are better markers for this occasion [10].

Treatment

A strict gluten-free diet is the most effective therapy for patients with DH; however, remission can take months to years [33]. The most effective pharmacologic agent is dapsone, which can resolve skin lesions and pruritus within 48–72 h [33].

Dapsone therapy in adults is started at 25–50 mg daily and slowly increased to 2 mg/kg daily based on response and tolerance to therapy, in association with adherence to a gluten-free diet. Adults on unrestricted diets may need between 50 and 150 mg daily for complete response [34]. Even at optimal dosing, mild eruptions of one to two new lesions per week are possible. These events do not warrant an increase in dose, rather a potent topical corticosteroid may be applied to the new lesions.

As dapsone is a sulfone antibiotic, mild asymptomatic side effects of this medication are expected in nearly all patients. Dapsone competitively inhibits dihydropteroate synthase, an enzyme involved in folic acid reduction; therefore, most patients will have some red blood cell (RBC) hemolysis. Patients with glucose-6-phosphate deficiency

(G6PD) should not take dapsone, as they are at increased risk for severe hemolytic anemia from oxidative stress. Testing for this deficiency is recommended before starting therapy.

Methemoglobinemia is another dose-related side effect of dapsone therapy. Methemoglobin, an oxidized form of hemoglobin, has a reduced ability to carry oxygen, leading to symptoms of hypoxia and anemia. Cardiovascularly-compromised patients may have symptoms even at low doses. Supplementation of these patients with antioxidants such as Vitamin E may theoretically help mitigate these effects.

Additional potential side effects include agranulocytosis and a hypersensitivity reaction. Agranulocytosis generally appears 2–12 weeks after commencement of dapsone. Surveillance through lab monitoring will detect early signs of this potentially lethal side effect, allowing discontinuation of the drug. Dapsone-associated hypersensitivity symptoms are a morbilliform cutaneous eruption with flu-like symptoms, including fever, lymphadenopathy, hepatitis and eosinophilia.

Because dapsone is associated with potential serious side effects, careful laboratory monitoring is crucial. Initial lab screening should include a complete blood count (CBC), liver and renal function panels, and G6PD screening. After initiation of dapsone, interval lab screening is recommended: CBC every 1–2 weeks for 1 month then every 1–3 months for 6 months; thereafter CBC and liver function should be checked every 3–6 months if dapsone is not increased during this time frame.

After 2–3 months of dapsone therapy and a gluten-free diet, dapsone can be tapered slowly. Reduction of 12.5 mg every 2–4 weeks is recommended.

For patients who are intolerant of dapsone, there are other sulfa-based drugs available, sulfasalazine and sulfapyridine. Sulfapyridine is not available commercially in the United States, but can be obtained through compounding pharmacies. Sulfasalazine is metabolized to sulfapyridine in the intestine; however, the level of active metabolite is more predictable when sulfapyridine itself is given [35]. Sulfapyridine dosing should be started at 0.5 g three times per day and increased up to 6 g daily for control of symptoms. Sulfasalazine should be dosed between 1 and 2 g daily [35]. Both medications have the potential adverse effects of agranulocytosis and hypersensitivity reactions, but not hemolysis. Adequate fluid intake and possible alkalinization of the urine with oral bicarbonate is also recommended to reduce the risk of drug-induced nephrolithiasis. As with dapsone, lab monitoring (CBC, LFTs and urinalysis) is recommended periodically.

Although adherence to a strict gluten-free diet is challenging, as gluten is hidden in many common foods, patients will benefit from this and will be able to decrease or discontinue medications. A 36-month study of 81 patients with DH on a gluten-free diet for 6–36 months and 49 patients with DH on a normal diet reported that 93 % of patients on a gluten-free diet achieved reductions in dapsone dosing versus 16 % of patients on a normal diet [36]. There are many resources for patients about gluten-free diets, including the Celiac Disease Foundation (www.celiac.org) and the Gluten Intolerance Group (www.gluten.net).

An additional adjunctive treatment for the pruritic skin lesions of DH is potent topical corticosteroids. These topicals are not effective monotherapy, and systemic glucocorticoids are generally ineffective.

Treatment of Children

Management of children is the same as for adults. They should follow a gluten-free diet and if necessary take dapsone 0.5–2 mg/kg/day [35]. As with adults, dapsone may be tapered as the gluten-free diet controls the disease.

Prognosis

Dermatitis herpetiformis is a chronic condition that requires life-long adherence to a gluten-free diet or treatment with dapsone. When gluten is re-introduced into the diet, symptoms may recur within weeks to months and when dapsone is discontinued, symptoms may appear within 2 days [37]. A small percentage of patients (10–15 %) may maintain remission despite the discontinuation of both dietary and pharmacological therapy [38].

Linear Immunoglobulin A Bullous Dermatosis

Linear IgA bullous dermatosis (LABD) or linear IgA disease is a rare, acquired idiopathic or drug-induced autoimmune subepidermal blistering disease. The main feature of this disease is the linear deposition of IgA at the dermo-epidermal junction. Distinguishing this from DH can be difficult, since similar clinical and histopathologic findings may occur; however, LABD is rarely associated with gluten-sensitive enteropathy and its sharp linear IgA deposition is easily distinguished from the granular IgA of DH by the experienced immunopathologist [39].

LABD occurs in both adults and children. In children, the disorder once known as chronic bullous disease of childhood is now recognized as the childhood form of LABD [40]. Adults may present with tense vesicles and bullae within erythematous annular plaques (Fig. 34.1b) or like DH as excoriated papules on the extremities, buttocks and face. Different from adults, children present clinically with

Table 34.1 Medications implicated in precipitating linear IgA bullous dermatosis

Drugs associated with linear IgA bullous dermatosis	
Common	Vancomycin
Multiple reports	Captopril
	B-lactams
	Cephalosporins
	NSAIDs
	Phenytoin
Isolated reports	Acetaminophen
	Amiodarone
	Atorvastatin
	Benazepril
	Candesartan/eprosartan
	Carbamazepine
	Furosemide
	Gemcitabine
	Interleukin-2
	Lithium
	Somatostatin
	Trimethoprim/sulfamethoxazole

widespread annular lesions with peripheral vesiculation on the lower abdomen, thighs and groin area [41]. The oral mucosa can be involved in both adults and children.

Epidemiology

Reported incidence rates range from less than 0.5–2.3 cases per million individuals yearly [42]. No predilection based on ethnicity or gender for LABD has been established [42]. LABD rarely occurs in neonates [43]. It can develop in children between the ages of 6 months and 10 years. The average age of onset in 25 affected children was 4.5 years [40]. Adults present with LABD later in life, with many cases occurring after age 60 [40, 42]. Drug-induced cases may occur at any age.

Risk Factors

There is no known inciting factor for most cases of LABD. Medications are suspected in multiple cases. The most common drug implicated is vancomycin [42]. Other reported associations are listed in Table 34.1.

Pathogenesis

A search for target autoantigens in LABD has been complicated, as studies of patient sera have shown varied results. LABD can be initially divided into subtypes based on ultrastructural location of IgA (sublamina densa type and lamina lucida type); however, overlap between the two types may occur. These can be established using basement membrane zone human salt-split skin and indirect immunofluorescence in cases where there is circulating IgA basement membrane antibody, since such separation occurs in the lower lamina lucida. Lamina lucida antigens will adhere to the epidermal side of the basement membrane separation and sub-lamina densa reactive antibodies will bind to the dermal side. In cases without circulating IgA basement membrane antibody, immunoelectron microscopy or basement membrane separation of the DIF-positive biopsy can be used to identify the type. In most cases this is unnecessary since treatment for both variants of LABD is the same.

The lamina lucida type predominantly targets a 97-kDa antigen and a 120-kDa antigen that are the proteolytic fragments of the extracellular portion of the bullous pemphigoid antigen 2 (BP180), a key epidermal-dermal adhesion transmembrane protein [44, 45]. Less frequently, LABD is associated with the NC16a epitope of BP180 [46–48]. The sub-lamina densa type of LABD has been reported to be predominantly type VII collagen, although a number of other antigens have been proposed [49]. Lastly, there is a subset of patients with features consistent with LABD who have both IgA and IgG antibodies against the basement membrane zone. A Japanese review of 213 patients with LABD found both antibodies in approximately 20 % of the cases [50].

Both humoral and cellular immunity may contribute to the pathogenesis of the lesions. Skin and mucosal findings may be the result of an antibody-induced local inflammatory response from the release of proteolytic enzymes by neutrophils and other inflammatory cells [42].

Clinical Manifestations

Patients generally present with lesions on the skin, or on the mucous membranes, or on both locations. Blister formation is sub-epidermal; therefore, the vesicles and bullae are typically tense, rather than flaccid as is found in pemphigus. Similar to DH, pruritus and scratching may leave only excoriations and erosions.

Adults and Children Manifest the Disease Differently (Fig. 34.3a–d)

Children often present with acute development of vesicles or bullae on sites of erythematous or normal skin. The distribution of these lesions is generally widespread, involving the face (usually the perioral area), trunk, genitalia, hands and feet. The lower abdomen, perineum and inner thighs may be the most intensely involved areas [40, 51, 52]. At the periphery of resolving lesions, new blisters often form, resulting in an arciform or annular configuration. These lesions are classically known as "string of pearls", "crown of jewels" or "rosettes" [53]. Children usually have pruritus, which can be severe, while other affected children may be asymptomatic. For some children, intense pruritus heralds relapse of their condition [54, 55].

Adults with LABD present typically with acute onset of skin lesions, rather than a gradual onset [41]. The lesions arise on uninvolved skin or within erythematous plaques. Adults generally do not develop the string of pearls lesions with peripheral vesiculation, but this may occur [41]. The general distribution of lesions in adults involves the face (also the perioral area, like children), trunk, extensor extremities and buttocks [42]. With the distribution including the

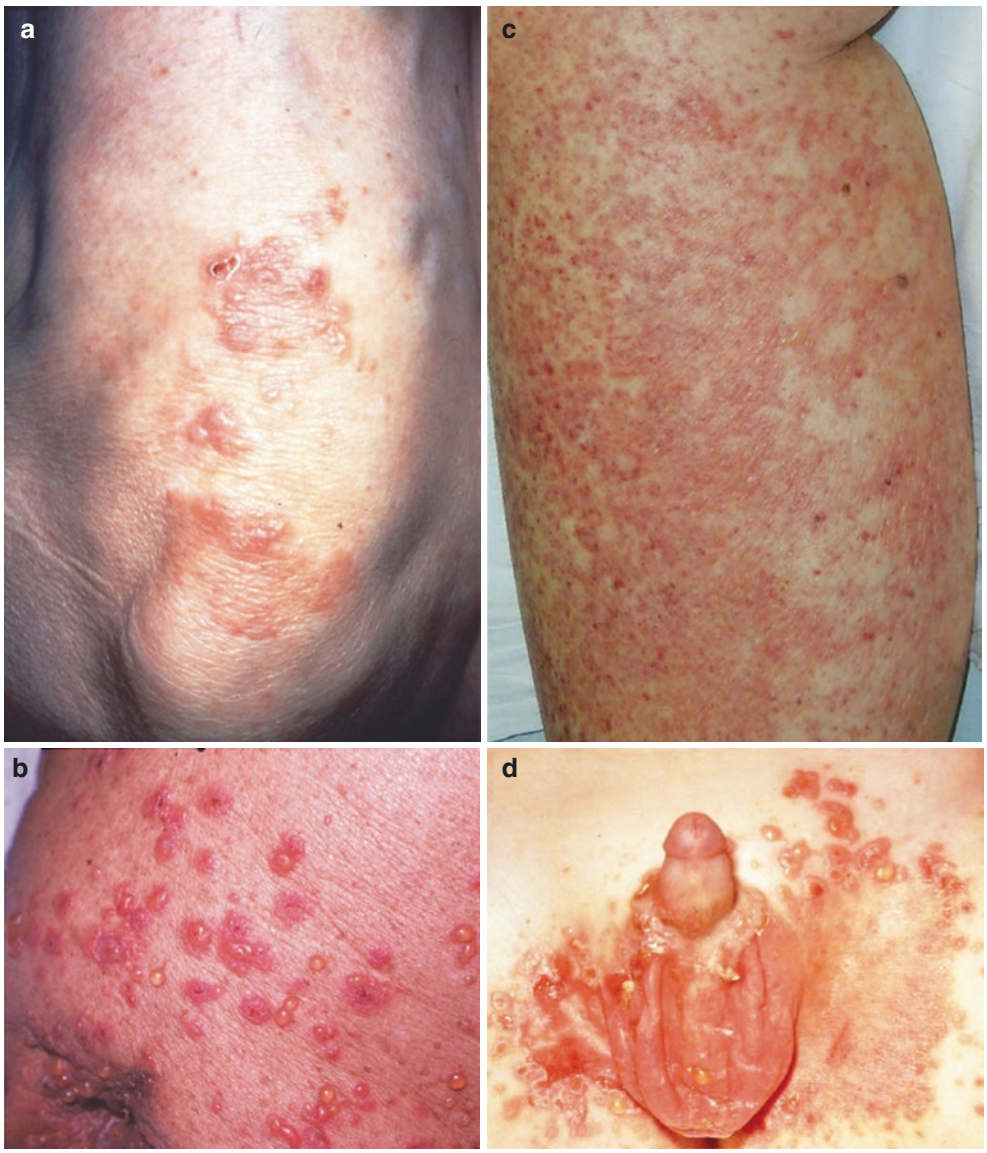

Fig. 34.3 Linear IgA bullous dermatosis. (**a**) Papulo-vesicles on the extensor forearms resembling DH. (**b**) Scattered tense vesicles with associated urticarial lesions. (**c**) Diffuse erythema and superficial vesicles in a case of vancomycin-induced LABD. (**d**) Scrotal and inguinal vesicles in childhood LABD

extensor extremities, this disease can be difficult to distinguish from DH. Additionally, localized variants of LABD presenting as limited eruptions of bullae or annular erythematous plaques have been reported in several different case reports [20, 56–62]. As with children, adults can also experience intense pruritus resulting in the development of excoriated papules or prurigo nodularis-like lesions [63, 64].

Mucosal Involvement

Adults and children can develop mucous membrane involvement. One study reported that up to 80 % of adults have mucosal lesions [40]; however, in children, estimates of mucosal involvement are varied. One study with 25 children from the United Kingdom reported 64 % had mucosal lesions [65]. Alternatively, two retrospective studies of similar sample size from Tunisia and Japan reported only 8–3 % involvement, respectively [66].

Infrequently the mucosa is the sole manifestation of LABD [67–70]. In these cases, mucosal-predominant LABD is considered a form of cicatricial mucous membrane pemphigoid [48].

Erosions or ulcers are the primary mucosal lesions as it is rare to find intact vesicles or bullae. Affected sites include any mucosal surface of the body, including the ocular conjunctivae, oro- and nasopharynx, larynx, esophagus, vagina and anus, with the ocular and oral mucosae being the most common sites [40–43]. Within the oral mucosa, the lesions are frequently found on the palate, palatine arches and buccal mucosae [42]. Additionally, erosive gingivitis and cheilitis may occur as manifestations of mucosal LABD [40, 70]. Ocular symptoms manifest as erythematous conjunctivae, discharge, pain or a foreign body-like sensation [65]. Occasionally, patients develop symblepharon and ectropion, thus the disease is essentially an IgA variant of ocular pemphigoid [71]. Mucosal scarring can lead to serious adverse sequelae, including corneal damage leading to blindness, airway obstruction and esophageal strictures [71–73].

Drug-induced and Idiopathic LABD

Medications are often causes of LABD. Vancomycin is the most common offending medication; however, over 20 other medications have also been linked to it. Other antibiotic classes in this list are beta-lactams and cephalosporins. Nonsteroidal anti-inflammatory medications and acetylcholinesterase inhibitors, such as captopril, have been cited in case reports (Table 34.1). The idiopathic form of LABD does not differ significantly from the drug-induced form [42]. Presentations of both of these forms of LABD can be with localized involvement rather than widespread. They may or may not involve the mucosa [42]. They can be morbilliform eruptions. They can even resemble erythema multiforme or toxic epidermal necrolysis [29, 74–79]. Therefore, one should consider drug-induced LABD in the differential diagnosis when evaluating a patient with possible toxic epidermal necrolysis.

The similarities between drug-induced LABD and idiopathic LABD were evaluated in a retrospective study of 16 patients presumed to have spontaneous LABD and 12 patients presumed to have drug-induced LABD. This study found closely associated frequencies in both groups of erythematous plaques, string of pearls-like configurations, target lesions and mucosal involvement [29]. The drug-induced group contained a higher frequency of patients with atypical presentations of large erosions and positive Nikolsky signs.

Adults are more frequently afflicted with drug-induced LABD; however, it has also been reported in children [20, 40, 42]. The onset of lesions generally begins within the first month of drug administration and then they resolve gradually over the ensuing several weeks [20, 80, 81]. Some patients may have the lesions persist beyond this timeframe. Furthermore, if the patient is re-exposed to the inciting medication, rapid reappearance of the lesions can occur [82].

Associated Disorders

The most common non-malignant disorder associated with LABD is ulcerative colitis (UC) [50, 83–87]. Two retrospective studies reported this possible association, one from the United Kingdom and one from Japan. The study from the UK found that of 70 patients, 5 patients (7 %) had LABD, and UC preceded the diagnosis of LABD by an average of 6 years [85]. The review from Japan of 213 cases of LABD found four patients with UC [50]. This association is not well elucidated. Some authors suggest that abnormal IgA1 production by the inflamed bowel may contribute to the development of LABD; however, even after removal of the colon, some patients continue to have recurrences of LABD, while others have the disease completely resolve [83, 85, 87].

Malignant disorders, lymphoproliferative and solid organ types have also been associated with LABD in multiple case reports [31, 58, 88–104]. Despite these reports, no retrospective analyses have been performed to confirm this association. Further studies may confirm this suspected link between LABD and certain malignancies.

Other conditions with reported associations to LABD include systemic lupus erythematosus [50, 105] and psoriasis [106, 107]. Lastly, there are reports of LABD following exposure to ultraviolet light [108].

Diagnosis

The histopathologic findings with hematoxylin and eosin staining of involved skin are identical to those of dermatitis herpetiformis, showing lymphocytes, eosinophils and papillary microabscesses [42, 55] (Fig. 34.4a). Other characteristic findings are a subepidermal blister with a diffuse underlying neutrophilic infiltrate in the dermis.

A biopsy of perilesional skin demonstrating linear deposits of IgA along the basement membrane zone on direct immunofluorescence (DIF) testing is the gold standard test for diagnosis (Fig. 34.4b) [55]. Indirect immunofluorescence

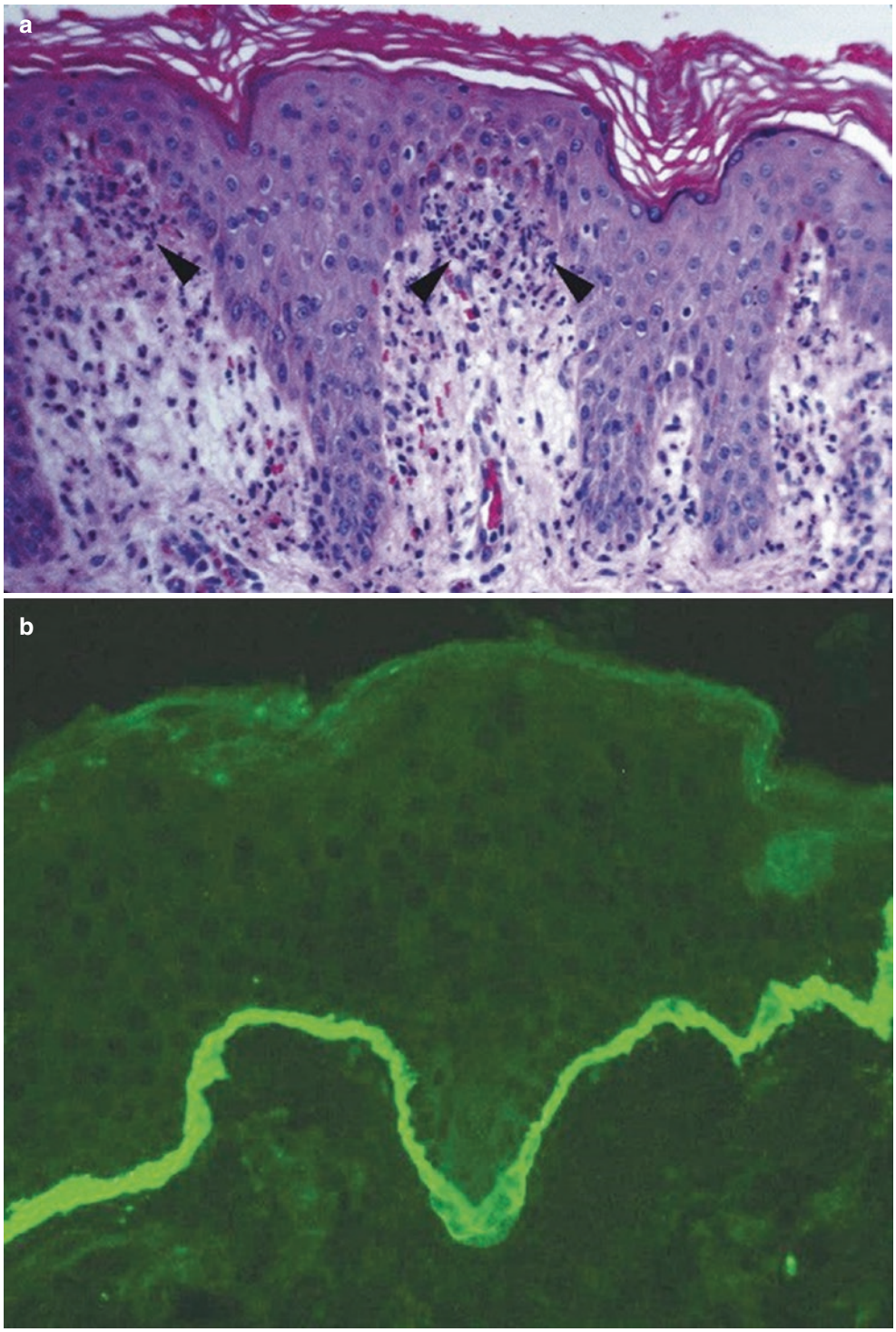

Fig. 34.4 Linear IgA bullous dermatoses. (**a**) Dermal papillary edema with a neutrophilic infiltrate (*arrowheads*) similar to DH. (**b**) Characteristic sharp linear IgA antibody deposition along the basement membrane zone

may be positive. Antibody binding on indirect immunofluorescence with basement membrane zone human salt-split substrate to the epidermal side of the induced cleavage zone is the most common location, while binding to the dermal side may occur in older patients or those who have both IgA and IgG deposits on the epidermal side of the basement membrane zone [50].

Treatment

The first-line medication therapy for LABD, as with DH, is dapsone [42, 52, 54, 55, 109]. Dapsone is started at a low dose, less than 0.5 mg/kg daily for children and 25–50 mg in adults. Gradually this dose is titrated upward over several weeks, depending on tolerance and treatment response [110]. This response can be immediate, resolving lesions within a few days of initiating therapy; however, some patients with more extensive disease and incomplete response to dapsone may need oral corticosteroids to accelerate improvement and effectively suppress the lesions [41, 54, 66, 111]. When patients need longer periods of immunosuppressive therapy or patients with predominantly mucosal involvement need more aggressive therapy, steroid-sparing agents can be effective, such as mycophenolate mofetil, azathioprine, intravenous immunoglobulin (IVIG), cyclophosphamide and rituximab [57, 111–122].

For patients who cannot tolerate dapsone, sulfapyridine or sulfamethoxypryridazine may be effective second-line therapies. Evidence for this is limited to reports from specialists in the field, as prospective therapeutic trials have not been performed to confirm this [42, 54, 55, 110, 123]. Sulfamethoxypyridazine is not available in the United States and sulfapyridine is only available through compounding pharmacies. The adult dosing range is 1000–1500 mg daily of either agent [54]. The sulfapyridine dosing range for children is 15–60 mg/kg daily [42, 55]. There is not an established dosing range for sulfamethoxypyridazine in children [42]. Both of these medications have been used in combination therapy with dapsone [55, 123, 124].

Colchicine can be effective in children with LABD. Some case reports and case series have shown it to be a reasonable substitute therapy for dapsone [111, 125–127]. In a series of eight children with systemic glucocorticoid-refractory LABD, the addition of colchicine led to dramatic improvement in five patients within 4–6 weeks. Furthermore, these children were able to taper off steroid therapy. The typical dosage in children is 0.6 mg twice daily.

Adults have also responded to colchicine according to some reports [56, 128]; however, other authors have not seen such reported results [54]. The adult dose range for colchicine is 0.6–1 mg two to three times daily [42, 52, 111, 127].

Additional therapies that have been used for LABD include tetracycline in combination with nicotinamide and topical tacrolimus ointment. This therapeutic combination has been effective for the treatment of bullous pemphigoid and has been applied to LABD. Three adult patients with LABD reported disease resolution within a few weeks of starting therapy [129–131]. The dosing range for tetracycline and nicotinamide are 1000–1500 mg daily and 900–2000 mg daily, respectively. Children under age nine cannot take tetracycline due to the adverse effect on developing teeth. Additional studies will help to confirm the efficacy of this treatment.

Children with LABD may respond to systemic antibiotic therapy. The mechanism for efficacy is not known, whether it is due to anti-inflammatory or antibacterial properties, or another unknown action remains to be clarified. One case series reports that in seven children treated with flucloxacillin, all had complete resolution, but only four children stayed in remission off of therapy [132]. Additional antibiotics reported to effectively treat LABD in children are oxacillin, dicloxacillin, erythromycin, micocamycin and trimethoprim-sulfamethoxazole [48, 66, 133–139].

Potent topical corticosteroids may be used adjunctively to accelerate resolution of lesions on the trunk or extremities, while low potency topical steroid creams can be used on the face, genitals or intertriginous areas.

Lastly, drug-induced LABD typically resolves when the inciting agent is stopped; however, in severe or persistent cases, dapsone and/or prednisone may be used to achieve faster resolution. Therapy should be tapered off early in the treatment course, within 4–6 weeks, to ascertain whether the disease is still active, warranting continuation of systemic therapy. A prolonged treatment course rarely occurs in this disease.

Prognosis

Idiopathic LABD can persist from months to several years in adults, whereas in children, it typically resolves before puberty [40, 52, 54]. This disease can also prevail for a decade or even longer. It can also recur after long periods of remission [54]. This is in contrast to drug-induced LABD, which usually improves within a few days of cessation of offending drug and resolves within several weeks [80].

The treatment duration for idiopathic LABD is variable. Therapy is generally continued for several weeks after complete resolution of lesions and then gradually tapered off. If at any point lesions recur, the treatment medication should be restarted [54].

Cutaneous lesions typically heal without scarring; however, mucosal lesions may lead to stricture formation or conjunctival and corneal scarring. These sequelae can have a significant impact on patients' oral hygiene and nutritional status.

Immunoglobulin A Pemphigus

IgA pemphigus, with subtypes subcorneal pustular dermatosis-type IgA pemphigus and intraepidermal neutrophilic IgA dermatosis, is a blistering disorder characterized by autoantibodies against the desmosomal components of keratinocytes. Subcorneal pustular dermatosis (SPD) appears clinically to resemble Sneddon-Wilkinson disease and IgA cell surface staining locates to the upper epidermis on DIF. The intraepidermal neutrophilic IgA dermatosis (IEN), as the name implies, exhibits IgA cell surface staining throughout the entire epidermis [140].

Epidemiology

Because of the rarity of these disorders, not many cases have been reported and epidemiologic information is scant. Reports suggest that they can occur at any age and that females might be slightly more predisposed [140]. Most cases have appeared in the United States, Europe and Japan [141].

Pathogenesis

In contrast to IgG-mediated pemphigus vulgaris, IgA pemphigus is an IgA-mediated anti-keratinocyte cell surface autoantibody disorder [142]. As stated above the target antigen in the SPD type is desmocollin 1, a calcium-dependent transmembrane glycoprotein of the cadherin family within the desmosomes [142, 143]. The target antigen in IEN has not been completely elucidated and studies have shown varying targets. Several patients have shown autoantibodies against desmoglein 1 and 3; however, when viewed through immunoelectron microscopy, the targets have been unidentified non-desmosomal transmembrane proteins [142, 144–147].

Clinical Features

Both types of IgA pemphigus are characterized by the subacute development of vesicles that evolve into pustules on erythematous plaques, usually distributed across the trunk and proximal extremities [140]. Other sites of involvement are the scalp, postauricular skin and intertriginous areas, while the mucous membranes are generally spared [48]. Pruritus may or may not be present, but can be pronounced if it is. Patterns of configurations may be herpetiform, annular or circinate [140]. Because the SPD type of IgA pemphigus so closely resembles Sneddon-Wilkinson disease, DIF must be performed to distinguish the two disorders.

Diagnosis

The diagnosis of IgA pemphigus is made through all tools available to the clinician, including clinical, histological, immunopathological and serological findings. Histological features are intraepidermal pustules and clefts with microabscesses in the subcorneal region for SPD and throughout the epidermis for IEN. There is a neutrophilic infiltrate in the epidermis and dermis with sparse or no acantholysis, especially in IEN [27].

DIF studies of perilesional skin reflect H&E findings in that intercellular IgA staining is located in the upper epidermal layers in SPD and throughout the epidermis in IEN. Weak intercellular IgG and/or C3 deposits may also be present [140]. IIF studies on monkey esophagus show expected intercellular IgA deposits, with testing positive about 50% of the time [140].

Additional tests used to identify circulating IgA autoantibodies to desmocollin 1 are immunoblotting, ELISA using recombinant desmocollin, and immunofluorescence molecular assay using desmocollin-transfected COS-7 cells (monkey fibroblast-like kidney cells), which are performed only in research laboratories at this time [48, 143, 148]. In one series of 22 patients, ELISA tests showed positive autoantibodies to desmoglein 1 in three patients and desmoglein 3 in one patient [142]. All four patients had either the IEN subtype of IgA pemphigus or even clinical and histological features of pemphigus foliaceus. Ten of the 22 patients had the SPD subtype, and they did not have autoantibodies to the desmogleins, rather they had autoantibodies to desmocollin 1 in COS-7 cells [142].

Treatment

As with all other IgA dermatoses, dapsone is the first-line therapy in both subtypes of IgA pemphigus; however, it is not always effective and response to treatment is varied, with the SPD type being more resistant to therapy [149]. Dapsone doses range between 75–100 mg for initial disease control and may be tapered down for maintenance therapy. Colchicine may also be used if dapsone is not well tolerated [150]. The SPD subtype is generally more recalcitrant to treatment. In these cases, isotretinoin or acitretin may be used alone or with dapsone as combination therapy [151]. For patients with the IEN subtype who fail dapsone monotherapy, oral steroids may be added in concert with dapsone. Occasionally, patients may need more aggressive immunosuppressive therapy with a monoclonal antibody, adalimumab, or mycophenolate mofetil [140, 152]. High potency topical corticosteroids may be used adjunctively with oral therapy.

Immunoglobulin A Vasculitis (Henoch-Schönlein Purpura)

Henoch-Schönlein Purpura (HSP), now also defined as IgA vasculitis (IgAV), is a leukocytoclastic small vessel vasculitis of the dermis that occurs most commonly in children after a precipitating infection or drug reaction [153]. The hallmark manifestations of the disease are the tetrad of palpable purpura, arthralgias, abdominal pain and glomerulonephritis. Although IgA deposition can occur in other isolated cases of small vessel vasculitis and secondarily in other associated diseases with small and large vessel vasculitis involvement, such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis and polyarteritis nodosa, HSP is the only disease that occurs because of the primary deposition of IgA complexes in the vessel walls [154–156]. Its manifestations are caused by this IgA deposition. The disease is generally self-limiting; however, protracted cases do occur and recurrences can happen. Adults can also manifest the disease after an inciting infection or drug reaction or for no identifiable reason.

Epidemiology

Children develop HSP most frequently between the ages of 3 and 12 years [157]. The annual incidence rates range from 3 to 26.7 per 100,000 for children and infants and from 0.8 to 1.8 per 100,000 for adults [157]. A population-based study from the United Kingdom showed a peak incidence for children between the ages of 4 and 7 years old. Adults appear to contract the illness between 45 and 50 years old; however, it has occurred in patients up to the age of 86 years [158–160].

HSP shows a slight predilection for males, with male-to-female ratios of 0.9–1.8 in children and 1.7–2.4 in adults [157–160]. Ethnic population studies reveal an increased frequency in white and Asian children and decreased frequency in blacks [157, 161]. Additionally, patients with familial Mediterranean fever have a significantly higher incidence rate for HSP [157, 162, 163].

Many epidemiological studies have shown a seasonality to the disease in children, with a prevalence for the fall and winter seasons and abatement in the summer [157]. This trend may be due to the association of HSP with increased winter-triggered upper respiratory infections in children [159]. Studies show that 30–65 % of IgA vasculitis cases develop after an upper respiratory infection [157, 164–168]. This seasonal association is not borne out in studies of adult cases; furthermore, no clear variations to the disease incidence have been illuminated [157]. Some epidemiologic studies have shown a possible association with preceding or concurrent malignancies; however, further studies are necessary to elucidate this possibility [157, 169–173].

Genetic factors have been investigated for predisposing and protective susceptibilities in the human leukocyte antigen (HLA) region. Two independent patient cohorts found an increased risk of HSP in patients with HLA-DRB1*01, HLA-DRB1*11, HLA-B35 and HLA-A11 alleles, while patients with HLA-DRB1*07 alleles in Spanish and Italian populations were found to be protected from HSP [6, 47, 157, 174–176].

Pathogenesis

IgAV is classified as a leukocytoclastic vasculitis due to the involvement of small vessels within the papillary dermis, generally the postcapillary venules. Neutrophils and monocytes are the main inflammatory infiltrate. Although the definitive role of IgA in the pathogenesis of HSP remains undefined, IgA deposition occurs within the small vessels of the affected organs, as evidenced on histological examination. Immunofluorescence staining reveals IgA, C3 and fibrin in these vessels. Additionally, infections and medications are the purported triggers for HSP; however, this has also not been completely clarified. Many studies have shown that immunologic, genetic and environmental components are involved [177–179].

IgA1, predominant in serum, is the main IgA antibody present in HSP. One theory regarding why this is found posits that abnormal glycosylation of the IgA1 hinge region would allow aggregation and large complexes to form [180]. Additional theories from research regarding the pathogenesis of IgAV include a role for IgA anticardiolipin antibodies, IgA rheumatoid factor antibodies and beta-2 glycoprotein 1 antibodies; however, more studies are needed to validate these potential contributors [181–185].

Clinical Features

The classic tetrad of HSP is palpable purpura, abdominal pain, arthralgias and glomerulonephritis. The palpable purpura generally occurs on the dependent areas of the body, starting on the bilateral lower extremities and extending up to the buttocks and back. Development of all the features is usually a subacute onset over days to weeks, with purpura and arthralgias presenting initially.

Skin manifestations are present in nearly 100 % of the presentation; however, the purpura may not always be the first sign. The rash generally appears with petechial, macular or even urticarial lesions that evolve shortly into palpable purpura, some with necrotic centers. Retiform plaques of purpura are common and characteristic (Fig. 34.5a, b). Sometimes, acral, scalp and facial edema accompany the rash.

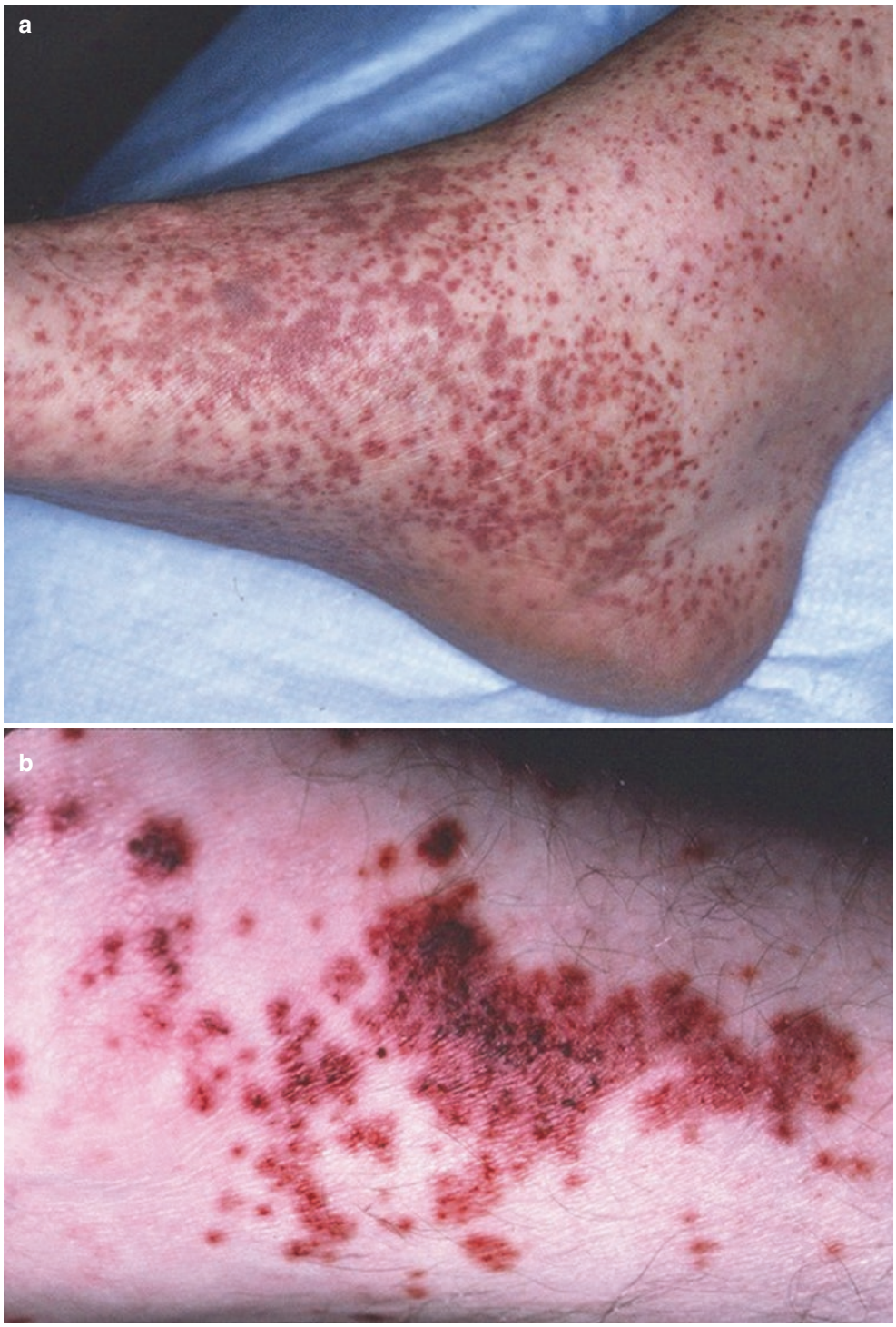

Fig. 34.5 IgA vasculitis (Henoch-Schonlein Purpura). (**a**) Confluent purpura on the foot. (**b**) Characteristic irregular plaques of retiform purpura

Some children have presented initially with abdominal pain and arthralgias, making the diagnosis of HSP more difficult. One review of the literature over a 5 year period reported this presentation occurring 15 % of the time [186]. The arthralgias typically involve the joints of the lower extremities and rarely the upper extremities [164, 186, 187]. Edema and pain accompany the joint pain. Chronic sequelae of the arthralgias are rare.

Gastrointestinal complications can range from mild pain, nausea and vomiting to extreme cases of gastrointestinal hemorrhage, ischemia and necrosis, as well as intussusception and perforation. If symptoms are not initially present, they manifest generally within a week of onset of the purpura. Rarely, abdominal symptoms are the sole manifestations of HSP without rash [188–190]. Although intestinal symptoms can be mild, testing confirms that mucosal injury is generally

present with heme-positive stools and other findings to suggest involvement [191]. In cases when intussusception is suspected, an ultrasound rather than a contrast enema is the initial screening test. Intussusception related to HSP involves the small bowel in about 60 % of cases, where as idiopathic intussusception usually involves the ileocolic region [192]. Contrast enemas can reliably detect idiopathic intussusceptions but not those involving the small bowel as well. Adults rarely develop intussusception.

Hematuria with or without proteinuria heralds renal involvement in HSP cases, which is seen in about 20–54 % of children and adults, with adults and older children manifesting it more frequently [187]. When renal involvement occurs it is a potential sign of long-term morbidity associated with HSP. One French study showed that of 250 patients with HSP, one-third had renal insufficiency within 4 months and that almost 15 years later, 80 patients (32 %) continued to have renal impairment, 27 (11 %) of whom had end-stage renal failure [160].

Diagnosis

Diagnosis of HSP or IgAV is usually a clinical one, owing to the classic tetrad of symptoms described above; however, confirmatory diagnostic tests with skin biopsies for H&E and DIF are recommended, especially with unusual presentations.

Histology of skin with H&E staining reveals classic leukocytoclastic vasculitic findings of fibrin deposition and a predominant neutrophilic inflammatory infiltrate in vessel walls. Direct immunofluorescence of lesional skin shows IgA and C3 deposition in the vessel walls. It is essential that early lesions be biopsied (less than 48 h old and pink in color rather than black or necrotic lesions), since severe inflammation in a lesion will destroy the diagnostic IgA epitopes [193].

When renal biopsies are performed, H&E staining demonstrates a range of isolated mesangial proliferation to crescentic glomerulonephritis. DIF reveals IgA deposition in the mesangial cells.

Laboratory testing should include a CBC with differential, renal function tests and a urinalysis to evaluate for hematuria and proteinuria. Abdominal imaging should be based on severity of symptoms. Because HSP is typically self-limiting, supportive or symptomatic care is usually all that is needed. Children and adults with renal involvement should be referred to a nephrologist for monitoring of resolution.

Treatment

There is much debate regarding the use of glucocorticoids in HSP; however, patients with severe enough symptoms that they cannot manage their oral intake, ambulate without difficulty or perform their activities of daily living may benefit. Dosing regimens should be between 1 and 2 mg/kg, with a maximum daily dose of 60–80 mg daily. Many studies, both retrospective reports and randomized trials, examined whether steroids were beneficial and the only benefits found were reduction of the duration of abdominal pain and development of chronic renal disease [194, 195]. Many authors do not recommend routine glucocorticoid therapy for all patients [196–198].

There are case reports demonstrating efficacy of dapsone in adults with chronic cutaneous involvement. Lesions disappeared with initiation of dapsone; however, they recurred once it was stopped [199–203].

Conclusion

Immunoglobulin A dermatoses represent a distinct but diverse category of immunologic skin diseases. They all have IgA deposition in the skin as their unifying characteristic, which leads to unique clinical presentations based on the pathophysiologic process within the skin. Because neutrophils are able to express the unique IgA receptor (FcaR1), they are the predominant infiltrate in the epidermis and dermis, which also leads to tissue damage mediated through their proteolytic actions. Lastly, owing to the effectiveness of sulfone therapy, dapsone and its related medications are the first-line treatment for this group of dermatoses.

Questions

1. What are the common findings in the skin for all of the IgA dermatoses?
   A. Cutaneous deposition of IgA at the histopathologic site of inflammation on direct immunofluorescence microscopy and a neutrophilic inflammatory infiltrate on hematoxylin and eosin staining of involved skin

2. Where is IgA found in the body?
   A. IgA is found in respiratory, gastrointestinal and genitourinary mucosal secretions, as well as human serum

3. What is the name of the IgA receptor common to these disorders?
   A. FcαR1

4. What are some of the cutaneous findings in these conditions?
   A. Bullae, vesicles, papules and pustules

5. What is the main therapy for the IgA dermatoses?
   A. Dapsone

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The Pemphigoid Spectrum

35

Donna A. Culton, Zhi Liu, and Luis A. Diaz

Abstract

The pemphigoid spectrum represents a group of blistering diseases of the skin defined by autoantibody formation against components of the hemidesmosome that collectively comprise the basement membrane zone. These autoantibodies along with complement are detected in perilesional tissue of affected individuals by direct immunofluorescence. Autoantibodies can also be detected in the sera in some individuals by indirect immunofluorescence and ELISA studies. Histology shows a subepidermal split with variable inflammatory infiltrate in the upper dermis. There are several variants of pemphigoid with unique clinical manifestations. Treatment is aimed at suppressing the misguided immune response through the use of topical and/or systemic steroids and immunosuppressive agents.

Keywords

Bullous pemphigoid • Cicatricial pemphigoid • Mucous membrane pemphigoid • Herpes gestationis • Pemphigoid gestationis

Key Points

- Bullous pemphigoid is a subepidermal autoimmune blistering disease of the skin, and is predominantly a disease of the elderly in its classical presentation.
- There are distinct clinical variants of this disease (bullous pemphigoid, cicatricial pemphigoid, and pemphigoid gestationis).
- The autoimmune response in BP is directed against two hemidesmosomal antigens, BP180 (collagen XVII or BPAg2) and BP230 (BPAg1).
- The diagnosis is based upon clinical findings, histology and immunofluorescence (direct and/or indirect immunofluorescence).
- Therapy includes topical and/or systemic corticosteroids along with immunosuppressive agents depending on the extent of disease.

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Bullous Pemphigoid

Overview

Bullous Pemphigoid (BP) is a subepidermal autoimmune bullous disorder and is the most common adult bullous disorder. BP typically occurs in elderly patients and presents with generalized pruritic tense bullae, with rare involvement of the mucous membranes. The pathogenesis of BP has been clarified by advances in the field of immunodermatology, which have also aided in diagnosis of atypical cases. Therapy focuses on immunosuppression to attenuate the autoimmune response.

Historical Background

BP was first described by Lever in 1953 as a subepidermal bullous dermatosis seen in elderly individuals [1]. Based on histologic evaluation, BP was characterized by detachment of the epidermis and an intense inflammatory cell infiltrate in the upper dermis. Jordon and Beutner discovered anti-skin

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autoantibodies in BP patients, present in the serum and bound to the dermal-epidermal junction in a linear fashion [2]. Further specification of the anti-skin autoantibodies was provided by Stanley et al., with the characterization of antibodies against a 230-kD epidermal protein within the sera of BP patients [3]. Subsequently, Mutasim et al. described that BP autoantibodies localize to hemidesmosomes, and Labib et al. described the binding of BP autoantibodies to an 180-kD epidermal protein [4, 5].

Further molecular characterization of the BP180 antigen was performed by Diaz et al. [6], demonstrating that BP180 is a transmembrane hemidesmosomal glycoprotein with both an extracellular C-terminus projecting into the lamina lucida, and an intracellular N-terminus. Development of a passive transfer murine model, and progress towards an active immunization murine model by Liu et al. have provided further clarification of the pathogenesis of BP [7].

Epidemiology

With onset typically occurring after the sixth decade, BP is classically considered a disease of the elderly. Indeed, the relative risk of developing BP in patients older than 90 years of age compared to those less than 60 years of age is approximately 300 [8]. Recent studies have suggested that even when age is controlled for, individuals with neurological diseases including dementia, stroke, and Parkinson's disease show an increased risk of developing BP [9–13]. In one of the most robust studies, the association of BP and neurological disease was only observed when the diagnosis of neurological disease was present prior to the development of BP, suggesting a possible causal association [10]. Though there have been numerous case reports of bullous pemphigoid developing in patients with malignancy, case–control studies have shown no increase in the frequency of malignancy in BP patients compared to age-matched controls [12, 14, 15]. There are no ethnic or gender predilections for developing BP.

Previous estimates of the incidence of BP range from 7 per million per year to 14 per million per year in France, Germany, and Scotland [16–18]. However, recent studies from the United Kingdom and France suggest that the incidence of BP has increased over the last several decades and may be as high as 22 per million per year to 43 per million per year [19, 20].

Given the advanced age of most patients with BP, assessing survival in this patient population has provided variable figures, with one year mortality ranging from 23 to 62 % [20–29]. Increased patient age, poor general health, extent of bullous lesions, presence of anti-BP180 autoantibodies, and dosing of oral steroids have all been associated with increased mortality in BP to varying degrees [23, 25, 26, 30].

Various human leukocyte antigen (HLA) classes have been associated with BP, specifically in regards to patient race. In Caucasians, HLA-DQB1*0301, has been associated with not only classic BP, but also ocular and mucous membrane involvement [31, 32]. Among patients of Japanese descent, increased incidence of the HLA-DRB1*04, DRB1*1101, and DQB1*0302 alleles have been noted [33]. Focusing on the geographic region of northern China, comparison of patients with BP to controls revealed decreased frequency of HLA-DRB1*08 and HLA-DQB1*06 in patients with BP [34].

Pathogenesis

The autoimmune response in BP is directed against two hemidesmosomal antigens, BP180 (BP Antigen 2, BPAg2 or collagen XVII), and BP230 (BP Antigen 1 or BPAg1). BP180 has been characterized as a member of the collagen family, possessing both an intracellular N-terminus domain and an extracellular C-terminus domain, which projects into the lamina lucida [6]. In contrast, BP230 is a cytoplasmic protein with structural similarity to plectin and desmoplakins I and II [35, 36]. As components of the hemidesmosome, BP180 and BP230 promote adhesion of the basement membrane to the basal layer of keratinocytes (Fig. 35.1).

Characterization of autoantibodies reveals a polyclonal response, with the majority of antibodies recognizing a cluster of epitopes about the largest noncollagen domain (referred to as NC16A) of the BP180 antigen [37, 38]. The BP180NC16A specific autoantibodies are predominantly IgE, IgG1, and IgG4 [39–41]. Titers of IgG and IgE BP180NC16A autoantibodies correlate to the degree of clinical severity [40–42]. Furthermore, autoantibodies against the BP180NC16A domain are sufficient to induce subepidermal blisters in the neonatal passive transfer model [43].

B cell production of pathogenic autoantibodies likely begins with development of autoreactive T cells. Most patients with BP demonstrate circulating autoreactive BP180-specific CD4 T cells as well as T-helper 2 (Th2) and Th1 responses against the BP180 ectodomain [44, 45]. Furthermore, BP180-reactive Th cells and IgG autoantibodies recognize similar or identical epitopes clustered in distinct regions of the BP180 ectodomain; the majority of autoreactive Th2 and Th1 cells and B cells recognize epitopes within the NC16A, followed by reactivity against the COOH-terminal and central regions [45–48]. Interestingly, T and B cell reactivity against the BP180 NC16A ectodomain is associated with severe BP, with widespread blisters and erosions, while responses against the central portion is more common in limited BP, with few blisters and erosions [47]. Less than 50 % of BP patients also show a combined T and B cell response against the COOH- and NH_2-terminal globular domains of BP230 [48].

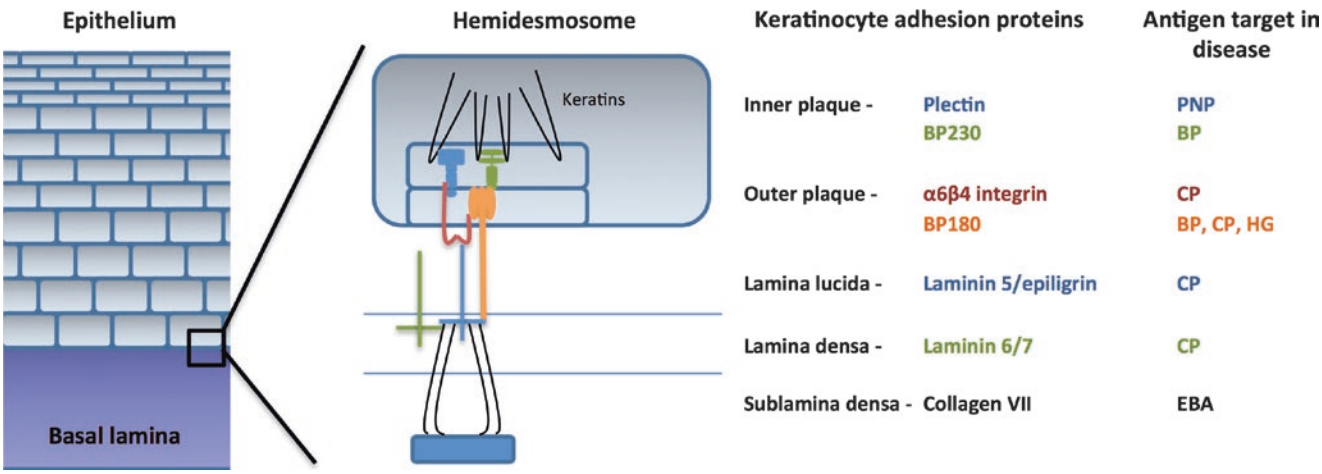

Fig. 35.1 Diagram of the hemidesmosome. Individual proteins comprise the hemidesmosome and many of these proteins serve as antigenic targets in clinical variants along the pemphigoid spectrum

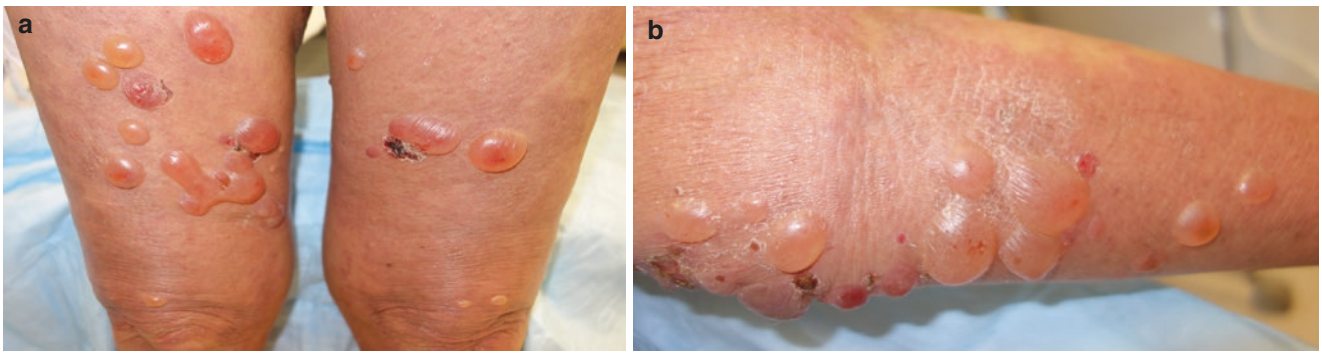

Fig. 35.2 BP classically presents with tense bullae overlying urticarial plaques on the trunk and extremities lower extremities (**a**) upper extremity (**b**) of elderly patients

The characteristic histologic and clinical features of BP are a result of the inflammatory cascade triggered by the binding of pathogenic autoantibodies to the antigen targets within the hemidesmosome structure. In vitro and in vivo studies show that antibodies against BP180 are pathogenic [7, 49]. In the in vivo model, neonatal mice injected intraperitoneally or intradermally with anti-murine BP180 antibodies develop BP-like skin lesions, including in situ deposition of IgG and complement C3 at the BMZ and an inflammatory cell infiltrate. Subepidermal blistering in this IgG passive transfer model of BP requires complement activation, mast cells and neutrophils [50–53]. Inflammatory cell products, such as neutrophil elastase, gelatinase and plasmin, result in tissue destruction at the dermal-epidermal junction, which manifests histologically as a sub-epidermal split [54–58].

Although the IgG autoantibodies in BP have been most studied to date, there is increasing evidence that IgE autoantibodies may also play a role in the pathogenesis of BP. As previously mentioned, over 90 % of BP patients have detectable circulating IgE anti-BP180 autoantibodies, and titers of IgE anti-BP180NC16A autoantibodies correlate with disease activity [40, 59]. IgE anti-BP180 autoantibodies likely play a role in the pathogenesis of BP by activation of mast cells and recruitment of eosinophils, as shown in murine models [60, 61]. Additional evidence comes from the dramatic improvement of several recalcitrant BP patients treated with the omalizumab, a monoclonal antibody that inhibits IgE binding to the high affinity IgE receptor [62, 63].

Clinical Features

Clinical manifestations of BP include pruritic, large, tense bullae overlying urticarial plaques (Fig. 35.2) [1]. However, over half of BP patients present with nonbullous lesions, as the prodromal phase is typically non-specific [64]. Intense pruritus, with or without an eczematous or urticarial eruption, are typically the first signs of the disease. In most patients, the prodromal phase is followed by the development of symmetric tense bullae, distributed primarily on the flexural aspects of the extremities and lower trunk. The

bullae are symptomatically pruritic, and may be filled with clear or hemorrhagic fluid. Rupture of the bullae results in shallow crusted erosions. Lesions heal without scarring, though milia formation may occur and postinflammatory pigmentation is quite common. Involvement of intertriginous areas may produce vegetative moist plaques. The mucous membranes are involved in a minority of patients (less than 10 %) and lesions are typically limited to the oral mucosa [1, 65, 66].

Diagnosis

Once the suspicion of BP is raised on the basis of clinical and historical patient information, definitive diagnosis is based not only on histologic evaluation, but also on further characterization of the autoantibody response through immunological techniques including direct and indirect immunofluorescence (IF) microscopy and enzyme linked immunosorbant assay (ELISA). Standard histologic evaluation via light microscopy reveals a subepidermal split with inflammatory infiltrate composed mainly of eosinophils and neutrophils in the upper dermis [1]. Occasionally, cell poor BP is observed where the inflammatory infiltrate is minimal or absent. Eosinophilic spongiosis has also been described in BP [67].

Direct IF (from perilesional, uninvolved skin) demonstrates a fine linear band at the dermal-epidermal junction of IgG and/or C3 with a sensitivity of 91 % [68] (Fig. 35.3a). Indirect IF (from patient's serum) demonstrates autoantibodies that bind the dermal-epidermal junction in a linear fashion with a sensitivity of roughly 70 %. The use of salt split skin as the substrate increases the sensitivity of indirect IF and also allows for distinction between BP (with localization of the autoantibodies to the epidermal side of salt split skin) and epidermolysis bullosa acquisita (with localization of autoantibodies to the dermal side of salt split skin) (Fig. 35.3b) [69, 70]. The BP180 NC16A domain ELISA is emerging as a more readily available test to evaluate for circulating autoantibodies in the serum and is being utilized in both the clinical and research setting. ELISA index values tend to correlate with disease activity with a sensitivity ranging from 72 to 89 % [68, 71]. However, approximately 7 % of the normal population has detectable anti-BP180 autoantibodies by ELISA without regard to age [68, 72]. The relevance of a positive BP180 ELISA in the absence of clinical findings is unclear, but the 7 % false positive rate suggests that ELISA should be used in the appropriate clinical context and with supporting direct and indirect IF studies rather than as the sole immunological criteria for diagnosis.

Therapy

Topical high potency corticosteroids are useful for limited areas of involvement in motivated patients. More generalized or severe manifestations typically require systemic corticosteroids, in initial dosing ranging from 0.5 to 1 mg/kg/day. After gaining control of development of new bullae, the dose may be tapered over 6–8 months, with dosing adjusted to account for the recurrence of disease. A recent study suggests that high potency topical steroids (clobetasol) may be an excellent approach to induce clinical remission in BP [24, 73].

In cases refractory to systemic corticosteroids, additional immunosuppressive medications may be employed. Methotrexate, azathioprine (dose adjusted for variability in thiopurine methyltransferase levels) and mycophenolate mofetil are all options, with selection based upon each medication's side effect profile [74–79]. Dapsone may be useful in some cases where systemic steroids or immunosuppressive drugs are contraindicated [80–82]. Plasmapheresis and intravenous immunoglobulin have been used successfully to induce remission in BP [83–85]. Rituximab, a monoclonal anti-CD20 antibody that targets B lymphocytes, has also shown efficacy in treating bullous pemphigoid [86]. In a small cohort of patients, low serum B cell activating factor (BAFF) levels and a higher proportion of memory B lymphocytes during B cell recovery post treatment were seen in those patients that flared post treatment [87]. While other

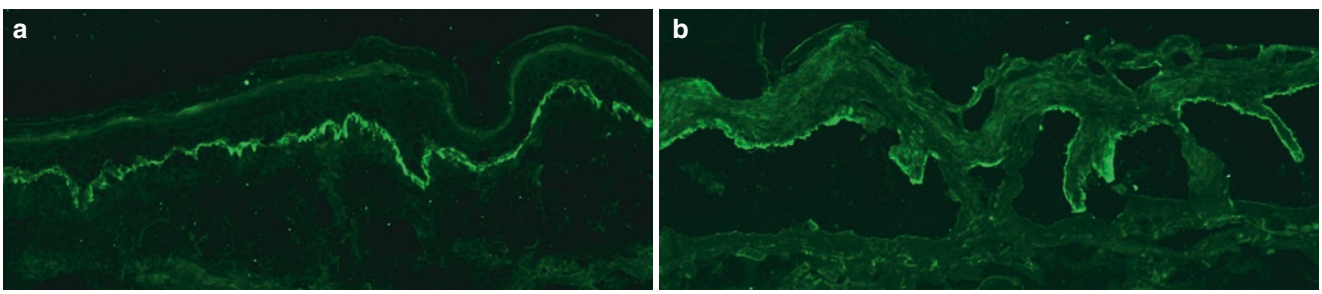

Fig. 35.3 Direct and indirect immunofluorescence (IF) findings of BP. (**a**) Direct IF shows C3 and IgG deposition in a linear pattern along the basement membrane zone. (**b**) Indirect IF shows IgG localization to the epidermal side of salt split skin

dermatologists have found tetracycline family members and nicotinamide useful in the control of BP, in our hands it has had a limited effect in some rare patients.

Cicatricial Pemphigoid

Overview

Cicatricial Pemphigoid (CP), also known as mucous membrane pemphigoid, is a rare chronic autoimmune blistering disorder, characterized by mucosal involvement and a high risk of scarring within the affected areas. Any or all mucous membranes may be involved in any individual patient. Direct IF demonstrates linear deposition of autoantibodies against various basement membrane zone components. The clinical course tends to be chronic and best managed with a multi-disciplinary approach to avoid major sequelae secondary to scarring [88].

Historical Background

Chronic blistering conditions with both skin and eye involvement were described throughout the 1800s [89, 90]. Up to the mid-1900s, CP was considered a variant of pemphigus, until Civatte and Lever separated the disorder based on histopathologic criteria [91, 92]. Lever suggested changing the name from "benign mucous membrane pemphigoid" to "cicatricial pemphigoid" based upon the tendency for scar formation [1, 93]. Tissue-bound immunoglobulins and complement components along the basement membrane zone were identified in the 1970s [94, 95] and circulating antoantibodies were demonstrated by Dantzig and Bean shortly thereafter [96, 97].

Epidemiology

CP has been documented to occur at any age, and is one of the most rare subepidermal bullous diseases, with an estimated annual incidence of approximately 1 per million per year [16, 18, 98]. There is a female predilection with females being affected twice as frequently as males with onset typically in the 60s [16, 18, 99]. In a recent review of patients with circulating antibodies recognizing laminin-5, over 30% of patients demonstrated internal malignancy, suggesting an increased relative risk of developing malignancy in this type of CP [100–102].

Pathogenesis

While CP is clinically characterized by the unifying clinical feature of mucosal involvement and scar formation and immunofluorescence finding of autoantibody deposition at the basement membrane zone, the antigenic targets can be quite heterogeneous. Commonly recognized target antigens in CP include BP180, BP230, laminin-332 (also known as laminin-5, or epiligrin), laminin-6, integrin $ß_4$ subunit, and integrin α subunit [103–106]. While autoantibodies are typically of the IgG class, IgA autoantibodies are also detected in some patients as well. The collective contribution of how the autoantibodies, the specific antigenic targets, and the microenvironment lead to mucosal-predominant inflammation and scar formation is still not understood.

Clinical Features

While any mucosal surface may be affected in CP, the oral site is most commonly involved, followed by, in decreasing order of involvement, the ocular, nasal, nasopharyngeal, anogenital, laryngeal, and esophageal sites [107–109]. Oral disease may present as erosive or desquamative gingivitis; intact blisters are rarely observed (Fig. 35.4a). Chronic erosions on the palate and lateral tongue are commonly seen, as well. Once healed, the areas may resemble lesions of lichen planus, with white reticulated striations.

Conjunctival involvement is also commonly seen, and, if not appropriately addressed, may result in blindness. Conjunctival inflammation, erosions, symblepharon, entropion and trichiasis may be present (Fig. 35.4b). Symptoms include dryness, burning and foreign body sensation of the eyes. Similar to the appearance of lesions in the oral mucosa, intact bullae are very rarely observed [107]. CP may be limited to the ocular mucosa in some patients without other sites of involvement. Examination by an ophthalmologist is critical for detection of early disease by slit-lamp evaluation.

Nasopharyngeal involvement may be associated with lesions of the upper aerodigestive tract [108]. Symptoms suggestive of nasopharyngeal involvement include nasal crusting, recurrent epistaxis, and nasal airway obstruction. A history of persistent pharyngalgia, dysphagia, odynophagia, dysphonia, or dyspepsia may suggest pharyngeal and laryngeal involvement [110]. Development of strictures or stenosis of the pharynx or larynx may prove life threatening, if not identified at an early stage.

Genital and anal involvement is relatively rare. Progressive disease may lead to narrowing of the introitus in women, or to phimosis in men. Anal involvement may lead to scarring, and potentially to stricture. The skin is involved in approximately a quarter of patients with CP with lesions most frequently appearing on the head, neck, and upper trunk. Erythematous plaques develop recurrent blisters, and heal with atrophic scarring.

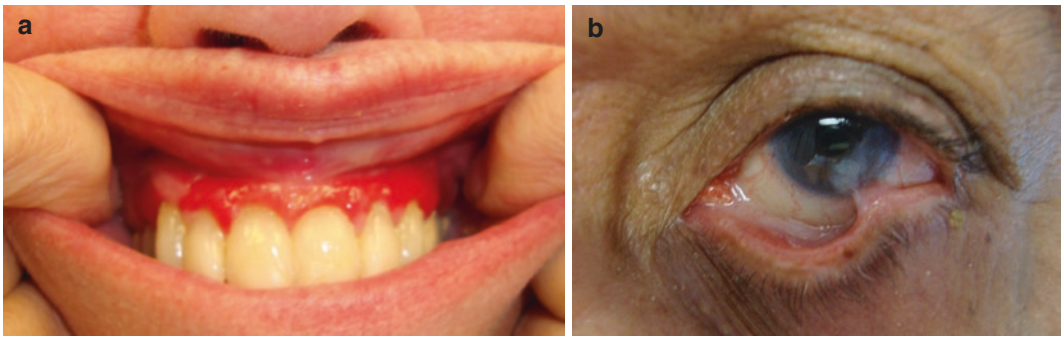

Fig. 35.4 Clinical manifestations of cicatricial pemphigoid (CP). (**a**) Oral manifestations of CP often include desquamative gingivitis. (**b**) Ocular manifestations of CP can include symblepharon formation

Diagnosis

Diagnosis of CP relies on immuofluorescent evaluation. Direct IF performed on perilesional mucosa or skin reveals IgG, IgA, and/or C3 in a continuous fine linear pattern along the basement membrane zone [88, 111–114]. Direct IF is most likely to provide conclusive information when performed on an area of mucosa adjacent to an area of inflammation [88]. In order to minimize the risk of conjunctival scarring, other anatomic areas of involvement should be biopsied preferentially, as injury to the conjunctiva may increase disease activity [88]. Indirect IF reveals autoantibodies at the basement membrane zone in 20–30 % of patients with clinical disease. Localization of autoantibodies, when performed on human salt-split skin substrate, is variable, depending upon the diversity of each patient's autoantibody milleu and the location of the autoantigens targeted [70, 115–119].

Therapy

In patients with mild, low-risk disease (defined as oral mucosa involvement, with or without skin involvement), potent topical corticosteroids may be sufficient. However, in patients with high-risk disease (defined as involvement of ocular, genital, laryngeal, nasopharyngeal, esophageal sites), initial therapy should involve systemic corticosteroids, with consideration of addition of cyclophosphamide or azathioprine [88]. Dapsone is also a consideration for stable or mild disease. Case reports document the use of various other modalities, including intravenous immunoglobulin, mycophenolate mofetil, and most recently, rituximab [120–125].

Herpes Gestationis (Pemphigoid Gestationis)

Overview

Herpes Gestationis (HG) is a rare dermatosis of pregnancy and the immediate post-partum period characterized by intensely pruritic urticarial lesions and eventually tense bullae. Most patients demonstrate autoantibodies to the BP180 antigen, with generation of a subepidermal separation on histologic evaluation. In pregnancies affected with HG, there is increased risk of prematurity and small for gestational age birth. The clinical course tends to be quite variable, and most cases resolve following delivery.

Historical Background

The term *herpes gestationis* was first cited by Milton in 1872, with demonstration of complement deposition along the basement membrane zone first documented by Provost in 1973 [126]. In 1976, Jordon et al and Katz et al characterized the "HG" factor as an IgG antibody that activates the complement pathway [127, 128]. Guidice et al identified the structural antigen for the "HG factor" as BP180, and provided further structural analysis of the antigen [37, 129].

Epidemiology

HG is a rare disorder exclusively seen during late pregnancy and the immediate post-partum period. Even more rare is its association with trophoblastic malignancy or molar pregnancy [130, 131]. Estimates of incidence for HG range from 1 in 10,000 to 1 in 50,000 pregnancies [132, 133]. HG often recurs in subsequent pregnancies, and may occur earlier and in a more severe form [134]. Also, with subsequent pregnancies, the time to resolution in the post-partum period may become progressively prolonged [135].

Pathogenesis

Generation of autoreactive T cells stimulate production of autoantibodies against the NC16A region of the BP180 antigen, prompting histopathologic and clinical manifestations of HG. Characterization of the autoreactive T cells has revealed

expression of a Th1 cytokine profile, supporting the production of IgG1 autoantibodies against BP180 [136, 137].

Deposition of the autoantibodies prompts complement activation, primarily through the classical pathway, followed by mast cell recruitment, degranulation, recruitment of inflammatory cells (primarily eosinophils), and production of destructive proteases [138–140]. The resultant damage at the dermal-epidermal junction manifests as a subepidermal separation on histologic examination.

HG has been associated with HLA-DR3, HLA-DR4, or both alleles [138–144]. Correspondingly, patients with HG are at increased risk for the development of other autoimmune disorders, such as Grave's disease [142].

Clinical Features

Typically, abdominal urticarial lesions appear abruptly during the second or third trimester, followed by the appearance of a generalized bullous reaction sparing the face, mucous membranes, palms and soles [133, 134, 145, 146]. However, initial onset in the immediate postpartum period has been described in 20 % of cases [147]. Most patients experience a flare with delivery, which spontaneously resolves over weeks to months following delivery. Flares with subsequent pregnancies, menstruation, or initiation of oral contraceptive use are common [145, 148].

Neonatal vesicles are present in approximately 10 % of cases, presumably secondary to passive transfer of pathogenic antibodies [145, 149, 150]. While the lesions are transient and typically mild, they are at increased risk of superinfection secondary to the infant's relatively immunocompromised status. Although associations have been reported in regards to preterm delivery and small for gestational age infants, there have been no reports of increased fetal morbidity or mortality [151, 152].

Diagnosis

Standard light microscopy of involved skin reveals a subepidermal vesicle with a lymphocytic and eosinophilic perivascular infiltrate. Direct IF of perilesional skin demonstrates linear C3 along the basement membrane zone; occasionally, IgG is also present, but to a lesser degree than C3 [140, 153]. While standard indirect IF is rarely positive, complement-added indirect IF is almost universally positive for pathogenic IgG antibodies [153].

Therapy

For limited disease, high potency topical corticosteroids along with oral antihistamines may provide sufficient control. However, more severe eruptions may require oral corticosteroids for adequate response. Intravenous immunoglobulin has also been effective for some patients [154]. More therapeutic options are feasible during the postpartum period, such as cyclophosphamide and methotrexate, although only sporadic reports of variable efficacy are available [155, 156].

Summary

The pemphigoid spectrum of diseases shares histological and immunofluorescence features of subepidermal blister formation with detection of autoantibodies and complement along the basement membrane zone. While BP180 is the antigenic target in the most classic form of the disease, clinical variants exist with more heterogeneous target autoantigens. The diagnosis of these diseases relies upon the triad of supporting clinical, histological, and immunological features. Treatment is aimed at suppressing the misguided immune response with topical and/or systemic corticosteroids in addition to other immunosuppressive agents. Progress continues to be made in understanding disease initiation, pathogenesis and more targeted treatment strategies.

Questions and Answers

1. What diseases are associated with bullous pemphigoid?
   Patients with neurological diseases including dementia, stroke, and Parkinson's disease show an increased risk of developing BP

2. Describe the stepwise events involved in the pathogenesis of bullous pemphigoid.
   The pathogenesis of bullous pemphigoid starts with autoantibody formation against BP180 and BP230. Bound autoantibodies fix complement at the basement membrane zone and elicit an inflammatory cell infiltrate of neutrophils, mast cells, and eosinophils. Inflammatory cell products, such as neutrophil elastase, gelatinase and plasmin, result in tissue destruction at the dermal-epidermal junction, which manifests histologically as a sub-epidermal split

3. What are the most common sites of involvement in cicatricial pemphigoid?
   While any mucosal surface may be affected in CP, the oral site is most commonly involved, followed by, in decreasing order of involvement, the ocular, nasal, nasopharyngeal, anogenital, laryngeal, and esophageal sites. The skin is involved in a minority of patients

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Epidermolysis Bullosa Acquisita

36

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Abstract

In 1895, two cases of a blistering disease with adult onset and features highly reminiscent of hereditary dystrophic epidermolysis bullosa (EB) were reported by Elliott. These clinical features included skin fragility, erosions, blisters, and a healing response characterized by scarring and the formation of milial cysts.

Keywords

Epidermolysis bullosa acquisita • EBA • Autoimmune disease • Blisters • Skin disease • Porphyria cutanea tarda • PCT • Bullous pemphigoid • BP • Cicatricial pemphigoid • CP • Immunoelectron Microscopy • IM • Anti–tumor necrosis factor-a • TNF-a • Pathogenesis

Key Points

- Epidermolysis bullosa acquisita (EBA) is an autoimmune, blistering skin condition, in which there is an autoantibody to type VII collagen, a component of the anchoring fibril complex of the basement membrane zone.
- There are a number of clinical presentations of this disease: the classic porphyria cutanea tarda (PCT)-like, noninflammatory mechanobullous disease; the bullous pemphigoid (BP) presentation of widespread inflammatory blisters; the cicatricial pemphigoid (CP)–like presentation with mucous membrane involvement; the Brunsting- Perry–like presentation (disease localized to the head and neck area); and the immunoglobulin A (IgA) bullous dermatosis–like presentation (inflammatory presentation with a neutrophil- rich infiltrate).
- Complications of EBA include: esophageal strictures, loss of nails, scarring, and contractures of the hands.
- Epidermolysis bullosa acquisita may be associated with underlying diseases such as inflammatory bowel disease, systemic lupus erythematosus, amyloidosis, and other inflammatory and autoimmune conditions.
- Clinical presentation and histological findings are used to confirm this diagnosis. Indirect and direct immunofluorescence mapping studies on NaCl split skin can facilitate distinguishing EBA from bullous pemphigoid. Western blotting confirms that the sera from EBA patients bind to a 290-kd autoantigen (type VII collagen).
- Epidermolysis bullosa acquisita can be challenging to treat. Colchicine and systemic glucocorticosteroids alone or in combination with cytotoxic drugs or cyclosporine have been used to treat severe disease. Recently, rituximab has shown promising results as a treatment option for EBA.

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In 1895, two cases of a blistering disease with adult onset and features highly reminiscent of hereditary dystrophic epidermolysis bullosa (EB) were reported by Elliott [1]. These clinical features included skin fragility, erosions, blisters, and a healing response characterized by scarring and the formation of milial cysts.

In the early 1970s, Roenigk et al. [2] summarized the epidermolysis bullosa acquisita (EBA) world literature, reported three new cases, and suggested the first diagnostic criteria: (1) a negative family and personal history for a previous blistering disorder, (2) an adult onset of the eruption, (3) spontaneous or trauma-induced blisters that resemble those of hereditary dystrophic epidermolysis bullosa, and (4) the exclusion of all other bullous diseases.

Kushniruk [3], Gibbs and Minus [4], and Nieboer et al. [5] showed that patients with EBA had immunoglobulin G (IgG) deposits at the dermal– epidermal junction identical to patients with bullous pemphigoid (BP). However, Nieboer et al. and Yaoita et al.[6] showed that the IgG deposits in EBA were within and below the lamina densa area of the basement membrane zone (BMZ), whereas BP immune deposits are within hemidesmosomes and high in the lamina lucida. Distinguishing EBA from the BP group is important because the clinical, pathologic, and immunologic presentations of EBA may be identical to BP and cicatricial pemphigoid (CP) but treatments may vary (see below) [7–12].

Clinical Findings

As noted above, the cutaneous lesions of EBA can be quite varied and can mimic other types of acquired autoimmune bullous diseases. The common denominator for patients with EBA is autoimmunity to type VII (anchoring fibril) collagen. Although the clinical spectrum of EBA is still being defined, there are at least five clinical presentations: (1) a classic presentation, (2) a BP-like presentation, (3) a CP-like presentation, (4) a presentation reminiscent of Brunsting- Perry pemphigoid with scarring lesions and a predominant head and neck distribution, and (5) a presentation reminiscent of linear IgA bullous dermatosis or chronic bullous disease of childhood [11–14].

Classic Presentation

This disease classically presents with non-inflammatory bullous lesions with an acral distribution that heals with scars and milia formation. When clinically mild, this form of EBA is reminiscent of porphyria cutanea tarda (PCT). However, when clinically severe, EBA will resemble the hereditary form of recessive dystrophic EB. The classic form of EBA is thus a mechanobullous disease marked by skin fragility. These patients have erosions, tense blisters within non-inflamed skin, and scars over trauma-prone surfaces such as the backs of the hands, knuckles, elbows, knees, sacral area, and toes. Some blisters may be hemorrhagic or develop scales, crusts, or erosions. The lesions heal with scarring and frequently form pearl-like milia cysts within the scarred areas. Additionally, a scarring alopecia and some degree of nail dystrophy may be seen. Although this presentation may be reminiscent of PCT, these patients do not have other hallmarks of PCT such as hirsutism, a photodistribution of the eruption, or scleroderma-like changes. Additional, their urinary porphyrins are within normal limits.

Although the disease is usually not as severe as that of patients with hereditary forms of recessive dystrophic EB, EBA patients with the classic form of the disease may have many of the same sequelae such as scarring, loss of scalp hair, loss of nails, fibrosis of the hands and fingers, and esophageal stenosis [15–28].

Bullous Pemphigoid-Like Presentation

A second clinical presentation of EBA is of a wide- spread, inflammatory vesiculobullous eruption involving the trunk, central body, skin folds, and extremities [7]. These tense bullous lesions are surrounded by inflamed or even urticarial skin. Large areas of skin may be without any blisters but with erythematous and urticarial plaques. These patients often complain of pruritus and do not demonstrate prominent skin fragility, scarring, or milia formation. This clinical constellation is more reminiscent of BP than of a mechanobullous disorder. Like BP, the distribution of the lesions may show an accentuation within flexural areas and skin folds. About 25 % of patients with EBA may present with a BP-like clinical appearance.

Cicatricial Pemphigoid-Like Presentation

Both the classic and BP-like forms of EBA may have involvement of mucosal surfaces. However, EBA also may present with such predominant mucosal involvement that the clinical appearance is reminiscent of CP [8]. These patients usually have erosions and scars on the mucosal membrane of the mouth, upper esophagus, conjunctiva, anus, or vagina with or without similar lesions on the glabrous skin. The clinical phenotype of EBA that is reminiscent of pure CP occurs in fewer than 10 % of all EBA cases.

Brunsting-Perry Pemphigoid-Like Presentation

Brunsting-Perry pemphigoid is a chronic recurrent vesiculobullous eruption localized to the head and neck and characterized by residual scars, subepidermal bullae, IgG deposits at the dermal–epidermal junction, and minimal or no mucosal involvement. The antigentic target for the IgG autoantibodies has yet to be defined; however, a patient reported with these findings had IgG autoantibodies directed against anchoring fibrils below the lamina densa [12]. It appears that EBA patients may present with a clinical phenotype similar to Brunsting-Perry pemphigoid.

Linear Immunoglobulin A Bullous Dermatosis-Like Presentation

This form of EBA manifests as a subepidermal bullous eruption with a neutrophilic infiltrate and linear IgA deposits at the BMZ when viewed by direct immunofluorescence (DIF). Clinically, it may resemble linear IgA bullous dermatosis (LABD), dermatitis herpetiformis, or chronic bullous disease of childhood (CBDC), and may feature tense vesicles arranged in an annular fashion with or without involvement of mucous membranes [29–34]. The autoantibodies are usually IgA, IgG, or both. Some clinicians regard these patients as having purely LABD [31], whereas others regard them as having a subset of EBA [32].

Childhood EBA

Childhood EBA is a rare disease with a variable presentation. Out of 14 patients reviewed, 5 presented with an LABD-like disease, another 5 presented with BP-like disease, and 4 presented with the classical manifestations [34]. Mucosal involvement is frequent in childhood EBA. The overall prognosis is more favorable than for adults with EBA [30, 34].

Additional Clinical Findings

In addition to the protean clinical manifestations of EBA, patients may suffer from a number of associated clinical problems that add to the morbidity of the disease. These include oral erosions, esophageal strictures, nail loss, milia formation, scarring, and a degree fibrosis of the hands. These are all associated clinical conditions that are shared (albeit usually milder) with hereditary dystropic EB.

Associated Systemic Diseases

Epidermolysis bullosa acquisita has been linked to several systemic diseases. Most commonly, inflammatory bowel disease (IBD) occurs in 20–30 % of all EBA patients. Patients with IBD, particularly Crohn's disease, have circulating antibodies to collagen 7 (C7) in approximately 70 % of cases [35, 37]. Recently, Ishii et al. have shown that injection of rabbit anti-murine C7 IgG (passive acquisition of EBA) or immunization with a fragment of murine C7 (active acquisition of EBA) not only produced cutaneous symptoms of EBA but also resulted in autoantibody deposition in the gastrointestinal tract with resultant blister formation [36, 38]. These findings in a mice model suggest that the correlation between IBD and EBA is more direct than previous thought. In addition to its relationship with IBD, anecdotal reports suggest that EBA may have other associated systemic diseases including systemic lupus erythematosus (SLE), amyloidosis, thyroiditis, multiple endocrinopathy syndrome, rheumatoid arthritis, pulmonary fibrosis, chronic lymphocytic leukemia, thymoma, diabetes, and other diseases in which an autoimmune pathogenesis has been implicated [37, 38].

Clinical Evaluation

Histopathology

Histopathology of an EBA lesion shows a subepidermal blister. In the classic mechanobullous presentation of EBA, there is fibrin in the blister cavity and an overall paucity of associated inflammatory cells within the blister cavity and dermis. Fibrosis and scaring may also be present in the underlying dermis. In the BP-like EBA presentation, the histopathology shows a more significant dermal inflammatory infiltrate of lymphocytes, macrophages, neutrophils, and eosinophils. In the IgA pemphigoid-like EBA form, there is often a predominance of neutrophils.

Direct Immunofluorescence

Krushnick et al. [3], Gibbs and Minor [4], Nieboer et al. [5], and Yaoita et al. [6] have shown that a positive direct immunofluorescence (DIF) is necessary for the diagnosis of EBA. Immunoglobulin G (IgG) and, to a lesser extent C3, deposits are present at the dermal–epidermal junction by DIF from a perilesional biopsy. However, the immunodeposits are almost identical in pattern to those seen in bullous pemphigoid. EBA may have stronger IgG deposition, while BP may have strong C3 deposition. Therefore, performing salt-split skin (SSS) DIF and SSS indirect immunofluorescence

(IIF) are necessary to distinguish EBA from the pemphigoid group of disorders. To perform the SSS technique, perilesional skin showing immunodeposits during routine DIF is incubated in 1 mol/L cold NaCl for 72 h. The procedure fractures the dermal–epidermal junction through the lamina lucida of the basement membrane zone. This places the bullous pemphigoid autoantigens associated with the hemidesmosome (i.e., BPAg 1 and BPAg 2, also known as type XVII collagen) on the epidermal roof of the separation [36–45]. The EBA antigen, type VII collagen, remains with the dermal floor [47]. The immunoreactants (usually IgG and C3) are again incubated with the tissue. If the patient has EBA, the immune deposits are detected on the dermal side of the separation.

Indirect Immunofluorescence

Many, but not all, EBA patients have an anti–BMZ IgG autoantibody circulating in their blood that can be detected by IIF. The EBA serum autoantibodies label frozen sections of human skin or monkey esophagus, producing a crisp linear fluorescent staining at the dermal–epidermal junction of the frozen sections after incubation with anti-IgG fluorescent-labeled antibodies. As with the routine DIF procedure, one cannot distinguish EBA from the pemphigoid group of diseases without doing salt-split IIF, which is always done on human skin substrate. Human skin is incubated in 1 mol/L NaCl, and the dermal–epidermal junction fractures cleanly through the lamina lucida zone, placing the BP antigens on the epidermal roof and the EBA antigen (type VII anchoring fibril collagen) on the dermal floor [41]. Salt-split skin substrate can be used to distinguish EBA and BP sera [42]. If the serum antibody is IgG and labels the epidermal roof, the patient does not have EBA, and BP should be considered. If, on the other hand, the antibody labels the dermal side of the separation, the patient usually has either EBA or bullous SLE. The latter can be ruled out by other serology and clinical criteria.

Rare Diseases that Give Dermal Staining by Salt-Split Immunofluorescence

It was thought that only EBA and bullous SLE show dermal staining of the salt-split skin on IIF or DIF. In recent years, other very rare autoimmune diseases have been shown to have IgG deposits in the lower lamina lucida space that map to the dermal side when the skin substrate is fractured by SSS technique. These diseases include anti-laminin 5 cicatricial pemphigoid [43], a BP-like disease in which the patients have autoantibodies to a 105- kd lamina lucida glycoprotein that is unrelated to laminin-5 [44], a newly discovered disease reported by Ghohestani and colleagues [46], with IgG autoantibodies directed against the α5 chain of type IV (lamina densa) collagen in association with renal failure, and another BP-like disease called protein 200 pemphigoid in which the autoantigen is a 200-kd glycoprotein in the lower part of the lamina lucida.

Electron Microscopy

Electron microscopy (EM) shows that the dermal–epidermal separation in an EBA lesion is associated with a paucity of normal anchoring fibrils and an amorphous, electron dense band beneath the lamina densa due to the IgG deposits over the anchoring fibrils [9]. Despite the sublamina densa deposits, EBA blisters frequently separate above the immune deposits within the lamina lucida [39].

Immunoelectron Microscopy

Immunoelectron microscopy (IEM) localizes the EBA IgG autoantibody deposits in the BMZ to within and below the lamina densa, the location of the anchoring fibrils. Immunoelectron microscopy showing these sublamina densa IgG deposits is the gold standard for the diagnosis, as first demonstrated by Nieboer et al. [13] and Yaoita et al. [6] This localization is distinct from BP IgG deposits which are localized to the hemidesmosomes of the basal keratinocytes and the IgG autoantibody deposits in CP and are confined to the lower lamina lucida.

Western Immunoblotting

Antibodies in EBA sera bind to a 290-kd band in Western blots of human skin basement membrane proteins containing type VII collagen, whereas sera from other primary blistering diseases do not [13].

This band corresponds to the alpha-chain of type VII collagen. Often a second band of 145 kd will be labeled with EBA antibodies. This band is the amino-terminal globular NC1 domain of the type VII collagen alpha-chain that is rich in carbohydrate and contains the antigenic epitopes of EBA autoantibodies, bullous SLE autoantibodies, and monoclonal antibodies against type VII collagen [13, 48].

Enzyme-Linked Immunosorbent Assay

Now that purified, recombinant, human, type VII collagen is readily available, an enzyme-linked immunosorbent assay (ELISA) for the diagnosis of EBA has been developed by Chen et al. [24, 49]. It has proven to be more sensitive for detecting anti-COL7 EBA autoantibodies in the sera of

patients than either IIF or Western blotting analysis. ELISA is performed quickly and easily with a very high sensitivity in determining which circulating autoantibodies are present.

Pathogenesis

Although EBA does not have a mendelian pattern of inheritance, African-American EBA patients in the Southeastern part of the United States have a high incidence of the human leukocyte antigen (HLA)-DR2 phenotype. The calculated relative risk for EBA in HLA-DR2+ individuals is 13.1 in these patients [27]. It is thought that, although it is not the primary cause, these patients have an immune profile that makes them susceptible to the disease.

While the etiology of EBA is unknown, it appears that when IgG autoantibodies bind to the patient's anchoring fibrils, a paucity of normal anchoring fibrils at the BMZ develops, and this is associated with poor dermal–epidermal adherence. This is exactly the same problem as hereditary dystrophic forms of EB due to a defect in the gene that encodes for type VII collagen.

Epidermolysis bullosa acquisita likely has an autoimmune etiology. Direct immunofluorescence of perilesional skin biopsies from EBA patients reveals IgG deposits at the dermal–epidermal junction [3–6]. The EBA antibodies bind to type VII collagen within anchoring fibrils [13, 14], structures that emanate perpendicularly from the BMZ and attach to the papillary dermis. This process results in decreased anchoring fibrils, but the pathway leading to this reduction is unknown. Type VII collagen has an affinity for fibronectin, a large glycoprotein in the papillary dermis and this interaction between the two may play a role in anchoring the basement membrane [22]. It is conceivable that EBA autoantibodies binding to type VII collagen interrupt the interaction between type VII collagen and fibronectin and a separation ensues.

Diagnosis

The diagnostic criteria for EBA developed by Yaoita et al. [6] still stand. These criteria, with slightly updated modifications, are as follows:

- A bullous disorder within the clinical spectrum outlined earlier
- No family history of a bullous disorder
- Histology showing a subepidermal blister
- Deposition of IgG deposits within the dermal–epidermal junction with a positive DIF on perilesional skin
- IgG deposits localized to the lower lamina densa or sublamina densa zone of the dermal epidermal junction when perilesional skin is examined by IEM. Alternatives also include indirect or direct salt split skin immunofluorescence, Western blotting, and ELISA.

Treatment

Epidermolysis bullosa acquisita is difficult to treat and can be refractory to many therapies. The therapy with the least associated risk known is colchicine at high doses. Colchicine is a well known microtubule inhibitor that also inhibits antigen presentation to T-cells and downregulates autoimmunity. The side effects of colchicine are relatively benign compared with other agents. Diarrhea is a common side effect, particularly at high doses. We do not use colchicine in EBA patients who also have inflammatory bowel disease [50, 51].

The noninflammatory, mechanobullous type of EBA is notoriously resistant to systemic steroids.

Immunosuppressants such as mycophenolic acid, azathioprine, methotrexate, cyclosporin A, and cyclophosphamide may be somewhat helpful in controlling EBA when it appears as an inflammatory BP-like disease. When using cyclosporin A high doses are needed, typically in the range of 6 mg/kg, and the nephrotoxicity of this drug sometimes limits its use [52, 53]. Some EBA patients improve on dapsone, especially when neutrophils are present in their dermal infiltrate.

Supportive therapy is warranted in all patients with EBA. This includes instruction on wound care and strategies for avoiding trauma. Patients should be warned not to over wash and overuse hot water or harsh soaps, and to avoid prolonged or vigorous rubbing of their skin with a washcloth or towel. In some, it appears that prolonged sun exposure may aggravate or promote new lesions on the dorsal hands and knuckles. Avoidance of prolonged sun exposure and the use of sunscreens may be beneficial. Patients should be educated to recognize localized skin infections and to seek medical care and antibiotic therapy promptly when they occur.

Rituximab, a monoclonal anti-CD20 antibody, has been used successfully to treat patients with EBA [54–60]. Many of the patients who have responded to rituximab have been refractory to numerous other intensive treatment regimens. Of interest, rituximab has resulted in complete or partial sustained remission of disease after cessation of the course of therapy. In many of the case reports, patients were maintained on other immunosuppressive therapies as well. One of these case reports was in a pediatric patient [54].

Photophoresis has been used anecdotally with success in various autoimmune bullous diseases. One reported case of life-threatening EBA had a remarkable recovery with photophoresis [61]. In a small trial of three EBA patients,

photophoresis lowered the circulating anti–type VII collagen antibodies in the patients' sera, increased the suctioning blistering times of the patients, and improved the disease [62]. Plasmaphoresis has been shown to reduce the burden of circulating anti-C7 antibodies in a patient with EBA [63].

Intravenous immunoglobulin (IVIG) has been reported to be effective in some patients with EBA [64]. The mechanism by which IVIG may invoke a positive response in EBA is unknown.

The anti–tumor necrosis factor-a (TNF-a) biologics have been tried in EBA with some success in limited open trials.

Conclusion

Epidermolysis bullosa acquisita is an autoimmune disorder characterized by autoantibodies to type VII collagen leading to blistering of the skin. There are a number of clinical variants that can be diagnosed with clinical findings, histopathology, direct immunofluorescence, and indirect immunofluorescence. When this disease is diagnosed, it is imperative to consider complications of the disease, as well as the associated autoimmune and inflammatory conditions that are frequently associated with EBA. Immunosuppressive therapy is usually required.

Questions

1. **Which of the following tests support the diagnosis of EBA:**
   A. Salt-split skin direct immunofluorescence (DIF) with the IgG deposits going to the epidermal roof
   B. Immuno-electron microscopy with the IgG immune deposits localized within and below the lamina densa
   C. Serum indirect immunofluorescence (IIF) showing an antibody that labels the roof of the epidermal- dermal separation
   D. Anti-endomysial IgA autoantibodies

2. **Autoantibodies in EBA patient sera are usually IgG and most commonly bind to which of the following structures in the skin?**
   A. Anchoring plaques
   B. Integrin a6b4 emanating from the hemidesmosome into the lamina lucida
   C. Anchoring filaments
   D. Anchoring fibrils

3. **Of the possible underlying systemic diseases associated with EBA, which one is the most common?**
   A. Amyloidosis
   B. Bullous amyloidosis
   C. Inflammatory bowel disease
   D. Lymphoma

4. **Regarding EBA treatment, which of the following is true?**
   A. The mechanobullous form of EBA usually responds nicely to high doses of systemic corticosteroids
   B. Colchicine is a good first line drug in EBA patients with underlying inflammatory bowel disease
   C. Cyclosporine A can be used to treat EBA at doses similar to those used in psoriasis patients
   D. Rituximab infusions are a useful therapy for EBA

5. **Patients with Brunsting-Perry Pemphigoid usually have:**
   A. Serum autoantibodies to the BP 180 antigen
   B. Serum autoantibodies to type XVII collagen
   C. Serum autoantibodies that label the floor of salt-split human skin substrate
   D. Serum antibodies that only bind to proteins within mucous membranes

Answers

1. B
2. D
3. C
4. D
5. C

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Granulomatosus

37

Sridhar M. Dronavalli

Abstract

The disease entities in the group of granulomatous disorders share some histological commonalities, however the etiology of most remain largely unknown. In previous editions, the authors offered a schematic by which to classify granulomatous inflammation based on appearance, precipitating factors, and degree of lymphocytic infiltration ('non-immunologic vs. immunologic') but warned us that the distinctions are more apparent than real. More than a decade later, this caveat holds true. Granulomatosus represents a distinctive reactive process in the spectrum of inflammation where the histiocyte is the key involved cell. A granuloma is a focal collection of activated and modified histiocytes, sometimes 'epithelioid' in appearance, usually surrounded by a rim of leukocytes. While histiocytes are now firmly established as monocyte/macrophage-derived cells based on surface immunohistochemical markers, past reliance solely on cell morphology has led to confusion as numerous diverse, reactive, and neoplastic cell populations can aggregate in the cutaneous microenvironment resembling histiocytes, commonly referred as 'histiocytoid'.

Keywords

Granulomatous • Monocytes • Macrophages • MGC • Multinucleated giant cells • Sarcoidosis • Non-infectious epithelioid granulomas • Inflamed blood vessels • Granulomatosis Disease

Introduction

Conceptually, the etiology of granulomatosus is thought of as an immune defense and reactive process. This is exemplified by various prototypic diseases such as tuberculosis and leprosy, representing infectious granulomas, and common conditions such as ruptured keratin cysts and suture granulomas, respectively representing endogenous and exogenous foreign-body reactions. Either undiscovered or undetectable by current investigative modalities several granulomatous entities, such as sarcoidosis and the group of necrobiotic granulomas, do not consistently demonstrate the presence of either infectious or foreign-body material.

Another classification distinguishes granulomas as immunologic vs. non-immunologic [1]. While both classifications involve macrophage activation admixed with leukocytes at the initiation of granuloma formation, the persistence of lymphocytes within the lesion are features of immunologic granulomas. In general, non-immunologic granulomas involve the introduction of large amounts of insoluble material while immunologic granulomas may result from introduction of a small amount of substances. These substances range from pathogenic stimuli such as bacteria, fungi, mycobacteria, or certain viruses and molds to chemical initiators. Still, even within the immunologic scheme, sarcoidosis and the necrobiotic granulomas cannot be neatly classified.

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Role of Monocytes and Macrophages

Some insight into the clinical spectrum of granulomatous diseases may be gained by examining the characteristics and function of the histiocyte or monocytes/macrophages (Mo/Mac). Mo/Mac are often regarded as the primary phagocytic population of the innate immune system with some antigen presenting capabilities. They express several classes of receptors and are able to produce a cadre of enzymatic and metabolic mediators, suggesting a broader role than microbial surveillance [3].

In its innate immunity role, the Mo/Mac bear multiple cell surface molecules that mediate diverse pathogen recognition and processing as well as those that mediate recruitment and trafficking of inflammatory cells to the site of infection, such as toll-like receptors (TLRs) and chemokine receptors [4, 5]. A cornerstone feature of the Mo/Mac is their dynamic modification in response to acute inflammation or infection. They display high cellular plasticity and are driven by local microanatomic and systemic factors [6, 7]. Resting monocytes comprise 2–10 % of peripheral blood leukocytes and can be marginated in response to chemotactic factors released as a result of systemic events. The cells can infiltrate most tissue where they transform into activated macrophages. Additionally, various organs have resident tissue-specific differentiated Mo/Mac, mostly myeloid in origin, comprising a network called the mononuclear phagocyte system [8].

Mo/Mac also occupy a unique niche in the inflammatory response. One bystander effect of inflammation is apoptosis, requiring swift and efficient engulfment without inciting further inflammation or allowing leakage of potentially immunogenic material [9]. It has recently been shown that macrophages use distinct processes to clear apoptotic cells vs. necrotic cells by a "zipper-like" phagocytosis vs. macropinocytosis, respectively [10]. Mo/Mac express and utilize various receptors including scavenger receptors, complement receptors, and integrins to recognize apoptotic bodies and mediate removal to control potential inflammatory responses [11]. Hence, Mo/Mac are slowly being appreciated to have a more sophisticated role in inflammation.

Multinucleated Giant Cells

In the face of infection, the presence of Mo/Mac may signal both a reactive, protective process and also a homeostatic, restorative process. As fusion and aggregation ensue, they form histologically striking structures such as multinucleated giant cells (MGC) and granulomas, respectively (Fig. 37.1) [12]. The process resulting in formation of these structures remains poorly understood. Mo/Mac can be stimulated to generate MGC in vitro by addition of various cytokine and conditioned media [13]. Interferon γ has been identified as a critical cytokine in MGC formation [14]. In specialized multinucleated giant cells, such as osteoclasts and foreign body giant cells, macrophage fusion receptor (MFR) and its ligand CD47 have been implicated to facilitate cell fusion and cell-to-cell recognition [15]. The latter is important for cell-cell reciprocity, allowing multinucleation rather than activation of intracellular degradative processes in the newly fused cell. Recently, a molecule called DC specific transmembrane protein (DC-STAMP) was shown to be required for fusion of Mo/Mac into giant cells [16]. The ligand for DC-STAMP is currently unknown; however, it is structural similar to chemokine receptors, suggesting the possibility that the ligand may be a soluble chemokine. Chemokines such as CCL2 have already been shown to play a role in foreign body giant cell formation [17].

Recently, it was demonstrated that during cell differentiation into MGC, high amounts of chemokines were induced in Mo/Mac [18]. However, once the MGC become fully differentiated, the expression of the chemokines such as CCL2 and CXCL10 become constitutive and can not be further upregulated by exposure to *Mycobacterium tuberculosis*. This suggests two distinct phases in MGC formation. First, in acute infection, the development of MGC induces rapid recruitment of Mo/Mac via chemokine release. In the second phase, the differentiated MGC stimulates a steady influx of Mo/Mac, presumably towards the development of granulomas, via a steady state of chemokines. The connection between granulomatous infections and chemokines is supported by reports demonstrating high amounts of chemokines in lung specimens of patients with tuberculosis [19, 20].

Granuloma Formation

Presence of granulomas may represent an intact immune protective process, an exaggerated reactive process, and/or an attempt at restoring homeostasis. The experimental model of granuloma formation traditionally relies on a murine model of persistent infection, usually with *Mycobacterium* species, to examine the interaction of pathogen with Mo/Mac. Research aimed at unraveling the inciting signals has implicated a role for TLRs, the pro-inflammatory cytokine TNF-α, and T-helper 1 (Th1) cytokines IL-2 and IFN-γ [21, 22].

The elicitation of the host's Mo/Mac TLRs response in the *M. tuberculosis* model is complex because both pro-inflammatory and anti-inflammatory cytokines have been reported following *M. tuberculosis* exposure [23]. It is unclear if this dichotomy represents an immune evasion strategy employed by the pathogen or a balancing act by the host in an attempt to control inflammatory responses. Surprisingly, the susceptibility to *M. tuberculosis* was low in numerous TLRs

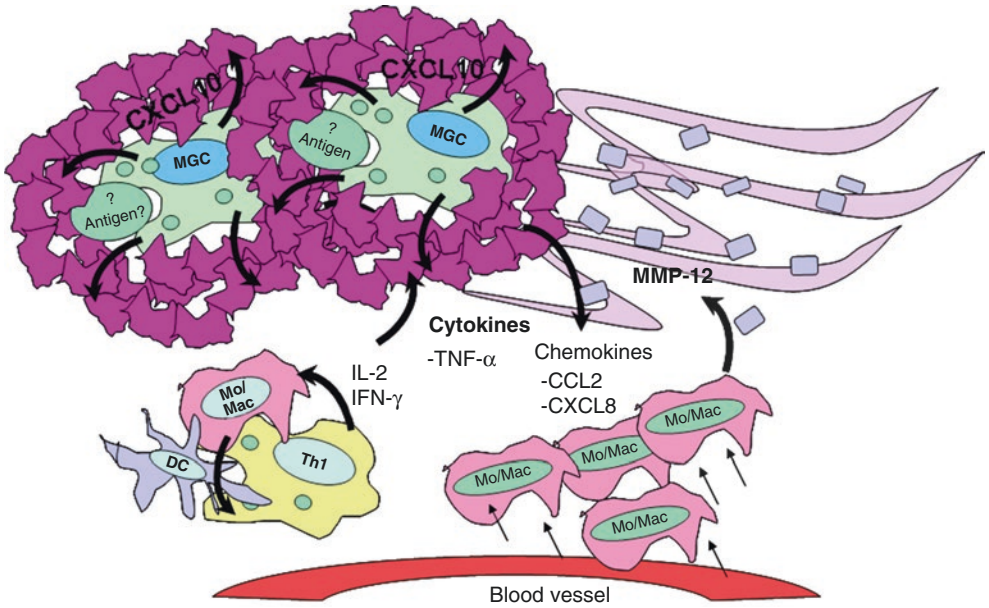

Fig. 37.1 Schematic for granuloma formation. Monocyte/macrophage (*Mo/Mac*) interaction with an antigen provokes an inflammatory response. Under influence of various cytokines and chemokines, some Mo/Macs undergo cell fusion to form multinucleated giant cells (*MGCs*). Once formed, MGCs secrete steady-state chemokines, attracting more Mo/Macs. Other activated Mo/Macs enlarge and differentiate into epithelioid histiocytes. Aggregation results in granulomas. Activation by tumor necrosis factor-a (*TNF-α*) and interferon-g (IFN-γ) is important in granuloma formation and maintenance

knockout (TLR2, TLR4, and TLR6) mouse models. However, the myeloid differentiation factor 88 (MyD88) knockout mice displayed high susceptibility to disease thus demonstrating the importance of TLRs in host defense against mycobacteria since MyD88 functions an adaptor molecule used by many TLRs following ligand activation. Studies using mycobacteria and other pathogens have demonstrated the importance of MyD88 in granuloma formation via IFN-γ induction [24–27].

More than a decade earlier, Flynn and colleagues reported that IFN-γ knockout mice infected with *M. tuberculosis* develop disseminated tuberculosis. They demonstrated that disease susceptibility followed granuloma necrosis due to the lack of macrophages activating signals needed for granuloma maintenance [28, 29]. Th1 cells play a role in both the formation and maintenance of granulomas by inducing and sustaining Mo/Mac recruitment. Similarly, mice deficient in TNF-α succumb to disseminated tuberculosis infection. In the absence of TNF-α, the granulomas formed are structurally disorganized leading to eventual necrosis [30, 31].

Matrix metalloproteinase (MMP), specifically MMP-12, also known as macrophage metalloelastase, has recently been reported to be abundantly expressed in granulomas [32]. Although not directly related to granuloma formation via macrophage activation but rather macrophage migration, MMP-12 has been shown in vivo to be important in macrophage penetration of basement membrane by digesting a variety of stromal substrates [33, 34]. Furthermore, surveys of several different human granulomatous skin disorders, including sarcoidosis, necrobiosis lipoidica diabeticorum, and granuloma annulare demonstrate that MMP-12 is abundantly expressed in co-localization with $CD68^+$ macrophages [35].

While the murine model provides a useful mammalian in vivo experimental tool, there are limitations. For example, cross strain murine infection with *Mycobacterium tuberculosis* produces multi-bacillary non-caseating granulomas, contrasting the usual course in human disease [36]. Furthermore, study of early events in granuloma formation, namely Mo/Mac recruitment, modification, and aggregation is limited by static ex vivo histological examination of tissue in this model.

Recent development of non-mammalian granuloma models have helped broadened our understanding of granuloma formation. This is possible through examination of host interaction with *Mycobacterium marinum* in species phylogenetically distinct from humans such as *Dictyostelium*, *Drosophila*, and zebrafish [37]. While extrapolation into human disease pathogenesis may not be immediately apparent, the observations suggest that pathogen recognition via pathogen associated molecule patterns (PAMPs) and macrophage aggregation may be evolutionary conserved processes. The *Dictyostelium* is a single cell amoeba that has similarities to the human phagocytic histiocyte. *Drosophila* is a known model of innate immunity. By utilizing the transparent zebrafish embryos, researchers have directly visualized migration and aggregation of macrophages, the hallmark of granuloma

formation, following infection with *M. marinum* [38]. Furthermore, at the embryonic stage, zebrafish lack circulating lymphocytes, suggesting that granuloma formation can be initiated in the absence of an adaptive immune contribution.

Granulomatous Disease

Except for infectious and foreign body granulomatosus, the factors determining the non-infectious granulomatous diseases are largely unknown. It likely results from the interplay between genetic susceptibility and environmental factors. Understanding the reaction patterns as well as the cellular and soluble mediators involved will be informative for understanding the etiopathogenesis of granulomatous diseases (Fig. 37.2). While this group of diseases involves infiltration and aggregation of immune cells, this does not necessitate that these disorders are immunologically mediated. Histiocytic aggregation may be seen in reactive processes.

Sarcoidosis is the most studied non-tuberculid granulomatous disease because of its affect on multiple organ systems and associated mortality and morbidity. Understanding the underlying molecular and cellular processes of this prototypic granulomatous disease may help elucidate the pathogenesis of other non-infectious granulomatous disorders.

Other diseases include cutaneous Crohn's disease, which is a spectrum of cutaneous manifestations associated with the inflammatory gastrointestinal disease, not all of which are granulomatous. The group of necrobiotic granulomas including granuloma annulare, necrobiosis lipoidica, and rheumatoid nodule share some common histological features but no consistent correlations with a single underlying systemic disease. Except for vasculitis and potential ulceration in the latter entity, the cutaneous conditions are largely benign. Annular elastolytic giant cell granuloma is a rare disease that is considered by some to be a variant of granuloma annulare, but with histologically distinct features. Similarly, it has a fairly benign course and may be self-limiting. Interstitial granulomatous dermatitis and palisaded neutrophilic and granulomatous dermatitis are two more recently classified granulomatous diseases that shares histologic features with granuloma annulare and necrobiosis lipoidica, though not much is known about their etiopathogenesis.

Non-infectious Epithelioid Granulomas

Sarcoidosis

Clinical Manifestations

Sarcoidosis is a multisystem disease characterized by non-caseating granulomas of unknown etiology [39]. The most commonly affected organ is the lung. Involvement of the skin is seen in up to one-third of patients, and may be the

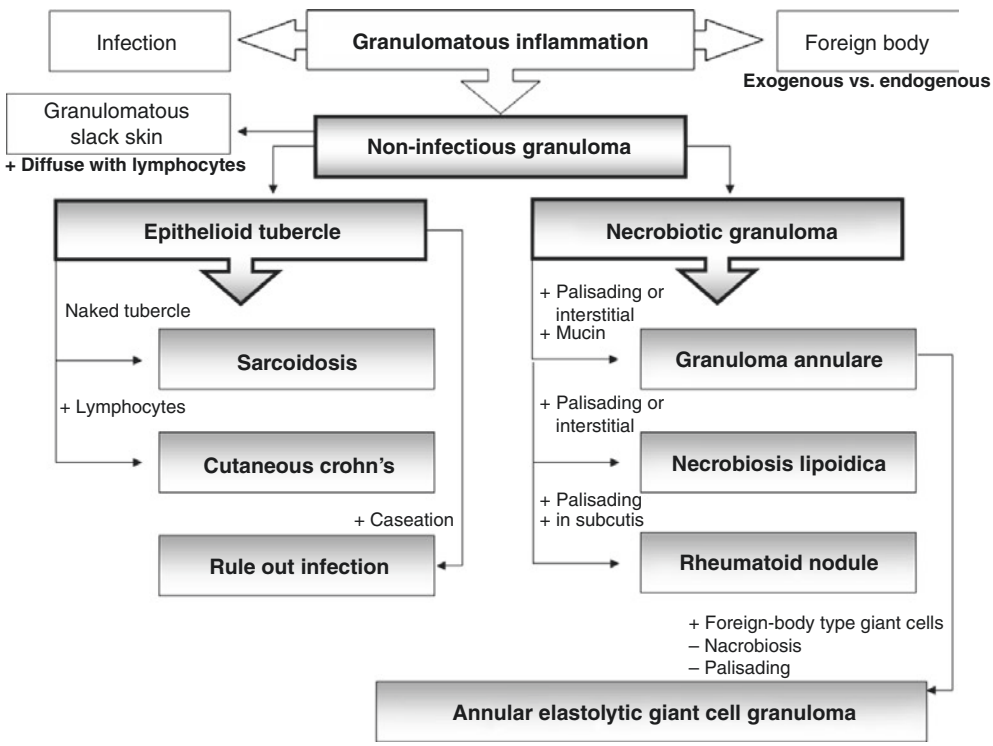

Fig. 37.2 An approach to the histologic diagnosis of granulomas

only clinical sign of the disease. Although the cutaneous lesions usually appear at onset of disease, it can occur at any time. Other organs affected include the liver, spleen, eyes, kidneys, glands, and less commonly, the central nervous system, heart, and musculoskeletal system. Several syndromes have been described in sarcoidosis depending on the constellation of symptoms and involved organs that include: Heerfordt-Waldenstrom syndrome (fever, parotid gland enlargement, anterior uveitis, and facial nerve palsy); Mikulicz's syndrome (infiltration of the parotid, submandibular, lacrimal, and sublingual glands) [40]; and Lofgren syndrome (fever, erythema nodosum, polyarthralgias, and bilateral hilar adenopathy). The latter is associated with a benign self-remitting course [41]. In general, while the mortality rate associated with severe pulmonary disease is low in sarcoidosis, 10–30 % of patients develop chronic debilitating disease.

Sarcoidosis affects all races worldwide with varying incidence. In the United States, African Americans have a 3.8-fold increased risk compared to whites, affecting women more than men [42]. The disease tends to be more chronic and fatal in African Americans [119].

Several studies have established that the skin disease does not correlate with prognosis or the extent of visceral involvement [42, 43]. However, there is correlation with erythema nodosum, usually a self-limiting condition seen most frequently in young women on initial presentation, with acute disease and large plaques with chronic disease [42, 44, 45]. Recent retrospective analysis of a small cohort of patients with the subcutaneous form of sarcoidosis suggests that this variant may be a subset associated with systemic disease [46].

The most common skin manifestations include non-painful reddish-brown papules and plaques often symmetrically involving the face, lips, neck, trunk, or upper extremities [47, 131]. Typically lesions lack scale or ulceration, though some lesions may develop these features. Cutaneous sarcoid is a great mimic and can have diverse clinical presentations. A variety of uncommon manifestions include an ichthyosiform, annular, angiolupoid (large telangiectatic), psoriasiform, or subcutaneous (Darier-Roussy) appearance. Atrophy can be seen in plaques. Scalp lesions have varying amounts of scales and may result in alopecia. Various non-specific nail and mucosal changes can also be seen.

Another manifestation of sarcoidosis is lupus pernio, indurated and violaceous papules and plaques on the nose, cheeks, and ears [48]. Ulceration can be seen in these lesions and can lead to scarring. Nasal alar lesions can extend into the nasal vestibule and nasal floor and is associated with granulomas in the upper respiratory tract and lungs in the majority of patients [49, 50]. The digits and toes may have sausage-shaped swelling due to underlying cystic lesions of the phalanges [51].

Histopathology

Langerhans, granulomas seen in sarcoidosis have the characteristic appearance of focal collections of epithelioid histiocytes associated with absent or sparse ring of lymphocytes composed of T and B lymphocytes. The term "naked tubercle" refers to a common observation of a granuloma devoid of lymphocytes and plasma cells. Multinucleated giant cells may be present, usually of the Langhans type, and these giant cells may contain asteroid and Schaumann bodies. There is typically no central caseation; however, there may be fibrinoid changes secondary to deposition of immunoglobulins, complement, and fibrinogen in up to 10 % of cases. The presence of caseation should prompt a search for infectious causes.

Both TNF-α and IFN-γ have been associated with granuloma formation [52, 53]. It is known that prolonged activation by these cytokines usually leads to apoptosis but the characteristic granulomas in sarcoidosis patients are non-caseating. It is unclear why there is the absence of apoptosis in the sarcoid granulomas. Recent studies have associated upregulated expression of an IFN-γ induced anti-apoptotic molecule p21Waf1, a cdk inhibitor, in sarcoidosis patients [54].

Pathogenesis

Numerous hypotheses have been put forth on the etiology of sarcoidosis, including infectious, autoimmune, and environmental. The identity of a causative antigen responsible for granuloma formation remains uncertain. The etiology and pathogenesis of sarcoidosis is complicated by highly varied disease presentation ranging from single organ to multisystem involvement, the lack of specific symptomology, and the waxing and waning nature of the disease. Both the clinical and tissue diagnosis of the disease require exclusion of other conditions such as mycobacterial or deep fungal infections, Wegener's granulomatosus, and malignancy.

A historic test for the diagnosis of sarcoidosis is the Kveim-Siltzbach skin test [55–57]. This was performed by injecting a patient with a suspension of sarcoid spleen material intradermally into the skin of a suspected patient. The formation of a non-caseating granuloma observed histologically 4 weeks later at the site of injection indicates a positive test. Studies of T cells at the Kveim-Siltzbach reaction sites have demonstrated an oligoclonal population of $CD4^+$ T cells which argues for sarcoidosis as an antigen driven disease [58]. This is supported by findings of similar oligoclonality, with a dominant V beta bias, in sarcoid lung T cells [59].

Infectious Etiology

Despite investigations for viral and mycobacterial causes of sarcoidosis, no consistency has been found. Human herpesvirus-8 (HHV-8) was postulated to be associated with sarcoidosis but has been met with many reports of negative

findings in sarcoidosis patients worldwide [60–63]. Mycobacterial DNA sequences have been found in various tissues in some sarcoidosis patients, while others report negative findings [64–67]. To date, no mycobacteria have been successfully cultured [68].

Recent reports have identified various mycobacterial components as potential pathogenic antigens in sarcoidosis. Serum from patients with sarcoidosis were found to have antibodies to Mycobacterium tuberculosis katG, M. tuberculosis heat shock protein 70, and Mycobacterium tuberculosis mycolyl transferase antigen 85A [58, 126, 127].

Environmental Exposure

Evidence for the role of environmental exposure in the pathogenesis of sarcoidosis comes from reports of higher incidence of disease in certain occupations, such as firefighters and aircraft carrier personnel [69, 70]. A recent study by Izbicki et al. noted an increased incidence among New York City Fire Department workers involved in the 2001 World Trade Center emergency [125]. Such disease clusters indirectly implicate an environmental etiology [71]. In a recent multi-center case control study, researchers did not find a single proximate cause. This group did report positive associations with insecticides, an agricultural environment, and microbial bioaerosols such as mold and mildew [72]. Interestingly, a negative association with cigarette smoking was found in the study, consistent with previous reports [73, 74].

Genetic Susceptibility

Familial predisposition of sarcoidosis is well-known: having a first-degree relative with the disease confers a five-times increased likelihood of having the disease. There are also reports of sibling discordance [84]. Several studies have reported disease susceptibility with HLA-1, HLA-B8, HLA-DRB1, HLA-DRB3, and HLA-DQB1 alleles [75, 118]. No definitive HLA genes have been uniquely established in African American sarcoidosis patients, a group identified to have 3.8-times increased annual incidence in the United States. Recent linkage studies suggest that more than one gene may be involved in disease susceptibility in African Americans [86]. A recent study by Valentonyte et al. reported an association of the butyrophilin-like 2 (BTNL2) gene on chromosome 6p with sarcoidosis but its precise function in sarcoidosis is still unknown [121].

CARD15/NOD2 has been linked to Blau syndrome, a granulomatous disease affecting the eyes, joints, and skin [76]. CARD15, expressed by mononuclear phagocytes, encodes NOD2 which recognizes a component of bacterial peptidoglycan [77]. The gene has also been associated with inflammatory bowel disease [78]. One report found an association of mutations in CARD15/NOD2 with early-onset childhood sarcoidosis, a distinct type of sarcoidosis in children younger than 4 years of age characterized by eye, joint, and skin involvement [79]. Attempts to find an association of CARD15/NOD2 in adult sarcoidosis have demonstrated no relationship [75].

Despite several proposals of correlative serum markers, including serum amyloid A and C-reactive protein, no consistent correlations have been demonstrated. Even serum angiotensin converting enzyme (ACE), initially promising, has not been a consistent marker of disease activity [80, 81]. Other markers currently being examined include macrophage inflammatory protein 1 (MIP-1) and vascular endothelial growth factor (VEGF) [82, 83].

Immune Regulation

There are numerous lines of research indicating that the involved lymphocytes are of the Th1 phenotype, producing cytokines such as IL-2, IFN-γ, and enhanced TNF-α [87, 88]. Findings of hypergammaglobulinemia in sarcoidosis patients leads to the question of B cell involvement in disease pathogenesis. It is now believed that the hypergammaglobulinemia is secondary to IL-2 and IFN-γ stimulation of B cells [89, 90]. Adding to the puzzle of sarcoidosis is that despite activation of networks of pro-inflammatory cytokines and robust recruitment of cells, there is often an associated state of either complete or partial anergy [91, 92]. This has been demonstrated by a lack of response to the tuberculin skin test or decreased sensitization to agents like the contact sensitizer dinitrochlorobenzene (DNCB) seen in up to two-thirds of sarcoidosis patients. Explanations offered for the observation of decreased delayed-type hypersensitivity was due to peripheral lymphopenia from sequestering and compartmentalization of T cells into granulomas. Recent data suggests that sarcoidosis patients have an unusually high number of innate T regulatory cells (T_{regs}), with constitutive $CD25^{bright}$/$CD4^{+}$ cells, both in the peripheral circulation and at the periphery of granulomas [93]. T_{regs} from sarcoidosis patients demonstrated similar capabilities of suppressing responder cell proliferation as compared to controls. The T_{regs} from sarcoidosis patients inhibit IL-2 but not TNF-α or IFN-γ production by responder cells compared to normal controls in which all three cytokines were completely inhibited. Since TNF-α is associated with sarcoidosis and granuloma formation, these findings may explain the concurrence of cell activation with a global state of anergy.

Th17, a CD4+ effector T cell population, has been linked to chronic inflammatory diseases involving Th1 cells including psoriasis, rheumatoid arthritis, and inflammatory bowel disease [117]. Recent studies have shown the presence of Th17 in the lung and peripheral blood of patients with active sarcoidosis [116]. Presence of these cells likely play a role in the chronic inflammatory state found in sarcoidosis [117].

It is known that 8–21 % of patients with common variable immunodeficiency (CVID) develop a granulomatous disease resembling sarcoidosis [94]. Since CVID is not a

single entity but rather a heterogeneous syndrome, a retrospective study correlating patients with granulomatous disease and specific immunologic derangement may provide some clues in the pathogenesis of sarcoidosis. Some CVID patients develop hyperproliferation of $CD4^+$ cells, yet some have increased apoptosis of $CD4^+$ cells. Sixty percent of patients have a diminished response to T cell receptor stimulation and expression of CD25, the receptors for IL-2.

Treatment

Many individuals require no treatment, though the decision to treat depends on the site and severity of organ involvement [122]. There are numerous anecdotal and case series that discuss the treatment of sarcoidosis but few clinical trials. The mainstay therapy includes corticosteroids. Other commonly used agents include minocycline (particularly for cutaneous sarcoidosis), anti-malarials, methotrexate, and TNF-alpha agents [95, 122]. Paradoxically, several reports have noted the appearance of cutaneous sarcoid lesions while on TNF-alpha therapy for other diseases [123, 124].

Cutaneous Crohn's Disease

Crohn's Disease (CD) is characterized by segmental granulomatous inflammation of the intestinal tract. Cutaneous manifestations of the disease occur in 14–44 % of patients, and include CD-specific, reactive, and associated conditions. CD-specific lesions involve the skin by the same mechanism as the GI tract and include fissures and fistulae, oral Crohn's disease, and metastatic Crohn's disease. The latter is a rare entity that denotes a cutaneous lesion distant from extension or fistulae formation from oral, anal, or ostomy sites. Reactive lesions involve the skin by distinct pathogenic mechanisms and include erythema nodosum, pyoderma gangrenosum, Sweet syndrome, and polyarteritis nodosa [96, 128].

The cutaneous manifestations of Crohn's Disease, including metastatic Crohn's disease, have inconsistent correlation with internal disease activity. Classically, extra-genital metastatic Crohn's disease presents with dusky erythematous plaques that may develop into ulcers. Histopathologic examination reveals a sarcoid-like epithelial granuloma, usually with a notable lymphocytic infiltrate and occasionally with necrobiosis [97, 98]. The inflammatory infiltrate can often surround dermal blood vessels, termed granulomatous perivasculitis [98].

A recent study attempted to elucidate the etiology of cutaneous Crohn's by examining the gastrointestinal and corresponding skin specimens in patients with Crohn's and cutaneous Crohn's disease for bacterial 16s rRNA. They report no bacterial 16S rRNA, examined by in situ real time vs reverse transcriptase polymerase chain reaction (RT-PCR), in skin lesions whilst being present in gastrointestinal biopsies, suggesting that bacterial dissemination may not be involved in cutaneous lesions. Reactivity to bacterial products may play a role in the cutaneous manifestation of Crohn's [99].

As discussed earlier, polymorphisms in CARD15/NOD2 have been associated with Crohn's disease but, the mechanism leading to predisposition of disease is unclear [100]. One hypothesis is that mutations in CARD15 leads to a defect in the acute inflammatory response to intestinal bacteria, leading to allowance of materials to breach the gut barrier. The subsequent response to the bacterial products leads to a granulomatous reaction [101].

Necrobiotic Granuloma

The group of necrobiotic granulomas consists of granuloma annulare (GA), necrobiosis lipoidica, and rheumatoid nodules. The etiology of these diseases is unknown. The common histological reaction pattern is palisading granulomas with areas of altered or degenerated connective tissue. Variations such as interstitial granulomatous inflammation can be see in GA and necrobiosis lipoidica, as well as interstitial granulomatous dermatitis and palisaded neutrophilic and granulomatous dermatitis, two recently categorized granulomatous diseases.

Granuloma Annulare

GA is a benign disorder limited to the skin. There have been many proposed inciting factors such as trauma, insect bites, and viral infection. A delayed-type hypersensitivity reaction to an unknown antigen may be the formative event; this hypothesis is supported by a study showing T-cell subpopulations in histologic specimens of GA lesions [129]. The association with systemic disease such as diabetes mellitus has been inconsistent. Atypical variants of GA have been described in patients with HIV/AIDS and lymphoma [102, 103]. The localized variant is most commonly seen, occurring as annular or arcuate plaques on the hands and arms [104]. It can also involve the extremities and trunk. Other variants include papular, generalized, perforating, subcutaneous, and patch forms of the disease. On histology, the recognizable pattern is of palisaded epithelioid histiocytes with a central acellular area of pallor, increased mucin, and altered collagen and elastic fibers. The most common pattern is the infiltrative or interstitial pattern where histiocytes are interspersed between collagen with subtle alteration of the fibers.

The entity annular elastolytic giant cell granuloma is regarded by some as a variant of GA. Lesions are also asymptomatic. On histology there are foreign body type giant cells without a palisading granulomatous inflammation. There is usually no altered collagen. An elastin stain shows loss of elastic fibers in granulomatous areas.

Rheumatoid Nodules

Rheumatoid nodules can be seen in the clinical setting of adult-type polyarticular rheumatoid arthritis and is a rare feature of rheumatic fever [105]. History and context of presentation will aid in the diagnosis. The etiology of rheumatoid arthritis (RA) remains unknown. Disease susceptibility is strongly associated with class II region genes, HLA-DRB1 [106–108].

Clinically, rheumatoid nodules present as firm papules or nodules along extensor surfaces of joints. Histology reveals a palisading layer of histiocytes and granulation tissue surrounding a central zone of fibrin [130].

There is high suspicion that RA is mediated by autoantibodies, specifically by a group that recognizes citrullinated proteins, including the antiperinuclear factor, antikeratin antibodies, and antifilaggrin antibodies [109]. These antibodies are commonly found in the sera and synovial fluid of RA patients [110]. In a small study of 26 patients, citrullinated proteins were observed in 70 % of the rheumatoid nodules [111].

Citrullination, a post-translation modification process, has been demonstrated to increase peptide and MHC affinity, suggesting that it can modulate immune responses [112]. The enzyme mediating citrullination is peptidylarginine deaminase, found in inflammatory cells including neutrophils, monocytes, and macrophages [113]. Hence, it is tempting to speculate a relation of the Mo/Mac and granulomatous reaction in the skin with citrullination as a potential source of "autoantigens". Recently, T cells recognizing the specific modification of antigens by citrullination was described [114]. It remains to be determined if they are the pathogenic mediators of RA.

Necrobiosis Lipoidica

Necrobiosis lipoidica "diabeticorum" (NLD) was originally described in patients with diabetes but demonstration of non-diabetic patients with the condition have lead to reconsideration of its name. The disease has a characteristic presentation of asymptomatic yellow-brown plaques involving the pretibial areas. The lesions over time become atrophic with development of telangiectasias. Ulceration can develop but the disease is generally benign. On histology the "cake layers" of granulomatous inflammation and parallel degenerated collagen is typically seen. The pattern can be palisaded or interstitial.

The etiology of NLD is unknown. Twenty years ago Ullman and Dahl suggested that the disease may be secondary to vasculitis based on findings of IgM and complement C3 deposition in blood vessels of affected skin [115]. Recently, NLD skin sections were shown to stain for GLI-1, a transcription factor in the hedgehog signaling pathway. The significance remains to be seen.

Closing Remarks

Traditionally the Mo/Mac is simply thought of as phagocytes. That frame of reference prompted investigations with the goal to identify causal pathogens in granulomatous diseases. Macrophages are very plastic cells found in most tissues and are responsive to their microenvironment. New understanding of the diverse homeostatic and regulatory functions of Mo/Mac should broaden our understanding of this inflammatory response.

Furthermore, diseases with overactive and chronic persistence of Mo/Mac and granulomatous inflammation can be the potential target of liposomes, specific uptake by Mo/Mac, or via targeting their unique receptors such as anti-Fc©RII receptor (CD 32). However, the degree to which macrophages play a role in promoting lesions and their persistence is not fully understood in some of these diseases. Agents that limit Mo/Mac response may play an important role in understanding the pathophysiology of these dermatoses.

Questions

1. Which of the following are involved in the mechanisms of macrophages forming multinucleated giant cells?
   A. Antigen recognition
   B. Inflammatory response
   C. Cell fusion
   D. Production of chemokines
   E. All of the above
   F. None of the above

Correct answer: (E) All of the above steps are involved in giant cell formation

2. What relevant immune factors are *not* involved in granuloma formation?
   A. Persistence of antigen
   B. Innate Recognition by TLR
   C. Th2 lymphocyte reactivity and associate cytokines
   D. Formation and maintenance of granulomas by reactive lymphocytes

Correct answer: (C) Th2 lymphocytes are not involved in granduloma formation (it is actually Th1 lymphocytes)

3. What role does immune regulation play in sarcoidosis?
   A. Sarcoidosis is an antigen-driven disease that is dependent on T-lynphocytes
   B. Autoantibodies are critical in the pathogenesis
   C. Infections are the commonest cause of sarcoidosis.
   D. The diagnosis is dependent on a positive skin test (Kveim-Siltzbach skin test)
   E. Mycobacterial infections are central to the pathogenesis.

Correct answer: (A) Sarcoidosis is an antigen driven disease that is T lymphocyte dependent

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Cutaneous Graft-Versus-Host Disease

38

Edward W. Cowen

Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially life-saving treatment modality. However, many patients who undergo allogeneic HSCT develop graft-versus-host disease (GVHD) of the skin, potentially resulting in significant long-term morbidity. Chronic GVHD may manifest on the skin in many different clinical presentations, including skin fibrosis, and represents a significant treatment challenge that is often compounded by co-morbidities due to GVHD involvement of other organ systems.

Keywords

Graft-versus-host disease • Hematopoietic stem cell transplantation • Morphea • Scleroderma • Fasciitis

Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative intervention for more than 15,000 patients each year suffering from cancer, primary immunodeficiency, and other serious heritable and acquired disorders (Table 38.1). However, allogeneic HSCT may lead to graft-versus-host disease (GVHD), a complex multi-organ disease that is a major cause of post-transplant non-relapse morbidity and mortality. Although it occurs most frequently in association with allogeneic HSCT, GVHD may also result from autologous HSCT, allogeneic liver transplantation, or blood transfusion. Practically every organ system may be affected by GVHD, but the skin is the most common site of involvement, and chronic cutaneous GVHD is perhaps most remarkable for its variable cutaneous manifestations.

The first step in allogeneic HSCT is the identification of a suitable stem cell donor. The donor is selected based on the similarity of his/her histocompatibility antigen (HLA) profile to that of the recipient. There are three major Class I HLA antigens, HLA-A, –B, and –C, and three major Class II HLA antigens: HLA-DP, –DQ, and –DR. Because the alleles in each HLA class tend to be inherited together, there is approximately a 25 % chance that a sibling donor will be a 6/6 HLA-identical match. If no related donor is available, then a bone marrow registry will be utilized to search for an unrelated donor. International registries now contain over 9 million potential donors and identify an unrelated donor for approximately 50 % of patients [1]. The risk of GVHD is directly proportional to the degree of mismatch in major HLA alleles between donor and recipient. In addition, mismatch of minor HLA antigens is more likely to occur in the setting of unrelated donor HSCT, also contributing to the development of GVHD. Once a suitable donor is identified, the stem cells are collected from the donor's bone marrow, or colony-stimulating factor (CSF) is administered to the donor in order to mobilize stem cells from the marrow prior to pheresis from the peripheral blood. Umbilical cord blood is a third source for stem cell transplantation but currently accounts for a small percentage of transplants performed worldwide. The source of the stem cell graft is an important factor in the development of GVHD as peripheral blood grafts may be associated with a higher risk of GVHD than bone marrow-derived grafts [2]. After harvesting, donor stem cells are selected by physical and immunologic sorting methods. Specific T-cell depletion of the graft may be utilized in order to decrease the risk of GVHD prior to transfusion.

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Table 38.1 Conditions treated with allogeneic HSCT

Autoimmune disorders
Autoimmune lymphoproliferative syndrome (ALPS)
Immune dysregulation, polyendocrinopathy, X-linked syndrome (IPEX)
Hematologic malignancy
Acute myeloid leukemia
Acute lymphoblastic leukemia
Chronic lymphocytic leukemia
Chronic myeloid leukemia
Hodgkin's lymphoma
Multiple myeloma
Non-Hodgkin's lymphoma
Bone marrow failure
Aplastic anemia
Diamond-Blackfan syndrome
Fanconi anemia
Dyskeratosis congenita/Hoyeraal-Hreidarsson syndrome
Myelodysplastic syndrome
Shwachman-Diamond syndrome
Immunodeficiency
Ataxia-Telangiectasia
Chediak-Higashi syndrome
Chronic granulomatous disease
Complete interferon-γ receptor 1 deficiency
DiGeorge syndrome
DOCK8 combined immunodeficiency
Familial hemophagocytic lymphohistiocytosis
GATA2 deficiency
Griscelli syndrome
Hyper-IgM syndrome
Kostmann syndrome
Leukocyte adhesion deficiency
Severe combined immunodeficiency
Wiscott-Aldrich syndrome
X-linked proliferation syndrome
Metabolic disorders
Fucosidosis
Gaucher disease
Mucopolysaccharidoses
Osteopetrosis
Other disorders
Congenital erythropoetic porphyria (Günther disease)
Essential thrombocytopenia
Histiocytoses
Idiopathic hypereosinophilic syndrome
Myelofibrosis
Polycythemia vera
Paroxysmal nocturnal hemoglobinuria
Sickle cell disease
Thalassemia
Waldenstrom macroglobulinemia

Pre-treatment of the recipient's marrow before transplantation is necessary in order to allow engraftment of the donor stem cells. Traditional myeloablative regimens utilize a combination of total-body irradiation and chemotherapeutic agents such as cyclophosphamide to permit engraftment. These regimens create an immunosuppressed state, preventing the host from rejecting the foreign stem cells. Myeloablative preparative regimens may also reduce tumor burden through a direct effect on the cancer; however, they are associated with a high rate of toxicity [3]. Over the last several years, reduced-intensity (nonmyeloablative) preparative regimens have resulted in less acute toxicity and have expanded the use of allogeneic transplantation to higher risk groups including older patients or those with significant organ dysfunction. Reduced-intensity regimens rely primarily on the transplanted graft for anti-cancer activity rather than the direct cytotoxicity of the preparative regimen [1].

Autologous stem cell transplantation, which utilizes the patient's own stem cells following ablation of the hematopoietic system, is an important treatment for certain malignancies such as non-Hodgkin's lymphoma and multiple myeloma. More than 30,000 autologous procedures are performed worldwide each year. Although the risk of GVHD and overall non-relapse mortality are greatly reduced following autologous transplantation when compared with allogeneic transplantation, autologous procedures are associated with an increased rate of malignancy relapse [4].

Acute Versus Chronic GVHD

Traditionally, the onset of GVHD symptoms before or after the 100 day mark following transplantation has been used to designate acute versus chronic GVHD, respectively. However, this temporal distinction is somewhat arbitrary, as patients may manifest classic signs of acute GVHD after day 100, and chronic manifestations may occur before 100 days post-transplantation. Whereas acute cutaneous GVHD typically presents as an exanthematous skin eruption with gastrointestinal and hepatic involvement, chronic cutaneous GVHD is remarkable for its protean skin presentation and is associated with variable but potentially widespread organ dysfunction and immunodsyregulation. Changes in transplant protocols have also impacted the onset of acute and chronic symptoms. Nonmyeloablative conditioning regimens may delay the onset of manifestations of acute GVHD until after 100 days following transplantation [5]. Similarly, the use of donor lymphocyte infusions (DLI), wherein additional stem cells are administered weeks or months following transplantation to augment the graft-versus-tumor response, may induce symptoms of acute GVHD after the 100 day period [18].

Clinical Manifestations of GVHD

Acute GVHD

Acute GVHD is a potentially life-threatening complication of allogeneic transplantation. The risk of developing acute GVHD depends on a number of factors, including HLA-compatibility, the age and sex of the donor and recipient, the GVHD prophylaxis regimen used, and the T cell composition of the graft. Without prophylactic immunosuppression, acute GVHD will develop in most allogeneic HSCT recipients. Therefore a calcineurin inhibitor (cyclosporine or tacrolimus) or other immunosuppressant regimen is commonly used in the first several weeks to months following transplantation during which time the risk of acute GVHD is greatest.

Despite prophylactic immunosuppressive therapy, nearly 30 % of HLA-identical related transplant procedures result in significant acute GVHD [6]. This risk is significantly higher in HLA-matched unrelated and mismatched transplants. The skin is often the earliest clinical sign of acute GVHD. Long-term survival from acute GVHD is directly related to the severity of skin, liver, and gut involvement. The 1994 Consensus Conference grading for acute GVHD is demonstrated in Table 38.2 [7].

Acute GVHD primarily involves the skin, liver, and gastrointestinal tract, although other organ systems may be affected less frequently. Skin involvement most often occurs within 2–4 weeks after transplantation. Cutaneous involvement may range in severity from an asymptomatic maculopapular erythematous eruption to widespread necrolysis, but most commonly presents with an exanthem-like eruption that preferentially involves the head, ears, palms, and soles (Fig. 38.1). In early GVHD, there may be involvement of the hair follicles creating a folliculocentric-appearance [8]. When severe, diffuse erythroderma or bullae with epidermal necrolysis may occur (Fig. 38.2).

Histologically, acute GVHD is characterized by widespread keratinocyte necrosis with a dermal lymphocytic infiltrate and basal cell hydropic degeneration (Fig. 38.3). Histologic changes mimicking GVHD may be found following high-dose chemotherapy or radiation therapy, in the setting of drug hypersensitivity, and with the eruption of lymphocyte recovery; therefore clinicopathologic correlation is often helpful [9]. In cases in which the clinical and histologic diagnosis of acute skin GVHD is non-diagnostic, the presence of hyperbilirubinemia or symptoms of nausea, vomiting, diarrhea, or abdominal pain are important indicators of hepatic and gastrointestinal involvement. Even in the setting of equivocal cutaneous and histological findings, the mortality associated with severe acute GVHD necessitates a low threshold for initiating empiric corticosteroid therapy.

Table 38.2 Staging and grading of acute GVHD

Stage	Skin	Liver	Gut
1	Rash <25 % BSA	Bilirubin 2 mg/dL to <3 mg/dL	Diarrhea 500–1000 mL/day or persistent nausea
2	Rash 25–50 % BSA	Bilirubin 3–6 mg/dL	Diarrhea 1000–1500 mL/day
3	Rash > 50 % BSA	Bilirubin 6–15 mg/dL	Diarrhea > 1500 mL/day
4	Erythroderma w/bullae formation	Bilirubin >15 mg/dL	Severe abdominal pain with or without ileus
Grade			
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III		Stege 2–3 or	Stage 2-4
IV	Stage 4 or	Stage 4	

Adapted from Przepiorka et al. [7]

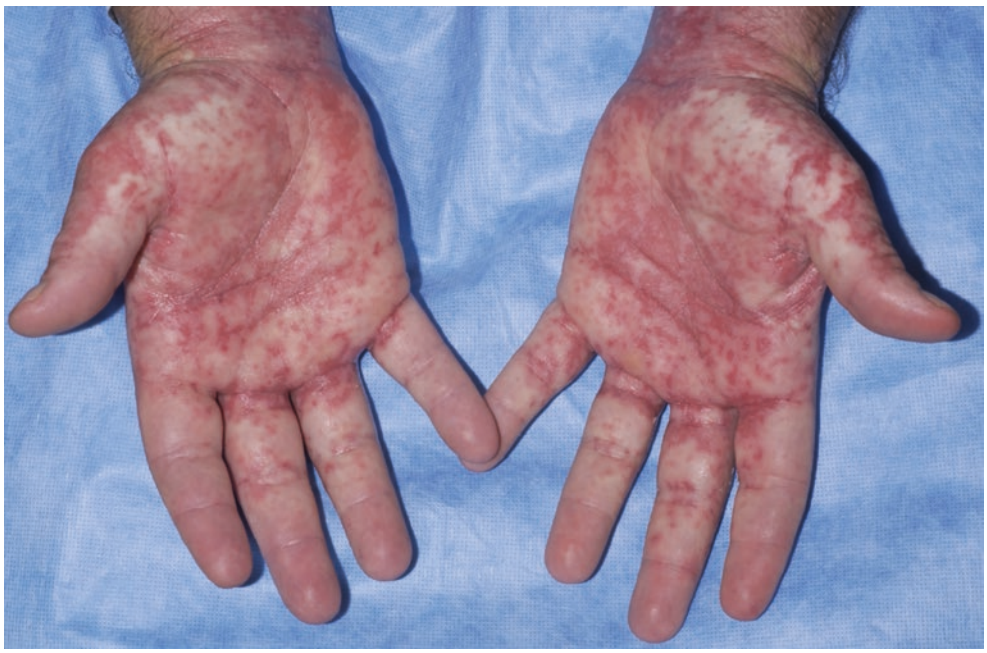

Fig. 38.1 Acute GVHD of the palms

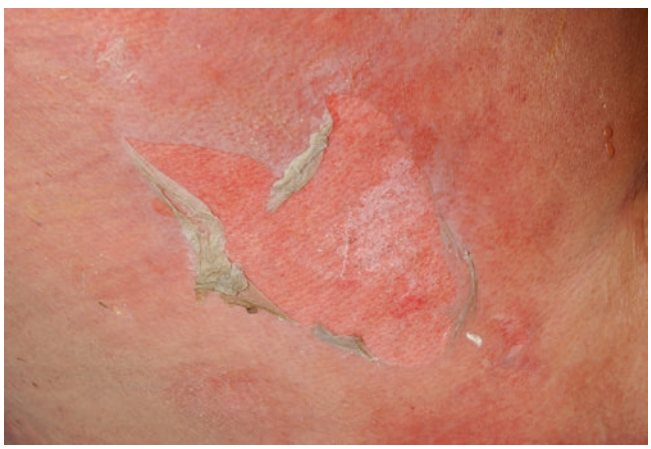

Fig. 38.2 Acute GVHD with necrolysis

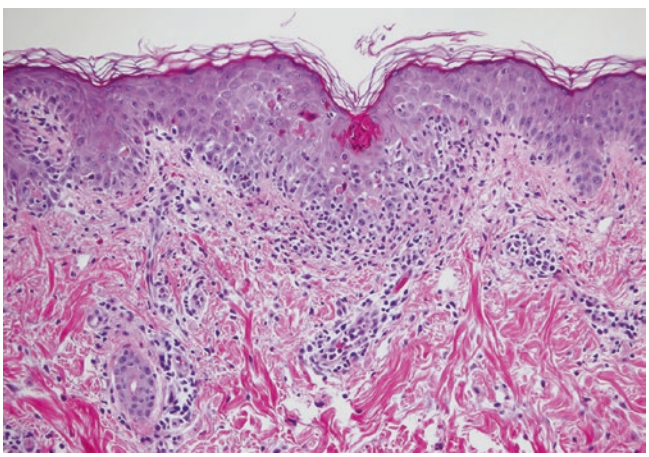

Fig. 38.3 Scattered necrotic epidermal keratinocytes with vacuolization of the basal cell layer and lymphocytic infiltration in the papillary dermis in a patient with acute GVHD (Hematoxylin and Eosin, 20×)

Chronic GVHD

Chronic GVHD occurs in 30–80 % of allogeneic HSCT recipients and is the leading cause of non-relapse mortality in survivors more than 2 years after transplantation [10]. Skin involvement may progress directly from acute disease, following a period of disease quiescence, or *de novo* without a history of previous acute involvement. The greatest predictor of chronic GVHD is a history of prior acute GVHD [11]. Other risk factors include older patient age, female donor for male recipient, mismatched or unrelated donor, peripheral blood graft, T-cell replete graft, and use of donor lymphocyte infusions. A flare of chronic cutaneous GVHD may be triggered by a number of factors, most commonly tapering of immunosuppression, but may also occur following the development of a drug eruption or sunburn, or in the setting of a cutaneous or systemic infection.

One of the greatest hurdles to improving chronic GVHD management stems from the clinical and immunological complexity of the disorder. In an effort to facilitate clinical research in the field of chronic GVHD, the National Institutes of Health Chronic GVHD Consensus Project published a series of articles providing a standardized approach for diagnosis and staging [12], histopathology [13], disease biomarkers [14], response criteria [15], supportive care [16], and clinical trial design [17]. Traditionally, chronic cutaneous GVHD has been described as either "lichenoid" or "sclerodermoid" involvement. However, these terms do not accurately portray the variability in the currently recognized cutaneous manifestations of chronic GVHD [18]. The Consensus Project diagnosis and staging guidelines provide a classification system of the clinical manifestations of cutaneous GVHD (Table 38.3). Poikiloderma, lichen-planus-like lesions, and sclerotic skin changes including fasciitis are considered diagnostic features of chronic cutaneous GVHD when they occur in the setting of allogeneic HSCT. Other cutaneous features, such as ichthyosis, dyspigmentation, and alopecia are also well-recognized manifestations but are not considered diagnostic of skin involvement [12].

Given the variety of epidermal changes associated with GVHD, the term "lichenoid" is preferred as a histologic descriptor of GVHD rather than as a clinical disease classification. Discrete lichen-planus-like violaceous papules are relatively uncommon in chronic GVHD, but may be seen most commonly on the palms and soles (Fig. 38.4). In the past, the term "lichenoid" has been used most commonly to describe cutaneous GVHD which manifests as poorly defined interconnecting erythematous papules and plaques with overlying scale. This eruption may localize to sites of previous UV exposure, such as the posterior and lateral neck, and may spare UV-protected areas such as the buttocks. Underlying sclerotic changes resembling lichen sclerosus or morphea may also be present. Resolution of this manifestation of chronic GVHD often results in a distinct reticulate pattern of hyperpigmentation, reflecting the pigment incontinence induced by the epidermal-dermal interface reaction.

Sclerotic changes associated with chronic GVHD may develop at any layer of the dermal and subcutaneous tissue. Chosidow et al. [19] estimated the incidence of sclerodermatous GVHD to be 3.6 % based on a review of 196 HSCT patients. However, the true frequency of sclerotic changes associated with GVHD may be higher if all forms of sclerosis are included. In a large NIH cohort of 206 patients enriched for severe and refractory chronic GVHD, sclerotic changes were detected in 109 (53 %) [20]. Sclerosis may be present on multiple levels on a single patient and the changes may or may not occur in the presence of overlying epidermal involvement. The most superficial level of sclerotic changes resembles lichen sclerosus and consists of atrophic gray patches with epidermal atrophy, often distributed symmetrically on the upper back. Morphea-like GVHD mimics morphea with patchy areas of prominent dermal sclerosis, often with overlying pigmentation, that results in a decreased ability to pinch the skin. Morphea-like GVHD often occurs preferentially at sites of skin friction or pressure such as the waistband area (Fig. 38.5) [21]. Scleroderma-like GVHD represents full-thickness sclerosis with the complete inability to pinch skin and with a hidebound appearance. Involvement over joints may significantly limit range of motion. In contrast to scleroderma, scleroderma-like GVHD does not begin with symmetric distal hand involvement and proceed proximally and Raynaud's phenomenon is uncommon [18]. Chronic sclerosis may be complicated by bullae formation as well as spontaneous erosions and ulcerations (Fig. 38.6). Benign angiomatous papules and nodules may develop in patients with chronic disease (Fig. 38.7) [22].

The histologic of "lichenoid" chronic GVHD resembles that of lichen planus with a bandlike lymphoplasmocytic infiltrate. Sclerotic GVHD resembles scleroderma with homogenization of collagen and loss of adnexal structures. The level and degree of fibrosis observed histologically reflects the clinical type of sclerosis. In patients with primary rippling to the skin resembling eosinophilic fasciitis, sclerosis and inflammation will be seen primarily at the interface between the reticular dermis and the subcutaneous fat and the deeper fascia.

Chronic GVHD-Related Fasciitis

GVHD-related panniculitis and fasciitis represent sclerosis of the deep subcutaneous tissue and fascia [23]. Although GVHD-related fasciitis is thought to be an uncommon presentation, it may present in an insidious manner with resultant marked functional limitations. Patients with GVHD-related fasciitis often manifest overlapping features of both panniculitis and fasciitis and the histologic diagnosis may depend on the depth and extent of the tissue biopsy.

Table 38.3 Clinical manifestations of chronic GVHD

Dermatologic and mucosal features	
Skin	Alopecia
	Angiomatous papules
	Bullae
	Erythema
	Hypo- or hyperpigmentation
	Ichthyosis-like
	Keratosis-pilaris-like
	Lichen-planus-like[a]
	Lichen-sclerosus-like[a]
	Maculopapular
	Morphea-like[a]
	Poikiloderma[a]
	Scleroderma-like[a]
	Sweat impairment
	Ulceration
Nails	Brittleness
	Longitudinal ridging or splitting
	Onycholysis
	Pterygium unguis
Subcutaneous tissue	Fasciitis[a]
	Panniculitis
Oral mucosa	Erythema
	Gingivitis
	Hyperkeratotic plaques[a]
	Lichen-planus-like[a]
	Mucocele
	Mucosal atrophy
	Mucositis
	Pseudomembrane
	Restriction of oral opening from sclerosis[a]
	Ulcer
	Xerostomia
Genital mucosa	Lichen planus-like[a]
	Vulvar erosions/fissures
	Vaginal scarring/stenosis[a]
Other organ system involvement in chronic GVHD	
Cardiovascular	Pericardial effusion
Ophthalmologic	Cicatricial conjunctivitis
	Sicca symptoms
	Confluent punctuate keratopathy
	Photophobia
	Blepharitis
Gastrointestinal	Esophageal web
	Esophageal stricture/stenosis
Hematopoeitic	Thrombocytopenia
	Eosinophilia
	Lymphopenia
	Hypo- or hypergammaglobulinemia
	Autoantibodies
Hepatic	Elevated total bilirubin
	Elevated alkaline phosphatase
	Elevated transaminases

Table 38.3 (continued)

Musculoskeletal	Myositis or polymyositis
	Edema
	Myalgia
	Arthralgia, arthritis
Neurologic	Peripheral neuropathy
	Myasthenia gravis
Pulmonary	Bronchiolitis obliterans ± organizing pneumonia
	Pleural effusion

[a]Diagnostic feature; other signs and symptoms listed are not considered sufficient to establish a diagnosis of chronic GVHD without further testing or other organ involvement

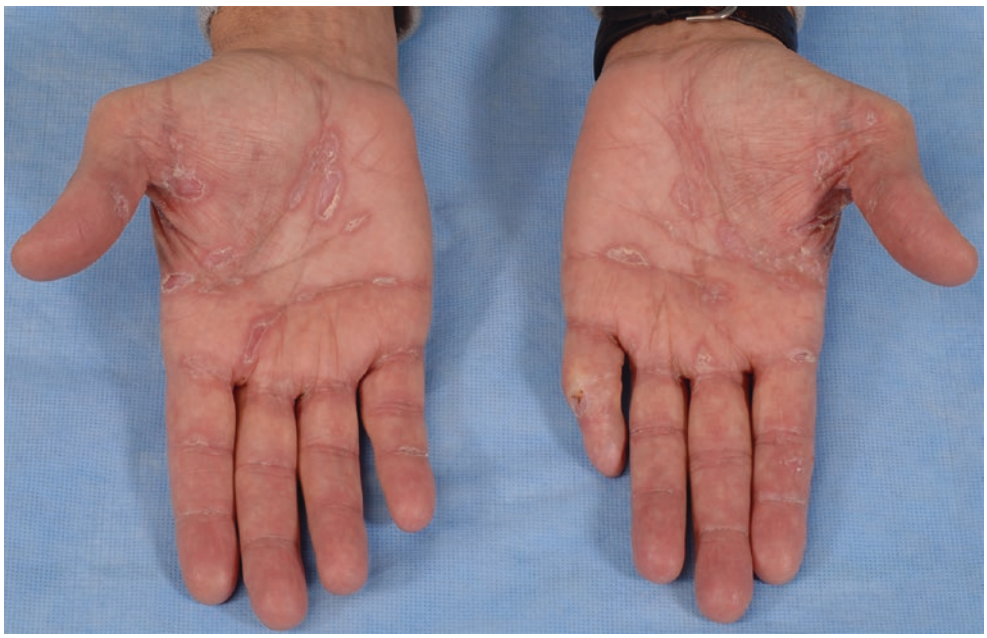

Fig. 38.4 Chronic GVHD; scaling violaceous papules and plaques on the hands

Patients may complain of muscle pain or weakness [24], or may demonstrate limited range of motion at affected joints at the time of presentation. GVHD-associated fasciitis shares many similarities with eosinophilic fasciitis, an uncommon disorder of unknown etiology first described by Shulman in 1974 [25]. GVHD-associated fasciitis presents with prominent induration and rippling of tissue, visible grooves demarcating fascial bundles or underlying superficial veins, and decreased range of motion (Fig. 38.8). The first indications of subcutaneous involvement may be edema of the affected limb. Magnetic resonance imaging may facilitate the diagnosis of subcutaneous involvement [26, 27].

Genital GVHD

Assessment of genital involvement is important in the management of GVHD patients. Genital involvement is most commonly associated with sclerotic cutaneous disease, but may occur with other forms of cutaneous involvement, or in the absence of other cutaneous involvement. Genital tract involvement may be present in as many as 49 % of female patients two years post-transplantation and may seriously impact the quality of life of affected individuals [28]. Manifestations include burning and irritation, discharge, erosions and fissures, or vaginal stricture (Fig. 38.9). In a recent review of 155 male allogeneic transplant recipients 1 year or more after transplant, 21 (13 %) manifested inflammatory lesions, most frequently balanoposthitis (12 patients), lichen-sclerosus-like lesions (6), and phimosis (5) [29].

Oral GVHD

The second most common organ system involved with chronic GVHD after the skin is the mouth. Chronic oral GVHD may affect the oral mucosa and salivary glands. Mucocele formation is very common but usually asymptomatic. Lichen-planus-like oral involvement manifests as erythematous hyperkeratotic plaques and erosions. Sclerosis of the skin surrounding the mouth or the frenulum can cause difficulty opening the mouth or protruding the tongue, respectively. Persistent erosions and fissures cause burning pain, particularly upon contact with acidic foods. Salivary

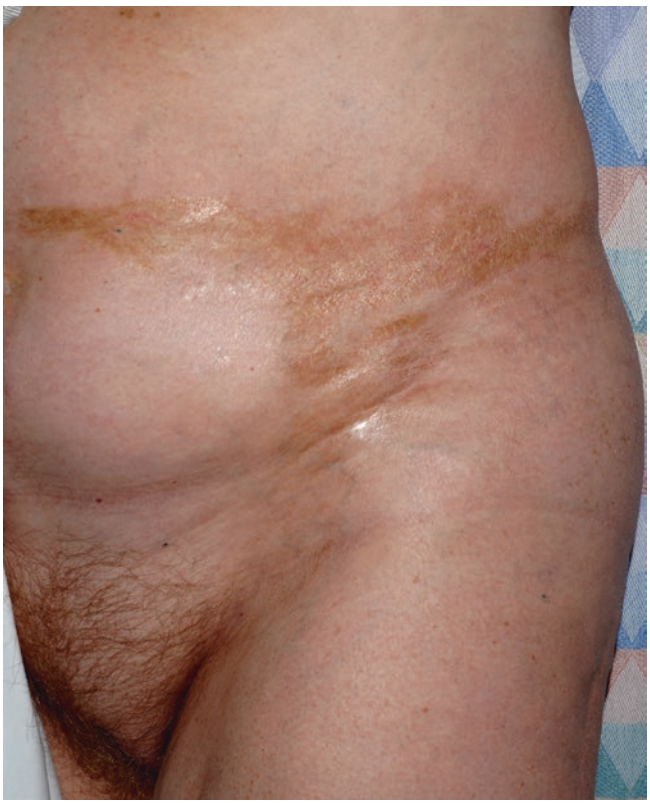

Fig. 38.5 Tan sclerotic plaques with koebnerization at site of waistband in patient morpheaform chronic GVHD

gland involvement from chronic GVHD results in decreased saliva production and sicca symptioms. Loss of taste is also commonly reported by patients [30].

Immunology of GVHD

Acute GVHD

Acute GVHD is a reaction of immunocompetent donor cells against the cells and organs of the host. Billingham [31] described three features necessary for the development of GVHD: (1) the transplanted graft must be immunologically competent; (2) the recipient must not be capable of rejecting the graft; (3) the recipient must express antigens that are recognized as foreign by the graft. Grafted cells recognize the host as foreign through differences between the donor and host in major and minor HLA expression.

Pro-inflammatory Environment

Ferrara and Reddy [32] proposed a three-step model for the immunopathophysiology of acute GVHD. The first phase occurs prior to transplantation of the graft during which time chemoradiotherapy, the underlying disease state, and other factors activate host antigen presenting cells (APCs). Total body irradiation in particular plays an important role in priming the immune response by inducing epithelial cell damage in the gastrointestinal tract,

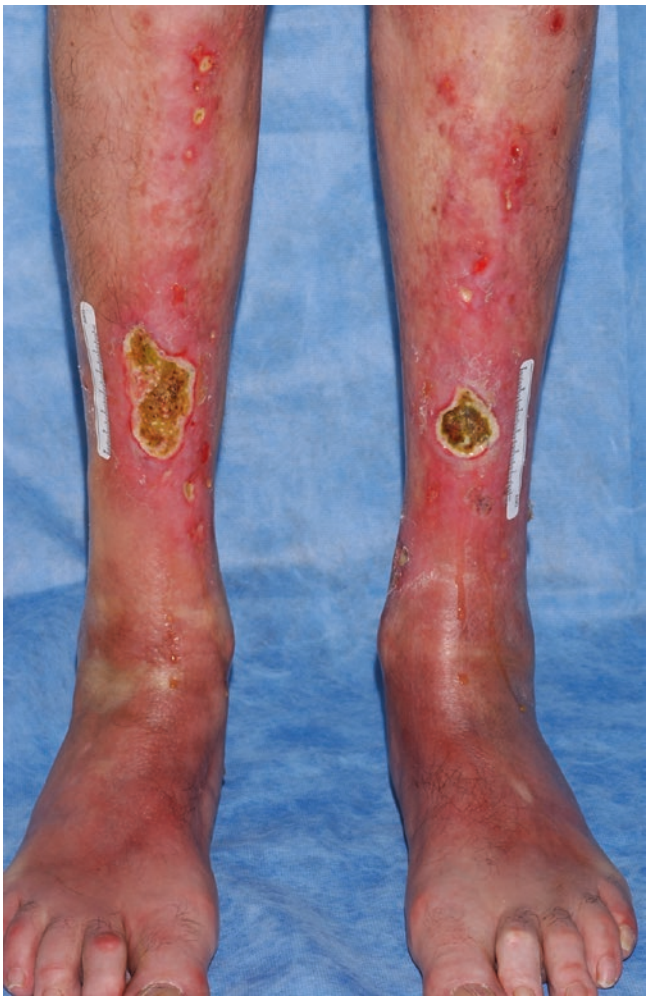

Fig. 38.6 Hidebound sclerosis with skin ulceration in a patient with scleroderma-like chronic GVHD

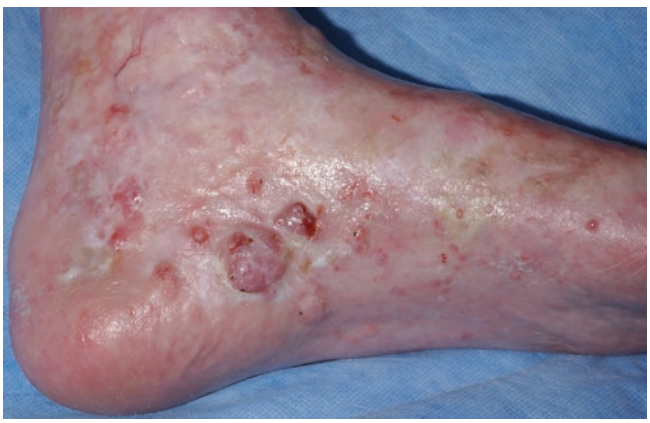

Fig. 38.7 Numerous angiomatous papules and nodules in a patient with chronic sclerotic GVHD of the legs

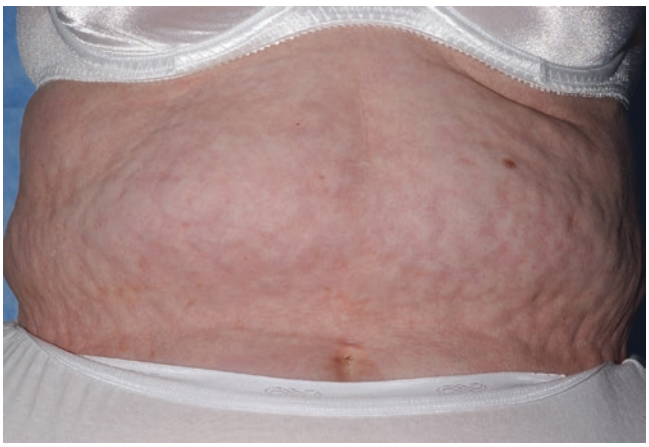

Fig. 38.8 Subcutaneous sclerosis from chronic GVHD; there is prominent rippling and nodularity of the subcutaneous tissue appreciable by deep palpation. The overlying skin is normal in texture and color

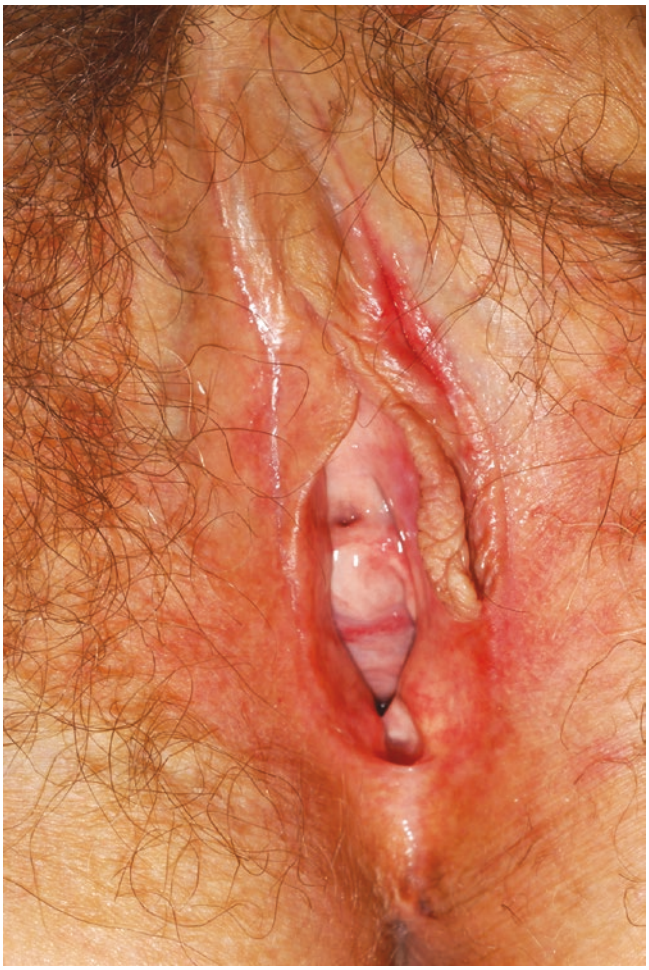

Fig. 38.9 Chronic vulvar GVHD; resorption of labia minora; pallor and sclerosis of vulvar vestibule; fissuring of the interlabial sulcus

which leads to host secretion of inflammatory cytokines (TNF-α and IL-1) and exposure to microbial products such as lipopolysaccharide. In fact, total body irradiation may contribute to chronic GVHD as well, particularly sclerotic skin disease, as pre-transplant conditioning was recently found to a be a risk factor for development of sclerotic chronic GVHD [20].

In the second phase of acute GVHD, host APCs expressing MHC class I and II molecules are recognized as foreign by donor T-cells. In murine studies, host dendritic cells are sufficient to activate donor T cells [33]. Differences in minor histocompatibility antigens (mHags), such as those encoded on the Y chromosome, may also play an important role in propagating acute GVHD, particularly in the setting of HLA-identical transplantation. Activation of natural killer (NK) cells, which eliminate APCs, abrogates the development of GVHD, suggesting a crucial role for host APCs in the propagation of acute GVHD [34]. The three major organ systems involved in acute GVHD – the skin, liver, and gut – contain large numbers of APC, which may in part be responsible for the localization of tissue damage to these organ systems [35].

In the final effector phase, inflammatory mediators and cell-mediated killing work together to induce the clinical effects typical of acute GVHD. $CD8^+$ cytotoxic T cells (CTLs) utilize perforin/granzyme mediated cytolysis, whereas $CD4^+$ T cells utilize Fas/FasL signaling, which may be particularly important for inducing hepatic damage [32]. TNF-α and IL-1 signaling play a prominent role in cellular damage in acute gastrointestinal GVHD. Cytokine gene polymorphisms may influence the expression of GVHD as a variance in the TNF-α gene has been associated with an increased risk of severe acute GHVD [36], whereas polymorphisms in IL-10, a potent suppressor of TNF-α, IL-1, and other inflammatory cytokines, has been associated with a decreased incidence of acute GVHD [37].

Regulatory T-Cells

Acute GVHD is mediated by donor T-cells that expand following transplantation in response to the recipient environment. Regulatory T-cells (T_{reg}) constitute a subset of the T-cell population which exert control over the allogeneic T-cell response against the host. T_{reg}s express FOXP3, a key transcription factor for T_{reg} function, as well as CD25, the IL-2 receptor α chain that is also expressed by activated T-cells. Donor grafts with a low percentage of CD4 + Foxp3+ T_{reg}s are associated with an increased risk of acute GVHD. In addition, the ratio of CD4 + FOXP3+ cells to CD4 + FOXP3-CD25+ in patients after transplant is significantly reduced in patients with acute GVHD, suggesting an important role for T_{reg}s in control of effector function [38]. Manipulation of specific T-cell subsets in donor grafts may allow for modulation of the GVHD and graft-versus-leukemia response.

Chronic GVHD

In contrast to acute disease, our understanding of the pathogenesis of chronic GVHD is somewhat incomplete. Chronic GVHD demonstrates a complex interplay of immunologic processes with features of alloimmunity, autoimmunity, and immunodeficiency. The heterogeneity of clinical manifestations and disease course in chronic GVHD makes identification of a murine model challenging. In addition, correlations between murine and human chronic GVHD are difficult because marked differences in immune reactions are observed by differences in the intensity of the conditioning regimen, disparity between strains, donor graft composition, and endogenous microbes of the animals [39]. The best characterized murine model of GVHD is the parent-into-F_1 mouse. In this model, parental lymphocytes are injected into the F_1 recipient offspring. Because the parental lymphocytes are genetically related to the recipient, they are not recognized as foreign. However, the donor lymphocytes recognize the F_1 mouse as foreign, inducing a GVHD reaction.

In chronic GVHD, autoreactive T-cells are thought to arise from impairment of negative thymic selection due to thymic damage incurred from chemotherapy, acute GVHD, or age-related atrophy [40]. The clinical similarity of chronic GVHD to autoimmune diseases such as Sjogren's syndrome and scleroderma and the reported benefit of treatment of chronic GVHD with anti-CD20 monoclonal antibody [41] also suggest a role for humoral immunity in chronic GVHD. Circulating autoantibodies were described in one of the first series describing the clinical features of chronic GVHD in 1980 [42]; however, the relevance of antibody formation to disease activity remains unclear. In a prospective study, the cumulative incidence of antinuclear antibodies (ANA) in patients with extensive chronic GVHD was 94 % after a median follow-up time of 26 months. The presence of nucleolar pattern ANA and other antibodies in association with ANA was associated with an increased risk of extensive chronic GVHD [43]. However, in this study, the presence and titer of specific autoantibodies did not predict the type of organ involvement, a finding in accord with a recent study comparing 106 patients with sclerotic GVHD to patients with GVHD without skin sclerosis which failed to find an association with ANA or other scleroderma-related antibodies [20, 43]. Nevertheless, antibodies targeting minor-HLA antibodies on the Y chromosome in male recipients of stem cell grafts from female donors correlate with the presence of chronic GVHD, suggesting a potentially important interplay between T and B cells in the pathogenesis of chronic GVHD [44].

Cytokine Dysregulation

Chronic GVHD is associated with elevated levels of IL-1 (β), IL-6, transforming growth-factor-β, TNF-α, and IFN-γ as well as decreased levels of IL-10 [45, 46]. TGF-β is the cytokine that has been most strongly implicated in GVHD-related fibrosis. Tissue fibrosis is a common manifestation in several organ systems involved with chronic GVHD, including the liver, pulmonary system (bronchiolitis obliterans), and skin. TGF-β is a pleiotropic cytokine that in the acute post-transplant period regulates donor engraftment and graft-versus-leukemia effect [47]. In the chronic period, TGF-β appears to the major driving force for collagen synthesis and the development of fibrosis. In the murine sclerodermatous GVH model, treatment with anti-TGF-β antibody prevented lung and skin fibrosis [48].

Platelet Derived Growth Factor

In scleroderma, platelet-derived growth factor (PDGF) appears to play a key role in the increased proliferative capacity of fibroblasts, an effect which is enhanced by the presence of transforming growth factor-β [49]. Increased gene expression of PGDF has been also detected in the skin in the murine sclerodermatous GVHD model [50]. Baroni et al. [51] reported stimulatory autoantibodies to the platelet-derived growth factor receptor (PDGFR) in a group of 46 patients with systemic sclerosis. Ten additional patients with scleroderma-like GVHD were also reported to have agonistic antibodies (this group was not further described in the paper). In this study, production of reactive oxygen species (ROS) and tyrosine phosphorylation was reversed with the use of PDGFR tyrosine kinase inhibitors, suggesting that agents such as imatinib mesylate with activity against the PDGFR may have potential utility for targeting this signaling pathway in the setting of scleroderma and sclerotic GVHD. However, to date the identification of stimulatory PDGFR antibodies in systemic sclerosis or GVHD has not subsequently been confirmed by other groups.

Donor Lymphocyte Infusion and Graft-Versus-Tumor Effect

An important barrier to the effective control of acute and chronic GVHD is the risk of inhibiting the activity of the stem cell graft against the donor's primary malignancy (graft vs. leukemia effect). Numerous studies have demonstrated that the risk of tumor relapse is lower in patients with GVHD than in those that do not develop GVHD [52]. Similarly, T-cell depletion of the donor graft and aggressive multiagent GVHD prophylaxis reduces the risk of developing GVHD, but does so at the expense of an antileukemic effect, resulting in an increased relapse rate [53]. Donor lymphocyte infusions (DLI) have been utilized to augment the antitumor response of the graft but are also associated with an increased rate of GVHD [54]. Ideally, our understanding of chronic GVHD will progress to the point where separation of the graft vs. host and graft vs. leukemia (tumor) effect

would be possible. In reality, it is a constant struggle to balance these competing forces in the management of these patients.

Treatment of Cutaneous GVHD

Acute GVHD

Acute GVHD is treated with systemic steroids, resulting in a 40–50 % response rate. There is no consensus as to the appropriate second-line agent in those patients who do not respond adequately to corticosteroid therapy. A variety of salvage therapies have been utilized in patients with steroid refractory GVHD, but no single agent has proven to be a superior option (Table 38.4) [110]. The major limitation of most acute GVHD therapies arises from the use of systemic immunosuppression and attendant risk of infection. Newer biological agents, such as those targeting tumor necrosis factor-α, have shown some benefit in acute GVHD but have been linked to invasive fungal infections [111].

Topical agents play a limited role in the management of acute cutaneous GVHD; however, high-potency topical corticosteroids may be of benefit in patients with limited skin involvement. Proper skin hygiene and surveillance for the development of cutaneous infections is needed.

For patients manifesting primarily cutaneous involvement, phototherapy is a potential treatment option in lieu of additional systemic immunosuppression. Psoralen in combination with UVA (PUVA) resulted in disease response of 15 out of 20 patients treated for acute skin GVHD [89]. Wetzig et al. [102] reported improvement with UVA1 in 5 out of 7 patients with acute skin GVHD. Complete response of steroid-resistant acute skin disease following narrowband UVB (NB-UVB) was reported in 7 out of 10 [107] and 8 out of 14 patients, respectively [108].

Extracorporeal Photopheresis

Extracorporeal photopheresis (ECP) is a leukopheresis procedure that has been approved by the US Food and Drug Administration for the treatment of cutaneous T-cell lymphoma. Following leukopheresis, the mononuclear cell sample is mixed with 8-methoxypsoralen and exposed to a UV-A light source before re-infusion into the patient. This procedure spares the patient of the risk of serious infection associated with systemic immunosuppression and is particularly effective for skin GVHD [71]. Greinix et al. [71] reported a 65 % complete response rate for acute skin GVHD after 3 months of treatment. In the pediatric setting, Messina et al. [72] reported a 76 % response rate for acute skin involvement in 33 patients. The optimal frequency and duration of ECP for GVHD is unclear. Apoptosis of alloreactive T cells is the presumed mechanism for the efficacy of ECP in GVHD, despite the fact that only a small percentage of circulating lymphocytes are treated at each ECP session [112]. ECP requires a large, double-lumen pheresis cathether and is not available at all medical centers. In addition, small children are not able to undergo this procedure because of the fluid volume extracted for the procedure. Recent modifications in the process are allowing the therapy be used more safely in small children by exchanging smaller fluid volumes.

Chronic GVHD

Skin-Directed vs. Systemic Therapy

Infection is the leading cause of death in patients with chronic GVHD. Patients are immunosuppressed from their disease state as well as from the immunosuppressive regimens required to treat their disorder. Treatment recommendations for chronic cutaneous GVHD must include a consideration of the type and extent of skin involvement, the potential for long-term morbidity (e.g. sclerotic disease), and the presence of other organ system involvement. Other important factors include the presence of high-risk features such as thrombocytopenia and progressive onset of disease, risk of infection, and the status of the underlying disease state/malignancy.

Limited cutaneous involvement in the absence of high-risk features or other systemic involvement may be addressed with topical measures. Non-sclerotic disease may respond to mid- to ultra-high potency topical steroids (triamcinolone 0.1 %-clobetasol 0.05 % cream/ointment), but may lead to skin atrophy with prolonged use [16]. Topical emollients and anti-pruritic agents may provide additional symptomatic relief. Choi and Nghiem [97] reported improvement in pruritus and erythema in 18 patients treated with tacrolimus 0.1 % ointment. However, all patients eventually required systemic therapy or phototherapy in order to control their skin disease. Subsequent reports have also described a modest benefit from tacrolimus ointment at 0.1 % and 0.03 % as well as with pimecrolimus [88, 98]. These agents may have utility for the treatment of superficial involvement, particularly in areas at high risk of skin atrophy with topical steroids. A response to topical tacrolimus 0.01 % has also been reported for oral GVHD [99]. However, intraoral use of tacrolimus 0.1 % ointment markedly elevated the serum drug level in a patient taking systemic tacrolimus [113]. Systemic toxicity after widespread application of topical tacrolimus was also reported in a pediatric patient with acute GVHD who was not on systemic tacrolimus [114]. The risk of cutaneous malignancy following long-term treatment with topical calcineurin inhibitors in the setting of chronic GVHD is unknown.

Table 38.4 Treatment for acute and chronic mucocutaneous GVHD

Treatment	Type of GVHD	
	Acute	Chronic (L/Sc/Oral)
Antithymocyte globulin	Remberger et al. [55]	–
Azathioprine	–	Penas et al. [56] (Sc)
		Epstein et al. [57] (Oral)
Basiliximab	Funke et al. [58]	–
Clofazimine:	–	Lee et al. [59] (L/Sc)
Corticosteroids (systemic)	Doney et al. [60, 61]	Goerner et al. [62][a]
		Ruutu et al. [61]
Cyclosporine	Aschan [63]	Goerner et al. [62][a]
		Epstein and Reese [64] (Oral)
Dacluzimab	Bordigoni et al. [65]	Teachey et al. [66][a]
Denileukin diftitox	Shaughnessey et al. [67]	–
Etanercept	Uberti et al. [68]	Chiang et al. [69][a]
Etretinate	–	Marcellus et al. [70] (Sc)
Extracorporeal photopheresis	Greinix et al. [71, 72]	Couriel et al. [73] (L/Sc) [74]
	Messina et al. [72]	Seaton et al. [74] (L/Sc)
		Flowers et al. [75][a]
Hydroxychloroquine	–	Gilman et al. [76] (L)[b]
Imatinib mesylate	–	Olivieri et al. (Sc) [77]
		Olivieri et al. (Sc) [78]
		Magro et al. (Sc) [79]
		Chen et al. (Sc) [80]
Infliximab	Patricarca et al. [81, 82]	Baird et al. (Sc) [83]
	Couriel et al. [82]	
Mycophenolate mofetil	Basara et al. [84, 85]	Basara et al. [84][a]
	Baudard et al. [85]	Baudard et al. [85] (L/Sc)
Pentostatin	Bolanos-Meade et al. [86]	Vogelsang et al. [87] (L/Sc)
Pimecrolimus (topical)	–	Schmoook et al. [88] (L)
PUVA:	Wiesmann et al. [89]	Leiter et al. [90] (L/Sc)[c]
		Vogelsang et al. [91] (L/Sc)
Rapamycin (sirolimus)	Benito et al. [92]	Johnston et al. [93] (L/Sc)
Rituximab	Kamble et al. [94]	Cutler et al. [41] (L/Sc)
Tacrolimus [systemic]	Furlong et al. [95]	Carnevale-Schianca et al. [96] (L/Sc)
Tacrolimus [topical]	–	Choi and Ngheim [97] (L)
		Elad et al. [98] (L/Sc)
		Eckardt et al. [99] (Oral)
Thalidomide	–	Kulkarni et al. [100][a]
		Browne et al. [101][a]
UVA1:	Wetzig et al. [102]	Wetzig et al. [102] (L/Sc)
		Calzavara et al. [103] (L/Sc)
		Ständer et al. [104] (Sc)
		Grundmann-Kollman et al. [105] (Sc)
UVB:	Grundmann-	Enk et al. [106] (L/Sc)
	Kollman et al. [107][d]	
	Feldstein et al. [108]	Brazzelli et al. [109] (L)

L lichenoid or erythematous skin involvement, *Sc* sclerotic skin involvement

[a]Type of chronic cutaneous GVHD not specified

[b]In this study, both lichenoid and sclerotic-type cutaneous GVHD was treated; however, none of the sclerotic patients responded to therapy

[c]Bath PUVA

[d]Narrowband UVB

Preventative strategies include sun avoidance, the use of sunblock, and protective clothing to decrease the risk of a UV-associated flare in symptoms. Surveillance for cutaneous malignancies should be performed at regular intervals. Diffuse or patchy post-inflammatory pigmentation is a frequent sequelae of chronic GVHD, particularly in darkly pigmented individuals. Following resolution of GVHD activity, this pigmentation fades gradually. Topical hydroquinone with or without retinoids or corticosteroid-containing compounds offer very limited benefit [16].

Treatment of eroded tissue and skin ulcerations in the setting of skin sclerosis is challenging. Aggressive wound management utilizing protective films, dressings, and wound healing products such as becaplermin may be beneficial [16]. Regular supervision in a dedicated wound care clinic may maximize the likelihood of achieving wound healing. As patients may be relatively immunosuppressed by the presence of GVHD or by therapies aimed at controlling GVHD, it is important to rule out an infectious source. Bacterial, viral, mycobacterial, and fungal cultures should be performed as indicated. Similarly, consideration should be given to non-infectious sources of skin breakdown other than GVHD, including vasculitis, bullous drug reaction, neuropathy, primary cutaneous malignancy, or metastatic disease.

Systemic treatment of chronic GVHD utilizes many of the same systemic modalities as for acute GVHD. Generally, first line treatment consists of continuation of the calcineurin inhibitor used for GVHD prophylaxis with prednisone initially at 1 mg/kg/day [115]. Similar to acute GVHD, there is no consensus regarding second line treatment. Therapeutic options include ECP, mycophenolate mofetil, rituximab, sirolimus, and imatinib mesylate. Unfortunately, interpretation of therapeutic responses in clinical trials is hampered by the lack of standardized evaluative indices for skin involvement. In addition, responses to epidermal (lichenoid) and sclerotic skin disease are sometimes reported together without the use of response criteria specific for the different skin manifestations [116].

Extracorporeal Photopheresis

Extracorporeal photopheresis has emerged as a major second-line treatment for patients with steroid refractory chronic GVHD. Rates of partial or complete remission of skin disease range from 31 to 100 % in clinical series [117]. However, in a randomized blinded comparison of standard chronic GVHD therapy (n = 47) vs. standard therapy + ECP (n = 48), the primary endpoint (total skin score) was not significantly different between the two groups [75]. The precise mechanism by which ECP treatment affects chronic GHVD is still unclear. ECP causes an increase in the plasmacytoid DC2 dendritic cell population and a corresponding decrease in the monocytoid DC1 population, which may result in a shift from a primarily Th1 to a Th2 cytokine profile [118]. Increased production of IL-10, in particular, may play an important role in the mitigation of the GVHD response through inhibition of antigen presentation and promotion of regulatory T cell differentiation [119, 120]. Although GVHD-related fasciitis resembles eosinophilic fasciitis the former does not respond well to steroid therapy and may result in significant long-term functional disability. ECP has been used successfully for the treatment of eosinophilic fasciitis [121], and also appears to be an attractive treatment option for GVHD-related fasciitis [122]. Several questions regarding ECP therapy remain unanswered, including the treatment schedule (weekly vs. every 2 weeks) and the potential for ECP as a first-line therapy in the management of chronic GVHD [117]. Nevertheless, several consensus groups now recommend ECP as second-line therapy, particularly for skin, oral and liver chronic GVHD [123–125].

Phototherapy

As with acute GVHD, chronic GVHD may respond to PUVA therapy. Lichenoid chronic GVHD has been reported to respond to psoralen plus UVA (PUVA) therapy in several small series [126]. Improvement in both lichenoid and sclerotic chronic GVHD has been reported in a small series following treatment with PUVA-bath therapy [90]. It must be kept in mind that chronic GVHD results in immunodeficiency that is often further compounded by iatrogenic immunosuppression and may result in an increased risk of skin neoplasia. Multiple squamous cell carcinomas have been reported following PUVA treatment for chronic GVHD [127]. The risk of melanoma in patients following HSCT is significantly elevated [128].

UVA1 is a form of phototherapy in which long wavelength (340–400 nm) UV light is used without a photosensitizer. UVA-1 radiation has the potential to reach the deeper layers of the dermis and even the subcutis. For this reason, UVA-1 has been employed for a number of sclerosing skin conditions, particularly localized scleroderma, with improvement in skin thickness in several small series [129]. Experience with UVA-1 for chronic GVHD is more limited, but has been effective in a small number of patients with lichenoid and sclerotic disease [102, 104, 105] although relapse after therapy has been seen in both types of cutaneous involvement [103]. UVA-1 is available at several major medical centers, but is not widely available in the community and questions remain regarding the optimal treatment frequency as well as whether the risk/benefit ratio supports use of low, medium or high dose therapy [129].

NB-UVB has also been reported in both the acute and chronic GVHD setting, primarily in small cases series.

NB-UVB shares with UVA-1 the advantage of avoiding exposure to oral or IV psoralen, is more easily performed in children, and is also more widely available in the community than UVA-1 phototherapy and ECP. Brazzelli et al. [109] reported an 80 % response in 10 pediatric patients with overlap acute/chronic GVHD or chronic GVHD.

Systemic Retinoids

Marcellus et al. [70] described the Johns Hopkins GVHD group's experience with etretinate for treatment-refractory sclerotic GVHD. Twenty out of 27 evaluable patients had a subjective response. Six patients could not tolerate the medication due to scaling and/or skin breakdown. Further prospective studies are needed to determine the utility of systemic retinoids for superficial disease.

Imatinib Mesylate

Imatinib mesylate is a multikinase inhibitor with activity against BCR-ABL, c-kit, and PDGFR, among other tyrosine kinases. The drug is FDA-approved for the management of Philadelphia chromosome + chronic myelogenous leukemia and has a significant track record of safety. In addition, imatinib mesylate has demonstrated significant anti-fibrotic properties in a number of murine models of lung [130] and skin fibrosis [131], suggesting that it may represent a 'targeted' anti-fibrotic therapeutic option in patients with sclerotic chronic GVHD. To date, several retrospective reviews [78, 79] and prospective studies [44, 77, 80] suggest that the drug may have efficacy in a subset of patients with steroid-refractory sclerotic skin disease. However, known side effects of imatinib mesylate, including cramping, fluid retention and fatigue, have been frequently reported in chronic GVHD patients and for this reason the drug is usually dosed at 100 mg and increased up to 400 mg if tolerated [77].

Treatment of Genital GVHD

As mentioned earlier, assessment of genital involvement by history and physical examination should be included in the dermatologic evaluation of all female patients. Standard recommendations in all female patients regardless of the presence of GVHD include the use of a vaginal topical estrogen to decrease atrophy of mucosal tissue, discussion of the risks/benefits of systemic hormonal therapies, education regarding self-examination, regular gynecological symptom review, pelvic examination, and cervical cytology. External genital involvement may be treated with high-potency topical glucocorticoids (betamethasone dipropionate cream 0.05 % gel or ointment) applied once or twice daily for up to 12 weeks [16]. Topical calcineurin inhibitors (cyclosporine, tacrolimus) are also beneficial in mild-moderate disease [16, 132]. The presence of vulvar disease and/or symptoms of vaginal involvement should prompt consultation with a gynecologist experienced in the evaluation and management of genital GVHD for a comprehensive internal examination. Hydrocortisone acetate rectal foam 100 mg/g for 4–6 weeks may be used for intra-vaginal application [28]. Severe vaginal stenosis may result in hematocolpos and requires the use of aggressive topical steroids and vaginal dilator insertion. Surgical lysis of extensive vaginal synechiae may be necessary in severe cases [16]. Transplant patients may also be at higher risk for cervical dysplasia and require close surveillance for the presence of vulvar HPV infection along with regular cervical cytology [133].

Treatment of Oral GVHD

Localized oral disease should be treated with alcohol-based high potency corticosteroid gels. Tacrolimus ointment is preferable for lip involvement due to the risk of atrophy with topical steroid use. Widespread oral disease may be treated with dexamethasone rinse formulation (0.5 mg/mL) 4–6 times/day. Cyclosporine [64] or azathioprine [57] rinses have been used in steroid-resistant cases. Oral phototherapy (PUVA, UVB, narrowband UVB) may be effective but the specialized equipment required for oral phototherapy is not readily available [16]. Patients with oral sicca symptoms are treated with salivary stimulants (e.g. sugar-free gum), saliva substitutes, and frequent sipping of water [16]. Patients with oral dryness are at increased risk of tooth decay thus meticulous oral hygiene is advised as well as surveillance for candidal infection. An increased incidence of squamous cell carcinoma of the oral cavity has been reported in patients with chronic GVHD following HSCT [134], and therefore, a high degree of suspicion is required for oral lesions that do not respond appropriately to therapy.

Questions

1. A 25 year old male with acute myelogenous leukemia requires hematopoietic stem cell transplantation. Which of the following donor/recipient characteristics is the most important risk factor for the development of GVHD?
   A. Female gender donor
   B. Advanced donor age
   C. Advanced recipient age
   D. Donor/recipient HLA incompatibility
   E. Recipient history of previous chemotherapy exposure

2. Which of the following manifestations are most consistent with acute GVHD?
   A. Lichen sclerosus-like changes, mucocele, esophageal stricture
   B. Alopecia, sclerotic skin changes, episcleritis
   C. Oral lichenoid changes, cutaneous lichenoid changes, nephritis
   D. Mucositis, bronchiolitis obliterans
   E. Hepatitis, erythema, diarrhea

3. Which of the following is NOT a diagnostic cutaneous feature of chronic GVHD?
   A. Morphea-like fibrosis
   B. Subcutaneous fibrosis
   C. Lichen planus-like changes
   D. Erythema
   E. Scleroderma-like fibrosis

4. Which is a potential complication of sclerotic skin changes of chronic GVHD?
   A. Restrictive lung disease
   B. Skin ulceration
   C. Range of motion restriction
   D. Alopecia
   E. All of the above

5. Which of the following treatment modalities has demonstrated the most potential for improvement in sclerotic skin disease?
   A. Oral PUVA
   B. Bath PUVA
   C. UVB
   D. NB-UVB
   E. UVA1

Answers

1. D
2. E
3. D
4. E
5. E

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Iatrogenic Immunodeficiency and Skin Disease

39

Ramya Kollipara, Elizabeth Shane, Sheevam Shah, and Stephen K. Tyring

Abstract

Skin cancer in organ transplant recipients (OTR) is an ongoing problem as the number of living OTR grows due to an increasing number of transplants performed and longer patient and graft survival time. Kidney transplants, followed by liver transplants, account for the majority of cases. Chronic immunosuppression, along with other risk factors, places these patients at higher risk of developing malignancies, the most common being cutaneous malignancies. The dose and duration of immunosuppression are more important as risk factors than any one immunosuppressive agent in particular. It is thought that immunosuppressive drugs accelerate the development of skin cancer by being directly carcinogenic and by creating a state of compromised immune surveillance. Management of these patients requires a multifaceted approach involving the transplant team, dermatologists, other care providers and the patients. Treatment and follow up may then be determined on an individual basis on the patient's risk factors and the relative risk of the skin cancer. Patients should perform monthly skin self-examinations and have their skin examined by a physician annually.

Keywords

Immunosuppression • Organ • Transplant • Kidney • Liver • Skin • Cancer • Human Papillomavirus

Key Points

- The number of living OTR continues to grow due to an increased number of transplants performed and a longer patient & graft survival time, thereby increasing the number of patients on immunosuppression
- Immunosuppression required to maintain allografts leads to a significantly increased rate of both internal and cutaneous malignancies with skin cancer being the most common
- The skin cancer seen in immunosuppressed patients, primarily NMSC, occurs earlier and behaves more aggressively compared to NMSC in the general population
- Of the various types of skin cancers seen in OTR, SCC is the leading cause of mortality

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- It is important to be aware of the associated risk factors so that OTR who are at increased risk can be identified and followed even more closely by dermatologists and the transplant physicians
- Immunosuppressive drugs accelerate the development of skin cancer by being directly carcinogenic and by creating a state of compromised immune surveillance
- HPV infection has been recognized as a putative risk factor in NMSC of OTR

Introduction

The number of living OTR continues to grow due to an increased number of transplants performed and a longer patient & graft survival time. As the number of organ transplant recipients (OTR) continues to increase so too does the need for a thorough understanding of these patients and the complications they are likely to encounter. There are over 140,000 OTRs currently living in the United States and between January and August 2009 there were 19,114 transplants performed [1]. Kidney transplants account for the majority of these, followed by liver transplants. Heart and lung are the two next most commonly transplanted organs [2]. The immunosuppression required to maintain these allografts leads to a significantly increased rate of both internal and cutaneous malignancies with skin cancer being the most common [3]. It is therefore likely that dermatologists will be caring for an increasing number of organ transplant recipients.

Types of Skin Cancer

Skin cancer is the most common malignancy for which OTR are at risk [4]. They are at particularly increased risk of nonmelanoma skin cancers (NMSC) with a rate of 50 times that of the general population [5]. Compared to immunocompetent individuals, transplant recipients are 15 years younger at the time of NMSC diagnosis [6]. Comparable results from various studies show that NMSC is diagnosed in 15–43 % of OTR 10 years post-transplantation [7]. These NMSC occur earlier and behave more aggressively compared to NMSC in the general population. The lesions are frequently multiple, have a more rapid rate of growth, and have an increased rate of recurrence and metastasis than seen in nontransplant patients [8]. The mean age of diagnosis of first NMSC since transplant varies with type of transplant but is reported as 4–9 years for kidney transplants. The most common location for NMSC in OTRs is the head, neck, and other locations include upper limbs, trunk and sun-exposed areas [9].

In the general population, basal cell carcinomas (BCC) occur approximately four times more frequently as squamous cell carcinomas (SCC). This ratio of BCC to SCC is reversed in OTRs [8]. In addition to increased rates of NMSC, studies have also found an increased risk of malignant melanoma, particularly in the pediatric population [10]. Kaposi's sarcoma incidence is increased by 84-fold in transplant recipients compared to the general population [11]. Merkel cell carcinoma, a rare neuroendocrine skin cancer, has also been found more commonly in OTR (approximately 24-times more common than in healthy patients) and is more aggressive than in the general population [12]. Of the various types of skin cancers seen in OTR, SCC is the leading cause of mortality. Data from the Cincinnati Tumor Registry showed that 63 % of deaths in OTR who died from skin malignancies were due to SCC [13].

Risk Factors

Several risk factors have been identified to help determine which patients are most likely to develop skin cancer post-transplantation. One clear contributor to the development of NMSC in both the general population and OTRs is ultraviolet radiation (UVR) [14]. This is supported by the tendency of lesions to develop in sun-exposed areas and by the increased risk of NMSC in OTRs reported in parts of the world with high levels of sun exposure [15]. The role of UVR as a risk factor is also supported by the fact that recipients with Fitzpatrick skin types I, II, or III have been shown to be at increased risk which is also true of the non-transplant population as well [16, 17]. Additionally, older patients are more likely to develop skin cancer which is in part attributed to greater cumulative sun exposure prior to transplantation [18, 19]. Finally, regular use of sunscreen and protective clothing has shown to reduce the incidence of NMSC in OTRs [20]. Ultraviolet radiation acts as both an initiator and promoter of skin cancer. It is directly mutagenic to epidermal keratinocytes and also has local immunosuppressive effects by reducing the number of Langerhans cells, thus impairing antigen presentation and recognition [21, 22].

Another well recognized important risk factor for NMSC is immunosuppression. An increasing number of patients are on long-term immunosuppression due to the increased number of organ transplants and the increasing survival of both the organs and their recipients. The duration and intensity of immunosuppression are directly related to the degree of cancer risk [23]. It is thought that immunosuppressive drugs accelerate the development of skin cancer by being directly carcinogenic and by creating a state of compromised immune surveillance [24–27]. The various immunosuppressive agents have different mechanisms of action which will be discussed later in this chapter.

Genetic polymorphisms have been shown to increase the risk of NMSC in OTRS. Variations in the methylenetetrahydrofolate reductase increase the risk of NMSC by increasing the sensitivity of DNA to ultraviolet radiation damage and by altering the DNA methylation process. Polymorphisms in glutathione-S-transferase have also been linked to increased incidence of NMSC in OTRs as they can interfere with the process of metabolizing reactive oxygen species. Other genes implicated include those that control melanin production, the interleukin-10 gene and the retinoblastoma gene [20].

Differences in skin cancer incidence have also been reported depending on type of organ transplantation, with heart and lung transplantation having the greatest risk due to older age at transplantation and a need for more intensive immunosuppression regimen [1]. Furthermore, the risk of a second SCC after an initial SCC post-transplantation is very high (reported at 80 % at 4 years in one study) [28]. Kidney transplant recipients and then liver recipients have a less significant risk [29]. The relative differences in risk may be due to a varying level of immunosuppression required to maintain each organ type [30]. In kidney transplant recipients, an effect of pretransplant end-organ disease has been identified. Incidence of NMSC was increased in patients who received a transplant for polycystic kidney disease and decreased in patients with diabetic nephropathy as primary cause of renal failure. This is hypothesized to be due to poor immunosuppressive drug absorption seen in diabetics due to gastroparesis and autonomic neuropathy [31].

It is important to be aware of these risk factors so that OTR who are at increased risk can be identified and followed even more closely by dermatologists and the transplant physicians. Knowledge and identification of these risk factors both before and after transplantation is vital to determining the appropriate level of follow up. In addition, discussion of these risk factors with the patients may encourage patients to practice safer sun habits and contact a physician earlier should they have a lesion of concern.

HPV and Its Role in Skin Cancer in Organ Transplant Recipients

It is known that OTR have an increased incidence of both viral warts and NMSC post-transplantation [32]. Identification of human papillomavirus (HPV) within these lesions suggests that HPV is pathogenic to the development of skin cancer in transplant recipients. Up to 90 % of transplant recipients have HPV-induced warts. Although considered benign lesions in immunocompetent individuals, warts in transplant patients have been shown both clinically and histologically to progress into dysplastic lesions and invasive SCC [33]. This implies that warts may be of prognostic significance to OTR [34].

An increasing number of HPV viral types are being identified in skin lesions of OTR. This is mostly attributed to the improved methods of detection of the virus using PCR with degenerate primers instead of consensus primers [34, 35].

Studies show that among the numerous HPV types that have been isolated from SCC of OTRs, there is a predominance of HPV types 5 & 8 [34]. Of note, these two types were also found to predominate in SCC of patients with epidermodysplasia verruciformis (EV). EV is a rare inherited disorder characterized by widespread warts and associated with a deficiency of cellular immunity. Approximately 30 % of EV patients develop skin cancers. A diverse range of HPV types have been identified in these lesions which is now referred to as the EV-HPV type supergroup which includes types 5 & 8 [35].

Studies have detected HPV DNA more frequently in SCC of OTRs compared to SCC of non-immunosuppressed individuals. In contrast, OTRs and non-immunosuppressed individuals had similar rates of detection of HPV DNA in BCC [34, 36]. The prevalence of HPV in BCCs of OTR and immunocompetent individuals has varied in several studies [37, 38]. Whereas, it was a common finding of all related studies among immunosuppressed patients, HPV DNA was more frequently detected in SCC than BCC. This indicates that HPV infection is more closely associated with SCC than BCC development.

The extent to which HPV plays a role in NMSC development in transplant patients is still unclear. A recent study found HPV DNA in the eyebrows and antibodies against HPV in the blood in OTRs with SCC [20]. In addition to SCC, HPV DNA has also been identified in benign tumors, normal skin of immunocompetent individuals, and normal skin of OTRs [39]. Immunosuppression may lead to a chronic state of HPV infection in these patients but is not alone sufficient to cause tumorgenesis. Current data shows that HPV and ultraviolet radiation are co-carcinogens as HPV interferes with cell-cycle arrest and DNA repair [20, 40].

One factor which might argue against the theory of HPV infection as a direct risk factor is the relatively low viral load found in skin cancers. Although a slightly higher amount of viral DNA was found in skin cancers of OTR compared to immunocompetent individuals, the level of viral DNA was still far lower than that seen in genital & cutaneous warts [34]. Additionally, long-standing warts in transplant recipients do not inevitably progress to skin cancers. Despite the strong association between the number of HPV-induced warts and the development of skin cancer, studies have shown an equally high prevalence of EV-HPV DNA in keratotic skin

lesions in transplant recipients both with and without cancer. The detection rate and spectrum of HPV infection between these same two groups in hyperkeratotic papillomas, actinic keratoses, and SCC was similar [41].

HPV and its role in cervical cancer has been well established [42]. It is generally accepted that integration of the viral genome into the host genome confers increased aggressiveness [43]. One study analyzed 181 specimens ranging in severity from condyloma to invasive cervical carcinoma. Only 3 % of biopsy specimens of cervical intraepithelial neoplasia showed integrated HPV DNA. In contrast, 81 % of cervical carcinomas ($P < 0.001$) showed integrated HPV DNA. All HPV 18-containing carcinomas had integrated HPV DNA which may be related to its greater transforming efficiency in vitro and its reported clinical association with more aggressive cervical cancers [44]. Studies have also shown that HPV16 DNA is not always present in the integrated form in tumors, suggesting that integration and subsequent inactivation of the transcriptional regulator, E2, are not essential steps for the development of HPV16 associated carcinoma [45]. Further studies specifically addressing the relative risk of episomal versus integrated viral DNA in the cutaneous malignancies of OTR would be of interest.

The recognition of HPV infection as a putative risk factor in NMSC of OTR has led to the investigation of synthetic immune response modifiers such as imiquimod as possible treatment of these lesions. A randomized, blinded, placebo-controlled study which looked at the safety and efficacy of 5 % imiquimod cream showed it to be a safe treatment in OTR on skin areas up to 60 cm^2. The study showed imiquimod 5 % cream may decrease cutaneous dysplasia and the frequency for squamous tumors to develop in high-risk patients. Of significance, renal graft function, assessed via serum creatinine measurement was unaffected. Furthermore, one study showed that imiquimod when used in conjunction with 5-fluorouracil led to complete clearance of SCC in situ in renal transplant patients. Of note, multiple studies have demonstrated that imiquimod does not decrease graft function even though it is an immune system stimulator [46]. Despite the promising effects of this drug, larger confirmatory studies are still necessary [47, 48].

Immunosuppressive Agents Used in Organ Transplant Recipients

The use of systemic immunosuppressive agents in organ transplant recipients is a well established risk factor for the development of NMSC. Multiple immunosuppressive agents exist and more are continuing to be developed. The impact of each agent in the development of NMSC is difficult to discern since more than one agent is usually used in each OTR. Intervening factors such as UVR exposure, skin type, and HPV burden among others exist, which further cloud the situation. The various immunosuppressive agents may contribute to NMSC development by two mechanisms: impairment of immune surveillance and direct carcinogenesis [24–27]. Studies show conflicting results indicating which agents seem to carry the greatest risk. However, there is a consensus that the dose and duration of immunosuppression are more important as risk factors than any one agent in particular [23]. This is supported by the fact that increased incidence of NMSC is seen in patients on normal dose immunosuppression compared to low-dose immunosuppression [49]. Additionally, patients receiving a triple regimen are at higher risk of NMSC development than those on double regimens [50]. Finally, reduction of immunosuppressant doses has been shown to be a reasonable adjuvant therapeutic strategy in OTRs with multiple or high risk skin malignancies [51].

Various trends in the use of the different immunosuppressants have developed over time. The Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients Annual Report (OPTN/SRTR) has divided the use of immunosuppressants into several "eras" (see Table 39.1) [52]. During the "experimental era" (1954–1962) prednisone was the only available agent and the only routine transplants performed were those of kidneys of identical twins. The "azathioprine era" (1962–1983) began with the development of azathioprine (AZA) as an adjunct to prednisone and allowed for cadaveric renal transplants. The FDA approval of cyclosporine (CsA) in 1983 led to the "cyclosporine era" lasting until early 1990s. The use of CsA led to increased graft survival and routine extra-renal organ transplantation. The "modern era" (1990s to present) includes the development of new immunosuppressive agents with even greater survival rates.

Table 39.1 Immunosuppressants and organ transplantation

Immunosuppressant eras	Time period	Agent(s) used	Organs transplanted
Experimental era	1954–1962	Prednisone alone	Kidneys of identical twins
Azathioprine era	1962–1983	Prednisone & azathioprine	Cadaveric kidneys
Cyclosporine era	1983–1990	Cyclosporine	Extra-renal transplants
Modern era	1990–present	Tacrolimus & sirolimus	Extra-renal transplants with increased organ survival

Source: Helderman et al. [52]

Immunosuppressive agents can be divided into induction agents and maintenance agents. Induction agents are antibodies given peri-operatively to induce tolerance to the graft by depleting host T-cell activity. Newer agents, basiliximab and daclizumab, rabbit antithymocyte globulin and anti-interleukin-2 receptor antibodies, respectively, are used in the majority of inductions [53]. Maintenance immunosuppressives can be classified as antimetabolites, calcineurin inhibitors, and rapamycin each of which have different mechanisms of action allowing for synergistic effects when used in combination.

Azathioprine acts as an antimetabolite. AZA, a purine analog, blocks B and T-cell proliferation through the inhibition of purine synthesis and metabolism. Adverse effects of AZA such as bone marrow suppression and hepatitis result from its broad inhibition of purine synthesis [53]. AZA was recently reported to increase photosensitivity to ultraviolet A light (UVA), and also enables UVA to directly damage DNA [54]. Another antimetabolite, mycophenolate mofetil (MMF), is a prodrug that is metabolized into the active compound mycophenolic acid which inhibits de novo purine biosynthesis. MMF, approved in 1995 for use in renal transplant recipients, is now being used widely in place of AZA [55]. A switch to MMF can normalize photosensitivity to UVA and may contribute to reducing additional DNA damage and thus SCC [54]. In addition to bone marrow suppression, MMF also causes gastrointestinal distress. Improved gastrointestinal tolerability has been shown with the use of an enteric coated formulation in stable renal transplant recipients [56].

Cyclosporine (CsA), a calcineurin inhibitor, blocks activation of T-cells by preventing the expression of cytokine interleukin-2 (IL-2). CsA binds to cytoplasmic nuclear factor of activated T cells (NFAT), a family of transcription factors, preventing transcription of growth factors such as IL-2 [53]. CsA is also known to enhance the expression of transforming growth factor-β (TGF- β). TGFβ- is known to inhibit IL-2-stimulated T-cell proliferation and generation of cytotoxic T lymphocytes [55]. The carcinogenic effect of CsA has been shown in a study where patients treated with corticosteroids, azathioprine, and CsA had a three-fold increase in risk of skin cancer when compared to patients on corticosteroids and azathioprine alone [11]. Other studies have found lesions occur earlier in CsA-treated patients [11, 50]. CsA may have a direct cellular effect which promotes the progression of cancer independently from its effects on host immune cells. An ex vivo study showed that CsA-treated adenocarcinoma cells transformed non-invasive cells to invasive cells with pseudopodia and increased cell motility [24]. These changes were dose-dependent and reversible. Monoclonal antibodies directed against TGF-β prevented these changes indicating CsA-induced TGF- β production as a mechanism.

Tacrolimus (TAC), another calcineurin inhibitor, binds the cytoplasmic protein, FK-binding protein (FKBP), and prevents production of IL-2 by inhibiting phosphatase activity of calcineurin. TAC is 100 times more potent than CsA [57] and, in addition to nephrotoxicity, its side effects include glucose intolerance and reversible alopecia [55]. Tacrolimus has been shown in vitro to promote tumor growth in human hepatoma cells [58]. It has been suggested that tacrolimus may be less oncogenic than CsA based on a lower prevalence of enhanced TGF-β transcription [59].

Sirolimus, also known as rapamycin, is a relatively new antitumor agent, which shows promise in decreasing the risk of NMSC in OTRs. The cellular target of sirolimus, mTOR or mammalian target of rapamycin, is considered a member of P13K family kinases [60]. Sirolimus binds the intracellular protein FK-binding protein-12 (FKBP12) forming a high affinity complex which in turn binds mTOR. The binding of mTOR, also called FRAP (FKBP-rapamycin associated protein), ultimately results in cell cycle arrest at G1/S phase through the dephosphorylation and inactivation of p70 ribosomal protein S6 kinase. Consequently, this leads to the inhibition of IL-stimulated lymphocyte division and antibody production [55]. More specifically, sirolimus inhibits the response to interleukin-2 (IL-2) thereby blocking activation of T- and B-cells [56].

Sirolimus has been shown to have antineoplastic properties in both in vitro and in vivo studies [61–63]. Studies have shown a decrease in metastatic area in mice treated with rapamycin and an increase in tumoral area in mice treated with CsA. The decrease in tumor growth in mice treated with rapamycin is attributed to a decrease in neovascularization whereas the increase in tumor growth in CsA-treated mice was associated with extensive neovascularization. Sirolimus has been shown to inhibit vascular endothelial growth factor (VEGF) both in vitro and in vivo [63]. Another study using a human renal cell cancer pulmonary metastasis model showed sirolimus reduced, whereas CsA increased, the number of pulmonary metastases. Circulating levels of VEGF and TGF-B were found to be lower in rapamycin-treated mice than in control or CsA-treated mice [64].

Despite the relatively recent introduction of sirolimus, its use as an immunosuppressive agent in OTR has been studied. The incidence of skin cancer in sirolimus-treated organ transplant recipients was assessed at 2 years post-transplantation and comprised 1981 patients from five multi-center studies. All patients received CsA and corticosteroids and had varying combinations of sirolimus (SRL), AZA or placebo [65]. The study showed that patients receiving SRL immunotherapy without CsA have a lower incidence of malignancy than patients receiving both SRL and CsA. However the patients on combination therapy showed significantly lower incidence of skin cancer compared to CsA and placebo. The

study also found that use of SRL concentration-controlled immunotherapy and early elimination of CsA resulted in significantly lower rates of malignancy.

Sirolimus is well tolerated and has the advantage of less nephrotoxicity and elevating blood pressure compared to calcineurin inhibitors. Side effects of sirolimus include hyperlipidemia, thrombocytopenia, leucopenia, and anemia [66]. Although sirolimus, as well as preliminary data of its derivative CCI-779 (everolimus) look promising, it is important to recognize that further studies will need to take place to further assess its effects due to the relatively new development of the drug and the latency of onset of NMSC in OTRs.

HLA Subtypes and NMSC

Recipient HLA type has been suggested as a possible risk factor for NMSC in OTRs. Several theories on how HLA type may play a role in increasing the risk of NMSC exist. Studies investigating HLA types and the risk of NMSC in OTR have been done with conflicting results [67]. Two of the largest studies have found HLA-A11 to increase post-transplant risk of NMSC [67, 68]. Of these two studies, one was able to successfully identify a subset of Caucasian renal transplant recipients in a northern climate who were at increased risk at both short and long-term follow up after transplantation. This increased risk associated with HLA-A11 is only conferred in patients with lighter, sun-sensitive skin [67]. This study suggests a role for more aggressive monitoring in patients with HLA-A11 type. There are recurring findings in different studies showing HLA- DR1, HLA-DR4 and HLA- B27 and their association with non-melanoma skin cancer, but no definitive conclusions have been reached [69]. Further studies need to be conducted to confirm these findings and perhaps identify other HLA types which may have significance to OTRs and their risk of NMSC.

Treatment and Follow-Up Recommendations of NMSC in OTR

Management of OTR as a dermatologist is challenging due to the chronic immunosuppression and progressively increasing risk of NMSC. The American Society of Transplantation (AST) recommends that patients perform monthly skin self-examinations and have their skin examined by a physician annually [70]. A survey that weighed the advantages and disadvantages of various clinical settings of OTR concluded that regardless of the clinical design, certain principles are key to providing the best care [71]. The survey stressed the importance of: close communication with the transplant team, education of other care providers regarding OTRs' unique dermatologic concerns, patient education as a key to prevention, and close follow-up determined by the risk of skin cancer.

The International Transplant-Skin Cancer Collaborative (ITSCC) has combined data from many studies to develop useful clinical guidelines for the treatment of skin cancer in OTR [72]. Patients should be followed according to their individual risk factors. For low-risk individuals with no history of skin cancer, a yearly follow-up is recommended. For higher-risk individuals with a history of sunburns, fair skin type, or of older age, a 6–12 monthly schedule is advised. If there is any history of NMSCs, AKs or warts, then follow-up should be scheduled at 3–6-month intervals. For both high-risk SCCs and multiple NMSCs, a follow-up should be scheduled for every 3 months. Follow-up can be as frequent as once a month for patients with metastatic SCC [73, 74]. Precancerous lesions such as warts and actinic keratoses should be recognized and treated early to reduce the viral burden and the extent of intraepithelial neoplasia (See Figs. 39.1, 39.2, and 39.3). Treatment of precancerous lesions includes: cryosurgery, topical 5-fluorouracil, topical imiquimod, and electrodesiccation and curettage (ED&C). Photodynamic therapy may be used in OTRs for the treatment of actinic keratoses not responsive to conventional treatments, and superficial NMSC [20]. Topical and systemic retinoids may be used as chemoprevention of skin cancer but are only effective while the retinoid is being used [75, 76]. NSAIDs such as topical diclofenac are being used to treat AKs in those patients that prefer not to use 5-FU [73].

Some emerging treatments include epidermal growth factor (EGFR) inhibitors, ingenol mebutate (IM) and afamelanotide. There is increasing evidence showing amplification and overexpression of EGFR in SCCs. Cetuximab, a monoclonal antibody to the EGFR receptor, has been used primarily in metastatic SCC of the head and neck. Ingenol

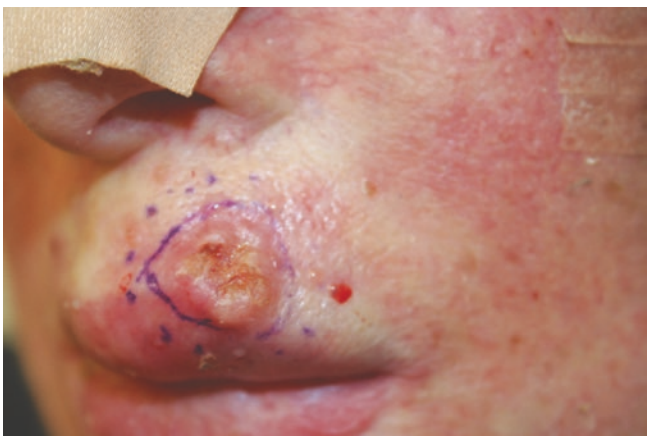

Fig. 39.1 Fifty-eight year old heart transplant patient who was previously treated with radiation to the circumoral area for multiple cutaneous squamous cell carcinomas. This is a recurrent moderately differentiated squamous cell carcinoma within the radiation field

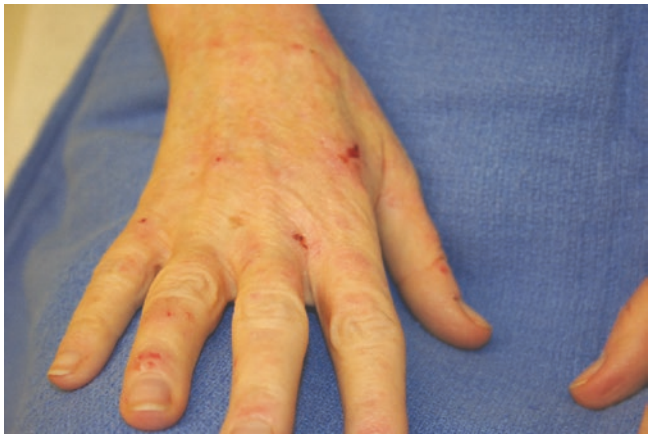

Fig. 39.2 Fifty-eight year old heart transplant patient with multiple actinic keratoses of the dorsal hands

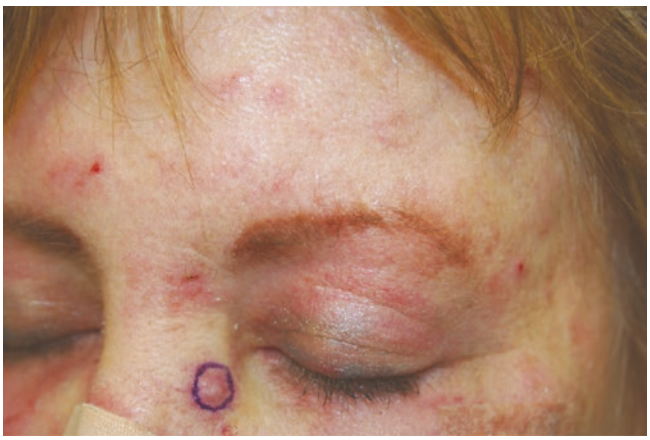

Fig. 39.3 Fifty-eight year old heart transplant patient with multiple actinic keratoses of the forehead and a lesion on the left nasal sidewall which was biopsied to reveal well-differentiated squamous cell carcinoma

mebutate (IM) is a new topical medication that has been recently approved by the FDA for treating AKs of the scalp, trunk and extremities. It has the added benefit of requiring fewer applications than 5-fluorouracil, diclofenac and imiquimod. The mechanism of action is not known, but two proposed mechanisms are direct damage to the mitochondrial membrane, leading to rapid cellular necrosis and the release of pro-inflammatory mediators. The other mechanism may involve the maturation of B cells that produce antibodies that lead to neutrophil-mediated, antibody-dependent cellular cytotoxicity. Afamelanotide is a synthetic analog of melanocyte stimulating hormone (α-MSH) that may protect skin from UVB damage [20].

Less aggressive SCC can be managed with destructive modalities such as: ED&C, cryosurgery, Mohs micrographic surgery, or excised with postoperative margin assessment (See Table 39.2).

Aggressive SCCs should be removed completely using excisional techniques including Mohs, excision with intraoperative frozen section control, or excision with postoperative margin assessment. Additional modalities may be useful in some instances. Radiation therapy may be considered as adjunctive therapy or as a primary modality for inoperable tumors (Fig. 39.1). Although not routinely used, small studies are beginning to support the role of sentinel lymph node biopsy (SLNB) in the evaluation of high risk NMSC [77].

Chemoprophylaxis with oral retinoids such as acitretin has been shown to be effective in reducing the rate of development of premalignant and malignant lesions in OTR [76, 78]. This effect is only exhibited while the patient is actively taking the retinoid. After cessation of the drug, the rate of development of lesions returns to baseline or may even exceed the prior rate of development. Retinoids have several side effects which are often very difficult for patients to tolerate on a

Table 39.2 Cutaneous squamous cell carcinomas in organ transplant recipients

Characteristic	Less aggressive SCC	More aggressive SCC
Size:		
'Mask' areas of face[a], genitals, hands, feet	<0.6 cm	>0.6 cm
Cheeks, forehead, neck, scalp	<1.0 cm	>1.0 cm
Trunk & extremities	<2.0 cm	>2.0 cm
Rate of growth	Static or slow-growing	Rapid
Ulceration	No	Yes
Clinical margins	Distinct, well-defined	Indistinct
Satellite lesions	No	Yes
Neurotropism	Absent	Present
Histology:		
Invasiveness	In situ/invasion limited to papillary dermis	Deep extension into subcutaneous fat
Perivascular or intravascular invasion	No	Yes
Differentiation	Well-differentiated	Poorly-differentiated

Source: Christenson et al. [71]

[a]Mask area includes: central face, eyelids, eyebrows, periorbital, nose, lips, chin, mandible, pre- & post-auricular areas, temple, ear

long-term basis. These side effects include: dry skin, dry lips, significant hair loss, pruritus and arthralgias [76].

Areas of multiple SCC, such as the dorsum of the hand, have been successfully treated with excision and split-thickness skin grafting. Although the grafted area remains lesion-free for an extended period, this procedure has significant morbidity and requires an extensive recovery [79, 80].

In cases of life-threatening skin-cancers, reduction of immunosuppression may be considered. Studies have shown that renal transplant recipients with very aggressive SCC have an improved prognosis following dose reduction compared to those whose immunosuppression was left unchanged [81]. It has also been shown that graft function may continue despite reduction of immunosuppression [82, 83]. It is important when considering reduction of immunosuppressive therapy that it is done in consultation with the transplant physician. If the need to reduce the level of immunosuppression is warranted, transplant physicians often prefer to lower the dose of AZA first as CsA confers better allograft survival [84]. Introducing mTOR inhibitors, or substituting them for a calcineurin inhibitor, may further reduce SCC formation [54].

Conclusion

Skin cancer in OTR is a continuing problem as the number of living OTR grows due to an increasing number of transplants performed and longer patient & graft survival time. This, in turn, has lead to a growing number of patients on chronic immunosuppression. Chronic immunosuppression, along with other risk factors, places these patients at higher risk of developing malignancies, the most common being cutaneous malignancies. Further investigation of these risk factors and the identification of others will hopefully lead to improved prevention, management and treatment of these patients. In addition, further investigation of current immunosuppressive regimens and the development of new immunosuppressive agents, will hopefully lead to decreased morbidity and mortality, particularly from cutaneous malignancies.

Management of these patients requires a multi-faceted approach involving the transplant team, dermatologists, other care providers and the patients. It is important as dermatologists to make all those involved in the care of these patients aware of their unique dermatologic concerns. Treatment and follow up may then be determined on an individual basis based on the patient's risk factors and the relative risk of the skin cancer.

Questions

1. Which of the following risk factors for the development of non-melanoma skin cancer is most important in both the general population and organ transplant recipients?
   A. Fitzpatrick skin type
   B. Ultraviolet radiation
   C. Family history
   D. Immunosuppression

Answer: B

Explanation: Lesions tend to develop in sun-exposed areas. There is also an increased risk of non-melanoma skin cancer in OTRs reported in parts of the world with high levels of sun exposure

2. Which of the following immunosuppressive agents inhibits the phosphatase activity of calcineurin?
   A. Tacrolimus
   B. Sirolimus
   C. Cyclosporine
   D. Azathioprine

Answer: A

Explanation: Tacrolimus binds FK-binding protein and prevents production of IL-2 by inhibiting phosphatase activity of calcineurin. Sirolimus binds FK-binding protein-12, forming a complex that binds mTOR. Cyclosporine blocks activation of T-cells by preventing the expression of cytokine IL-2. Azathioprine blocks B and T-cell proliferation through the inhibition of purine synthesis and metabolism

3. Which of the following HLA subtypes increases the risk of post-transplant non-melanoma skin cancer?
   A. HLA-DR1
   B. HLA-B27
   C. HLA-DR4
   D. HLA-A11

Answer: D

Explanation: Studies have found HLA-A11 to increase post-transplant risk of non-melanoma skin cancer. This increased risk is conferred in patients with lighter, sun-sensitive skin

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Autoinflammatory Diseases

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Abstract

The term *autoinflammatory syndrome* was first proposed in 1999 to describe diseases with a lack of apparent provocation for inflammation and absence of high titer autoantibodies or antigen-specific T lymphocytes. The term *autoinflammation* was proposed in order to draw a distinction between diseases caused by dysregulation of the adaptive immune system (autoimmune) versus innate immune system (autoinflammation). Since that time, the definition of autoinflammatory diseases has evolved to include dysregulatory disorders characterized by significant excessive inflammation mediated predominantly by components of the innate immune system. As our understanding of immune dysregulatory diseases evolves, we are now beginning to understand that diseases characterized by excessive inflammatory response lie on an immunological disease continuum, in which both innate and adaptive immune system components may play pivotal roles in disease propagation.

Keywords

Autoinflammatory diseases • Autoinflammatory syndrome • Autoimmune disease • Familial Mediterranean Fever • Fever syndromes • Periodic Syndrome • Muckle-Wells Syndrome • Psoriasis • Skin diseases • Majeed syndrome • Dermatologic manifestations

Introduction

The term *autoinflammatory syndrome* was first proposed in 1999 to describe diseases with a lack of apparent provocation for inflammation and absence of high titer autoantibodies or antigen-specific T lymphocytes [1]. The term *autoinflammation* was proposed in order to draw a distinction between diseases caused by dysregulation of the adaptive immune system (autoimmune) versus innate immune system (autoinflammation). Since that time, the definition of autoinflammatory disease has evolved to include dysregulatory disorders characterized by significant excessive inflammation mediated predominantly by components of the innate immune system (Table 40.1). As our understanding of immune dysregulatory diseases evolves, we are now beginning to understand that diseases characterized by excessive inflammatory response lie on an immunological disease continuum, in which both innate and adaptive immune system components may play pivotal roles in disease propagation.

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Innate Immunity

The innate immune system provides an immediate and non-specific host defense against infection while the adaptive immune system provides a more complex, antigen-specific

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Table 40.1 Clinical features of autoinflammatory disorders

Autoinflammatory disease	Inheritance	Gene/Protein	Age of onset	Flare duration	Mucocutaneous manifestations	Musculoskeletal manifestations	Systemic manifestations	Treatment
FMF	AR	*MEFV*/pyrin	First three decades of life	1–3 days	Erysipeloid-like rash on dorsal hands and feet	Arthritis	Fever Polyserositis AA amyloidoisis	Colchicine IL-1 antagonists
TRAPS	AR	*TNFRSF1A*/TNFR1 receptor	Childhood or adolescence	7–14 days	Migratory erythema Periorbital edema Conjunctivitis	Arthralgia, arthritis	Fever Pleuritis Abdominal pain AA amyloidosis	TNFα antagonists IL-1 antagonists
MKD	AR	*MVK*/mevalonate kinase	First year of life	3–7 days	Urticaria-like eruption Morbiliform eruption Petechiae Vasculitic purpura	Arthralgia, arthritis	Fever Cervical lymphadenopathy Abdominal pain Vomiting, diarrhea Splenomegaly	NSAIDs TNFα antagonists IL-1 antagonists
FCAS	AD	*NLRP3/CIAS1*/NLRP3	Usually in childhood	24 h	Cold-induced neutrophilic urticaria Conjunctivitis	Arthralgia	Fever Rare secondary amyloidosis	IL-1 antagonists
MWS	AD	*NLRP3/CIAS1*/NLRP3	Childhood	1–3 days	Neutrophilic urticaria Conjunctivitis Uveitis Scleritis	Arthralgia	Fever Sensorineural deafness Papilledema Aseptic meningitis AA amyloidosis	IL-1 antagonists
NOMID	AD	*NLRP3/CIAS1*/NLRP3	First weeks of life	Continuous	Neutrophilic urticaria Conjunctivitis Uveitis	Erosive arthritis Joint deformities	Fever AA amyloidosis Papilledema Aseptic meningitis Seizures Cognitive impairment Sensorineural deafness	IL-1 antagonists
DIRA	AR	*IL1RN*/IL1RA	At birth or within first weeks of life	Continuous	Sterile pustulosis Oral ulcerations	Joint swelling Hyperostosis Osteolysis	Low-grade fever Premature birth HSM Respiratory insufficiency Thrombosis	Anakinra
DITRA	AR	*IL36RN*/IL36RA	Childhood, adulthood, Peri-partum	Days to weeks	Sterile pustulosis	Arthralgias	Fever	Acitretin IL-1 antagonists
SAPHO/CRMO	Unknown	Unknown	Childhood or young adulthood usually	Weeks	Palmoplantar pustulosis (60%) Acne (25%) Neutrophilic dermatoses	Hyperostosis Osteolysis Synovitis	Fever	NSAIDs Bisphosphonates Methotrexate TNFα antagonist IL-1 antagonists

Autoinflammatory disease	Inheritance	Gene/Protein	Age of onset	Flare duration	Mucocutaneous manifestations	Musculoskeletal manifestations	Systemic manifestations	Treatment
Majeed syndrome	AR	*LPIN2*/lipin-2	Neonatal	Continuous	Neutrophilic dermatoses	Hyperostosis Osteolysis	Congenital dyserythropoetic anemia	IL-1 antagonists
CAMPS	AD	*CARD14*/CARD14	Infancy	Continuous	Generalized pustulosis	Arthralgia	Fever	IL-12/23 antagonist
PAPA	AD	*PSTPIP1*/PSTPIP1	Childhood	Weeks	Pyoderma gangrenosum Acne	Pyogenic arthritis	Fever	TNFα antagonist IL-1 antagonists
PRAAS	AR	*PSMB8*/PSMB8	First weeks of life	Days to weeks	Violaceous annular plaques Heliotrope rash, periorbital swelling Lipodystrophy	Variable arthritis Myositis	Fever HSM Microcytic anemia Lymphadenopathy Metabolic abnormalities	No established therapy
PLAID	AD	*PLCG2*/phospholipase Cγ_2	Early childhood	Hours	Cold-induced urticaria Atopy Granulomatous rash	None	Autoimmune thyroiditis Sinopulmonary infection	No established therapy
APLAID	AD	*PLCG2*/phospholipase Cγ_2	Infancy	Continuous, triggered by sun and heat exposure	Recurrent vesiculobullae Corneal bullae and ulcerations	Arthralgia	Recurrent sinopulmonary infection Enterocolitis	No established therapy Corticosteroids, IL-1 antagonists have been used
DADA2	AR	*CECR1*/ADA2	Early childhood	Continuous	Livedo racemosa	None	Fever Vasculopathic changes HSM Ophthalmologic involvement Mild immunodeficiency	No established therapy

immune response. Effector cells of innate immunity include phagocytes, such as macrophages, dendritic cells and other antigen presenting cells, whereas in autoimmune diseases, B and T lymphocytes are the primary mediators of the inflammatory response.

The innate immune system acts through pattern recognition receptors (PRR) which recognize highly conserved pathogen motifs called pathogen-associated molecular patterns (PAMPs) and damage motifs known as damage-associated molecular patterns (DAMPs). There are three recognized types of PRRs employed by the innate immune system: Toll-like receptors (TLRs), NOD-like receptors (NLRs) and retinoic-acid-inducible-gene-1-like receptors (RLRs). Recognition of foreign material by PRRs leads to activation of signal transduction pathways which signal gene expression of pro-inflammatory cytokines, including interleukin-1 (IL-1) family cytokines, interferon α (IFNα), interferon γ (IFNγ), and tumor necrosis factor α (TNFα). Chronic, prolonged, unmitigated, excessive activation of PRRs can lead to autoinflammation and autoinflammatory diseases [2].

The Inflammasome

The first-described autoinflammatory diseases, the classic periodic fevers syndromes and the cryopyrin-associated periodic syndromes (CAPS) – were characterized by dysregulation of inflammasome activation. Activation of the NLRs leads to the formation of large multimeric complex protein structures, known as inflammasomes, which are critical for host defense against infection. Two main inflammasomes have been described: the NALP1 inflammasome and the NALP3 inflammasome, also known as the cryopyrin inflammasome [3]. In response to microbial components or endogenous metabolic stress, inflammasomes mediate procaspase activation which in turn catalyzes the cleavage of pro-IL-1β and pro-IL-18 into activated IL-1β and IL-18, respectively. IL-1β is produced primarily by myeloid cells and is the primary cytokine implicated in several autoinflammatory diseases. The IL-1 receptor (IL-1R) is ubiquitously expressed, and binding of IL-1β to the IL-1R results in proinflammatory signaling through NFkB-mediated transcription of proinflammatory genes [4]. The most well-described inflammasome, the NALP3 inflammasome, plays a key role in the pathogenesis of Familial Mediterranean Fever (FMF), the TNF receptor-associated periodic syndrome (TRAPS) and CAPS [3] (Fig. 40.1).

Since the initial characterization of the cryopyrin-associated fever syndromes, numerous other monogenic autoinflammatory disorders have been described, including those which lead to pustular and other neutrophilic skin manifestations, and which further contribute to our understanding of IL -1 and non-IL-1-mediated innate immune pathways driving inflammatory skin disease. Characterization of several monogenic diseases has also led to use of targeted therapeutics for their management. Importantly, these insights have also helped to begin to dissect pathogenic mechanisms of phenotypically similar autoinflammatory diseases with as yet no known genetic etiology, and identify appropriate targeted therapeutics for their management, thereby improving human health.

This chapter will introduce monogenic autoinflammatory diseases and discuss pathogenic mechanisms, clinical features and therapeutic options for these disorders.

Classic Fever Syndromes

Familial Mediterranean Fever

The term Familial Mediterranean Fever (FMF) was first proposed in 1958 to describe a periodic fever syndrome which predominated in individuals of Eastern Mediterranean descent [5]. It is the most prevalent autoinflammatory disease worldwide [6]. Although FMF is classically recessively-inherited [7, 8], dominant inheritance and clinically symptomatic heterozygotes have been reported [9]. Genetic mutations in the *MEFV* gene are responsible for FMF and were first reported in 1997 [7, 8]. Almost one-half of the mutations described to date have been identified on exon 10, underscoring the importance of this exon in the function of *MEFV* [10].

The *MEFV* gene encodes the protein pyrin which binds the apoptotic speck (ASC) adaptor protein in the NALP3 inflammasome. Binding of wildtype pyrin and ASC inhibits assembly of the NALP3 inflammasome [11]. Mutations in *MEFV* are gain-of-function mutations affecting pyrin, thereby leading to increased inflammasome activity and subsequent IL-1β production. This hypothesis has been confirmed in a pyrin knock-out murine model as well as a murine model with truncated pyrin protein in which increased caspase-1 activation and increased IL-1β maturation is observed [12]. In a pyrin knock-in murine model, it was demonstrated that pyrin could form an inflammasome of its own, independent of NALP3, which could activate proinflammatory cytokine IL-1 [13].

Individuals with FMF typically present within the first three decades of life with acute episodes of fevers, erysipelas-like rash, arthritis and polyserositis. Flares can last 1–3 days in duration, and occur as frequently as weekly or as rarely as every few years [14, 15]. The erysipelas-like rash of FMF tends to involve the hands and dorsal feet, lasting up to 72 hours and recovering with recrudescence of fever (Fig. 40.2a). Approximately 30% of affected individuals will experience acute nonerosive arthritis during flares. The majority of patients develop monoarthritis (70%), and fewer develop oligo- (26%) and polyarthritis (4%). The most commonly affected joints include the knees, followed by ankles and hips. Arthritis is typically self-limited and not associated with permanent periarticular or cartilage damage [16]. Polyserositis can manifest as an acute abdomen, pleuritis, pericarditis, scrotal pain and more rarely, aseptic meningitis [17]. Gastrointestinal disease

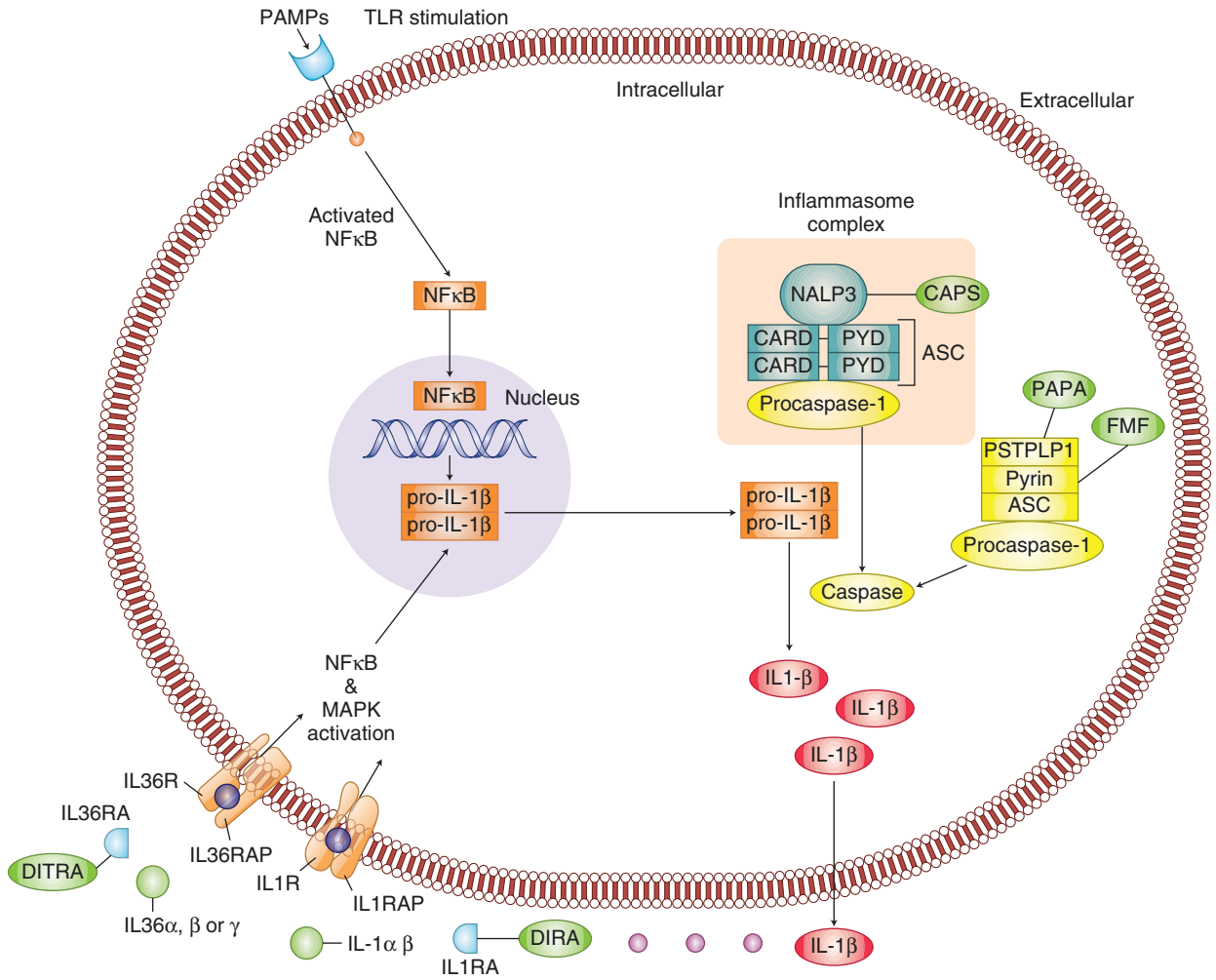

Fig. 40.1 Pathogenic mechanisms of autoinflammatory diseases. *PAMP* pattern recognition receptor, *TLR* toll-like receptor, *NALP3, NACHT* LRR and PYD domains-containing protein 3 (NALP3) or cryopyrin, *CARD* caspase recruitment domain, *PYD* pyrin domain, *ASC* apoptosis-associated speck-like protein containing a CARD, *PSTPIP1* proline serine threonine phosphatase interacting protein 1, *IL-1R* IL-1 receptor. *IL-1RAP* IL-1 receptor accessory protein, *IL-36R* Il-36 receptor, *IL-36RAP* IL-36 receptor accessory protein, *CAPS* cryopyrin associated periodic fever syndrome, *PAPA* pyogenic arthritis, pyoderma gangrenosum, acne. *FMF* familial Mediterranean fever, *DIRA* deficiency of the IL-1 receptor antagonist, *DITRA* deficiency of the IL-36 receptor antagonist

manifestations, ranging from mild abdominal pain to noninfectious peritonitis, occur in 95 % of FMF patients [18]. Prolonged myalgias in the setting of fevers [19], elevated inflammatory markers likely secondary to vasculitis [20], as well as HLA-B27-independent sacroiliitis have also been reported [21].

Secondary AA amyloidosis is a prominent feature of chronic and uncontrolled FMF [22]. In the setting of chronic inflammation, AA amyloid is subject to protein misfolding and subsequent deposition in extracellular matrices of various tissues leading to organ impairment. In FMF, amyloidosis typically involves the kidneys. Affected individuals present with proteinuria and subsequently develop nephrotic syndrome and uremia, ultimately leading to renal impairment and death. Biomarkers for FMF include elevated serum amyloid A as well as elevated levels of DAMP protein S100A12. The latter has been shown to correlate with joint disease severity [23, 24]. Elevated systemic inflammatory markers have been during attacks as well as persist during attack-free periods [25].

Skin histopathology of FMF erysipelas-like erythema demonstrates superficial dermal edema and sparse perivascular infiltrate composed of neutrophils and few lymphocytes. Deposits of C3 in the vessel walls of the superficial vascular plexus can be seen on direct immunofluorescence [26–30]. Synovial fluid from affected joints ranges from non-inflammatory to septic-appearing [16].

Oral colchicine is the mainstay of therapy for the management of recurrent attacks and secondary amyloidosis associated with FMF [31]. In addition to achieving remission of inflammatory flares in the majority of patients, oral colchicine 1–2 mg daily has been associated with improvement of renal function in 95 % of affected individuals with proteinuria but not nephrotic syndrome. Furthermore, colchicine use has been shown to prevent the development of amyloid renal disease in

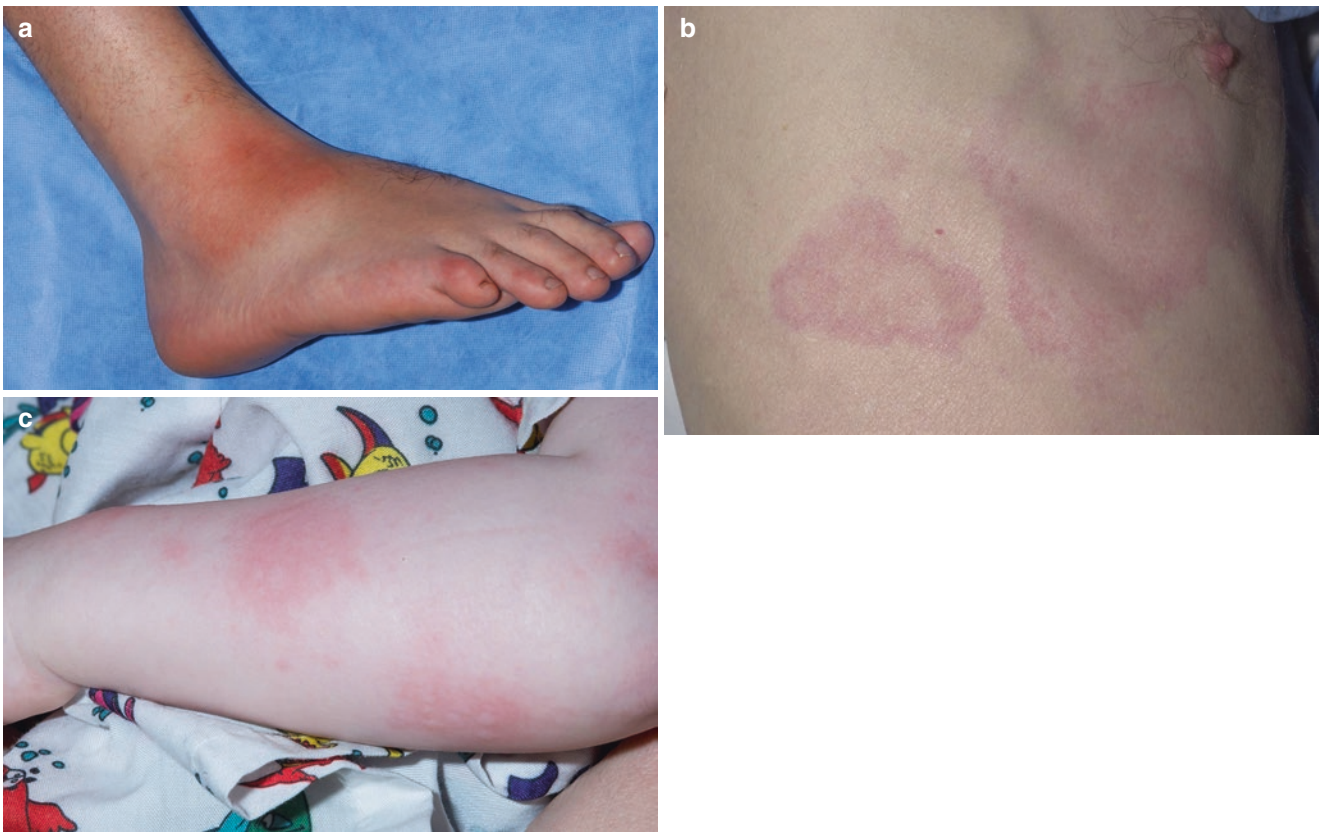

Fig. 40.2 Dermatologic manifestations of classic periodic fever syndromes. (**a**) Erysipeloid erythema involving the anterolateral ankle of a child with Familial Mediterranean Fever (FMF). (**b**) Migratory erythema with serpiginous borders and central clearing on the right lateral chest of a young man with TNF receptor-associated periodic syndrome (TRAPS). (**c**) Urticaria-like plaques involving the left arm of a child with mevalonate kinase deficiency (MKD)

FMF patients [16]. IL-1 antagonists should be considered in individuals who do not respond to or continue to have progressive renal amyloidosis despite oral colchicine therapy, as well as in patients who are intolerant to oral colchicine [32–36].

TNF Receptor-Associated Periodic Syndrome

Shortly after the discovery of the genetic cause of FMF, the genetic basis of TNF receptor-associated periodic syndrome (TRAPS) was reported in 1999 [37]. TRAPS is a dominantly-inherited disorder caused by mutations in the *TNF receptor superfamily member 1A (TNFRSF1A)* gene which encodes the TNFR1 receptor protein. Over 100 different mutations in *TNFRSF1A* have been reported to date [38].

The TNFR1 receptor is ubiquitously expressed. Mutations in *TNFRSF1A* lead to TNFR1 receptor protein misfolding. As a result, TNFR1 is unable to be transported to the cell membrane and therefore is sequestered in the endoplasmic reticulum at levels tenfold higher than the wild type protein [39, 40]. Mutations in TNFR1 lead to constitutive activation of MAP-kinases with subsequent uncontrolled production of IL-1 and TNFα.

The majority of TRAPS patients present in childhood or adolescence with a constellation of symptoms that can include recurrent fevers, abdominal pain, pleuritis, arthralgias, myalgias and periorbital edema and/or conjunctivitis. Attacks last for approximately 7–14 days but they may persist for up to 4 weeks. Abdominal pain occurs in 88 % of TRAPS patients, ranging from mild to moderate pain to acute abdomen [41]. Cutaneous manifestations occur in 69-87 % of TRAPS patients, most commonly as centrifugal migratory erythematous patches overlying sites of myalgia. Serpiginous patches and plaques and urticaria-like eruptions are less common [42] (Fig. 40.2b). The rash tends to progress from proximal to distal sites concurrent with myalgia symptoms. Arthralgia occurs in two-thirds of patients, involving peripheral joints in a monoarticular or oligoarticular pattern. Arthritis is less common [43, 44]. Periorbital edema is a hallmark feature of TRAPS, and conjunctivitis and uveitis has also been reported in approximately one-half of affected individuals [45]. Like FMF, renal deposition of AA amyloid, and subsequent renal impairment, is a prominent feature of TRAPS, affecting 8–10 % of patients with TRAPS [23]. Serum amyloid A (SAA) can be used as a biomarker in TRAPS [23].

Skin histopathology of the migratory rash associated with TRAPS is notable for superficial and deep perivascular and interstitial infiltrate of lymphocytes and monocytes. Small vessel vasculitis and recurrent panniculitis have also been

reported [42]. Myalgias have been associated with monocytic fasciitis [46].

Definitive management of TRAPS remains elusive. Initially, it was thought that utilization of TNFα antagonists would be highly successful in ameliorating the signs and symptoms of disease. A prospective study demonstrated that etanercept, the soluable p75 TNFR:Fe fusion protein, reduced the frequency and intensity of attacks but did not lead to complete resolution [47]. Furthermore, a diminished effect of etanercept over time has been reported [47]. Paradoxically, anti-TNFα monoclonal antibodies have also been reported to cause an acute worsening of a patient's clinical disease [48–51]. The IL-1 antagonists anakinra and canakinumab have also been used for the management of TRAPS with variable response [52, 53].

Mevalonate Kinase Deficiency

Mevalonate kinase deficiency (MKD) (formerly known as *Hyperimmunoglobulinemia D and periodic fever Syndrome (HIDS)* and *Hibernian fever*) is a recessively inherited condition caused by mutations in the *mevalonate kinase (MVK)* gene which encodes the mevalonate kinase protein [54, 55]. The most commonly reported mutation is V377I but however more than 30 distinct mutations have been reported [56].

Mevalonate kinase catalyzes the conversion of mevalonic acid to 5-phosphomevalonic acid in the biosynthesis of cholesterol and nonsterolisoprenoids. Mutations in *MVK* lead to decreased mevalonate kinase enzymatic activity which leads to reduction in isoprenoids, products of cholesterol biosynthesis pathways [57]. In vitro data from MKD patients demonstrate that isoprenoid biosynthesis leads to decreased IL-1β secretion in MKD leukocytes [58]; however, the mechanism by which depletion of isoprenoids results in increased circulating proinflammatory IL-1β is an area of ongoing investigation.

Affected individuals present within the first year of life with recurrent fevers, rash and arthralgias that can last 3–7 days and recur every 4–6 weeks. Flares can be triggered by childhood vaccinations, trauma, infection and stress. Cutaneous manifestations include an urticaria-like eruption, morbiliform eruption, petechiae and vasculitic purpura [59, 60] (Fig. 40.2c). Approximately two-thirds of affected individuals develop polyarthralgias and/or a nonerosive polyarthritis but it is not uncommon for only a single joint to be involved. The most commonly affected sites of joint involvement are large joints such as the knees and ankles [43, 44]. Bilateral cervical lymphadenopathy is a prominent feature of HIDS. Abdominal pain, vomiting, diarrhea, mucosal ulcerations and splenomegaly can also been seen. Unlike FMF and TRAPS, amyloidosis is an atypical finding [60].

Elevated serum ESR, CRP, IgA, IgD and urine mevalonic acid levels can be detected during disease flares. Urine mevalonic acid levels are currently used for diagnosis of MKD. Elevated IgD levels can also be seen in FMF and TRAPS and, therefore, should not be used for diagnosis [18]. Patients with MKD may also have normal IgD levels. Histologic examination of skin lesions reveal perivascular lymphocytic infiltrate, and may demonstrate vasculitic features and a neutrophilic infiltrate [59, 60].

The management of MKD is primarily supportive. NSAIDs may be used for the management of mild disease. Colchicine is of little utility in managing MKD. Successful management of MKD with TNFα antagonists [61] and IL-1β antagonists [62, 63] in individual cases has been reported.

IL-1 Family-Mediated Autoinflammatory Diseases

Cryopyrin-Associated Periodic Syndromes

Cryopyrin-associated periodic syndromes (CAPS), or the *cryopyrinopathies,* comprise a spectrum of 3 disorders characterized by autosomal dominant gain-of-function mutations in the *NLRP3/CIAS1* gene which encodes the NLRP3 protein [64]. Ranging from mildest to most severe in presentation, the three disorders include familial cold-induced autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal onset multisystem inflammatory disorder/chronic infantile neurologic, cutaneous and articular syndrome (NOMID/CINCA).

The majority of MWS and FCAS cases are familial [64], while the majority of NOMID cases reported are sporadic [65, 66]. The latter is thought to be due to the severity of the NOMID phenotype with death prior to reproductive age. Among approximately 130 mutations identified in *NLRP3/CIAS1*, greater than 90% have been associated with exon 3 [38, 64]. Mutations in NLRP3 result in inappropriate activation of the inflammasome and elevated IL-1 production. Approximately 50% of NOMID patients have germline mutations in NLRP3 by Sanger sequencing while far fewer germline mutations have been found in MWS and FCAS. In those for whom germline mutations cannot be identified, approximately 70% have somatic mosaicism identified through deep sequencing methods [67]. Given this information, diagnosis of CAPS is made on a clinical basis, with rapid response to trial with an IL-1 antagonist used for confirmation of clinical diagnosis. Genetic testing may be used for confirmation of diagnosis.

Common features in patients with CAPS include fever, leukocytosis, transient neutrophilic urticaria, conjunctivitis, arthralgias, and elevated inflammatory markers. The urticaria-like rash tends to be non-pruritic and presents as rose-colored flat macules or slightly raised plaques on the trunk and extremities that resolve within 24 hours. Lesional skin histopathology is characterized by neutrophilic infiltrate with interstitial and perivascular neutrophils and lymphocytes without dermal edema or vasculitis [68, 69]. Disease

onset, severity, multiorgan involvement, morbidity and mortality differ between these three diseases.

FCAS

FCAS is the mildest of the 3 cryopyrinopathies. Patients usually present early in childhood, but presentations later in adulthood have been described. Febrile attacks are triggered by cold exposure, with development of fever and rash within 2 hours of exposure, peaking at 2–6 hours, and lasting 12–24 hours. Amyloid deposition is infrequent, and is found in only 2% of patients [23, 70].

Muckle-Wells Syndrome

Muckle-Wells syndrome is characterized by intermediate severity. Unlike FCAS, disease manifestations are not associated with cold exposure and febrile attacks tend to occur with greater frequency, with the characteristic rash often noted in the early afternoon [71]. Ocular inflammation is characterized by inflammation of the conjunctiva, sclera, and anterior chamber. Patients can present with headache secondary to papilledema and aseptic meningitis. In the second or third decades of life, patients can develop sensorineural deafness due to damage of the Corti organ secondary to chronic ear inflammation. Sensorineural deafness occurs in approximately 75% of untreated cases [72, 73]. AA amyloidosis is frequently seen in MWS, occurring in 25% of untreated cases [74].

NOMID/CINCA

NOMID/CINCA is the most severe of the cryopyrinopathies. NOMID is characterized by chronic systemic multiorgan inflammation and persistently elevated inflammatory markers. Patients present within the first few weeks of life with persistent low-grade fevers, neutrophilic urticaria, painful arthropathy and elevated inflammatory markers (Fig. 40.3a). Aseptic meningitis and subsequent elevated intracranial pressure leads to irritability, headaches, nausea, vomiting and seizures. Although the aforementioned symptoms can wax and wane, inflammatory markers in patients with NOMID tend to remain persistently elevated [75].

In untreated NOMID, significant end-organ damage is observed early in life [76]. Chronic aseptic meningitis leads to increased intracranial pressure, hydrocephalus and papilledema. Furthermore, chronic aseptic meningitis in combination with central nervous systemic inflammation can contribute to cognitive impairment. Chronic papilledema can result in optic nerve atrophy, leading to gradual vision loss in the third decade of life. Uveitis may also contribute to vision loss [75]. Sensorineural deafness due to chronic inflammation of the cochlea may develop within the first few years of life. Persistent joint inflammation resulting in premature and aberrant ossification, osteolytic lesions and cartilage hypertrophy can lead to permanent joint deformities in 50–70% of untreated NOMID patients [65, 77–79].

All cryopyrinopathies respond rapidly and completely to IL-1 blockade [75, 80–86]. To date, three IL-1 antagonists – short-acting anakinra and the longer-acting rilonacept and canakinumab – have been FDA approved for the management of CAPS. IL-1 antagonists should be initiated early in life to prevent end-organ damage in these conditions and doses should be titrated to resolution of CNS and cochlear inflammation as continued benefit has been seen with prolonged use of these agents [4, 76, 79, 80, 83–85, 87, 88].

DIRA

Deficiency of the IL-1 receptor antagonist (DIRA) was first described in 2009 [89, 90]. Since that time, approximately 25 affected individuals in United States (including Puerto Rico), Canada, the Netherlands, and Brazil have been described [89–95]. Homozygous loss-of-function nonsense and missense mutations in the *IL1RN* gene, which encodes the IL-1 receptor antagonist (IL1RA), are responsible for the DIRA phenotype.

IL1RN is constitutively expressed in healthy human hosts and serves to inhibit proinflammatory IL-1-mediated signaling by competitively binding the IL-1 receptor. In DIRA, *IL1RN* mutations result in nonfunctional IL1RA, leading to unopposed IL-1 proinflammatory signaling and widespread systemic inflammation [96]. Because the IL-1 receptor is ubiquitously expressed, the disease manifestations of this condition are seen in multiple organ systems.

Affected individuals present at birth or within the first few weeks of life with fetal distress, pustulosis, oral ulcerations, joint swelling, and pain with movement (Fig. 40.3b). Premature birth has been observed. The pustular eruption of DIRA resembles pustular psoriasis, ranging from discrete crops of pustules to generalized pustulosis. The spectrum of bony changes seen includes epiphyseal ballooning of long bones, anterior rib-end widening, periosteal elevation of long bones and multifocal osteolytic lesions. High fevers are not a typical feature of DIRA. Leukocytosis and markedly elevated systemic inflammatory markers are found [89, 90]. Stomatitis, hepatosplenomegaly, respiratory insufficiency and thrombotic events have been less commonly observed. In untreated patients, mortality secondary to multiorgan failure in the setting of systemic inflammatory response syndrome (SIRS) and pulmonary hemosiderosis with progressive interstitial fibrosis has been reported.

Affected skin histopathology demonstrates epidermal acanthosis, hyperkeratosis and extensive epidermal and dermal neutrophilic infiltrate with pustule formation along hair follicles [89].

DIRA responds dramatically to IL-1 antagonist therapy, which effectively provides a recombinant replacement of the dysfunctional protein. Anakinra, a recombinant IL-1 recep-

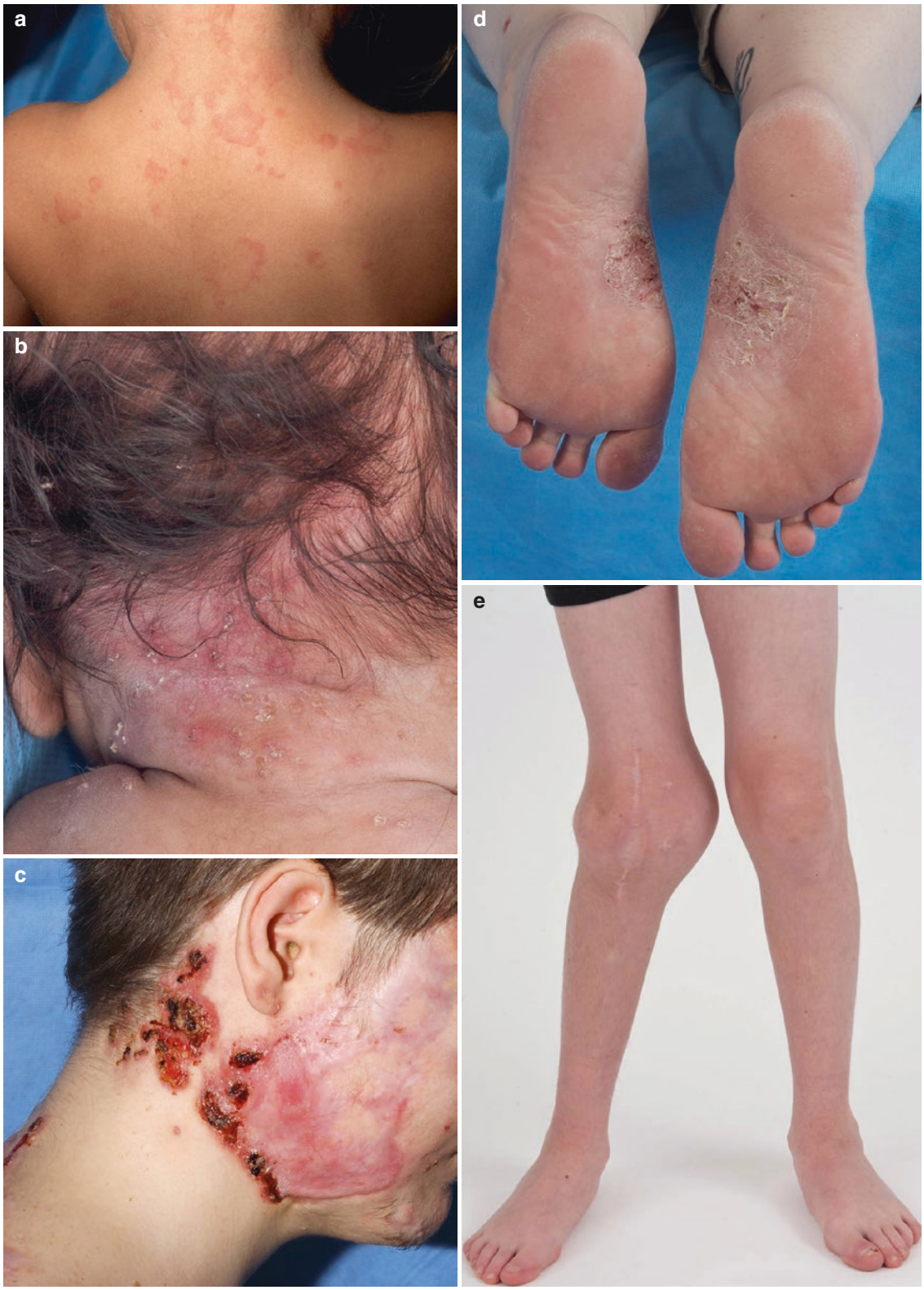

Fig. 40.3 Dermatologic manifestations of autoinflammatory diseases thought to be mediated by IL-1 pathways. (**a**) Urticaria-like plaques on the posterior neck and upper back of a child with neonatal onset multisystem inflammatory disease (NOMID). (**b**) Crop of pustules in the setting of background erythema on the posterior neck of an infant with deficiency of the IL-1 receptor antagonist (DIRA). (**c**) Pyoderma gangrenosum-like ulcers in the setting of acne and significant scarring involving the lateral and posterior neck and cheek of a young man with pyogenic arthritis, pyoderma gangrenosum and acne (PAPA). (**d**) Plantar pustulosis in a patient with SAPHO. (**e**) Hyperostosis of the right knee in the setting of CRMO

tor antagonist, leads to rapid improvement in skin manifestations, normalization of acute phase reactants and resolution of bony inflammation [89]. Given the severity of the disease and the availability of effective therapy, early disease recognition and prompt implementation of IL-1 blockade therapy prior to development of bony deformities, pulmonary sequelae and SIRS is critical.

DITRA

Deficiency of the IL-36 receptor antagonist (DITRA) was first described in 2011 in familial and sporadic cases of pustular psoriasis in 9 Tunisian families [97] and 3 unrelated English patients [98]. DITRA is an autosomal recessive condition caused by homozygous and compound heterozygous inactivating mutations in the *IL36RN* gene, which encodes the IL-36 receptor antagonist protein (IL36RA). Following the initial description of DITRA, *IL36RN* mutations have also been reported in individuals of varied ancestry, including European [99], Chinese [99], Japanese [100] and Lebanese [101], suggesting that these mutations may be found in other populations.

IL-36 is an IL-1 family cytokine that binds to the IL-36 receptor, enabling the recruitment of the IL-1 receptor accessory protein and subsequent signal transduction involving nuclear factor kappa-light chain-enhancer of activated B cells (NFkB) and mitogen-activated protein (MAP) kinases. The IL-36 receptor antagonist is encoded on chromosome 2 and competitively binds the IL-36 receptor, thereby providing negative feedback to IL-36 signaling [97, 98]. In DITRA, mutations in the IL36RN gene result in dysfunctional IL36RA protein, leading to unopposed proinflammatory signaling through the IL-36 receptor. Deficiency of the IL-36 receptor antagonist leads to an exaggerated inflammatory response by a mechanism analogous to that observed with dysfunctional IL-1 receptor antagonist protein in patients with DIRA. Unlike the IL-1 receptor in DIRA expression of the IL-36 receptor is limited to epithelial surfaces that have contact with the outside environment [98], thus limiting the disease phenotype predominantly to mucocutaneous surfaces.

The age of onset of DITRA is quite variable, ranging from onset in infancy through adulthood. Two cases of onset during pregnancy have also been reported [97]. Clinical characteristics include recurrent and sudden onset pustular eruptions on a background of erythematous plaques, with associated fevers, neutrophilia, leukocytosis and elevated inflammatory markers. While clinical features of DITRA are confined to the mucocutaneous surfaces, the spectrum of cutaneous involvement can be quite varied. While many reported cases demonstrate generalized pustulosis, patients have also been reported to have limited involvement including acrodermatitis continua of Hallopeau, palmoplantar pustulosis and migratory glossitis [97, 98], suggesting that the same gene may be responsible for a spectrum of phenotypes. Patients may have a history arthropathy, cholangitis, or plaque psoriasis [97]. Skin histopathology demonstrates epidermal acanthosis with elongation of rete ridges, parakeratosis and spongiform pustules [97].

Targeted therapeutic options for DITRA do not exist. The majority of reported DITRA patients have been managed with systemic and topical corticosteroids and systemic retinoids with variable success. Methotrexate and biologic agents, including adalimumab, infliximab and etanercept, have also been utilized with variable success [97, 98]. Recent reports of successful management of DITRA patients with IL-1 antagonists suggest that IL-1 might play an important role in disease pathogenesis [102, 103].

PAPA

Pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) was first coined in 1997 [104]. It is an autosomal dominant disease with incomplete penetrance. Disease-causing mutations in PAPA syndrome are due to gain of function mutations in the *proline-serine-threonine-phosphatase-interacting-protein-1* (*PSTPIP1)* gene, also known as *CD2BP1*.

PSTPIP1 is a cytoskeletal protein that interacts with PEST-type protein tyrosine phosphatases (PEST-PTPs), Wiskott-Aldrich syndrome protein (WASP), and pyrin. While the pathogenesis of PAPA syndrome is not completely understood, mutations in *PSTPIP1* are thought to decrease the interaction of PSTPIP1 with PEST-type proteins, thereby increasing phosphorylation of *PSTPIP1* and increasing *PSTPIP1* interaction with pyrin, ultimately leading to unopposed IL-1 proinflammatory signaling [105].

PAPA patients typically present within the first decade of life with aseptic monoarthritis of the large joints such as knees, elbows and ankles. Persistent untreated chronic inflammation can lead to joint destruction. Cutaneous features of PAPA include acne and pyoderma gangrenosum. Although pyoderma gangrenosum lesions can present early in childhood, cystic acne tends to manifest at puberty (Fig. 40.3c). Hidradenitis suppurativa, psoriasis and rosacea have also been described [106–108]. Given the incomplete penetrance of this disease, a spectrum of disease severity is seen with PAPA, ranging from mild acne to explosive acne fulminans, pyoderma gangrenosum, and debilitating erosive joint disease. Pathergy is a prominent feature of PAPA.

Laboratory studies reveal persistently elevated systemic inflammatory markers and leukocytosis but are otherwise nonspecific. Histopathology of cystic acne lesions is similar to that seen with cystic acne in other settings: distended

follicles with cystic spaces and follicular openings filled with keratinaceous debris and numerous bacteria. Ruptured cystic contents induce a brisk perifollicular neutrophilic inflammatory infiltrate. The histopathology of pyoderma gangrenosum is similar to that of pyoderma gangrenosum in other settings. Early lesions are characterized by a neutrophilic vascular infiltrate. Actively progressing lesions demonstrate neutrophilic infiltrates with leukocytoclasia. Pyoderma gangrenosum ulcers demonstrate marked tissue necrosis with surrounding mononuclear cell infiltrates. The synovial fluid from affected joints is characterized by sterile neutrophil-predominant infiltrate [108].

Management of PAPA with biologic agents has been met with mixed results. TNFα antagonists appear to be more effective for cutaneous manifestations while IL-1 antagonists seem to be more effective for managing articular disease manifestations. The reason for this is not known. Systemic and intra-articular corticosteroids have been used for articular disease, but steroid use can exacerbate acne so should be used with caution. Severe acne in PAPA patients can be managed with tetracycline antibiotics or isotretinoin. Effective management of PAPA syndrome is often a challenge and patients may require multiple systemic agents, including multiple biologic agents [109]. Patients should be closely monitored for infection when on multiple immunosuppressive agents.

SAPHO/CRMO

Although reports of acne and osteoarticular manifestations date back to the 1960s, the unifying term Synovitis Acne Pustulosis Hyperostosis Osteitis (SAPHO) was first described in 1987 [110, 111]. SAPHO is a rare disease, with a prevalence of fewer than 4 in 10,000 [112] and a female predilection [113–116]. This condition usually presents in childhood or young adulthood. Chronic Recurrent Multifocal Osteomyelitis (CRMO) was first described in 1972 and predominates in children [117]. It is unclear if SAPHO and CRMO are distinct diseases – many consider them to be the same entity. The genetic etiology of SAPHO/CRMO is unknown; however, a CRMO murine model does exist caused by homozygous mutations in the murine gene *pstpip2* [118].

The etiology of SAPHO is poorly understood. A number of familial cases have been reported, suggesting a genetic component in disease pathogenesis [119–123]. In addition, SAPHO shares some clinical features with several monogenic autoinflammatory diseases, including DIRA and PAPA, in which pro-inflammatory IL-1 pathways are thought to play a critical role. The efficacy of IL-1 blockade in these other diseases suggests that IL-1 pathways may play an important role in SAPHO pathogenesis. *Propionibacterium acnes,* a skin saprophyte, has been isolated in bone biopsies of affected bone lesions [124–126], suggesting a possible role as an opportunistic pathogen that activates innate and T-cell immunity through increased complement, IL-1 [127], IL-8, and TNFα levels [128, 129].

Cutaneous manifestations of SAPHO and CRMO may present concomitantly with, prior to, or after the onset of osteoarticular disease. Approximately 85 % of adults with SAPHO develop cutaneous manifestations of disease while only 30 % of children with CRMO manifest cutaneous disease [130]. Cutaneous features are predominantly neutrophilic in etiology, with palmoplantar pustulosis predominating in 60 % of affected individuals, followed by acne seen in 25 % of patients [115] (Fig. 40.3d). Other reported cutaneous disease manifestations include hidradenitis suppurativa, dissecting cellulitis of the scalp, pilonidal cyst/sinus, generalized pustular psoriasis, psoriasis vulgaris, subcorneal pustular dermatosis, erythema nodosum, and more rarely, pyoderma gangrenosum or Sweet's Syndrome [113, 131–134].

Osteoarticular manifestations are the hallmark of SAPHO/CRMO and are required for diagnosis. The osteoarthropathy is characterized most prominently by osteitis and hyperostosis. In adults, anterior chest wall osteitis is most common, affecting 65–90 % of individuals. The next most commonly affected site is the spine, affecting approximately 30 % of individuals, with the thoracic spine most frequently involved. Sacroiliitis develops in approximately 52 % of affected individuals. In adults, appendicular skeleton abnormalities are rarely seen, with long bone involvement in 5–10 %, and mandibular involvement reported in 1–10 % [135, 136]. In contrast, long bones are commonly involved in children, with distal and proximal tibia being most commonly involved, followed by proximal and distal femur [137, 138] (Fig. 40.3e). Affected individuals may have arthritis at joints adjacent to bony lesions. In adults, distant synovitis can also be seen.

Systemic features of SAPHO/CRMO include fevers and malaise. Elevated inflammatory markers can sometimes be seen. Inflammatory bowel disease, most often Crohn disease, has been reported in 10 % of patients with SAPHO syndrome [115].

First-line therapy for SAPHO/CRMO-related bony disease includes NSAIDs [139]. In the setting of NSAID failure, bisphosphonates [140–143] or methotrexate [139] should be considered for management of bone inflammation. Bisphosphonates are quite effective for the management of osteoarticular inflammation, but must be used with caution in females of childbearing age given the potential risks of fetal skeletal defects. In addition to efficacy in the management of bone inflammation, methotrexate has also demonstrated efficacy for skin manifestations. TNF-α antagonists [144] and IL-1 antagonists [145, 146] have also

been shown to be highly effective for the management of refractory cases of SAPHO/CRMO skin and bone lesions in case reports and case series, however neither have been systematically studied and neither are FDA-approved for this indication.

Majeed Syndrome

Majeed syndrome was first described in a Jordanian family in 1989 [147]. It is a rare, autosomal recessive condition resulting from loss of function mutations in the *LPIN2* gene [148, 149]. To date, 3 families with Majeed syndrome and 4 distinct *LPIN2* mutations have been reported [147, 150–152].

The *LPIN2* gene encodes lipin-2, a phosphatide phosphatase important in glycerol biosynthesis which acts as a transcription co-activator regulating lipid metabolism genes [153]. Although the function of lipin-2 is not fully understood, it may also play an important role in mitosis and cellular response to oxidative stress [154, 155]. The *LPIN2* mutations in Majeed syndrome abolish the enzymatic activity of lipin-2 [156, 157]. How loss of lipin-2 function leads to cutaneous and osteoarticular inflammation and hematologic abnormalities in Majeed syndrome is not fully understood. It has been hypothesized that loss of lipin-2 activity might lead to diminished IL-1RA production, as the latter has been observed in one described Majeed patient to date [152], however this remains an area of ongoing investigation.

Affected individuals present with the triad of: neutrophilic dermatosis, neonatal onset recurrent multifocal osteomyelitis (CRMO), and congenital dyserythropoeitic anemia (CDA) [158]. The most common cutaneous manifestation described is Sweet's Syndrome [147], however pustular dermatosis and psoriasis-like lesions have also been reported. CRMO in these patients develops in infancy and persists relentlessly, resulting in painful bone inflammation, erosive joint disease and joint contractures. The most common sites of bony inflammation include the clavicles, sternum, and long bones. Unlike isolated CRMO, mandible and vertebral body involvement is rare. The CDA associated with Majeed syndrome is characterized by microcytosis and both peripheral and bone marrow involvement, which is unique among the described types of CDA, however the severity of CDA in Majeed syndrome is variable [147, 150, 151]. Elevated proinflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α have been reported in affected patients [152].

Majeed syndrome has been treated with NSAIDs [149], corticosteroids [147], colchicine [159], interferon gamma, bisphosphonates [160] and TNF antagonists [152] with variable success. Recent case reports have demonstrated resolution of cutaneous and bony inflammation as well as inflammatory markers and anemia with IL-1 inhibition with both anakinra and canakinumab, confirming that IL-1 pathways may play a critical role in the pathogenesis of Majeed, and by extension, potentially also in SAPHO/CRMO [152].

NFkB-Mediated Autoinflammatory Diseases

CARD14-Mediated Pustular Psoriasis

Rare, highly-penetrant, autosomal dominantly-inherited, gain of function mutations in the *caspase recruitment domain 14 (CARD14)* have recently been reported in familial and sporadic cases of psoriasis vulgaris [102, 103, 161, 162] and familial cases of pityriasis rubra pilaris (PRP) [163]. A sporadic *de novo* mutation in exon 4 of *CARD14* has also been reported in a 3 year-old Haitian child with generalized pustular psoriasis (GPP), termed *CARD14*-mediated pustular psoriasis (CAMPS) [162]. These reports suggest that *CARD14* mutations can be associated with clinical and histological features of plaque psoriasis, pityriasis rubra pilaris and pustular psoriasis, indicating that these phenotypes may lie on a spectrum and share common pathogenic pathways.

The *CARD14* gene resides at the PSORS2 psoriasis susceptibility locus on chromosome 17. The CARD14 protein is thought to play a role in NFkB activation. *CARD14* mutations found in the reported psoriasis, PRP and CAMPS patients have been associated with upregulation of IL-8, a neutrophil chemotaxin, and CCL20, a chemotactic factor for CCR6+ immature dendritic cells and T cells [164–166]. The novel mutation found in CAMPS additionally led to upregulation of *IL36G*, a proinflammatory gene, and *SOD2*, a potential antinecroptosis gene [162]. These genes have previously been shown to be upregulated in response to injury, suggesting that CARD14 may play a role in maintaining skin homeostasis in the setting of injury [164].

In the single case of CAMPS reported, disease onset occurred at 6 months of age. Clinical features of CAMPS included generalized pustulosis, palmoplantar keratoderma, fevers and nail dystrophy. Neither arthropathy nor arthritis was present. Clinical laboratory assessments were notable for leukocytosis and elevated inflammatory markers. The patient's disease was refractory to therapy with systemic corticosteroids, cyclosporine, infliximab and anakinra [162]. Ultimately, her disease responded to IL-12/23 inhibition with ustekinumab, suggesting that IL-12/23 may play an important role in driving the clinical phenotype seen in

CAMPS. Furthermore, ustekinumab may play an important therapeutic role in managing other CARD14-mediated dermatoses.

Autoinflammatory Diseases Mediated via Other Pathogenic Pathways

Proteasome Associated Autoinflammatory Syndrome

Proteasome-associated autoinflammatory syndrome (PRAAS) was first described in 1939 by Nakajo and colleagues. Since that time, this disorder has been known by various names including Nakajo-Nishimura syndrome (NNS) [167, 168]; Japanese autoinflammatory syndrome with lipodystrophy (JASL) [169]; joint contracture muscular atrophy, microcytic anemia and panniculitis like lipodystrophy (JMP) [170]; and chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) [171]. PRAAS is characterized by autosomal recessive mutations in proteasome subunit b type 8 (*PSMB8*). Four disease-causing mutations (T75M, A92T, C135X, and G201V) have been described to date [172].

Proteasomes are evolutionarily-conserved, ATP-dependent protein structures that are critical for protein degradation. Proteasomes target intracellular polyubiquitinated proteins and cleave them into peptides which are then available for antigen presentation [173, 174]. PSMB8 encodes the inducible β5i subunit of the proteasome. In the setting of interferon (IFN) stimulation, the critical β1, β2 and β5 proteasome subunits are replaced with inducible subunits β1i, β2i and β5i forming immunoproteasomes [175]. Immunoproteasomes generate peptides for antigen presentation and also help to maintain homeostasis by removing accumulating proteins from cells [176, 177]. Patients with PRAAS have a persistent dysregulated IFN signature on microarray analysis and increased STAT-1 phosphorylation in monocytes, suggesting cellular stress. Mutations in *PSMB8* lead to defective assembly of the proteasome complex, which in turn leads to accumulation of polyubiquitinated proteins in the cells, and subsequent cellular stress resulting in further increased IFN signaling [172]. Cytokine studies have demonstrated that PRAAS patients have increased levels of IL-6, IL1RA, and IFN-inducible chemokines CXCL10 and CCL2 [172, 178].

Onset of PRAAS occurs within the first weeks of life and is characterized by fever and erythematous and violaceous annular eruptions involving the trunk and extremities which persist for several days or longer and may leave residual purpura. Later in infancy, patients develop periorbital erythema resembling a heliotrope rash but with prominent associated edema (Fig. 40.4a). Violaceous nodules and plaques of variable morphology are common on the trunk and extremities, as well as hepatosplenomegaly and elevation in inflammatory markers. Within the first years of life, individuals with PRAAS develop progressive lipodystrophy predominantly involving the face and extremities, failure to thrive, microcytic anemia and lymphadenopathy (Fig. 40.4b). Other reported features include widening of the digits, arthritis, joint contractures, myositis, basal ganglia calcification, dyspnea, seizures, short stature and low body weight [167, 168, 171, 172, 179, 180]. Truncal obesity and hyperlipidemias, insulin resistance, and acanthosis nigricans suggest additional metabolic abnormalities [172, 179]. Severe inflammatory attacks can result in systemic inflammatory response syndrome (SIRS), organ failure, and death. Calcifications in vessels and soft tissues, as well as cardiomyopathy and cardiac arrhythmia, have also been reported [179]. Skin histopathology of PRAAS is distinct from other autoinflammatory diseases and demonstrates dense perivascular and interstitial infiltrates comprising atypical mononuclear cells and neutrophils in the papillary and reticular dermis.

Systemic therapies for PRAAS, including systemic steroids, NSAIDs, colchicine, methotrexate, azathioprine and tacrolimus have been met with mixed results [172]. Partial responses have been seen with biologic agents including TNF-α, IL-1, and IL-6 antagonists [172]. Lipodystrophy remains a prominent and persistent disease characteristic despite therapeutic intervention [172]. Dysregulation of IFN pathways suggests that this pathway may be a promising therapeutic target for future intervention.

PLCG2-Associated Diseases

PLCG2-associated antibody deficiency and immune dysregulation (PLAID) and *PLCG2*-associated antibody deficiency and immune dysregulation (APLAID) are two recently described autoinflammatory diseases which are paradoxically characterized by both immune activation and immunodeficiency. Both PLAID and APLAID are dominantly inherited [181, 182].

PLCG2 encodes phospholipase $C\gamma_2$, a phospholipase responsible for cleaving phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol triphosphate (IP3) and diacyloglycerol (DAG). Phospholipase $C\gamma_2$ regulates inflammatory and immune signaling pathways in hematopoietic cells. The carboxy terminal SRC-homology 2 (cSH2) domain of *PLCG2* is evolutionarily conserved and plays a critical role in the regulation of the gene as it contains the elements of gene autoinhibition. Mutations affecting this region may directly destabilize the region and affect ligand binding and interaction of residues interacting with the domain. Mutations in the autoinhibitory cSH2 component of the *PLCG2* gene are responsible for

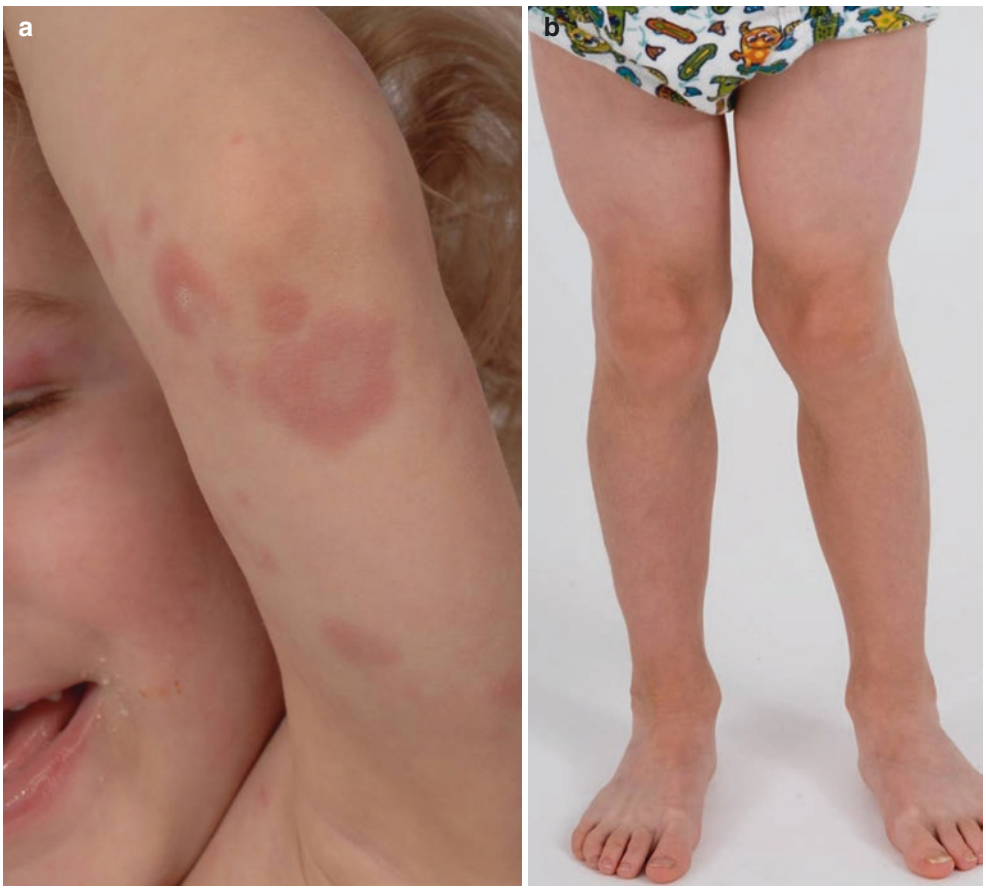

Fig. 40.4 Dermatologic manifestations in PRAAS. (**a**) Periorbital erythema and deep red round and annular plaques involving the upper extremity and trunk in a child with proteasome associated autoinflammatory syndrome (PRAAS). (**b**) Lower extremity lipodystrophy in a child with PRAAS

development of both PLAID and APLAID. In PLAID, large activating in-frame heterozygous deletions of the *PLCG2* cSH2 domain cause failure of autoinhibition and result in constitutive phospholipase activity. This results in temperature-dependent function of the phospholipase $C\gamma_2$ enzyme. Specifically, at 37 °C, mutant *PLCG2*-expressing cells are anergic, however when cooled, these cells are activated [181]. In APLAID, a missense mutation in the *PLCG2* cSH2 domain leads to a Ser707Tyr substitution which directly affects the autoinhibitory function of this region [182].

PLAID was first reported in 2012 in 3 families of PLAID patients and is clinically characterized by familial cold-induced evaporative urticaria [181, 183]. Other disease manifestations that were described include atopy, granulomatous rash, autoimmune thyroiditis, sinopulmonary infection, antinuclear antibodies and common variable immunodeficiency and variable B cell immunodeficiency. Affected individuals had low levels of serum IgM and IgA, circulating CD19+ B cells, IgA+ and IgG+ class-switched memory B cells, and natural killer cells. In contrast, IgE levels were elevated.

APLAID was initially reported in one family in 2012 and is clinically characterized by recurrent inflammation of multiple organ systems including the skin, eyes and lungs. The reported individuals presented in infancy with an epidermolysis bullosa-like eruption which progressed to erythematous and vesicopustular lesions in childhood. Systemic features included corneal bullae, arthralgia, sinopulmonary infections, enterocolitis, absence of autoantibodies, and mild immunodeficiency. B cells in these patients were notable for enhanced surface ligand mediated cellular signaling and absence of cold sensitivity, in contrast to PLAID patients. These patients had a paucity of circulating IgA and IgM antibodies and also lacked peripheral class-switched B cells. Skin histopathology demonstrated a dense dermal mixed inflammatory cell infiltrate composed of lymphocytes, histiocytes, eosinophils, and karyorrhectic debris [182].

Management of PLAID has been largely supportive, including prophylaxis and treatment of recurrent infections in immunocompromised individuals. NSAIDs and TNF-α antagonists were ineffective in both APLAID patients, however both had partial disease response to

IL-1 antagonists. These individuals also had significant improvement of systemic inflammation with corticosteroids, but side effects limit the use of these agents [182]. It has been hypothesized that phospholipase inhibitors might be beneficial for the management of PLAID and APLAID [184].

Deficiency of Adenosine Deaminase 2

Deficiency of Adenosine Deaminase 2 (DADA2) (also known as fever with early onset of stroke (FEOS)) was first described in 2014 in 9 individuals [185]. Recessively-inherited loss of function mutations in cat eye syndrome chromosome region, candidate 1 (*CECR1*), which encodes adenosine deaminase 2 (ADA2), are responsible for this condition.

The ADA2 enzyme is expressed predominantly in myeloid cells and secreted into the blood. It acts by converting adenosine to inosine and 2′-deoxyadenosine to 2′-deoxyinosine. Loss of function missense mutations in *CECR1* in DADA2 affect the stability and impact the catalytic and dimerization domains of ADA2. This leads to reduced ADA2 levels and enzyme activity in the blood. Studies in zebrafish paralog *cecr1b* suggest that mutations in this gene affect endothelial integrity and lead to a near complete absence of neutrophils [185]. Studies in both humans and zebrafish indicate that ADA2 may be necessary both for vascular integrity and leukocyte development in the zebrafish, and that the near absence of ADA2 in humans may lead to strokes and autoinflammation by similar mechanisms.

Patients with DADA2 present in early childhood with recurrent fevers and a spectrum of vasculopathic features including livedo racemosa, strokes, and polyarteritis nodosa (Fig. 40.5). Ischemic and hemorrhagic lacunar strokes occurred during episodes of inflammation with onset typically before the age of 5. Hepatosplenomegaly, portal hypertension, ocular inflammation, recurrent bacterial and viral infections, hypogammaglobulinemia, lymphopenia, low IgM levels, and anti-nuclear antibodies were variably seen in affected individuals. Positive lupus anticoagulant developed over time but anti-phospholipid antibodies were not detected. Skin histopathology predominately demonstrated perivascular lymphocytes and interstitial infiltrate comprising neutrophils and macrophages. Cutaneous vasculitis was observed in one individual [185].

Corticosteroids partially controlled episodes of inflammation and fever. Despite aggressive management with corticosteroids, cyclophosphamide and cytokine inhibitors, one affected individual continued to have stroke events into adulthood. Effective targeted therapeutic strategies for DADA2 require further investigation.

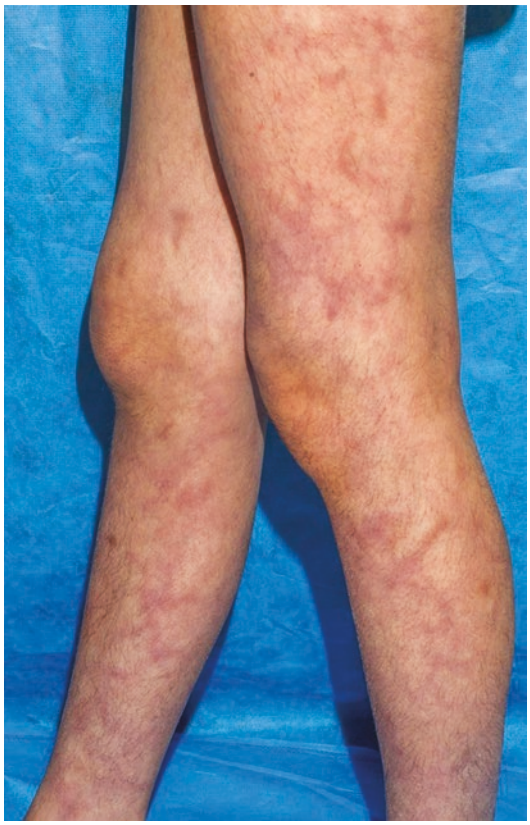

Fig. 40.5 Livedo racemosa in a patient with deficiency of adenosine deaminase 2 (DADA2)

Summary

Recent genetic insights into several rare monogenic autoinflammatory disorders have elucidated pathogenic mechanisms of debilitating diseases resulting from dysregulation of the innate immune system. The study of these molecular pathways of sterile inflammation has pointed to potential targeted therapeutic strategies for these enigmatic diseases. The identification of novel autoinflammatory syndromes continues to provoke investigation for novel targets for therapeutic intervention.

Questions

1. Dysregulation of the NALP3 inflammasome is thought to play an important pathogenic role in which autoinflammatory diseases?
2. Osteoarticular inflammation and neutrophilic dermatoses are clinical features shared by which autoinflammatory diseases thought to be mediated by IL-1 pathways?
3. Mutations in which gene is responsible for a spectrum of pustular and papulosquamous dermatoses?
4. Dysregulation of what signaling pathway is thought to play an important role in PRAAS pathogenesis?

5. Which two recently-described diseases are paradoxically characterized by both autoinflammation and immunodeficiency?

Answers

1. Cryopyrin-associated periodic syndromes; familial Mediterranean fever; TNF-receptor-associated-periodic syndrome
2. SAPHO/CRMO
3. LPIN2
4. PSMB8
5. PLAID and APLAID

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Cutaneous T-Cell Lymphoma

41

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Abstract

Cutaneous T-cell lymphomas (CTCLs) are a group of extranodal non-Hodgkin's lymphomas (NHLs) that present primarily in the skin. The most common type of CTCLs, mycosis fungoides (MF) and Sézary Syndrome (SS), were first described over two centuries ago, and since that time, the clinical characteristics, pathophysiology, and immunobiology have been characterized in detail. Derived from skin-homing, mature, effector T-cells that usually express CLA/CD4/CCR4/CCR10 and lack T-cell markers CD7 and/or CD26, the malignant MF/SS cells typically have a Th2 phenotype. With more advanced disease, Th2 cytokines predominate and result in decreased host cell-mediated immunity that likely contributes to increased susceptibility to infection and disease progression. Early aggressive systemic chemotherapy has not been shown to improve overall survival in MF/SS and over the past 40 years [1] the therapeutic approach has undergone a paradigm shift, such that skin-directed therapies (SDTs) and systemic immune-modifying biologics play a central role in initial MF/SS management. This chapter will review MF/SS clinical presentation, staging work-up, pathophysiology, immunobiology, and how these have shaped current treatment strategies. In particular, MF/SS chemokine biology, immune defects, and immune modifying therapies, including the new frontier of hematopoietic stem cell transplantation will be specifically highlighted.

Keywords

Cutaneous T-Cell Lymphoma • CTCL • Non-Hodgkin's lymphomas • NHLs • Mycosis fungoides • MF • Sézary Syndrome • SS • Dermatitis • Psoriasis • Parapsoriasis • Skin condition • Skin disease • Tumor-node-metastasis-blood • TNMB

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Clinical Presentation

CTCLs are a heterogeneous group of extranodal lymphomas and were the subject of a new joint classification system in 2005 by the World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) (Table 41.1) [2]. MF/SS is relatively uncommon and comprises approximately 3.9% of all NHLs [3]. The incidence of CTCL has increased since 1973 with an annual age-adjusted incidence of 6.4–9.6 cases per million people in the United States [4]. The cause of the vast majority of MF/SS cases are unknown, despite numerous environmental and infectious etiologies having been investigated as potential sources of chronic antigenic stimulation. These include human T-cell lymphotrophic virus (HTLV), cytomegalovirus (CMV), Borrelia burgdorferi, human herpes virus-8 (HHV-8), *Staphylococcus aureus*, *Chlamydia* species, and chemical exposures such as aromatic or halogenated hydrocarbons [5, 6].

MF classically presents as scaly oval or annular patches/plaques (T1-2 tumor designation) on sun-protected areas such as the trunk, buttocks, axillae, groin, and proximal extremities, ("bathing trunk" distribution) with or without pruritus (Fig. 41.1a–c). Typically, it has indolent behavior and in early disease when host immunity is intact and lesions may wax and wane spontaneously. Because of this, early disease may take years before definitive diagnosis is made and skin biopsies may demonstrate non-specific dermatitis. Lesions may also less commonly affect sun exposed areas such as the face and can also result in localized hair loss (as seen in the follicular variant). They may also progress to thicker, more infiltrated plaques (Fig. 41.1c), ulcerated tumors (T3 disease) (Fig. 41.1d), or coalesce into a confluent erythema >80% body surface area ("erythroderma" or T4 disease) (Fig. 41.1d). In these cases, the disease can cause more symptoms and behave more aggressively.

In addition to the classic presentation, MF has myriad other clinical morphologies and histological variants (classic Alibert-Bazin subtype, erythrodermic/Sezary Syndrome, unilesional, hypopigmented, pagetoid reticulosis, follicular/follicular mucinosis, syringotropic, granulomatous/slack skin, bullous/vesicular, palmoplantar, pigmented purpuric dermatosis-like, interstitial, icthyosiform, hyperkeratotic/verrucous, vegetating/papillomatous) [7] and has been referred to as one of the great clinical imitators, similar to cutaneous syphilis and sarcoidosis. Subtypes may be characterized by more indolent behavior (hypopigmented MF) or by more aggressive behavior (follicular, granulomatous), depending on their clinical course. Follicular MF, even with limited lesions, can be recalcitrant to therapy given the depth of the malignant infiltrate.

SS typically has a more aggressive course than MF from its onset, and typically presents as de novo erythroderma with leukemic peripheral blood involvement by the atypical, hyperconvoluted, cerebriform, malignant T-cells known as Sézary cells (also previously referred to as Lutzner cells or cellules monstrueuses). This is in contrast to patients who have MF that evolves into erythroderma over time (erythrodermic MF). Originally, SS was described with the clinical triad of erythroderma, lymphadenopathy, and palmoplantar keratoderma (Fig. 41.1e). SS patients may also have severe pruritus, active scaling/desquamation, prominent conjunctival eversion of the eyelids (referred to as ectropion), diffuse alopecia, chills, low grade fevers, night sweats, or fatigue.

MF/SS patients, particularly erythrodermic patients, are heavily colonized by skin bacteria and are susceptible to recurrent infections with correct *Staphylococcus aureus*, which often are methicillin-resistant (MRSA). Patients with ulcerated tumors are also at high risk for bacterial sepsis and in general, infections have previously been the leading cause of mortality in MF/SS patients [8].

MF/SS patients may also present with other concomitant skin conditions, such as the chronic, recurrent primary cutaneous CD30+ lymphoproliferative disorders (LPDs) such as lymphomatoid papulosis (LyP) or CD30+ primary cutaneous anaplastic large cell lymphoma (ALCL) [9]. Patients with MF/SS may also be at higher risk for having secondary malignancies, such as other skin cancers (nonmelanoma and

Table 41.1 EORTC-WHO classification of cutaneous T- and NK/T-cell lymphomas (2005)

Mycosis fungoides and variants/subtypes
Sezary syndrome
Primary cutaneous CD30+ lymphoproliferative disorders
Subcutaneous panniculitis-like T-cell lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Adult T-cell leukemia/lymphoma
Primary cutaneous peripheral T-cell lymphoma, unspecified
Aggressive epidermotropic CD8+ T-cell lymphoma
Cutaneous γδ T-cell lymphoma
PC CD4+ small/medium-sized pleomorphic T-cell lymphoma
Peripheral T-cell lymphoma, other

Source: Reproduced with permission of American Society of Hematology from Willemze et al. [2]

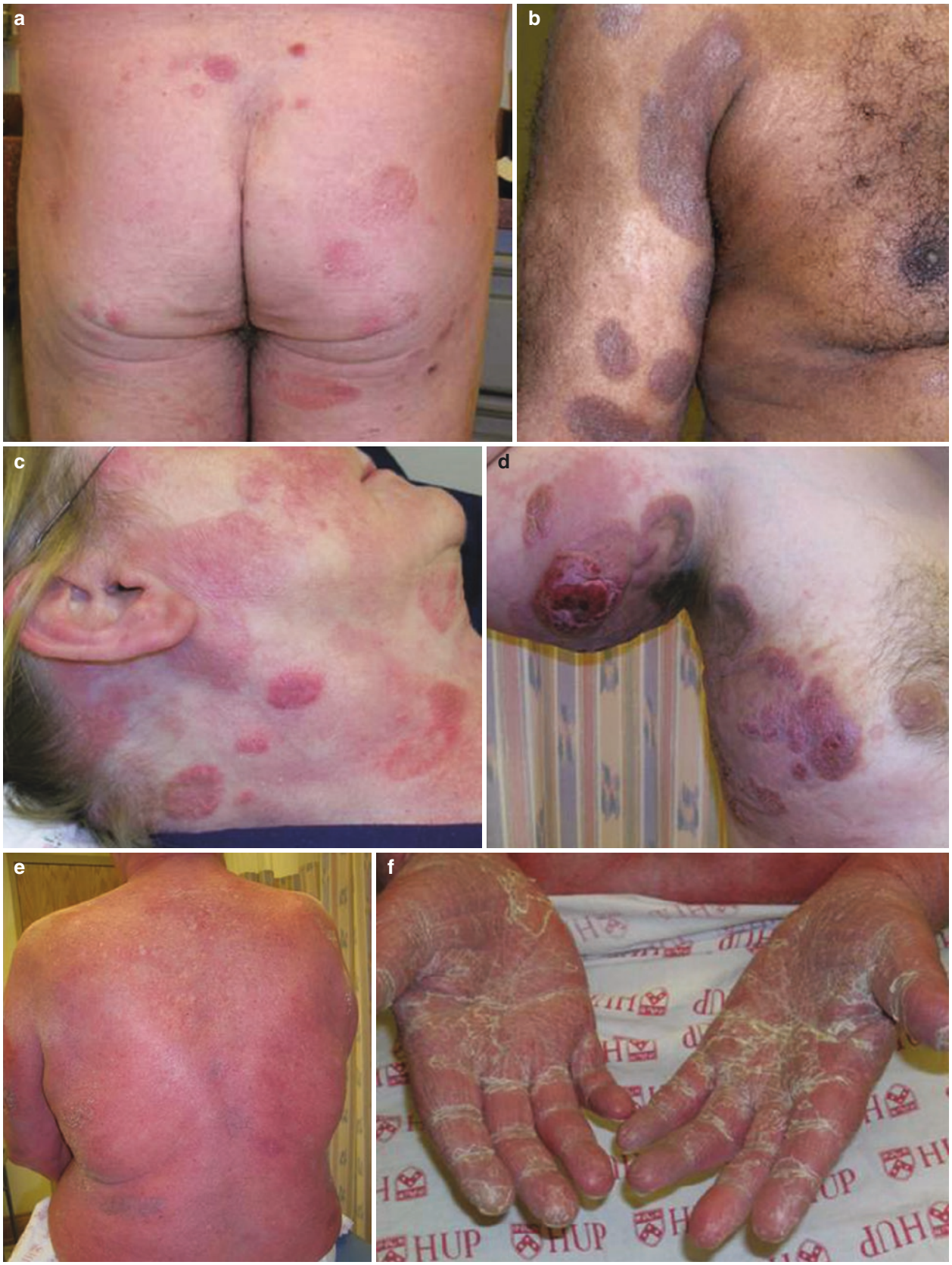

Fig. 41.1 Skin lesions in mycosis fungoides (MF)/Sézary syndrome (SS). Oval patches of MF in typical sun-protected areas of the buttocks (**a**) and proximal arms/axillae (**b**). (**c**) Raised, annular plaques of MF. (**d**) MF plaques and an ulcerated tumor. SS patient with erythroderma (**e**) and palmar keratoderma (**f**)

melanoma) and secondary lymphomas [10]. Young patients with MF/SS diagnosed before 30 years of age may experience an increased risk of secondary malignancies, particularly melanoma and lymphoma, but, despite this risk, have a favorable prognosis [11].

Diagnosis

Early diagnosis of MF can be elusive, as the clinical and histopathology of early MF lesions can mimic benign dermatoses, such as eczematous dermatitis, allergic contact dermatitis, psoriasis and parapsoriasis. Traditionally, the diagnosis is made when a patient presents with typical lesions and skin biopsy reveals atypical lymphocytes (with large, hyperchromatic and hyperconvoluted nuclei) within the epidermis, in the absence of epidermal spongiosis, and often associated with epidermal dendritic cells (DCs) to form the pathognomonic "Pautrier's microabcesses." Currently there are sophisticated laboratory tools to aid in diagnosis, which include tissue immunohistochemistry and flow cytometry (which can demonstrate CD3 expression, CD4>CD8 expression of greater than 4:1, as well as characteristic T-cell lineage antigen loss of CD5, CD7, CD26, and less often CD3). Molecular detection of clonal T-cell receptor (TCR) gene rearrangements (β and γ chains) in the skin or other tissue (such as the blood or lymph node) can also further strengthen the diagnosis [12]. These days, the standard evaluation of early MF often involves assessment of multiple criteria (clinical, histopathological and molecular) [13]. However, it must be noted that T-cell clonality can also be detected in benign and autoimmune inflammatory dermatoses and should not be automatically equated with neoplasia [14, 15]. Diagnosis ultimately rests on accurate clinicopathologic correlation with adjunctive molecular studies, if appropriate.

In SS, leukemic blood involvement was traditionally measured by the Sézary prep, visual examination of a peripheral blood buffy coat smear for the atypical Sézary cells (Sézary count >5 % total lymphocytes was considered significant). However, this method was laborious and was subject to considerable inter-observer variability. More sensitive and specific tools to measure peripheral blood involvement include anti-TCR Vβ-specific monoclonal antibodies, or T-cell surface marker antibodies (CD3, CD4, CD7, CD8, CD26) using fluorescence-activated cell-sorting (FACS) analysis/flow cytometry.

The identification of a clonal malignant TCR Vβ population aids in the diagnosis and allows for monitoring of the disease course in SS patients. However, as SS cells express molecules also present on normal activated CD4 T cells, diagnosis based on the phenotype of circulating malignant cells can be difficult [6]. SS patients without an identifiable circulating clone pose a particular diagnostic and therapeutic monitoring challenge, as loss of CD26 is suggestive but not definitive of the malignant population [16].

To that end, much work has been performed recently to identify additional diagnostic markers for SS. Wysocka and colleagues identified CD164 as a novel early detection marker for SS in patients with low-to-high tumor burden, demonstrating a statistically significant correlation between CD164 acquisition and loss of CD26 expression [17]. The studies further showed that while CD164 is present on CD4 T cells of SS patients with a wide range of tumor burdens, it is absent on CD4 T cells of healthy controls and patients with atopic dermatitis. Additionally, morphological examination of purified CD4+CD164+ T cells demonstrates the morphology of malignant Sézary cells. Lastly, CD4+CD164+ T cells were noted to disappear in SS patients who experienced clinical remission as a result of treatment, underscoring the potential for CD164 to serve as a marker for malignant cells in SS.

Other markers recently reported useful in flow cytometric analysis include vimentin, CD158k [18], T-plastin (PLS3) [19], Twist [20], and NKp46 [21]. Michel and colleagues demonstrated that combination of CD158k, PLS3, and Twist gene expression profiling by quantitative PCR can be used for an efficient molecular diagnosis of SS [22]. The authors showed that $CD4^+$ T cells from SS patients expressed significant PLS3, Twist, CD158k, and NKp46 mRNA levels and that analysis of expression of these four markers accurately classified 100 % patients in the study.

In SS, molecular testing for T-cell receptor gene rearrangements can often detect the identical clonal T-cell population in the blood and the skin. In general, matching T-cell monoclonality detectable in several different skin lesions or tissues (skin, nodes, blood) in a patient over time is suggestive of CTCL [23].

Disease Staging

Since 1979, a tumor-node-metastasis-blood (TNMB) classification and staging system has been used for MF/SS and TNMB stage has proven to be an important prognostic measure (Tables 41.2 and 41.3) [24]. In 2007, modifications were proposed by the International Society for Cutaneous Lymphomas (ISCL) regarding further stratification of blood involvement parameters to reflect the newer and more sensitive assays [25, 26]. The current B classification proposed by the ISCL includes B0 (no clinically significant blood involvement), B1 (clinically significant "minimal" blood involvement with Sézary cells <1.0 K/ul) and B2 (leukemic blood involvement detectable either as (a) Sézary cells >1.0 K/ul; (b) CD4/CD8 ratio >10 and CD4+CD7- population >40 % or CD4+CD26- population >30 %, (c) lymphocytosis with

Table 41.2 TNMB classification for mycosis fungoides and Sézary syndrome, 2014 National Comprehensive Cancer Network guidelines

T (skin)	
T1	Limited patch/plaque (<10 % body surface area)
T1a	Patches only
T1b	Presence of plaques with or without patches
T2	Generalized patch/plaque (≥10 % body surface area)
T2a	Patch only
T2b	Presence of plaques with or without patches
T3	≥1 tumors (≥1 cm in diameter)
T4	Generalized erythroderma (≥80 % BSA)
N (lymph node)	
N0	No clinically abnormal (palpable; ≥1.5 cm diameter) peripheral LNs
N1	Clinically abnormal LNs; histopathology Dutch grade 1 or NCI $LN_{0\text{-}2}$
N1a	Clone negative
N1b	Clone positive
N2	Clinically abnormal LNs; histopathology Dutch grade 2 or NCI LN_3
N2a	Clone negative
N2b	Clone positive
N3	Clinically abnormal LNs; histopathology Dutch grade 3–4 of NCI LN_4; clone positive OR negative
M (viscera)	
M0	No visceral involvement
M2	Visceral involvement (pathology confirmation of specific organ involved)
B (blood)	
B0	Absence of significant blood involvement (≤5 % of peripheral blood lymphocytes are atypical/Sézary cells)
B0a	Clone negative
B0b	Clone positive
B1	Low blood tumor burden (>5 % of peripheral `blood lymphocytes are atypical/Sézary cells but does not meet criteria of B2
B1a	Clone negative
B1b	Clone positive
B2	High blood tumor burden defined as one of the following: ≥1000 Sézary cells/μL with positive clonal rearrangement of TCR; CD4:CD8 ratio ≥10 with positive clone; or $CD4^+CD7^-$ cells ≥40 % or $CD4^+CD26^-$cells ≥30 % with positive clone

Table 41.3 Clinical staging system for mycosis fungoides and Sézary syndrome, based on the 2014 National Comprehensive Cancer Network guidelines

Clinical stages	T	N	M	B
IA	1	0	0	0 or 1
IB	2	0	0	0 or 1
IIA	1 or 2	1 or 2	0	0 or 1
IIB	3	0–2	0	0 or 1
IIIA	4	0–2	0	0
IIIB	4	0–2	0	1
IVA_1	1–4	0–2	0	2
IVA_2	1–4	3	0	0–2
IVB	1–4	0–3	1	0–2

molecular genetic evidence of a clonal T-cell population; or (d) chromosomally abnormal T-cell clone). The updated blood involvement parameters may affect staging and prognosis of patients. Currently, the ISCL proposes that in patients with erythroderma (T4 skin classification), the B2 classification be considered the equivalent to nodal involvement (hence T4N0-1M0B2 would be upgraded from Stage III to Stage IVA1). A retrospective study by Vonderheid et al. demonstrated that using modified B criteria improved prognostication in erythrodermic patients [26]. Several large studies have since validated the 2007 ISCL staging classification for prognosis and risk stratification [27–29]. National Comprehensive Cancer Network Clinical Practice Guidelines for MF/SS have been available since 2008 and include

recommendations for diagnostic and staging work up as well as current established treatments and treatment algorithms, and are updated yearly [30].

Once patients have an established diagnosis of MF/SS, a typical staging workup includes complete skin and physical examination, bloodwork including complete blood count with differential, comprehensive metabolic panel, lactate dehydrogenase, and either peripheral blood flow cytometry or Sézary cell prep (flow optional in T1a, limited patch disease with no high risk clinical or histologic characteristics). Computed tomography (CT) or positron emission tomography scanning (PET) is appropriate for patients with systemic symptoms, clinically palpable lymphadenopathy (>1.5 cm), or more advanced T classification (T1b follicular MF, extensive T2a/b, T3/tumors or T4/erythrodermic disease, patients with B symptoms). These tests are useful to assess nodal or visceral involvement by MF/SS. The role of staging bone marrow biopsy remains controversial [31]. In the United States, bone marrow biopsy is typically reserved only for patients with advanced disease with some evidence of other hematological abnormalities (i.e.: other cytopenias).

Overall survival in MF/SS is highly dependent on initial disease stage. Several studies have demonstrated that early stage disease (i.e.: skin disease only, limited to <10 % body surface area) if treated has overall median survival comparable to healthy control populations. However, with more advanced disease, overall survival also decreases with Stage IV disease having a 27 % 5-year survival and Sézary Syndrome patients having a 30 % 5-year survival [32]. In retrospective studies, the risk of disease progression ranged from 10 % (Stage IA) to 25 % (Stages IB or higher) over a 30 year period [33, 34]. While a 2003 study by Kim and colleagues [32] reported an overall survival (OS) of only 2.5 years in patients with SS, a study by Agar and colleagues [27] in 2010 reported a 3.1 year OS, and a study by Talpur and colleagues [28] from 2012 showed an OS of 4.64. As the major cause of death in patients with SS is line sepsis, which is often caused by *Staphylococcus aureus*, Talpur et al. report that *Staphylococcus* was prospectively cultured and was aggressively treated and prevented with antibiotics and skin care [35]. The authors thus postulate that the improved survival may have resulted in part from early treatment of coexisting infections, as well as earlier diagnosis of disease and advent of new therapies.

Various clinical prognostic factors have been demonstrated including patient age, initial stage, T classification, visceral disease, elevated LDH, eosinophilia, presence of Sézary cells, and B0b blood classification [27]. Important histological factors that indicate poor prognosis include "large cell transformation" (LCT, presence of >25 % large atypical T-cells in the infiltrate seen in the skin or lymph node or blood, may be either CD30+ or CD30-, with CD30- transformation having worse prognosis than CD30+) [36], folliculotropic or granulomatous infiltrate; in contrast, the presence of CD8+ tumor infiltrating cytotoxic lymphocytes (TILs) in the infiltrate has been demonstrated to be a favorable prognostic marker.

Recently, a new cutaneous lymphoma international prognostic index (CLIPi) for early and late stage CTCL has been developed and validated to aid with patient risk stratification [37]. Based on the proposed index, significant adverse prognostic factors at diagnosis included male gender, age >60, presence of plaques, folliculotropic disease, and stage N1 for early stage disease, and male gender, age >60, stages B1/B2, N2/3 and visceral involvement for late stage disease. Using these variables 3 distinct groups are designated for early and late stage patients: 0–1 (low risk), 2 (intermediate risk), and 3–5 factors (high risk). Using this prognostic index a 10 year OS for each group may be obtained (in the early stage model was 90.3 % (low), 76.2 % (intermediate) and 48.9 % (high) and 53.2 % (low), 19.8 % (intermediate) and 15.0 % (high) for the late stage model).

CLIPi thus supplements the current NCI Staging System and is a useful tool for individual prognostication and for making comparisons between different groups of patients in clinical trials.

Perhaps the most potentially powerful prognostic tool currently being developed in MF/SS is cDNA microarray analysis. Previously, MF/SS patients were shown to have a distinct gene expression profile that was distinguishable from other benign inflammatory dermatoses [38]. In addition, such profiles could also identify high risk patients with <6 month survival. The panel of genes identified included upregulated GATA3 (involved in Th2 differentiation), downregulated STAT4 (involved in Th1 differentiation), and CD26 (dipeptidylpeptidase IV, which regulates T-cell entry into the epidermis, see section "Chemokines"). Recently, several studies have confirmed that a similar panel of genes has been shown to be capable of molecularly diagnosing MF/SS patients [39, 40].

Pathophysiology

Chemokines and Their Receptors in CTCL

As mentioned previously, CTCL is a malignancy in which tumor cells exhibit an affinity for the skin. This affinity is dependent upon their expression of cell surface "trafficking" molecules which mediate their migration to the cutaneous microenvironment. Chemokines and their receptors are included in this family of molecules and play a critical role in mediating tissue-specific trafficking of leukocytes to the skin.

Chemokines are chemotactic cytokines that are produced by a wide array of tissues and range from 8 to 17 kDa in size.

They are divided into families in accordance with the positioning of cysteine residues in their chemical structure. Aside from their role in cell migration, they have also been shown to play a role in the mediation of inflammatory responses, angiogenesis, and cellular proliferation [41].

Chemokine receptors are seven transmembrane-spanning G-protein coupled proteins that are capable of recognizing more than one chemokine. Several chemokines and their receptors have been identified to play a critical role in the trafficking of immune cells to the skin, more specifically, thymus and activation-regulated chemokine (TARC), macrophage-derived chemokine (MDC), stromal derived factor-1 (SDF-1), and cutaneous T-cell-attracting chemokine (CTACK). These chemokines have been shown to be produced by multiple cell types (including keratinocytes, fibroblasts, and endothelial cells) and are upregulated at sites of cutaneous inflammation. Skin-trafficking T-cells have been shown to express receptors for these chemokines.

Lymphocyte trafficking to the skin requires that the cell undergo a sequential series of events that begins with an initial tethering interaction with the endothelial surface, followed by rolling, activation, firm adhesion, and diapedesis into the dermal microenvironment. The tethering and rolling events are mediated by interactions between cutaneous lymphocyte associated antigen (CLA) and E-selectin [42]. CLA is expressed on the surface of skin-trafficking T-cells and E-selectin is expressed by endothelial cells at sites of cutaneous inflammation.

Activation of the arrest stage of lymphocyte trafficking is mediated by interactions between chemokines and their receptors. Upon engagement of the chemokine receptor, signals are transduced which activate downstream events that include a change in cellular morphology, such that integrins on the surface of the leukocyte are sufficiently exposed to contact their ligands on the dermal microvasculature (e.g. LFA-1 binding to ICAM-1). This leads to the arrest of the rolling cell, which may then migrate into the dermis along a chemotactic gradient generated by chemokines produced in the skin.

The malignant cells in CTCL have been shown to express skin-trafficking molecules and includes CLA and an array of chemokine receptors. Although frequently elevated, we and others have observed that CLA expression may be more variable among circulating malignant cells [43], whereas chemokine receptors involved in skin-trafficking are more consistently expressed at high levels among this population.

It appears somewhat counterintuitive that cells expressing skin-trafficking molecules may remain confined to the circulation, as is noted among patients with advanced disease such as in SS. This confinement of malignant cells to the blood compartment may in part be accounted for by significantly elevated chemokine levels (such as TARC) in the skin and circulation of patients. It is plausible that TARC levels in the blood may exceed a threshold concentration required for effective chemotaxis, and thus abolish a skin-derived chemotactic gradient. This idea is supported by the work of Poznansky et al. who reported the potential for T-cells to exhibit movement away from a chemotactic gradient established at concentrations above which normal directional migration is detected [44]. This finding, in combination with the variable expression levels of other skin-trafficking molecules (such as CLA) among the malignant population in CTCL may provide an explanation for the high percentage of neoplastic cells detected in the circulation of patients with advanced disease.

Several studies have evaluated the expression of chemokine receptors by malignant cells in the skin and circulation of CTCL patients. Flow cytometric analysis and immunohistochemical studies revealed preferential expression of the chemokine receptors CCR4, CXCR3 and CXCR4 among MF cells and the surrounding reactive T cells in patients with early patch and plaque stage disease. Interestingly, patients with tumor stage disease exhibit a loss of CXCR3 (and rarely CCR4), while expressing high levels of the chemokine receptor CCR7 [45]. CXCR4 and CCR4 have both been shown to play a role in lymphocyte migration to the skin, while CCR7 mediates the migration of T cells to lymphatic tissue and may account for the nodal disease observed among patients with advanced disease. CCR7 recognizes secondary lymphoid tissue chemokine (SLC/CCL21) produced within the nodal microenvironment. Preferential expression of CCR10 has also been reported among tumor cells of MF patients. CCR10 is the receptor for the cutaneous T-cell attracting chemokine (CTACK/CCL27) which is constitutively produced by epidermal keratinocytes [46].

Similar to the MF cells in tumor stage disease, the malignant cells in patients with peripheral blood disease have been shown to express elevated levels of CCR4, CCR7, CXCR4, and CCR10 [47]. In addition, the circulating malignant cells are also characterized by their loss of the cluster of differentiation markers CD7 and/or CD26 [48]. The absence of CD26 is believed to contribute to the accumulation of malignant cells in the skin of Sézary syndrome patients [49].

Campbell and colleagues have recently demonstrated that MF and SS may arise from two distinct T-cell subsets. Their studies show that clonal malignant T cells from the blood of SS patients universally coexpress the lymph node homing molecules CCR7 and L-selectin as well as the differentiation marker CD27, a phenotype consistent with central memory T cells (T_{CM}) [50]. The authors also note that CCR4 was universally expressed at high levels, while there was variable expression of CCR6, CCR10, and CLA. In contrast, T cells isolated from MF skin lesions lacked CCR7/L-selectin and CD27, but strongly expressed CCR4 and CLA, a phenotype of skin resident effector memory T cells (T_{EM}).

Additionally, genomic analysis reveals differing molecular profiles characteristic of MF and SS. MF is characterized

by increased expression of FASTK and SKAP1 genes, and diminished expression of the RB1 and DLEU1 tumor suppressor genes [51], while SS is characterized by amplification of MYC oncogene in 75 % of patients with SS, which was detected in only a minority of patients with MF [52].

Although MF and SS were originally thought to represent points of progression in a disease continuum, the recent data suggest that these two entities might best be considered as separate lymphoproliferative diseases which originate from distinct T-cell subsets and have different genetic signatures.

CD26 is a dipeptidyl transferase expressed as both a soluble factor in the circulation and as a cell surface protein expressed by lymphocytes. CD26 mediates the cleavage of the chemokine stromal derived factor-1 (SDF-1), produced by inflamed skin (Fig. 41.2a). SDF-1 is the natural ligand for the chemokine receptor CXCR4, which is expressed by the malignant population in Sézary syndrome. The absence of CD26 leads to elevated levels of SDF-1, which in turn, contributes to the trafficking of malignant cells to skin (see Fig. 41.2b).

A CD26 negative population of greater than 30 % among erythrodermic patients is believed to be sufficient to support a diagnosis of Sézary syndrome, and thus differentiate such patients from individuals suffering from benign inflammatory dermatoses [16]. We have recently reported a correlation between the percentage of circulating CD4 + CD26- T-cells and changes in clinical status among Sézary syndrome patients. More specifically, a reduction in this population typically heralds improved clinical status, whereas, an increase frequently precedes disease progression characterized by a greater than 20 % increase in affected body surface area, or a greater than 10 % increase in body surface area accompanied by progressive lymphadenopathy and/or hepatosplenomegaly [53].

While chemokine receptors are necessary for tissue-specific lymphocyte trafficking to the skin, expression of their chemokine ligands plays an essential role in this process. In the absence of chemokines, engagement and activation of the chemokine receptor will not occur. The epidermotropism exhibited by neoplastic cells in CTCL has been attributed, in part, to the production of chemokines by epidermal cells. Whether their production arises secondary to the accumulation of malignant cells in the skin or precedes the infiltration has not be en established.

Elevated levels of the chemokine, cutaneous T-cell attracting chemokine (CTACK/CCL27), has recently been reported in the serum of patients with CTCL. The highest levels were detected among patients with more advanced disease (tumor stage and erythroderma), and improved in response to successful treatment [54]. CTACK has been shown to be produced by epidermal keratinocytes and is presented on the surface of dermal endothelial cells [46, 55]. It is recognized by the chemokine receptor, CCR10, which, as mentioned previously, is expressed by the malignant population [56].

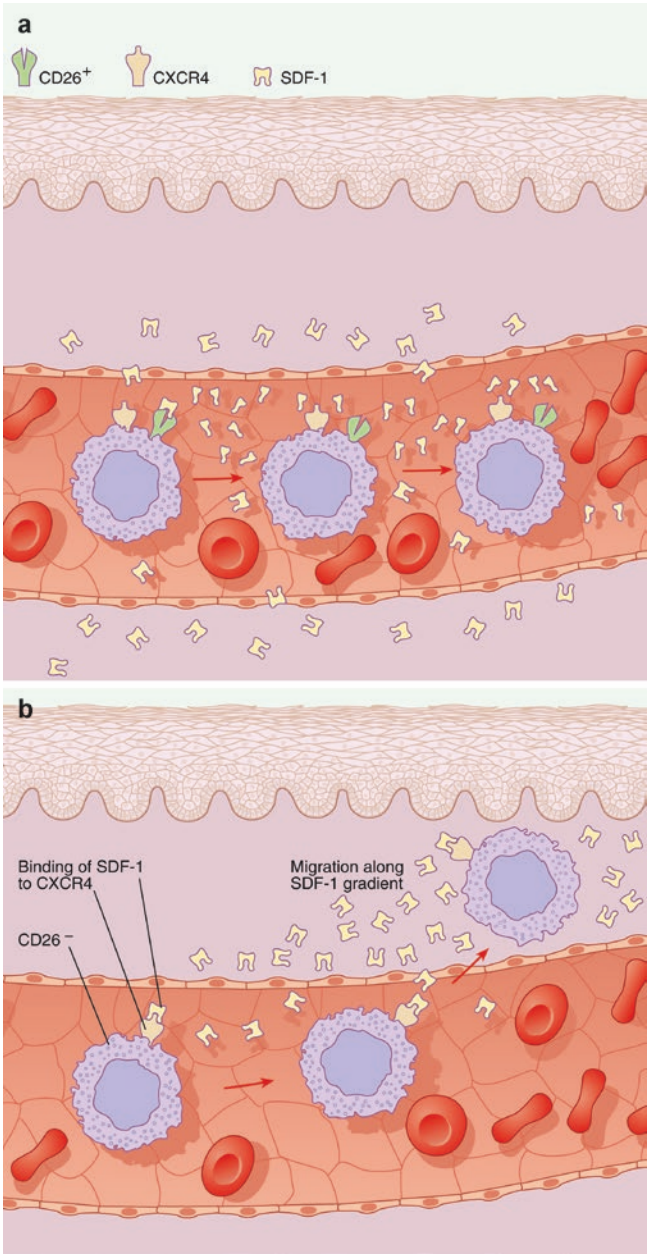

Fig. 41.2 (**a**) CD26 is a bipeptidyl transferase expressed by normal T lymphocytes that induces the cleavage and inactivation of the chemokine stromal-derived factor-1 (SDF-1). This cleavage prevents the binding of SDF-1 to its receptor, CXCR4, thus interfering with SDF-1/CXCR4-mediated chemotaxis into the skin. CD26+ T cells are resistant to the chemotactic effects of SDF-1 via CD26-mediated inactivation (*top*). (**b**) The absence of CD26 is reported among circulating malignant cells in Sézary syndrome. In the absence of CD26, SDF-1 may efficiently establish a chemotactic gradient that promotes the migration of malignant cells into the skin via engagement of CXCR4. Engagement of CXCR4 by SDF-1 in the cutaneous microvasculature and subsequent chemotaxis of the cell into the dermal microenvironment are demonstrated (*top*)

Thymus and activation-regulated chemokine (TARC/CCL17), the ligand for CCR4, was shown to be elevated in CTCL patients relative to normal individuals and patients with psoriasis vulgaris [57]. More specifically, an increase in serum TARC levels was shown to correlate with more advanced disease, being greatest among tumor stage patients as compared to patch and plaque stage patients. Immunohistochemistry revealed TARC expression by lesional keratinocytes at all stages of disease. These findings support a functional role for CCR4 in the accumulation of malignant cells in the skin of CTCL patients.

Preferential expression of mRNA for the chemokine interferon-gamma inducible protein 10 (IP-10) has been reported in the epidermis of patients with early stage epidermotropic CTCL [58]. IP-10 is the ligand for CXCR3, which is expressed by MF cells in early patch/plaque stage disease [45]. CXCR3 and its ligand, IP-10, are considered to be Th1 associated molecules given their ability to recruit Th1 cells to sites of inflammation. Thus, levels of IP-10 and CXCR3 positive cells may reflect the host anti-tumor response. Patients with more advanced disease exhibit a depression in CXCR3 levels, whereas, expression of the Th2 associated chemokine-receptor, CCR4, and its ligand (TARC), remain elevated and skew the cutaneous cytokine milieu in favor of Th2 cytokine production, which likely contributes towards the pathogenesis of disease in CTCL.

Once the malignant T-cells have been recruited to the skin, their growth appears to be supported by the in situ production of cutaneous cytokines. Yamanaka and colleagues have demonstrated that lesional skin of CTCL patients over produces interleukin-7 [59]. IL-7 itself clearly exhibits pro-proliferative effects on malignant T-cells derived from CTCL patients.

Chromosomal and Genetic Abnormalities

MF/SS is not considered a primary genetic disorder attributable to discrete mutation(s), however with disease progression, several genetic abnormalities have been detected. Cytogenetic studies have demonstrated chromosomal deletions (1p, 17p, 10q, 19) and gains (4q, 18, 17q) [60]. Even early MF lesions have been shown to have STAT 3 constitutively activated or defects in their apoptosis pathways (decreased Fas expression) [61–63]. In more advanced disease, such as tumor-stage MF or SS, other genetic perturbations include p15/16 and p53 defects, microsatellite instability, hypermethylation of tumor suppressor genes (p14, p15, p16, BCL7a, PTPRG, p73) [64, 65], and constitutive activation of NF-kB [66]. In addition to these, epigenetic factors (which affect gene expression through modification of histones and other chromatin-associated proteins) appear to play a role in CTCL development/progression, given the clinical activity of histone deacetylase inhibitors (HDACi) in CTCL [67].

MF/SS malignant T-cells also can variably express the interleukin-2 receptor (IL-2R) that binds the cytokine IL-2. IL-2R is comprised of 3 subunits: IL-2Rα (CD25/p55), IL-2Rβ (CD122/p75) and IL-2Rγ (CD132/p64). IL-2Rα has been viewed as a marker of cell activation and is significantly expressed in approximately 20 % of MF/SS patients, particularly in later stage disease [68], and soluble IL-2R can be detected in the peripheral blood [69]. The T-cells can also express other activation markers such as CD45RO and/or proliferating-cell nuclear antigen (PCNA).

Immunobiology

Immune Dysregulation in CTCL: Clues to Understanding Disease Progression

It has long been known that patients with advanced MF/SS typically exhibit abnormalities in a variety of parameters of cell-mediated immunity. Moreover, the malignant T-cells have been implicated as the cause of the endogenous immune deficiency. Earlier observations by Vowels and colleagues demonstrated that the malignant $CD4^+$ T cells observed in most cases of MF/SS appear to exhibit a Th2 phenotype (Fig. 41.3). In vitro stimulation of peripheral blood cells derived from SS patients routinely results in increased levels of measurable IL-4 expression [70]. Furthermore, Vowels et al. demonstrated levels of IL-4 and IL-5 mRNA in clinically involved skin, even among patients with early patches or plaques while uninvolved skin and the skin of normal volunteers did not have detectable levels of Th2 cytokine mRNA [71]. Increasing levels of IL-10 mRNA in parallel with an increasing density of the malignant T cell infiltrate as lesions progressed from patch to plaque to tumor has also been demonstrated [72]. cDNA microarray analysis of the malignant T-cells isolated from patients with SS have shown that the Th2 cell–specific transcription factors such as GATA-3 and Jun B are highly overexpressed [38]. Thus, despite evidence of a vigorous host response in skin lesions in early disease, characterized by the presence of IFN-γ–secreting $CD8^+$/TiA-1^+ T cells [73], the chronic production of Th2 cytokines such IL-4, IL-5 and IL-10 by the malignant T cell population likely represents one mechanism by which the tumor cells circumvent the anti-tumor immune response.

The host anti-tumor response, although effete, may play a role, albeit small, in containment of disease progression, even among those with advanced disease. This is supported by the observation of rapid progression of SS associated with the use of immunosuppressive agents such as cyclosporine [74]. In addition to enhanced Th2 cytokine production during disease progression, patients with circulating malignant

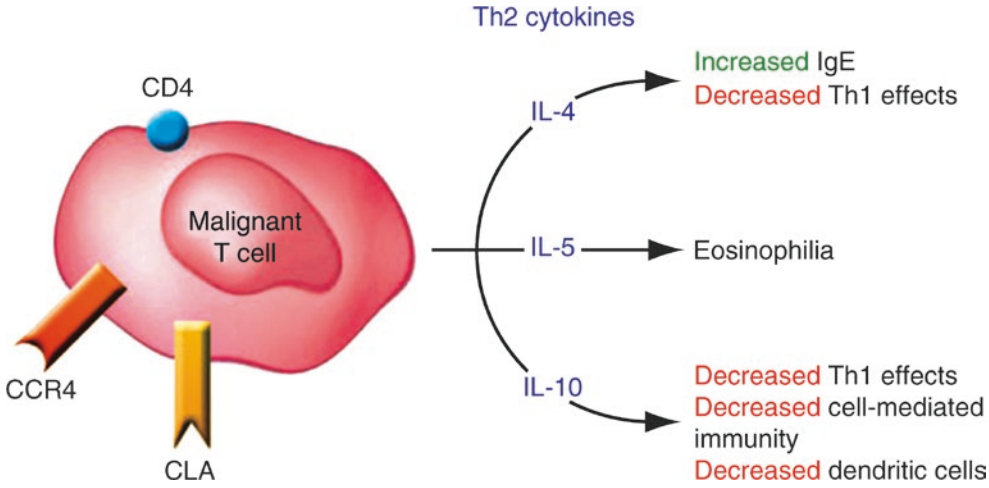

Fig. 41.3 The malignant T cell in MF/SS expresses CD4/CLA/CCR4/CCR10 and produces the cytokines IL-4/IL-5/IL-10, which results in Th2 predominance and subsequent multiple abnormalities in cellular immunity (From Kim et al. [5] with permission of the American Society for Clinical Investigation. Copyright 2005)

T cells also manifest defects in Th1 cytokine production. Wysocka et al. observed a progressive decline in the production of IL-12 and IFN-α by peripheral blood cells that correlated with an increase in the peripheral blood burden of malignant T cells [75]. Furthermore, the decline in production of these cytokines appeared to be linked to a decline in the numbers of peripheral blood myeloid and plasmacytoid DCs, respectively. Accompanying the decrease in DCs and a defect in IL-12 production is a deficit in production of other products of myeloid DCs, including IL-15 which is an important IFN−γ–stimulating agent and powerful booster of Th1 responses. In contrast, Yamanaka et al., observed increased serum IL-18 and IL-18 mRNA within CTCL skin samples suggesting alternative potential sources for this interferon inducing cytokine [76] Nevertheless, peripheral blood cells of SS patients clearly manifest marked decreases in IFN-γ production [70].

Intense recent interest has focused on the possible contributory role of regulatory T-cells (Tregs) in the endogenous immunosuppression in advanced CTCL. Berger and colleagues have demonstrated that under certain *in vitro* conditions that malignant T-cells may assume properties of Tregs, including the expression of Foxp3 and production of TGF-β and IL-10 [77]. Similarly, Walsh et al., was able to demonstrate increased Foxp3 expression among the peripheral blood cells of some patients with SS and among the circulating cells of all patients with HTLV-1 associated CTCL [78]. The cells of such patients were found to produce increased concentrations of TGF-β. Wong et al. has observed increased expression of CTLA-4 upon activation of the circulating cells of patients with CTCL, indicating that this molecule, which is typically associated with Tregs, was upregulated and a sign of increased numbers of Treg cells [79]. These findings remain controversial as Tiemessen observed that the Foxp3 positive Tregs of some CTCL patients are dysfunctional and exhibit a reduced ability to inhibit the proliferation of CD4+/CD25- T-cells [80] while Yamano and colleagues have made similar observations in regard to HTLV-1 infected CD4+ cells [81]. Despite these disparate findings, many investigators involved in studies of the immunobiology of CTCL feel that Tregs likely play some role in the pathogenesis of the immune suppression. Thus, as discussed below, elimination of Tregs is just one of several new therapeutic strategies under investigation.

Other mechanisms that could account for abnormalities in DC maturation, and thus, for diminished IL-12 production have been highlighted by recent studies by French et al., which demonstrated a defect in expression of CD40 ligand upon activation of malignant T cells derived from patients with SS [82]. CD40 ligand is not expressed on resting T cells but is normally upregulated on the cell surface upon engagement of the TCR. By contrast, malignant CD+/CD7- T cells fail to express CD40 ligand upon engagement of the TCR by anti-CD3. Clearly, the absence of CD40 ligand interaction with CD40 on APCs during an immune response can lead to a profound reduction in DC activation and cytokine production. Through the in vitro addition of recombinant hexameric CD40 ligand, French and colleagues demonstrated reconstitution of IL-12 and TNF production by the cells of patients with SS. These findings provide obvious insights regarding potential strategies for correcting defects in immune dysregulation related to diminished numbers and function of DCs associated with advancing MF/SS.

Derangements in T-cell diversity may also play a role in the immune suppression of CTCL. Yawalkar et al., using beta-variable complementarity-determining region 3 spectratyping determined that patients with both early as well as late disease exhibit loss of the normal T-cell repertoire [83, 84]. These findings are reminiscent of a chronic retroviral infection as similar observations have been made in HIV infection. Loss of T-cell repertoire may partially account for the failure of advanced stage CTCL patients to respond normally to a variety of antigens.

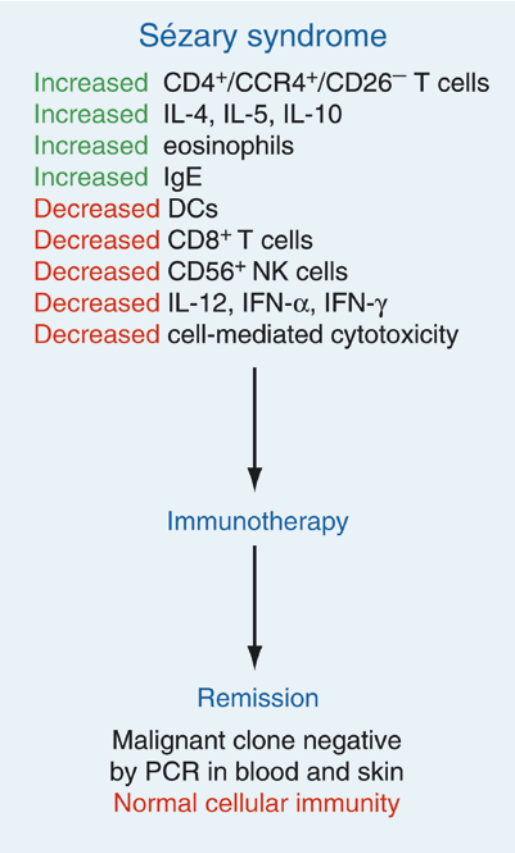

Fig. 41.4 Elimination of the malignant T-cell clone during immunotherapy leads to a restoration of a normal immune response. Studies of numerous patients with SS have demonstrated that induction of complete remission with clearing of the malignant T-cell clone during multimodality immunotherapy leads to a restoration of normal host immune function (From Kim et al. [5] with permission of the American Society for Clinical Investigation. Copyright 2005)

The result of the diverse abnormalities of DC and T-cell function during progressive MF/SS are multiple abnormalities in cellular immunity (Fig. 41.4). Marked defects in cellular cytotoxicity occur that correlate with the burden of circulating malignant T cells [85]. Progression from early to more advanced MF/SS is typically associated with a marked decline in NK cell numbers and activity which could partially be due to a decline in myeloid DC production of IL-15 which is critical for the normal growth of these cells. Similarly, a decline in the number of peripheral blood $CD8^+$ T cells accompanies an increasing burden of circulating malignant T cells. Furthermore, the percentage of these cytotoxic cells that express activation markers, such as CD69, is also significantly reduced compared to NK and $CD8^+$ T cells from MF patients without overt peripheral blood involvement [86]. A reduction in the number of functioning NK and $CD8^+$ T cells is almost certainly associated with a deterioration of both host anti-tumor immunity and immune surveillance against microbial organisms. Examples of these phenomena include infections such as disseminated herpes simplex/zoster or progressive multifocal leukoencephalopathy among SS patients who have never been iatrogenically immunosuppressed by chemotherapy or other immunosuppressive medications [8, 87, 88]. As mentioned earlier, other malignant neoplasms, including melanoma and non-melanoma skin cancers as well as Hodgkin's lymphoma, also appear to be more common in MF/SS patients independent of the history of previous predisposing therapy (such as phototherapy or radiation therapy) [10, 89, 90]. These findings reflect an overall impairment in immune surveillance against cancer.

Other characteristic immunological findings associated with the progression of MF/SS include development of peripheral eosinophilia and elevated levels of serum IgE [70, 91]. Peripheral eosinophilia has been determined to be an independent marker for poor prognosis and disease progression [92]. In one study, peripheral blood cells from patients with SS and eosinophilia produced markedly higher levels of IL-5 upon stimulation than did cells of patients or normal volunteers without eosinophilia [93]. A finding of importance in this study indicated that culture of the patients' cells with either recombinant IFN-α or IL-12 significantly inhibited the excess production of IL-5. These observations suggest that these cytokines could be useful therapeutic tools to prevent continued proliferation of eosinophils under the influence of IL-5, thus, possibly preventing at least some of the adverse effects associated with high eosinophil counts.

It is particularly noteworthy that after successful treatment of SS with immunotherapeutic agents, with resolution of evidence for skin disease and disappearance of the malignant clone from the blood, virtually all abnormal immune parameters are restored to normalcy (Fig. 41.4) [85]. One implication of these observations is that the malignant clone is likely responsible for much of the immune dysregulation that occurs in SS. Moreover, these findings indicate that SS patients who experience remission will have their immune system at least partially reconstituted. Thus, such patients should be less likely to experience severe consequences of microbial infection in comparison to those with advanced SS.

Treatment

Treatment of MF/SS can be divided into 2 major categories: skin directed therapies (SDTs) and systemic therapies. As listed in Table 41.4, there are numerous options. Therapeutic approaches can vary widely from center to center, and the availability of many newer therapies varies among countries. It is useful to divide the discussion of therapies in regard to early stage vs. late stage disease (Table 41.5).

Table 41.4 Treatment options in MF/SS

Skin directed therapy	Mechanism of action
Topical corticosteroids	Tumor cell apoptosis, decrease skin LCs
Topical chemotherapy (nitrogen mustard, BCNU)	Tumor cell apoptosis
Topical retinoids (bexarotene, tazarotene)	Tumor cell apoptosis
Topical imiquimod	TLR7 agonist, triggers innate & adaptive anti-tumor immunity
Phototherapy (UVB, PUVA, excimer laser)	Tumor cell apoptosis Decrease skin LCs
Electron beam therapy (EBT)	Tumor cell apoptosis
Biological therapy	
RXR retinoid (bexarotene)	Tumor cell apoptosis Inhibit tumor cell IL-4 production
RAR retinoid (isotretinoin, all-trans retinoic acid)	Tumor cell apoptosis Induce interferon-γ
Interferons (alpha, gamma)	Enhanced cell-mediated cytotoxicity Inhibit tumor cell Th2 cytokine production
GM-CSF	Enhanced circulating dendritic cell numbers and function
Extracorporeal photopheresis	Circulating tumor cell apoptosis Induction of DC differentiation
Fusion protein/toxin (denileukin diftitox[a])	Targets and kills CD25 (IL2 receptor) expressing tumor cells.
Histone deacetylase inhibitors (vorinostat, romidepsin)	Affects tumor cell gene transcription, non-histone effects.
Other systemic therapy	
Cytotoxic chemotherapy (MTX, pegylated doxorubicin, gemcitabine, etoposide, pentostatin)	Cytotoxic agents
Hematopoeitic stem cell transplantation (allogeneic)	Cytotoxic agents (induction)
	Graft-vs-tumor effect
Experimental therapy	
Transimmunization ECP	Enhances DCs antigen processing of apoptotic tumor cells
Targeted monoclonal antibodies (CD4, CD52, CD40, CCR4)	Targets tumor cells (CD4, CD52, CCR4) Activated dendritic cells (CD40)
Cytokines (IL12, IL2, IL15)	Augments cell mediated anti-tumor immunity
Toll-like receptor agonists (CpG-ODN, imidazoquinolones)	TLR agonists, augment innate and adaptive anti-tumor immunity
Tumor vaccines	Clonotypic TCR as antigen Dendritic cell based vaccines

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[a]Currently not available commercially

Early MF

It has become quite clear during the past several decades that traditional systemic chemotherapy has not resulted in durable remissions in MF/SS [1]. As a consequence, emerging therapeutic efforts have focused upon targeted biological agents and immune modifications using a multimodality approach. Numerous arms of the immune system must cooperate to generate a sufficient host anti-tumor response such that the proliferation of the malignant T cell population in MF/SS patients can be controlled and, ideally, eradicated (Fig. 41.5).

Patients with patches or plaques limited to less than 10% skin surface area (stage IA or T1 disease) tend to exhibit normal cellular immune responses. Therefore, use of skin-directed therapies, including topical chemotherapy [94, 95], superpotent topical corticosteroids, topical retinoid application [96], psoralen plus ultraviolet A phototherapy (PUVA) [97, 98], or electron beam radiation therapy [99], which target the tumor burden in the skin by directly inducing apoptosis of malignant T cells, is often sufficient to induce complete clearing of disease. At this stage, the systemic immune response is intact and it may contribute to controlling disease burden from going beyond the skin. In the event that clearing is not complete, the addition of a single agent systemic immunomodulator, such as recombinant IFN-α or the retinoid bexarotene (Targretin (R)), typically leads to a better clinical response.

For patients who do not yet manifest overt peripheral blood disease but who exhibit more extensively infiltrated cutaneous plaques or a greater extent of skin surface area involvement, multimodality therapeutic approaches appear to result in more rapid responses [100–103]. IFN-α, produced by plasmacytoid DCs, is a product of the innate

Table 41.5 Treatment of MF/SS by clinical stage

Stage	Initial therapy	Subsequent therapy	Therapy for refractory disease
IA (early stage)	Skin directed therapies		
IB/IIA	Topical chemotherapy Phototherapy Electron beam therapy (± low dose biological agents)	Interferons Retinoids Multimodality rx Topical chemo + biological agent Phototherapy + biological agent 2 biological agents Denileukin diftitox[a] Histone deacetylase inhibitors	Experimental therapies
IIB (advanced stage)	Few tumors Localized EBT Intralesional IFN Topical chemotherapy + biological agent Generalized tumors Total skin EBT Denileukin diftitox Multimodality therapies	Multimodality therapy Denileukin diftitox Histone deacetylase inhibitors Single agent chemotherapy	Experimental therapies
IIIA, B	PUVA Retinoids Interferons Methotrexate ECP Histone deacetylase inhibitors Multimodality therapies	Multimodality therapies Denileukin diftitox Histone deacetylase inhibitors Single agent chemotherapy	Experimental therapies
IVA, B	Single agent systemic therapy Multimodality therapy (+skin directed therapy)	Adjuvant palliative local radiation for extracutaneous disease Bone Marrow/Stem Cell Transplant Chemotherapy	Experimental therapies

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[a]Currently not available commercially

immune response, and appears to be one of the most highly active biologic agents used in the therapy of MF/SS [104]. It is now well accepted that PUVA plus IFN-α administration can produce higher clinical response rates than the use of PUVA alone [103, 105, 106]. Several small studies have also suggested that a combination of IFN-α with oral retinoids, including bexarotene or 13-cis retinoic acid, may induce rapid responses among patients with extensive skin lesions [100, 107].

Mechanistically, IFN-α induces a variety of beneficial effects on the host immune response that may lead to disease clearing. IFN-α directly enhances cell-mediated cytotoxicity; both $CD8^+$ T cells and CD56+ NK cells exhibit rapid activation as assessed by upregulation of CD69, and the cytotoxic effects of NK cells are significantly boosted [86]. IFN-α also suppresses Th2 cytokine production by malignant T cells, which may lead to abrogation of the suppressive effects of IL-4 and IL-10 on antitumor immunity. Recently Wood and colleagues demonstrated that methotrexate and IFN-α synergistically upregulates Fas expression on CTCL cells (methotrexate demethylates the Fas promoter and IFN-α increases Fas levels via STAT1) resulting in enhanced tumor cell apoptosis [108]. This is one potential mechanism to explain the clinical efficacy of this combination [109].

Bexarotene, a recently FDA approved retinoid X receptor–specific compound has also been determined to have valuable immunomodulatory effects that are of benefit in the treatment of MF/SS, particularly when used in combination therapy. Bexarotene has the ability to induce apoptosis within the malignant population of T cells [110, 111]. This effect may account for the nearly 50 % response rate of MF/SS patients when high-dose, single-agent oral bexarotene is administered [112]. Nevertheless, Budgin et al. have demonstrated that although the malignant cells of most patients with SS are susceptible to the apoptotic effects of bexarotene in vitro, purified Sézary cells from approximately one-third of patients demonstrate significant resistance to apoptosis [111]. This finding may account for the failure of a subset of patients to respond clinically to this compound. Furthermore, recent observations suggest that chronic bexarotene use may be associated with emergence of malignant clones of T-cells that lack the specific RXR-responsive receptor and which exhibit resistance to the apoptotic effects of this retinoid [113]. In regard to cytokine production, bexarotene has the capacity to inhibit IL-4 production, and

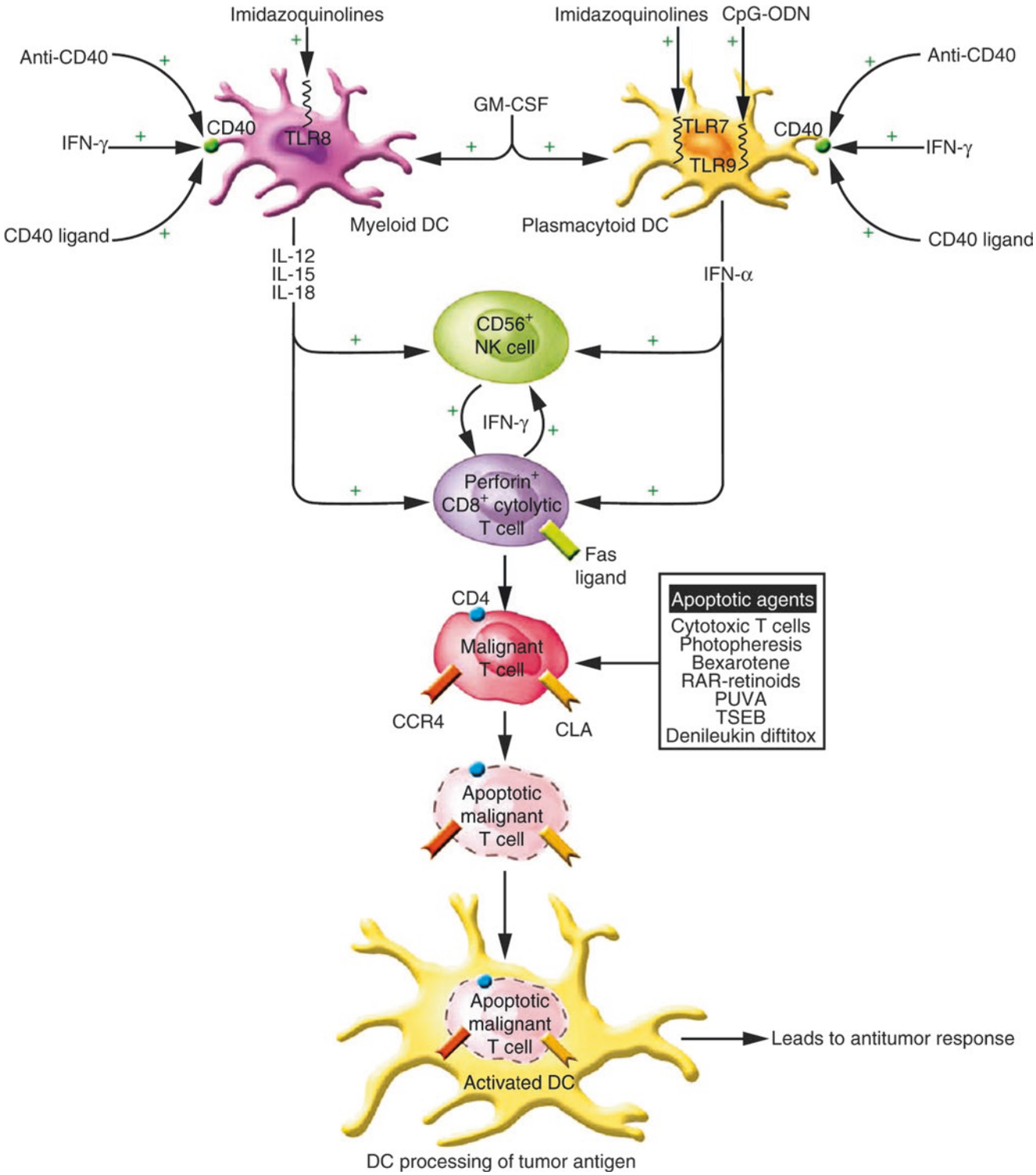

Fig. 41.5 Multimodality strategy for enhancing the antitumor response using immunotherapeutics. A multimodality approach encompasses the activation of multiple arms of the immune response through the use of agents to activate DCs, CTLs (CD8+), and NK cells (CD56+). Granulocyte-macrophage colony-stimulating factor (GM-CSF) may enhance the numbers of DCs while agents that enhance CD40 expression (IFN-γ) and activation (CD40 ligand; activating anti-CD40 antibody) and TLR ligands (CpG-ODNs; imidazoquinolines) lead to DC cytokine production and to enhanced DC processing of apoptotic malignant T cells. Cytokines produced by DCs as well as exogenously administered cytokines augment CD8+ T-cell cytokine production. Proapoptotic agents, including bexarotene, RARspecific retinoids, PUVA, photopheresis, topical chemotherapy, total skin electron beam irradiation (TSEB), and denileukin diftitox can assist in the development of an antitumor immune response by reducing the overall tumor burden and by providing a source of apoptotic malignant cells and tumor antigens for uptake by DCs. (From Kim et al. [5] with permission of the American Society for Clinical Investigation. Copyright 2005)

possibly other Th2 cytokines, in vitro by stimulated peripheral blood cells of SS patients. The net effect would be to negate the suppressive effects of these cytokines on cellular immunity.

In contrast to bexarotene, retinoic acid receptor (RAR)-specific retinoids may have modest direct immune potentiating properties. Using a number of different RAR-specific retinoids, including all-*trans*-retinoic acid and 13-*cis*-retinoic acid, Fox et al., demonstrated that these compounds exhibited the capacity to induce IL-12–dependent IFN-γ production [114]. Moreover, synergistic production of IFN-γ occurred when low concentrations of IL-2 were added to the RAR-specific retinoids. In contrast, bexarotene does not induce IFN-γ production [111]. These findings support the use of an RAR-specific retinoid as another component of the combined therapeutic approach.

Toll-like receptors (TLR) are a family of specialized immune receptors that induce protective immune responses when they detect highly conserved pathogen-expressed molecules. The major populations of human dendritic cells include myeloid dendritic cells (mDCs) which express TLR8 and plasmacytoid dendritic cells (pDCs) which express TLRs 7 and 9. Over the past decade, TLR agonists have been used to stimulate an antitumor immune response by activating and bridging the innate and adaptive immunity [115]. Substantial data has now emerged to suggest that TLR agonists may have a significant role in the therapy of MF/SS.

Wysocka and colleagues have demonstrated TLR 7, 8, and 9 have the capacity to potently activate innate immune cells of patients with advanced, refractory CTCL and that these effects can be synergistically enhanced in vitro by addition of IFNγ or IL-15 [116].

Imiquimod, a member of the imidazoquinoline family, which is FDA approved to treat basal cell carcinoma, actinic keratoses, and condyloma, can induce local immune activation that can be associated with lesion regression when applied as a cream directly to CTCL skin lesions [117, 118]. Imiquimod potently triggers TLR7, expressed on pDCs, which results in IFN-α and TNF-α production [119, 120]. Imiquimod may also directly induce cells of some tumor types to undergo apoptosis [121] and is currently listed as a standard of care skin directed therapy in current National Comprehensive Cancer Network clinical practice guidelines for MF/SS. However, it has a low bioavailability leading to unreliable clinical responses, which are dependent on numbers of resident pDCs within lesions that can be activated by imiquimod. Numerous factors can lead to diminished numbers and function of pDCs in the skin of CTCL patients including the use of potent topical steroids and administration of ultraviolet light, which can induce apoptosis of dendritic cells. In addition, application of topical tacrolimus may inhibit the ability of resident pDCs to further activate surrounding normal T cells which are necessary for the induction of an adaptive immune response against the tumor cells [122].

Advanced MF/SS

Current Therapies

In contrast to patients with early MF, patients with advanced MF/SS exhibit a broad array of abnormalities affecting every arm of the immune response that participates in anti-tumor immunity ($CD8^+$ T cells, NK cells, and DCs). Thus, more aggressive therapy is required at these later stages of MF/SS to adequately restore the host immune response. Accordingly, evidence is now emerging indicating that multimodality immunotherapy can frequently induce complete clinical responses in advanced disease that are both durable and sufficient to eradicate the malignant clone [85, 123, 124].

Central to the strategy for elimination of the malignant T cell population is the use of agents that can induce apoptosis of these cells while simultaneously enhancing the host's ability to process the apoptotic cells so that a robust cytotoxic T cell response can be generated. For patients with circulating malignant T cells, extracorporeal photopheresis (ECP) can result in massive apoptosis of cells within this compartment [125, 126]. ECP is a leukapheresis procedure FDA approved for the treatment of SS in which approximately 10^{10} peripheral blood mononuclear cells are collected from the patient, treated with 8-methoxypsoralen, exposed to 1–2 J of ultraviolet A light in the photopheresis machine, and re-infused back to the patient. In addition to inducing malignant T-cell apoptosis, ECP also induces monocyte differentiation into DCs capable of phagocytosing and processing the apoptotic tumor cell antigens [127]. Repeated cycles of ECP for 2 consecutive days every 3–4 weeks with re-administration of the treated cells is occasionally sufficient to induce a complete clinical response in SS. One potential modification to ECP called transimmunization involves prolonged co-incubation of the apoptotic malignant T-cells and the newly formed DCs prior to reinfusion to optimize the above antigen processing and more efficient induction of tumor targeted immunity [127].

Nevertheless, administration of large numbers of apoptotic cells as generated by ECP can compromise dendritic cell functions, including cytokine production, and therefore has the potential to exacerbate the pre-existing immune depressed state [128]. Such observations support the rationale for the adjunctive use of multiple agents that can enhance both the afferent immune response (events related to processing of apoptotic malignant cells) as well as the efferent response (direct cytolytic attack on the tumor cells).

In support of this approach, our group at Penn has recently demonstrated high response rates of SS patients when ECP was combined with the administration of multiple immune

adjuvants [123, 129]. As part of this regimen, IFN–α and bexarotene were routinely used in combination with ECP. In some cases, GM-CSF was administered following each ECP treatment to enhance APC function. The monthly administration of 125 μg of GM-CSF on 2 consecutive days resulted in significant increases in circulating DC numbers compared to DC numbers in ECP-treated SS patients who did not received GM-CSF [75]. In one patient, administration of GM-CSF three times per week for 6 months resulted in the persistent normalization of DC numbers indicating that APC functions might be markedly augmented in this patient population with the long-term use of GM-CSF.

In addition to IFN-α, additional approaches to enhance the effector phase of anti-tumor immunity are presently being utilized. Although limited results have been reported following the administration of recombinant IFN-γ, recent evidence suggests that it has significant potential for the treatment of MF/SS and it is clearly well tolerated, particularly by the elderly who experience frequent cognitive dysfunction and fatigue with IFN-α treatment [130–132]. In addition to enhancing the cytolytic lymphocyte function of patients with MF/SS, IFN-γ suppresses the excess Th2 production, enhances CD40 expression, and primes their abnormal DCs for IL-12 and IL-15 production, particularly in response to CD40 ligation [75] and Toll like receptor agonistic stimulation [133]. In our Cutaneous Lymphoma Program at the University of Pennsylvania, when possible we are presently routinely using IFN-γ for SS patients who appear to be refractory to IFN-α. In several cases, addition of IFN-γ to a multimodality regimen that included photopheresis and bexarotene appeared to be associated with the induction of a sustained complete clinical response [131, 132].

Alternative routes exist for the administration of IFN-γ. In a small pilot study, Dummer and colleagues demonstrated clinical efficacy of IFN-γ cDNA administered subcutaneously in an adenoviral vector [134]. The local intralesional injection resulted in significant responses rates of individual lesions in both MF and SS patients. Moreover, elevated serum levels of IFN-γ were observed and appeared to be associated with regression of lesions distant from the injection sites. This study further suggests that elevation of IFN–γ levels can beneficially alter disease progression.

Removal of the Immunological Brake: Elimination of Treg Activity

As indicated above, increasing evidence exists for the role of enhanced $CD4^+CD25^+$ Treg function in CTCL as a contributory mechanism for depressed cellular immunity [77, 78]. Although strategies for the elimination of suppressor activity in MF/SS remain untested, several potential approaches are possible. Previously, denileukin diftitox (Ontak), a diptheria toxin–IL-2 protein conjugate, was available for targeting IL-2 receptor–bearing T cells but currently is unavailable [135]. After binding to the IL-2 receptor, it undergoes endocytosis followed by release of the diptheria toxin, which results in arrest of protein synthesis and, ultimately, apoptosis of T cells. Intravenous administration of denileukin diftitox to patients with MF results in the regression of plaques and tumors [136]. Its major mechanism of action is thought to be mediated by direct killing of malignant T cells. However, it is entirely possible that at least a portion of its activity is mediated through the elimination of CD25-bearing Tregs as suggested during recent studies of ovarian cancer treatment [137].

Because Tregs express CTLA-4, another potential approach to their inhibition is through the use of anti–CTLA-4 antibody [138]. This therapeutic approach, although promising for metastatic melanoma [139], remains untested so far for CTCL. Tregs may also suppress immunoreactivity by production of IL-10 or TGF-β. Antibodies with neutralizing activity for these factors could be utilized to reverse their inhibitory effects on the immune response.

Monoclonal Antibodies

Alemtuzumab

Alemtuzumab (Campath) is a humanized monoclonal antibody directed against CD52 expressed on malignant and benign T cells, B cells, monocytes, and dendritic cells. It is currently approved for the treatment of chronic lymphocytic leukemia, and several studies have investigated its efficacy in CTCL. In an open-label trial of alemtuzumab in 19 pretreated patients with advanced erythrodermic CTCL (i.e., erythrodermic MF and SS) with a median follow-up of 24 months, the overall response rate was 84 %, with 47 % complete and 37 % partial remissions [140]. Several studies have recently shown that alemtuzumab can preferentially induce significant clinical responses in SS but not MF patients [141, 142]. Based on these observations, Clark and colleagues have proposed that human cutaneous T_{CM} recirculate into the blood, whereas T_{EM} are a non-recirculating skin resident population, postulating that the skin resident T_{EM} cells escape alemtuzumab-induced cell-dependent cytotoxicity due to absence of potential cytotoxic effectors such as monocytes, neutrophils, and natural killer cells in the skin [141]. Interestingly, the authors also report a marked lack of cutaneous infections in low dose (10 mg subcutaneously three times weekly for at least 6 weeks) alemtuzumab-treated SS patients despite profound lymphopenia, suggesting that skin resident T_{EM} cells can protect the skin from pathogens even in the absence of T cell recruitment from the circulation.

In contrast, in a retrospective analysis of long-term efficacy and safety of higher dose alemtuzumab (30 mg two to three times weekly in the induction phase followed by 30 mg weekly during the maintenance phase) in 39 patients, De Masson and colleagues again report profound lymphopenia in the majority of patients during a median follow-up of 24 months, associated with a 62 % rate of infections including

cytomegalovirus viremia, tuberculosis, *Mycobacterium chelonae* cutaneous infection, bacterial infection, toxoplasmosis, aspergillosis, and unspecified pulmonary infection [142]. Additional adverse effects included grade 3 or higher cytopenias, acute coronary syndrome, ischemic colitis, deep-vein thrombosis, serum-sickness-like reaction, and infusion reactions. These adverse events led to treatment discontinuation in 44 % of cases and death in 5 % of cases. There was no significant difference in terms of effectiveness and safety between patients treated with subcutaneous and intravenous alemtuzumab. Additionally, Faguer et al. reported LCT in the skin of a patient with CTCL treated with alemtuzumab, suggesting a lack of immune surveillance associated with alemtuzumab-related lymphopenia [143]. Furthermore, in some cases, CD52 may become down-regulated on the malignant cells after alemtuzumab therapy [144], therefore a flow cytometric evaluation of CD52 expression may be considered before starting alemtuzumab treatment, and particularly before re-treatment with alemtuzumab after good initial response followed by relapse.

Brentuximab Vedotin

CD30 is a pro-survival receptor, which belongs to the tumor necrosis factor receptor superfamily [145]. Initially identified on Reed–Sternberg cells in Hodgkin's Lymphoma (HL), and later systemic ALCL, it is now known that CD30 is also expressed in other B cell and T cell NHLs, including CTCLs, such as primary cutaneous ALCL (pcALCL), LyP, and LCT MF.

Brentuximab vedotin (BV) is an antibody-drug conjugate of anti-CD30 monoclonal antibody and the proapoptotic antitubulin agent monomethyl auristatin. It has been approved as a second-line treatment for HL and ALCL, with investigation of its use in other malignancies, including CTCL currently ongoing. Results from a Phase II open-label trial of BV in CD30+ CTCLs (LyP, pcALCL, and CD30+ MF) showed a 100 % in LyP/pcALCL, and 44 % in CD30+ MF which varied based on degree of CD30 expression [146]. A Phase III trial in CD30+ CTCL is currently recruiting patients.

Several cases of progressive multifocal leukoencephalopathy (PML), a frequently fatal JC virus-induced central nervous system infection, have been reported in association with BV therapy [147]. Prior immunosuppressive therapy and compromised immune system function were the initial postulated risk factors; however, later reports described two CTCL patients treated with BV who had developed PML who had not previously received chemotherapy [148]. Therefore, the decision to administer BV in patients with CTCL should be based on consideration of risk-benefit profiles and alternative therapeutic options.

Histone Deacetylase Inhibitors

Histone deacetylase inhibitors (HDACi) represent a novel class of anticancer drugs, with two members of the group, vorinostat and romidepsin, approved for the therapy of CTCL. HDACi modulate chromatin structure has been shown to promote growth arrest, differentiation, and apoptosis of tumor cells [149]. While HDACi have been shown to have a significant response rate and a high rate of pruritus relief with vorinostat in particular in heavily pretreated, refractory CTCL patients [150], there is also emerging evidence for the potent immunosuppressive properties of HDACi. Studies have shown that HDACi can have therapeutic benefit in autoimmune disease models [151–153] perhaps owing to the enhancement of regulatory T-cell functions [154]. Moreover, we have previously reported a patient with CTCL with refractory bullous pemphigoid, who experienced rapid resolution of her autoimmune disorder following initiation of therapy with vorinostat [155]. Furthermore, *in vitro* studies have shown anti-inflammatory properties of HDACi via suppression of cytokines such as TNF-α and IL-1β [156]. Our group has reported significant blunting of functional NK activity in a SS patient treated with vorinostat, as well as potently suppression of multiple arms of the immune system by vorinostat in vitro [157]. Kelly-Sell and colleagues serially examined the cellular immune function of eight CTCL patients undergoing treatment with romidepsin, showing similarly decreased NK cell cytolytic activity and DC activation, as well as increased specificity for romidepsin induced CD4+ tumor cell apoptosis and dose dependent increases in cellular apoptosis of healthy cells in multiple lineages [158].

These findings have raised concern that HDACi may suppress immune function in CTCL patients, may lead to greater susceptibility to opportunistic infections [159], and support the concurrent use of multiple immune stimulatory agents to preserve the host immune response. In particular, addition of interferon gamma to HDACi has been reported to be a safe, well tolerated, and successful combination in several case reports [160, 161]. The proposed mechanism of action supporting use of this combination therapy involves stabilization of Th1 cytokine profiles by interferon gamma by enhancing cytotoxic T-cell activity and promoting a more robust antitumor response to counteract the immunosuppressive effects of HDACi. As attempts at preservation of cellular immunity are critical in the management of CTCL, the recent data highlight the complexity of the effects of HDACi and the need to balance their anti-tumor effects and immunosuppressive capabilities.

Experimental Therapy

Resiquimod, which activates both TLR7 expressed on pDCs, and TLR8 expressed on mDCs, is a promising member of the imidazoquinoline family which is currently in a clinical trial for therapy of CTCL. Its activity results in production of a more extensive array of immune activating cytokines, including IL-12, 15, 18, and IFN-α. Because Tregs also express TLR8, activation of this receptor can lead to inhibition of these suppressive cells, resulting in further immune augmen-

tation. Additionally, as the bioavailability of resiquimod as a topical gel is tenfold greater than that of imiquimod cream, and its potency may be up to 100 times higher, it may have great enough efficacy to induce systemic immune activation after cutaneous application, to potentially be used as a single agent in treatment of patch to plaque stage lesions of CTCL. Use in combination with IFNγ with which it can synergize to broadly activate Th1 type cellular immunity might be particularly efficacious.

Additionally, recent clinical trials have continued to test the effects of an alternative class of DC activating agents, synthetic oligodeoxynucleotides that contain CpG motifs (CpG-ODNs). CpG-ODNs have been recognized as immune stimulatory agents through their activation of DCs following binding to TLR9 expressed on plasmacytoid DCs [162]. The immunostimulatory potential of CpG-ODNs has been tested in murine tumor models and has been observed to lead to the generation of strong antitumor T cell responses resulting in complete remission of certain established solid tumors [163]. Thus, there is substantial rationale to study the activity of CpG-ODNs in human tumor systems. In this regard, in vitro data indicate that CpG-ODNs can potently activate CTCL patients' DCs, leading to IFN-α production, increased expression of critical immune accessory molecules, and enhanced cell-mediated cytotoxicity [86]. Moreover, Kim and colleagues have shown in a phase I clinical trial for refractory advanced CTCL that CpG-ODNs administered subcutaneously as a single agent led to significant clinical responses in 32% of patients, with three patients having complete responses. The majority of responses were maintained long after study conclusion [164]. A subsequent trial by the same authors showed promising results of intratumoral vaccination of intralesional, subtherapeutic dosing of CpG-ODNs combined with local radiation therapy in 15 patients with relapsed or refractory MF [165]. Following the administration of a previously demonstrated subtherapeutic dose of CpG, nonirradiated skin lesions regressed in one-third of patients, suggesting that CpGs prime the local APCs to be more efficient. It was also noted that skin lesions of responding patients demonstrated decreased numbers of CD25+ Foxp3+ T-regs cells. Therefore, application of CpG-ODNs in a multimodality therapeutic approach that uses photopheresis might also yield significant therapeutic benefit. It is noteworthy that anti-viral vaccination strategies that incorporate the use of CpG-ODNs along with viral antigen appear to be markedly superior to those that use antigen alone [166]. Since ECP represents an immunization procedure utilizing apoptotic tumor cells, such findings support the use of CpG-ODNs at the time of re-infusion of the treated tumor cells in an effort to directly target the tumor antigens to DCs for processing.

Other mechanisms for activating the DCs of patients with MF/SS that are under pre-clinical investigation include strategies for the engagement of CD40. As stressed above, there is substantial evidence that a defect in CD40 ligand expression by malignant T cells plays some role in the depressed production of DC-dependent cytokines [82]. Furthermore, co-culture of peripheral blood cells of SS patients with hexameric recombinant CD40 ligand resulted in substantial production of IL-12. Clinical trials using this approach have yet to be undertaken. Another strategy that appears promising for the treatment of solid tumors but which awaits clinical testing for CTCL, involves the use of an activating anti-CD40 antibody. Animal models using this approach indicate that enhanced generation of tumor-specific T cells can occur [167, 168].

IL-12 is a cytokine known to induce IFN-γ release and to enhance cytolytic T cell and NK cell activities, and thus has the potential to enhance the anti-tumor immune response of MF/SS patients. In a small phase I study followed by a limited phase II study, the subcutaneous administration of recombinant IL-12 to a total of 32 patients with MF resulted in approximately a 50% response rate [169, 170]. Since malignant T cells lack the IL-12 β2 receptor [171] and are thus incapable of signaling in response to IL-12, it is presumed that the clinical response was not due to the direct effects of the cytokine on the malignant cells. Indeed, serial biopsies of cutaneous plaques during treatment revealed dense infiltrates of $CD8^+$ T cells that appeared near the time of initial signs of lesional regression [169]. Thus, it is believed that $CD8^+$ T cells with augmented cytolytic activity are the predominant "work-horses" activated in response to IL-12. Whether IL-12 administration has advantages over IFN-γ use is presently unknown, but it is hoped that in the future, IL-12 will also find its place in a multimodality therapeutic approach. Currently it is being studied in a phase 2 clinical trial for refractory CTCL using IL-12 DNA delivered by electroporation (Oncosec).

Future Strategies for Enhancing the Host Immune Response

Tumor Vaccines

Most vaccination strategies for MF/SS utilize the clonotypic TCR as a source of tumor-specific antigen. Immunogenic epitopes are found within both the variable (V-region) and the constant (C-region) regions of the clonotypic TCR-α and TCR-β receptor [172, 173]. In some experiments, immunogenic peptides have been directly isolated from the MHC class I molecules on the surface of the malignant clone. This confirms that the antigen-processing pathway for endogenous proteins remains intact in the malignant clone, and that the clonotypic TCR is subjected to antigen processing and presentation by MHC class I. Consequently, the TCR peptide-MHC complex on the surface of a malignant cell can serve as a target for recognition by CD8+ CTL's. This has been demonstrated in both normal donors and patients with MF/SS, where immunogenic peptides derived from the clo-

notypic TCR induced tumor-specific CD8+ T-cells which were capable of secreting TNF-α [172], as well as lysing autologous tumor cells *in vitro* [173].

Dendritic-cell based vaccines have also been developed for CTCL [174]. Sources of antigen used to pulse DC's prior to vaccination may include tumor-cell lysates, peptides, "mimotopes," tumor-derived DNA or RNA, apoptotic tumor cells and even tumor cell-DC fusions. Maier et al. reported on ten CTCL patients treated with weekly intra-nodal injections of autologous DC's pulsed with tumor lysate [175]. In this study, 50 % of patients had a clinical response to the vaccine, accompanied by an infiltration of CD8+ and TIA+ cytotoxic cells at the site of regressing lesions as well as molecular remission in some cases. Of note, clinical responses in this study were associated with a low tumor burden, which underscores the importance of instituting immunotherapy early in the course of the disease and prior to the development of significant immune dysregulation [175].

The addition of an immune adjuvant(s) may be used in an attempt to enhance the efficacy of the vaccine. Cytokines such as IL-12 [169, 176], IL-15 [86, 177], IL-18 [178], and IL-21 [179], can augment the development, the effectiveness, and/or the maintenance of anti-tumor CTL responses. Moreover, these same cytokines have also been shown to enhance NK-cell activity which may play an important role in controlling tumor growth *in vivo*. GM-CSF is another cytokine which has been used as a cancer vaccine adjuvant to enhance both the number and function of dendritic cells [180, 181]. As discussed earlier, other immune activating agents including TLR agonists (i.e. imidazoquinolines, CPG-ODN's), anti-CD40, and anti-CTLA-4 could be used in conjunction with a tumor vaccine for patients with CTCL.

Programmed Cell Death 1

Programmed cell death 1 (PD-1) is an inhibitory receptor expressed by activated T cells. Engagement of PD-1, a member of the B7-CD28 family, by its ligands (PD-L1 and PD-L2) transduces a signal that leads to inhibition of T-cell function, including proliferation and cytokine production, therefore leading to attenuation of the immune response [182]. PD1 is expressed in activated T cells, B cells and antigen-presenting cells, and the expression of its ligands has been described in aggressive solid tumors [183].

Recent evidence suggests that CTCL may have differential PD-1 expression in MF and SS. Kantekure and colleagues have shown that PD-1 was frequently expressed at the early, patch and plaque stages of MF, but that expression seemed to decrease with disease progression [184]. Several other studies have shown that PD-1 expression is upregulated on malignant T-cells in the skin and CD4 T-cells of SS patients to a greater extent than in MF patients [185, 186].

Blockade of PD-1 can increase immune activating cytokine production and may enhance functioning of cytotoxic T cells, which would lead to enhanced disease eradication. Samimi et al. have shown that blockade of the PD-1/PD-L1 pathway yielded a relative increase in IFN-γ production by PBMCs of patients with CTCL, suggesting improved antitumor immunity. Furthermore, a strong decrease in PD-1 expression with improvement of disease was observed.

In studies of metastatic melanoma, it has been shown that PD-1 blockade can mediate tumor regression in a substantial proportion of patients, and more recently a favorable survival, durable tumor remission, and long-term safety has been demonstrated in patients with advanced melanoma receiving a PD-1 antagonist nivolumab [187]. Clinical trials of PD-1 antagonists in CTCL are needed to evaluate efficacy of this promising targeted therapy in this patient population.

Chemokines

Chemokine receptors and their ligands may serve as potential targets for anti-tumor therapy in MF/SS. Antibody-based therapeutics targeting chemokine receptors and compounds with the potential to down-regulate chemokine receptor expression hold great promise as potential therapies. A novel humanized-CCR4 monoclonal antibody has recently been developed that has been approved for use in Japan for adult T-cell leukemia, lymphoma and is currently being investigated as a potential therapeutic for the treatment of patients with CCR4+ T-cell leukemia/lymphoma [188]. The CCR4+ cells in these conditions have been shown to exhibit features of regulatory T-cells with the ability to suppress anti-tumor immune responses. In addition to lysing $CCR4^{+}$ T cells in vitro, this antibody reduced the expression of Foxp3 mRNA suggesting a possible role in depleting Tregs [189]. It is currently being studied in a large Phase 3 randomized two-armed cross over trial in refractory MF/SS.

As mentioned earlier, IFN-γ can be combined with other biologic response modifiers to achieve high clinical response rates among SS patients [123]. In addition to its ability to enhance cell-mediated cytolytic activity and suppress Th2 cytokine production, IFN-γ may also significantly increase production of the Th1 associated chemokine, monokine induced by gamma-interferon (Mig), a CXC chemokine recognized by CXCR3 [190]. Aside from its expression by MF cells in early stage disease (as discussed earlier), it is also expressed by cytotoxic T-cells, and thus contributes towards the accumulation of tumoricidal cytolytic T-cells in lesional skin.

In recent work, we have observed the ability of the retinoid bexarotene to preferentially decrease CCR4 levels among malignant cells in-vitro [191]. This reduction was associated with a decrease in malignant cell chemotaxis in response to TARC. Our findings, may in part, explain the marked clearing of cutaneous erythroderma we have noted among patients treated with this therapeutic agent [192], and suggest a potential role for such compounds in the management of inflammatory and neoplastic diseases of the skin in the near future.

Interleukin-31

CTCL patients often suffer from intense pruritus, which can cause significant morbidity and decreased quality of life [193]. IL-31, is a Th-2 cytokine, found in pruritic conditions such as atopic dermatitis [194] and mastocytosis [195], and has been shown to be associated with the level of pruritus and severity of disease. Increased IL-31 protein was recently identified in the serum of patients with CTCL [196]. Subsequently, Singer et al. demonstrated that IL-31 is specifically produced by $CD4^{+}CD26^{-}$malignant cells in CTCL patients [197]. Additionally, the authors showed that in this patient population, IL-31 mRNA levels significantly correlate with pruritus severity, and that clinical resolution of pruritus correlates with decreased IL-31 levels in the circulation [197]. Given this strong association, IL-31 may be considered as a potential therapeutic target for anti-itch treatment in CTCL. Current established systemic medications used for pruritus include antihistamines, gabapentin, mirtazapine and other SSRIs, naltrexone.

Hematopoietic Stem Cell Transplant Therapy

Despite numerous therapeutic strategies reviewed above, no regimen has been shown to increase survival of patients with CTCL [198], therefore younger patients with advanced disease, with multiple tumors, erythroderma, or involvement of the reticuloendothelial system who are refractory to the above therapies are increasingly being considered for allogeneic hematopoietic stem cell transplantation (HSCT).

Originally, HSCT was conceived as a replacement for a patient's own diseased hematopoietic system, which theoretically had been destroyed by a pre-transplant regimen of chemoradiation. This concept of eliminating the abnormal cells and supporting hematopoiesis with transplanted stem cells continues to be the basis for autologous stem cell transplants, in which patients' own reserved stem cells are returned after chemoradiation. In fact, some authors support "high-dose chemotherapy with hematopoietic progenitor cell support" as a more appropriate term for autologous stem cell transplant [199].

However, allogeneic HSCT has evolved conceptually into an immunologic therapy with the observation that donor transplanted T-cells can elicit a graft-versus-tumor effect against the host's tumor cells [200]. The immunologic basis of the disorder graft versus host disease (GVHD), which causes a significant amount of transplant-related morbidity and mortality, also supports the concept that the engrafted immune system, even when HLA-matched, is active against host tissue [199]. Based on this model, investigators have employed strategies of pre- and post-transplant immunosuppression that are less toxic to the hosts' other organ systems and have had success with nonmyeloablative conditioning regimens for allogeneic stem cell transplants [201]. A truly nonmyeloablative conditioning regimen results in mixed chimerism of both donor and host hematopoiesis post-transplant, and allows for transplantation in elderly patients and those with co-morbidities who would otherwise not be candidates for myeloablative transplant.

Stem cell transplants have achieved long-term complete responses in many types of hematologic malignancies, and several case studies and retrospective analyses of autologous and allogeneic, as well as myeloablative and nonmyeloablative, stem cell transplants for advanced CTCL have been published. In the two largest series of advanced MF and SS patients undergoing autologous stem cell transplant, almost all patients did achieve a complete response. However, out of 13 reported patients with a complete response, 12 relapsed, with the longest time to relapse being 14 months, and the median time to relapse in the larger study being 7 months [202, 203]. Several other case reports of autologous stem cell transplant for advanced CTCL have been published, and although overall, patients do achieve a safe and complete response, more than half of reported cases have relapsed within 6 months [199].

In contrast to autologous stem cell transplant, retrospective analyses of allogeneic bone marrow transplant in advanced CTCL suggest that if the patient and the graft are able to survive the transplant period, complete responses can be achieved and sustained [204–209]. A patient-level meta-analysis performed by Wu et al. to compare the outcome of allogeneic versus autologous SCT in patients with MF/SS using 39 cases from the literature showed favorable overall and event free survival, and a more durable response in patients who received allogeneic SCT [210]. In this group, the majority of patients experienced persistent GVHD, mostly with mild to moderate severity. Meanwhile, the majority of the deaths in the autologous group were due to progressive disease.

A retrospective analysis by Duarte and colleagues of allogeneic SCT in advanced CTCL patients showed decreased relapse rates (38 % after 1 year, 47 % after 3 years post-transplantation) and increased overall survival (66 % after 1 year, 53 % after 3 years post-transplantation) when compared to published data of conventional treatments [211]. Duvic and colleagues have reported that 11 of 18 advanced CTCL patients who underwent total skin electron beam therapy followed by non-myeloablative allogeneic stem cell transplant were in complete remission after median follow up of 19 months, with morbidity and mortality attributable to sepsis and infections, as well as acute and chronic GVHD [212].

These responses are evidence for a graft versus tumor effect, which in most cases is accompanied by some degree of GVHD. In all allogeneic stem cell transplant patients, a balance between disease relapse and GVHD must be achieved using some degree of post-transplant immunosuppression. Disease relapse can thus often be controlled by reducing the level of immunosuppression, or by administration of donor lymphocyte infusions. In some cases, patients have been able to discontinue immunosuppression entirely, without evidence of recurrent CTCL or GVHD [208].

Nonetheless, treatment-related mortality (i.e., life-threatening infections and GVHD) occurs in approximately 30 % of cases [213]. Thus, more extensive studies are needed to optimize conditioning regimens, donor types, disease status and timing of allogeneic SCT for advanced CTCL, which would be considered the ultimate immunotherapy.

Questions

Question 1: Advanced mycosis fungoides/Sezary Syndrome results in increased serum levels of:
A. Th1 cytokines
B. Th2 cytokines
C. Th17 cytokines
D. IgE
E. B and D

Question 2: Advanced MF/SS patients are at increased risk for which infections?
A. Atypical Mmcobacterial
B. Gram negative bacterial
C. HSV and VZV reactivation
D. Syphillis
E. Tuberculosis

Question 3: An initial treatment approach for newly diagnosed Sezary Syndrome includes all EXCEPT
A. Extracorporeal photopheresis
B. Interferons
C. TNF alpha inhibitors
D. Retinoids
E. Skin directed therapies

Answer key
Question 1: E
Question 2: C
Question 3: C

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Immune Environment of Cutaneous Malignancies

42

Channa G. Ovits and John A. Carucci

Abstract

The ability of cutaneous malignancies to develop and progress involves a complex interplay with the local immune environment. Multiple immunomodulatory mechanisms underlie the ability of squamous cell carcinoma, basal cell carcinoma and melanoma to evade immune detection. These mechanisms include a modulation of the gene profiles of these cancers, which populations of immune cells are present and which cytokines are produced in the immune microenvironment. With squamous cell carcinoma, the gene expression and cytokine profile show an immunosuppressed microenvironment, along with functionally compromised dendritic cells and tumor-associated macrophages being present in the tumor microenvironment. Basal cell carcinoma also achieves immune evasion with an immunosuppressed microenvironment, as seen by the Th2 dominant cytokine profile and the presence of regulatory T cells. Immunosuppressed transplant patients have increased incidence of and more aggressive non-melanoma skin cancers due to the altered immune microenvironment, with modified T cell populations and ratios and pro-tumoral cytokines. Melanoma, despite its immunogenicity, displays a number of immune suppressive mechanisms, such as impaired antigen-presenting cell maturation, T cell anergy, the induction and recruitment of regulatory T cells and myeloid-derived suppressor cells. The understanding of the immune microenvironment of cutaneous malignancies is crucial, as it affords many potential targets for therapeutic options.

Keywords

Basal cell carcinoma • Squamous cell carcinoma • Melanoma • Macrophages • Regulatory T cells • Dendritic cells • Langerhans cells • Imiquimod • Th1 immune response • Th2 immune response

Cutaneous malignancies are the most common human cancers, with basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma comprising the majority of skin cancers. The public health burden of these cancers is significant, with basal cell carcinoma being the most common human cancer, squamous cell carcinoma being the second most common and melanoma being the fifth and sixth most common cancer in men and women respectively [1, 2]. Each year in the US, about 5 million people are treated for skin cancer and the cost of this treatment is approximately 8.1 billion dollars. 2.8 million cases of BCC alone are diagnosed each year in the US [3]. Understanding these cancers and their context within the immune environment can lead to better treatment options and novel therapeutic approaches being developed for the patients diagnosed each year.

The development and progression of these malignancies involves complex interactions with the local immune

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microenvironment. The tumor microenvironment includes tumor and non-tumor cells at the dynamic interface of neoplasia [4]. The immune cells include Langerhans cells, dermal dendritic cells, CD4 and CD8 T-cells, T-regulatory cells, macrophages, natural killer cells and mast cells. T cells, both CD4 and CD8, and dendritic cells are often shown to be infiltrating various tumors, and tumor-associated macrophages are often found surrounding and infiltrating the tumor as well. These cells, when functioning properly, work both in concert and independently towards immunosurveillance, the prevention of malignant progression and regression of primary malignancies. Immunosuppression, such as in transplant recipients, increases the risk of skin cancer, in particular the non-melanoma skin cancers. Additionally, melanoma behaves more aggressively in immunosuppressed patients. The malignancy's ability to evade immunosurveillance, progress and metastasize is the result of multiple immunosuppressive mechanisms.

Our understanding of cutaneous cancers' interaction with the immune environment has grown over recent years and continues to be explored. The attempts to translate these basic science developments into clinical applications have had varying levels of success, with immunotherapeutic agents, such as cytokines, anti-cytokine antibodies and vaccines, proposed, tested and sometimes approved for use. These treatments options for melanoma are outlined and discussed in Chapter 51.

Nonmelanoma Skin Cancer (NMSC)

Squamous Cell Carcinoma

Cutaneous SCC is the second most common human cancer, affecting greater than 300,000 individuals in the US annually [1, 5]. While most cases are treated successfully with local removal, aggressive cases can metastasize to local lymph nodes and distant organs. These aggressive cases are responsible for the approximately 10,000 non-melanoma skin cancer deaths in the US each year [1].

SCC is a malignant proliferation of the keratinocyte that tends to occur on sun-damaged skin. It often progresses from noninvasive precursor lesions, such as actinic keratosis (AK). It presents in a variety of forms, from a crusted patch or nodule, to an ulcer, to a hyperkeratotic indurated papule.

SCC Gene Expression and Immune Cell Profile

The immune microenvironment of SCC is unique, both in the cells that are local to the tumor and their ability to be stimulated, as well as the presence of various cytokines (Fig. 42.1). The gene expression profile of SCC shows a relatively immunosuppressed microenvironment [6]. In studies of immune response gene expression in SCC as compared to normal skin and psoriasis, a number of stimulatory genes are downregulated. These genes include the gene for cytotoxic T cell product granzyme B, the activated T-cell marker CD69 and the proinflammatory mediator inducible nitric oxide synthase (iNOS), an enzyme that is key in tumor immunity [6]. Additionally, invasive SCC showed a change in cytokine gene expression, with an increase in IL-24 expression as compared to AK or SCC in situ. SCC was also found to have increased expression of protumoral factor matrix metalloprotease 7 (MMP-7), which was shown to be induced by IL-24 in culture. IL-24 is thought to contribute to SCC invasion through the upregulation of MMP7 [7].

The evidence of immunosuppression in SCC continues with the lack of particular immune cells in the local environment; NK cells, B cells and monocytes are rarely detected around SCC [8]. Each of these cells has been shown to play a role in tumor cell eradication. NK cells are lymphocytes that were first identified for their ability to kill tumor cells without deliberate immunization or activation [9]. Monocytes exhibit considerable selective cytocidal activity against tumor cells through the generation of reactive oxygen species (ROS) [10]. Though some studies have shown resting B cells to inhibit T-cell mediated regression of tumor cells, other studies have shown the key role that activated B cells play in T cell activation and creation of long term responses against cancer [11]. The collective scarcity of these cells in SCC may play a role in SCC's ability to establish itself, as well as grow and metastasize.

The presence of the myeloid-derived suppressor cells (MDSCs) provides further evidence and mechanisms for SCC- related immunosuppression. MDSCs are potent suppressors of T-cell mediated responses, partially due to the downregulation of E-selectin on vascular endothelial cells. The lack of E-selectin on endothelial cells restricts T-cell entry into tumors and is caused by the presence of nitric oxide (NO) in the microenvironment. SCC-infiltrating MDSCs have been shown to produce NO, thus contributing to the immune evasion of the tumor. A possible therapy of suppressing NO production through inhibiting inducible NO synthase (iNOS) has been shown to induce E-selectin expression in vitro and may be effective as a future therapy for SCC [12].

Tumor-Associated Macrophages in SCC

Macrophages are a major population of leukocytes that are found surrounding and infiltrating solid tumors [13]. In SCC, macrophages have been shown to be far more abundant than in normal skin [14]. These peritumoral and penetrating

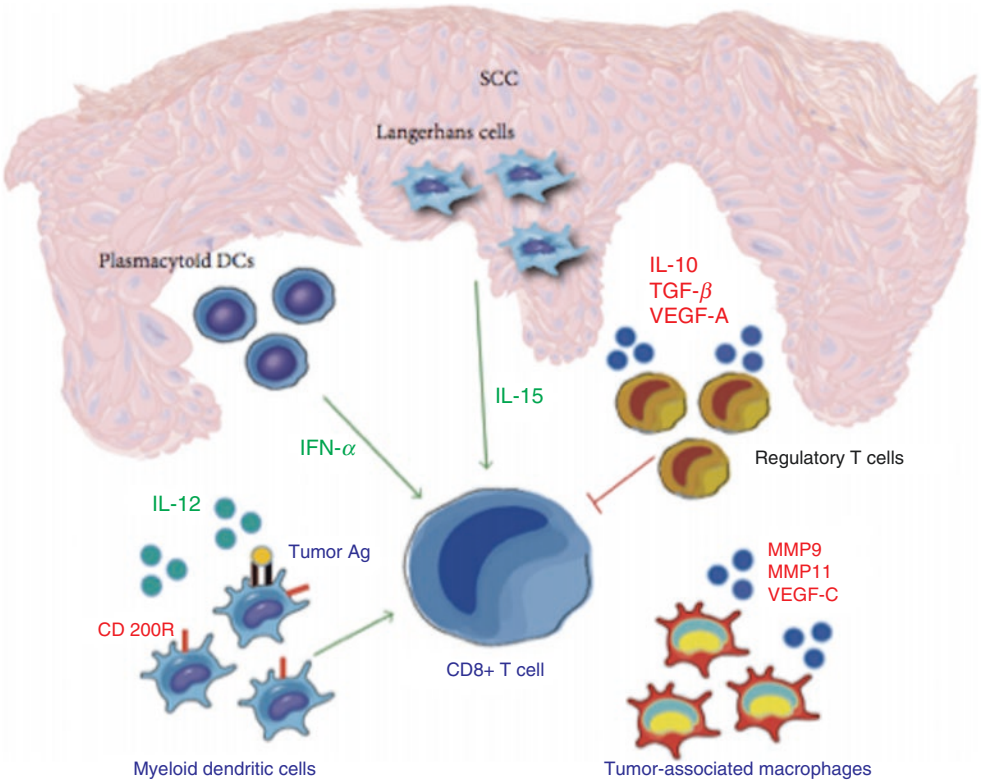

Fig. 42.1 The SCC immune microenvironment. The SCC microenvironment involves a complex interplay of immunoinhibitory and immunostimulatory cells and cytokines. While Langerhans cells and plasmacytoid DCs are producing proinflammatory cytokines IL-15 and IFN-α respectively to enhance the immune response, the presence of tumor associated macrophages and regulatory T cells contribute to immune dysfunction and tumor invasion through the production of matrix metalloproteases (MMP9 and 11), pro-angiogenic factors (VEGF-C) and immunoinhibitory cytokines (IL-10, TGF-β, VEGF-A). These protumoral cytokines can also functionally compromise the other immune cells of the microenvironment, leading to immature myeloid dendritic cells, which express CD200, and Langerhans cells which express a mixed gene profile of immune activation and immune tolerance genes

macrophages, as mentioned earlier, are defined as tumor-associated macrophages (TAMS) [15]. TAMS play a significant role in tumor behavior, with potential to inhibit or stimulate tumor growth. Some studies have shown TAMs are capable of eradicating tumor cells in vitro, yet others have correlated a poor prognosis with increased numbers of TAMS [16–18]. In their role in aiding and abetting the tumor, TAMS can fail to identify tumor antigens and can release angiogenic and tumor stimulating factors [19, 20]. It is suspected that the tumors themselves may be creating a dynamic microenvironment that transforms the TAMS into macrophages that allow for tumor growth [21].

Despite the potential ability of TAMS to inhibit tumor progression, an increased number of TAMS is correlated with a negative prognosis. This can be explained by a number of factors. In studies, TAMS have been shown to make matrix metalloproteinases (MMP)9 (gelatinase B) and 11 (stromelysin-3), zinc-dependent proteinases that participate in extra-cellular matrix degradation, which allow direct tumor invasion, as well as release pro-angiogenic factors otherwise sequestered within the extra-cellular matrix [14, 22]. This is in contrast to macrophages in normal skin, which produce these factors at lower levels than TAMS in the SCC microenvironment.

The macrophages of the SCC microenvironment have also been shown to be predominantly M2 macrophages, or alternatively activated macrophages. These macrophages are induced by IL-4, in contrast to M1 macrophages, or classically activated macrophages, which are induced by IFN-γ. A strong M1 macrophage response is thought to prevent tumor growth. In contrast, M2 macrophages have a lower antigen presenting capacity and have been positively correlated with tumor genesis and progression through inflammation. Recent studies have shown SCC- associated macrophages to be heterogeneously activated, with some expressing M1 markers, some expressing M2 markers and some expressing both M1 and M2 markers simultaneously [14]. This heterogeneous activation of TAMS in SCC gives rise to a potential therapy in driving TAM activation to the M1 anti-cancer phenotype.

TAMS have been shown to play a role in lymphangiogenesis, with the expression of vascular endothelial growth factor-C (VEGF-C) [23]. VEGF-C, a critical lymphangiogensis mediator, promotes increased lymph vessel density and has

been correlated with increased risk of metastasis in squamous cell carcinomas of the oral cavity and melanoma [24, 25]. The increased lymph vessel density spurred by VEGF-C is an important factor in cancer's development and spread.

With production of high levels of MMPs, expression of lymphangiogenic mediator VEGF-C and M2 characteristics, TAMS in SCC are failing to prevent tumor creation and progression and are rather promoting its survival and advancement. The overall behavior and subtype of TAMS in SCC show the immunosuppressive potential of immune cells when influenced by the tumor environment.

Dendritic Cells and SCC

Dendritic cells (DCs), the most potent antigen-presenting cell, exist in a variety of subtypes, and regulate the adaptive immune system [26, 27]. They are key players in cancer immune surveillance, with their ability to stimulate tumor-specific T-cell responses, along with having been shown to infiltrate various human tumors [28–31]. DCs are abundant in the skin immune system, existing mainly as epidermal Langerhans cells (LCs) and dermal myeloid DCs. LCs, with their epidermal localization, should be the first antigen presenting cell to encounter SCC tumor antigen, as SCC is a malignant proliferation of epidermal keratinocytes.

However, studies have shown that DCs from human cancers are often functionally compromised, with a decreased ability to induce IFN-γ and stimulate T-cells [32, 33]. Further studies have shown that the tumor microenvironment contains immunosuppressive cytokines which impair DC differentiation and function [26, 34].

With regards to DCs, SCC have been shown to have a relatively immunosuppressed microenvironment, with a lack of mature dendritic cells in comparison to normal skin (Fig. 42.2) [35, 36]. This is seen by the down-regulation of mature dendritic cell marker gene CD83 and decreased numbers of CD83 cells in SCC [6].

Additionally, SCC-associated mature myeloid dendritic cells (mDCs) have been shown to be functionally compromised and deficient in their ability to produce IFN-γ and stimulate T-cells, an important indication of the immunosuppressed microenvironment [37]. Even when cultured with mDC-maturing cytokines like IL1b, IL-6,TNF-α, and PGE2, SCC-associated mDCs remained impaired in their T–cell proliferation stimulation. This is despite their phenotypic maturity that is comparable to the mDCs of normal skin. This is thought to be due to the fact that the SCC cytokine milieu has been shown to be composed of immunosuppressive cytokines that suppress myeloid DCs, such as VEGF-A, TGF-β, IL-10, and IL-6 [37].

Langerhans cells (LCs) from SCC seem to be more capable of immune response than their dermal counterparts. LCs from SCC, in contrast to mDCs, have been shown to elicit a type 1 immune response when activated. Moreover, LCs from SCC have been shown to be more powerful stimulators of CD4 and CD8 T cell proliferation than those from normal skin and elicit a more powerful type 1 T-cell response. They also express higher levels of surface markers CD40, CD80, CD83, and CD86 than LCs from normal skin, making them more mature and is an important factor in immune response induction [38].

However, SCC-derived LCs still may have some limitations based on their gene profile. They show a mixed gene profile of upregulated immune activation genes, such as STAT4, IL15, and CD80 as well as upregulated immune tolerance genes, such as CD200 and receptor activator of NF-KB [38]. This mixed activation and tolerance profile is indicative of the tumor environment's effect on the LCs and more is still to be learned about how this impacts their behavior.

Basal Cell Carcinoma

Basal cell carcinoma (BCC) is the most common human malignancy, with a higher prevalence than all other malignant tumors combined. BCC is a malignant proliferation of keratinocytes from the basal layer of the epidermis. Sun exposure is the most important risk factor for BCC, but other risk factors include age and fair skin. BCC is slow-growing and highly curable, but can be extremely disfiguring if allowed to progress without intervention. BCC has a number of clinical presentations, spanning cystic, ulcerated, nodular, superficial, sclerosing, pigmented, and keratotic variants.

Cytokine and Chemokines in BCC

In BCC, a number of cytokines and chemokines are associated with its avoidance of immunosurveillance and subsequent development. Of the proinflammatory cytokines, IL-6, IL-8, IL-17, IL-22, and CXCL12 have been linked to BCC tumor progression. IL-6 has been shown to increase anti-apoptotic activity within BCC cell lines [39] and promote angiogenesis through the PI3k/Akt pathway, as well as through increasing the expression of the pro-angiogenic cytokine IL-8 [40, 41]. CXCL12 has also been shown to promote angiogenesis by binding to the CXCR4 receptor, which is expressed to a higher degree in more aggressive forms of BCC [42]. CXCL12 also upregulates the activity of matrix metalloprotease 13 (MMP-13), allowing for BCC invasion [43].

IL-17 and 22 have also been shown to increase proliferation and migration of BCC in vitro and tumor progression in vivo, through both their intrinsic signaling pathways and the induction of IL-6 and 8 production [44]. IL-10, an immunosuppressive cytokine, is upregulated in BCC and may

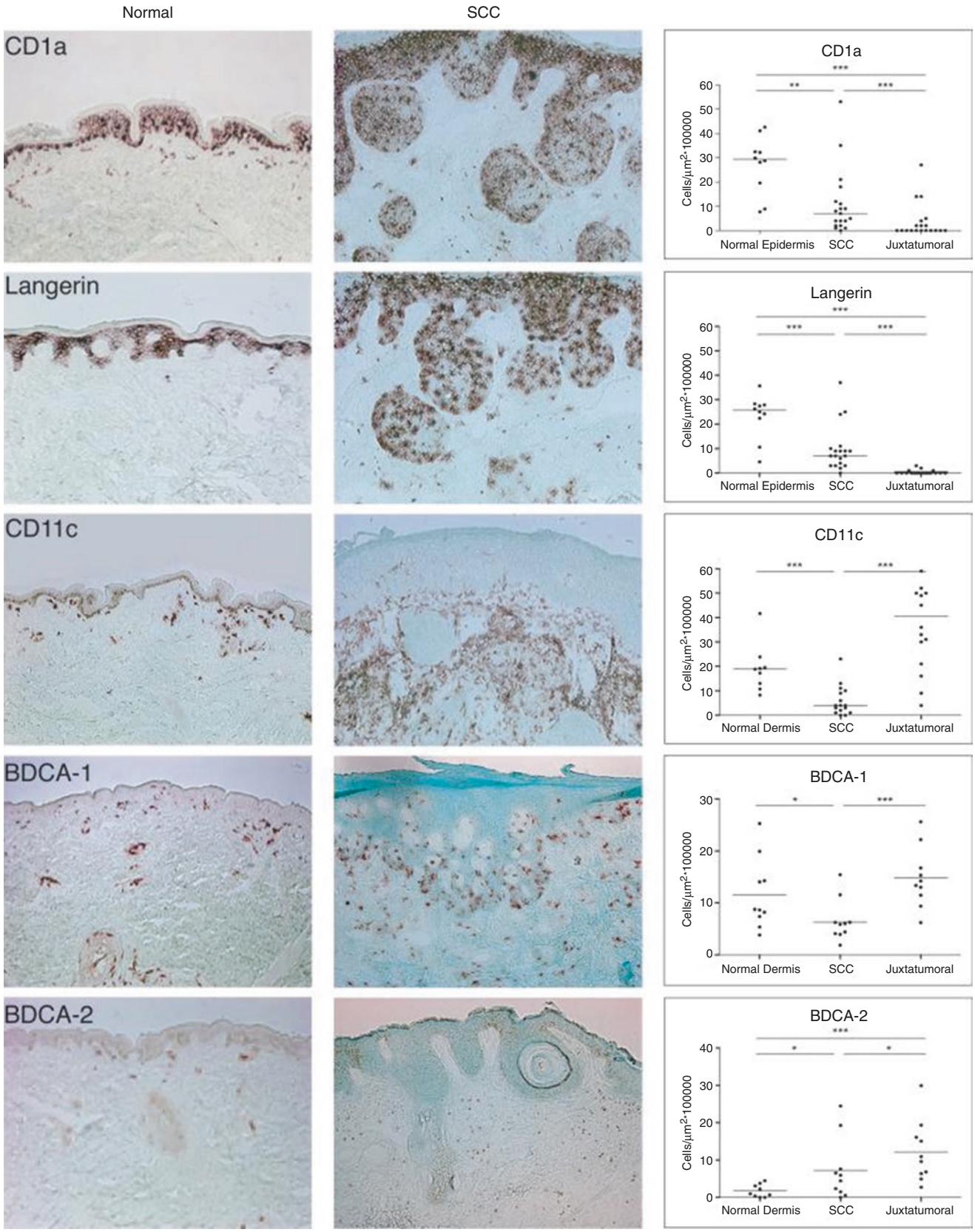

Fig. 42.2 SCC and dendritic cells. Immunohistochemical staining of normal skin and SCC for dendritic cell markers showed that SCC is associated with intratumoral CD1a+Langerin+Langerhans cells, juxtatumoral CD11c+myeloid dendritic cells and BDCA2+ plasmacytoid dendritic cells. (**a**) CD1a, (**b**) Langerin, (**c**) CD11c, (**d**) BDCA-1, and (**e**) BDCA-2 cells in normal skin, SCC, and juxtatumoral skin

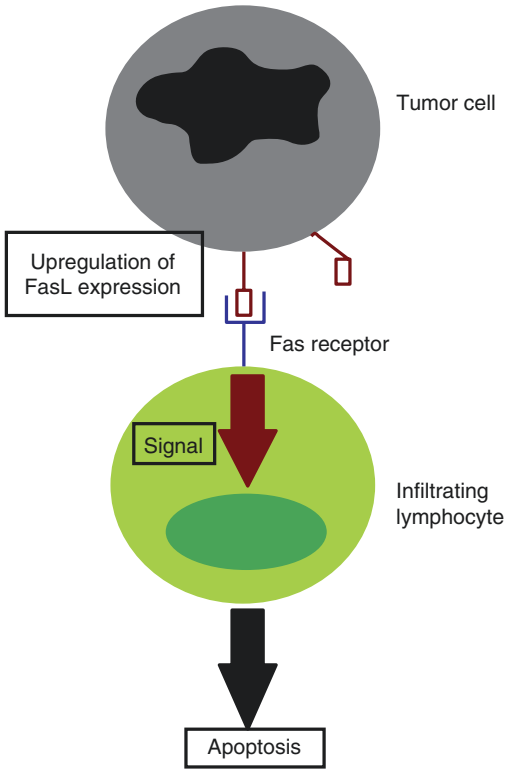

Fig. 42.3 BCC immune evasion through FasL. To evade immune detection, BCC cells upregulate expression of Fas ligand, which binds to the Fas receptor on the infiltrating lymphocyte. This binding inducing a signaling cascade within the lymphocyte, resulting in apoptosis

contribute to the immunosuppressed microenvironment of the tumor [45]. Its presence has been correlated with lack of HLA-DR, ICAM1, CD40, and CD80 expression, surface markers that aid in immune detection, as well as being correlated with immune cell maturity [46].

IFN-γ is a cytokine that has been shown to stimulate immune responses against cancer. In concordance with a permissively immunosuppressed microenvironment, BCC has decreased expression of IFN-γ receptor, which may play a part in the lack of cell-mediated immune response to BCC [47]. Conversely and logically, IFN-γ is elevated in actively regressing BCC [48]. Interestingly, IL-23, a cytokine which induces IFN-γ production as well as inducing antitumor effects in vitro, has also been shown to be elevated in BCC [49].

Fas ligand (FasL) is an apoptosis-inducing factor that by binding to its receptor on the cell surface, begins the apoptotic cascade of signaling within the cell. It is a member of the tumor necrosis factor family of receptor-ligand binding. When FasL is expressed by cancer cells, it induces the apoptosis of infiltrating lymphocytes, allowing the cancer to evade immune surveillance (Fig. 42.3). Some studies have shown BCC to express FasL, while others have had the opposite results, making the expression of FasL on the part of BCC still debatable [50–52].

In general, BCC is associated with a Th2 dominant microenvironment, which is the immune response correlated with immune tolerance. It is capable of significantly inhibiting the Th1 anti-tumor immune response. The Th2 environment is shown with increased expression of IL-4, IL-10, and CCL22, a chemokine responsible for regulatory T cell chemotaxis. However, the BCC microenvironment has also been shown to have an increased expression of interferon-associated genes and IL-23 expression, favoring a Th1 microenvironment [49]. This conflicting microenvironment, shown by the immunostimulatory and immunsuppressive cytokines in the BCC tumor milieu, is consistent with a dynamic state within the immune microenvironment.

Immune Cells and BCC

Dendritic cells, as mentioned earlier, are key in cancer immune surveillance and often have altered behavior in the unique microenvironment of the tumor. In the skin, they exist mainly as epidermal Langerhans cells (LCs) and dermal myeloid DCs. The presence and density of DCs in BCC is controversial, as studies have shown conflicting results. Some studies have shown a decrease and even absence of mature LCs, particularly in tumors of the face, and immature myeloid DCs were found to be present [49, 53], similar to the dendritic cell profile seen in SCC. This decrease in LC density in BCC is linked to increased aggressive behavior on the part of the tumor [54]. Additionally, in BCC cells, the depletion of macrophages and LCs resulted in enhanced tumor progression [55].

Other studies have shown an increased density of LCs within the BCC lesion, as well as in adjacent epithelium [56, 57]. An increase LC density within the BCC lesion is particularly associated with smaller tumor size and tumor location to the face [58]. One explanation for the contradiction in presence or absence of DCs in BCC that has been suggested is that the density of DCs in BCC changes over time, with an initial reduction allowing initiation and subsequent host response increasing DC density [59]. This is also consistent with the dynamic microenvironment suggested by the mixed cytokine profile of BCC.

Regulatory T cells (Tregs) are immune suppressive T cells, important in self-tolerance and immune homeostasis. Created in the thymus, they normally comprise 5–10% of the CD4 T cells in the periphery. They can also be induced in the periphery from naïve T cells [60]. Both induced and natural Tregs express the transcription factor forkhead box P3 (FOXP3), an important controller of suppressor protein expression and used to identify Tregs [61]. These cells have been found to surround BCC, which is consistent with the increased expression of the chemokine CCL22 (Fig. 42.4). This surrounding of BCC by Tregs can contribute to the

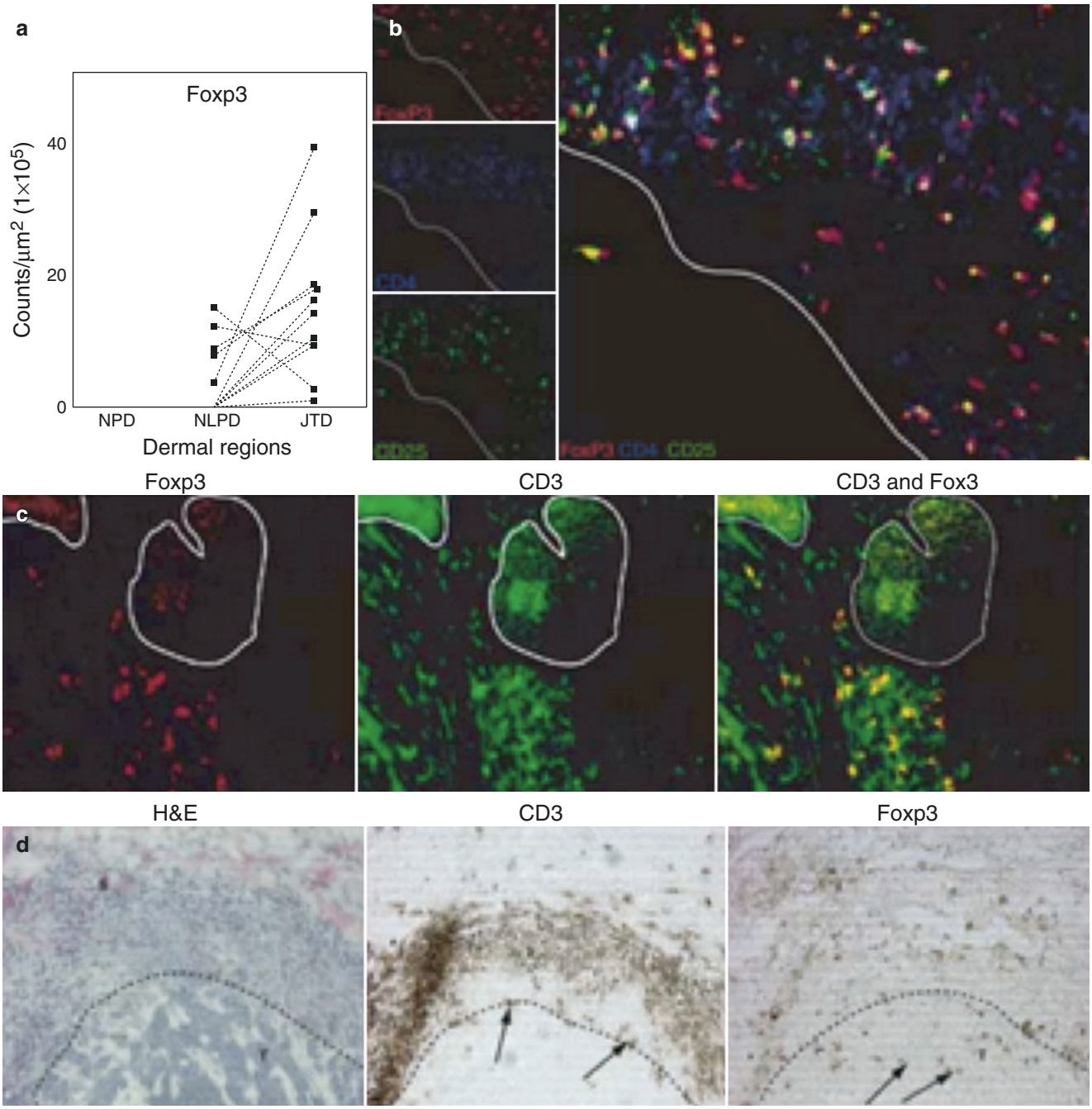

Fig. 42.4 BCC and regulatory T cells. (**a**) FoxP3+ cells were present in the "pseudocapsule" of inflammatory cells surrounding BCC. (**b**) Double label immunofluorescence confirmed that FoxP3+ cells expressed CD3. (**c**) Triple label immunofluorescence showing CD4+CD25+Foxp3+ cells associated with BCC. (**d**) Cell counts show increased numbers of Foxp3+ cells in juxtatumoral dermis (*JTD*) *versus* non-lesional papillary dermis (*NLPD*) *versus* normal papillary dermis (*NPD*)

immune evasion of the tumor, possibly through the impaired maturation of DCs [49].

Mast cells are also thought to contribute to the immunosuppressed microenvironment of BCC. They have been shown to accumulate at the periphery of BCC, especially in aggressive BCC tumors [62, 63]. Additionally, they participate in matrix degradation through the release of proteases, which allow tumor spread and contribute to tumor angiogenesis as a source of VEGF [64, 65], similar to the TAMS of SCC.

Imiquimod

With all the cytokines and immune cells contributing to an immunosuppressed BCC microenvironment,

immunomodulatory therapy options were a logical next step to explore. Imiquimod, one of the most successful therapies, is a topical treatment for BCC that has been shown to induce apoptosis in BCC [66]. It is an immune modifier and, more specifically, a Toll-like-receptor-7 (TLR-7) agonist, which induces multiple cytokines, stimulating an innate and adaptive cell-mediated immune response [67].

Imiquimod treatment results in various immune cells invading in stages. The tumor is initially infiltrated by CD4 T cells, followed by a massive intratumoral and peritumoral infiltration of macrophages, as well as activated DCs [68]. The plasmacytoid DCs are recruited by imiquimod's stimulation of Th1 cytokines, including IL-12. Imiquimod treatment efficacy has been linked to pretreatment DC density in the tumor, with a greater efficiency being shown with a higher density of pretreatment DCs [69]. Imiquimod also induces IFN-α, which in turn induces cell surface expression of FasR on the tumor cells, causing apoptosis of the tumor cells through the CD95 receptor ligand interaction [70–72].

Immunosuppression and Transplant-Associated NMSC

As evidenced by the previous points, the immune system plays a crucial role in the development and progression of non-melanoma skin cancer (NMSC). The most important risk factor for non-melanoma skin cancers is UV radiation. In addition to their mutagen properties, UVA and UVB have been shown to be immunosuppressive in humans. Studies have shown that UVA irradiation suppresses memory immunity to the seven antigens in the delayed-type hypersensitivity test Merieux [73, 74]. Additionally, irradiation with half the UVA in minimal erythema dose (MED), the amount of sunlight necessary to cause a sunburn, suppressed recall immunity to nickel [75]. In animal studies, lymphocytes from UV irradiated mice were unable to prevent malignant formation on UV-irradiated skin grafts in unirradiated mice [76]. Transplanted tumor cell lines, including SCC, which would be immunologically rejected in immunologically competent mice, were able to grow in UV immunosuppressed mice.

Another example of immunosuppression leading to increased risk of NMSC, as well as more aggressive behavior by the NMSC, is the occurrence of these cancers in patients with HIV infection. Immunosuppressed HIV-positive patients can develop rapidly growing cutaneous SCCs at a young age, with an increased risk of recurrence and metastasis [77].

The most obvious and important evidence linking immunosuppression and NMSC is seen in immunosuppressed

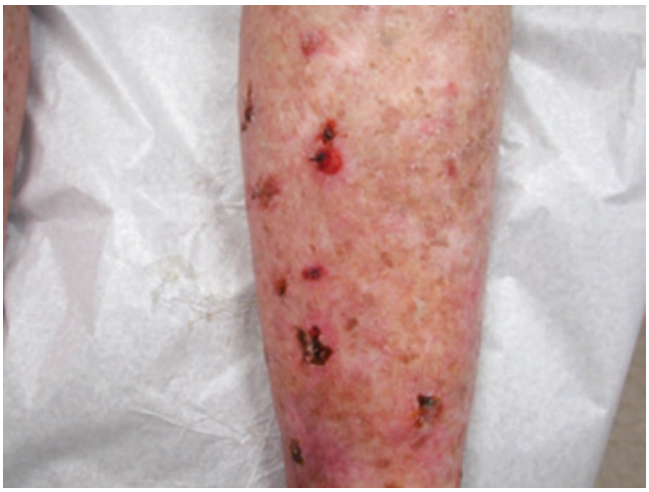

Fig. 42.5 Transplant-associated NMSC. Transplant recipient on long term immune suppression with multiple agents including calcineurin inhibitors presenting with multiple SCCs

organ transplant recipients (OTR), with SCC incidence being 60 to 100 times greater in this population than in the age-matched immunocompetent population [78]. The OTR population also has a greater likelihood of multiple skin cancers at presentation (Fig. 42.5) [79].

Furthermore, SCC in OTR is more aggressive, with a higher probability of recurrence and metastasis [80]. The risk of SCC in OTR seems to be directly proportional to the level of immunosuppression, with lower numbers of CD4 cells found in OTRs with NMSC versus OTRs without NMSC [81, 82].

This altered incidence and behavior of SCC in immunosuppressed patients can be attributed to the altered immune microenvironment. With regards to the modified microenvironment, transplant associated SCC (TSCC) has increased populations of immune cells that are immunosuppressive and produce pro-tumoral cytokines, as well as decreased populations of immune cells that initiate a Th1 (cell–mediated immunity) antitumor immune response [83].

The immunosuppressed TSCC immune microenvironment is altered in its T-cell populations and ratios. More specifically, TSCC has been shown to have increased IL-22-producing CD8+ cytotoxic T cells compared to immune competent SCC, as well as increased expression of IL22 receptors [84]. IL-22 is a pro-inflammatory cytokine typically involved in wound healing, which causes activation of genes involved in cell cycle progression and the prevention of apoptosis [85]. IL-22 has been shown to have pro-tumoral activity in multiple cancers. IL-22 has been shown to drive SCC proliferation in culture in a dose-dependent manner, most dramatically under starvation conditions [84]. This is in concert with the idea that IL-22 may drive SCC proliferation within the tumor,

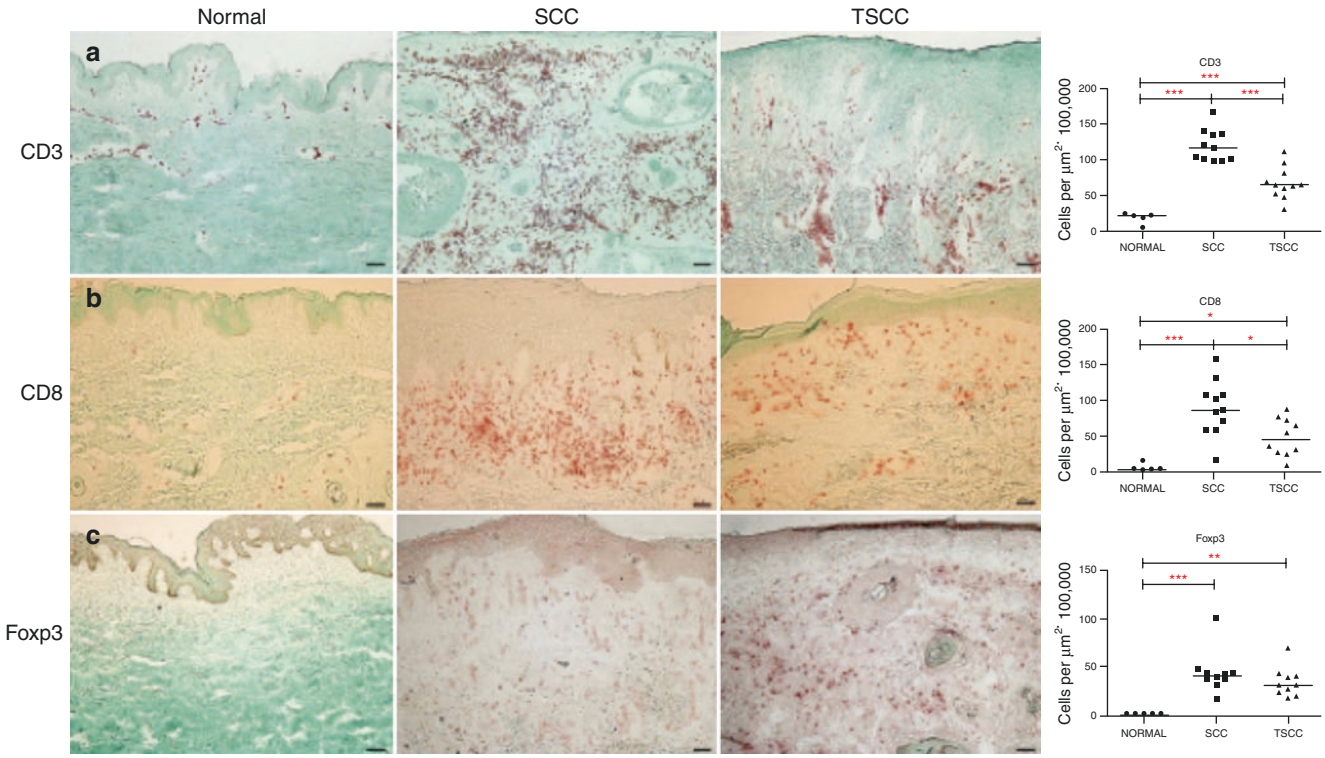

Fig. 42.6 T cell populations in SCC and TSCC. Immunohistochemical staining of normal skin, SCC and TSCC for T cell markers showing the increased amounts of CD3(**a**), CD8(**b**) and Foxp3(**c**) T cells in SCC and TSCC as compared to normal skin. Additionally, the ratio of Foxp3+ Tregs to cytotoxic CD8 T cells was increased in TSCC as compared to SCC, showing a tumor permissive environment in TSCC

where there are high metabolic demands and diminished enrichment.

Additionally, the CD8+ IFN-γ producing T cells in TSCC are decreased compared to SCC, which is associated with aggressive tumor behavior and increased metastasis [86]. TSCC also has an increased proportion of regulatory T cells (Tregs), which has been correlated with a poor prognosis in other carcinomas, such as breast and gastric (Fig. 42.6) [87, 88]. Tregs are important in preventing autoimmunity, but may suppress beneficial antitumor activity and aid in immune evasion [88–90].

TSCC has also been shown to have decreased CD4+ helper T (Th) cells infiltrating the tumor and reduced mRNA for IL17A. IL17A is a Th17-specific cytokine that is involved in inflammation and the recruitment of the innate immune system [91, 92]. The decreased IL17A favors graft tolerance but may weaken the host's anti-tumor response against the TSCC [93–96].

The skin adjacent to TSCC in OTRs has been shown to be Th2 immune response weighted, with decreased IFN-γ levels [83]. The Th2 gene expression is correlated with transplant tolerance and long-term allograft survival [97]. However, as mentioned earlier, the Th2 response is capable of significantly inhibiting the Th1 response, which is the anti-tumor immune response. This allows for the increased aggressiveness and recurrence rates of SCC in OTRs [83].

These changes in the immune microenvironment suggest a compromised inflammatory response in the SCC of OTRs. This alters the behavior and prognosis of TSCC but is a necessary component of immunosuppression for graft tolerance. Though a minimization of immunosuppression in heart and kidney transplant recipients showed reduction in the development of new SCC at 5 years [98], this reduction is not always feasible. A change in immunosuppression regimen should be considered, with calcineurin inhibitors being linked to higher incidences of cutaneous carcinomas, and sirolimus or everolimus being a better choice with respect to cutaneous tumorigenesis [99]. Lastly, the current knowledge of the immune microenvironment of TSCC opens new avenues of study, with cytokines like IL-22 being possible targets in future TSCC prevention and treatment.

Melanoma

Melanoma incidence rates have been increasing over that last three decades, and greater than 75,000 new cases are projected to be diagnosed in 2014, with over 9500 deaths estimated to occur. Although melanoma is only 4 % of all skin cancers, it accounts for greater that 80 % of skin cancer deaths. About a quarter of melanoma patients will experience recurrence and advanced stage disease [2].

The Immunogenicity of Melanoma

Melanoma is considered an extremely immunogenic tumor, with the capability of triggering host immunologic response. The immunogenicity is seen in the immune cell infiltrates that are often seen in melanoma tumors, as well as the relatively high rate of spontaneous regression with concurrent vitiligo. Many melanoma-specific antigens have been identified in triggering a T-cell immune response. Additionally, a number of immune therapies, such as IFN-α and IL-2, have been shown to be effective in patients, as discussed in the chapter 51 [100, 101].

Despite the established immunogenicity of the tumor, there are a number of immune suppressive mechanisms demonstrated to occur in melanoma, such as impaired antigen-presenting cell maturation, T cell anergy, the induction and recruitment of Tregs, and myeloid-derived suppressor cells [100, 102]. Thus, boosting or stimulating an immune response against melanoma has been and is still a promising avenue for therapy, and its exploration has resulted in some success with regards to disease-free and overall survival [103].

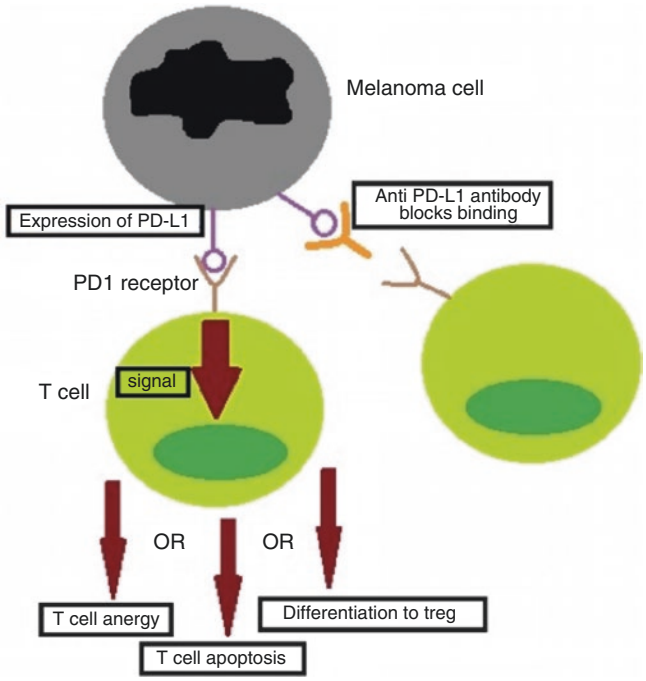

Fig. 42.7 Melanoma immune evasion through PD-L1. One method of immune evasion exhibited by melanoma is the expression of PD-L1 on its surface, which binds to the PD1 receptor on the surface of T cells. The binding of PD-L1 to its receptor causes intracellular signaling, leading to several different immunoinhibitory outcomes: T-cell anergy, apoptosis or differentiation of the T cell into a regulatory T cell (Treg). One of the immune therapies being explored is an anti-PD-L1 antibody, such as nivolumab, which blocks the PD-L1 on the melanoma cells from binding and inhibiting T cells

BRAF

In approximately half of human melanomas, activating mutations in the protein kinase BRAF allow the tumor cells to survive and proliferate [2]. BRAF is a proto-oncogene that is part of the RAF kinase family of growth signal transduction. This has been shown to be through the MEK/ERK pathway, which is important in cell division and differentiation. The most common mutation is the V600E BRAF, where a valine at position 600 is replaced by a glutamic acid. This mutated BRAF also has been linked to a deactivated AMPK, or AMP activated protein kinase, which inhibits cell growth in low energy states [104]. With AMPK deactivated, the melanoma cells can then grow despite lack of nutrients.

T Cell Anergy

Melanoma has multiple mechanisms for inhibiting the activation, proliferation and effector status of T cells. This primarily includes stimulating receptors on T cells, including those for PD-L1 and the B7-H4, both members of the same family of co-stimulatory molecules [101].

PD1 is an immunoinhibitory receptor on T cells that plays a crucial role in melanoma's immune escape. The PD1 ligand (PD-L1 or B7H1) is a member of the B family of costimulatory molecules that provides either an inhibitory or stimulatory secondary signal to T cells primarily binding HLA molecules on tumor cells. More specifically, the B7 family has been shown to control the effector phase of T-cell responses [105]. Specific tumors, such as prostate and renal cell carcinoma, have been shown to express B7 co-stimulatory molecules, which are involved in their escape from immunosurveillance [106].

PD-L1 has been found to be expressed by melanoma tumor cells. When the PD-L1 on the melanoma cell binds to the PD1 receptor on T cells, it causes an inhibition of T cell proliferation, survival and effector functions, such as cytotoxicity and cytokine release. It also induces apoptosis of tumor-specific T cells and promotes differentiation of CD4 T cells into regulatory T cells. Lastly, it increases the resistance of tumor cells to cytotoxic T cell attack [105]. Increased PD-L1 expression by melanoma is negatively correlated with survival (Fig. 42.7) [107].

This pathway is therefore an important therapeutic target, with anti-PD-L1 antibodies, such as nivolumab, tested as immunotherapeutic agents, either alone or in combination with other immune therapies. These have shown some success, but await further testing and demonstration of clinical effectiveness.

Another member of the B family of co-stimulatory molecules, B7-H4, has also been indicated in immune evasion of melanoma. Melanoma tumor cells have been found to express B7-H4, and its presence was shown to also have an inhibitory effect on T-cell cytokine production, in particular IFN-γ, TNF-α and IL-2 [101]. These cytokines are all integral to the T-cell mediated anti-tumor response. As mentioned above, high levels of PD-L1, or B7-H1, have been shown to correlate with reduced patient survival in melanoma. B7-H4 has also been associated with poor patient outcomes, with lower expression levels correlated with better survival [107].

Regulatory T Cells and Melanoma

Tregs, as mentioned earlier, are a dominant mechanism of tumor immune escape. Tregs are overrepresented in the peripheral blood of patients with melanoma and are found in the tumor microenvironment and affected lymph nodes as well [108]. The percent of Tregs infiltrating the melanoma lesions has been shown by some studies to negatively correlate with survival [109]. An even better predictor of survival is the ratio of CD8 T cells to Tregs in the tumor microenvironment [110].

Tregs are thought to accumulate in the melanoma tumor microenvironment through a number of mechanisms. As mentioned earlier, Tregs are both being induced by PD-L1 expressing melanomas and selectively surviving, as melanomas expressing PD-L1 are causing apoptosis in effector T cells. Secondly, chemokine secretion and integrin ligand expression of the melanoma tumor cells attracts Tregs from the periphery. Thirdly, immunosuppressive cytokines locally secreted by melanoma, such as TGF-β and IL-10, can induce and expand Treg populations [100].

Tregs' immunosuppressive capabilities are enhanced through molecular mechanisms, such as melanoma's expression of indoleamine 2,3 dioxygenase (IDO), IDO is an immunomodulatory enzyme which allows tumor cell immune escape through the depletion of tryptophan [111].

As Tregs are clearly an important part of melanoma's immune microenvironment and its ability to progress despite melanoma's immunogenicity, the removal or suppression of Tregs would seem to be a target ripe for therapeutic intervention. Despite many promising studies, clinical efficacy has not yet lived up to its potential, with agents based on IL-2 suppression (a crucial cytokine for Treg activation and proliferation) and FOXP3 vaccination not consistently showing improvement. Other emerging therapies that modulate Tregs, such as CTLA-4 blocking agents (CTLA-4 serves as an inhibitory molecule constitutively expressed by Tregs) and PD-1 blocking agents, have shown some success and need to be explored further [100].

Other Immunomodulatory Mechanisms of Melanoma

Some of the most important cells in melanoma immune modulation are myeloid-derived suppressor cells (MDSCs). As mentioned earlier, these cells are potent suppressors of T-cell mediated responses and contribute to immunosuppression in SCC. MDSCs contribute to melanoma tumor immune tolerance by releasing adenosine, an important immune regulator that is known to hamper the adaptive immune response [112, 113]. The adenosine receptor subtype A2a inhibits T-cell functions. Additionally, the A2b receptor subtype, activated by high adenosine levels in the hypoxic tumor microenvironment, has been shown to promote the expansion of MDSCs and accumulation in melanoma tissue, leading to an immune suppression cycle. MDSCs are also attracted by other inflammatory mediators produced during tumor progression [114].

Another common immune evasion mechanism is the downregulation or alteration of the HLA class I molecule necessary for antigen presentation, immune recognition, and anti-tumor response [115, 116]. A mechanism of HLA molecule expression downregulation noted in metastatic melanoma is through the loss of beta2-microglobulin (B2m), an important component of the HLA molecule. The B2m deficiency is mediated by a mutation of one copy of the B2m gene, followed by the loss of the other copy, i.e. loss of heterozygosity. The decrease in B2m and subsequent decrease in HLA class 1 molecule expression is correlated with decreased CD8 T cell tumor infiltration [117]. This loss may be an early event in tumor progression, leading to melanoma cells immune evasion of T cells and eventual metastasis [118].

Immune Therapy

Though melanoma is extremely immunogenic, in cases where it persists and even metastasizes, it employs multiple methods of immune evasion. Therefore, immune modifiers are important therapeutic avenues of exploration. IL-15 is a cytokine that stimulates innate and adaptive immunity, making it a logical therapeutic option to be explored in melanoma. It shares a receptor with IL-2, a similarly immunostimulatory cytokine, and in studies both IL-15 and IL-2 augment NK cell cytotoxic activity and increase transcription of perforin in vitro [119]. In trials, IL-15 delivered to melanoma resulted in tumor regression and increased long term survival, as well as resulting in an influx of NK and memory CD8 T cells [120, 121].

Diphencyprone (DPCP) is a topical immunotherapeutic agent that is currently in clinical trials as a therapy for

melanoma. It has demonstrated melanoma regression with treatment in early studies. Melanoma's regression with DPCP treatment is thought to be due to TH17 lymphocytes, the immune modulator subset of T helper cells, possibly through TLR4 signaling [122].

Many other immune therapies for melanoma have been and are currently being explored, including, as mentioned earlier, vaccines to certain cell markers, immunostimulatory cytokines and modulations of populations of immune cells present in melanoma's immune microenvironment. All of this is discussed further in Chapter 51.

Responsiveness to Immune Therapy

The presence and distribution of tumor-infiltrating lymphocytes (TILs) may be prognostically useful in melanoma. Their quantity has been qualified as absent, brisk, or non-brisk with a brisk grading in some studies predicting better disease-free and overall survival outcomes, independent of sex, age, and tumor stage. This has been contradicted by other studies showing the presence of TILs promoting tumor outgrowth and metastasis [123–125].

These differences in outcome can be explained by a difference in the cohort of patients examined, and more importantly, a lack of differentiation between different phenotypes. With CD4 and CD8 T cells infiltrating, the distinctions are crucial, as CD8 T cells can be inactivated and anergic, and CD4 T cells are a heterogeneous group and can be composed of Th1, Th2 or Tregs [123].

The genetic expression profile of melanoma is also a good predictor of its responsiveness to immune therapy. Melanomas with an upregulation of immune-related genes have been shown to have a favorable clinical response. The specific immune genes upregulated are the IFN-stimulated genes, the CXCR5/CCR5 ligands, the chemokine genes and the genes associated with immune effector functions. Besides a favorable response to immune therapy, the gene expression profile of immune activation was correlated with a good prognosis in melanoma patients [126].

Conclusion

Cutaneous malignancies are among the most common human cancers and compose a very significant health burden. The interaction between the immune microenvironment and the tumor is crucial in determining the tumor's ability to establish itself, grow and metastasize. The interplay between the tumor cells and the immune system has demonstrated the different mechanisms of immune evasion and suppression employed by both NMSCs and melanoma. These include a modulation of the cancer gene profile, such as the downregulation of stimulatory genes key in tumor immunity. Additionally, the mechanisms involve changing which populations of immune cells are present in the tumor microenvironment, such as having fewer NK cells and more Tregs present. Lastly, the cytokine profile surrounding these cancers is altered, enhancing tolerance, such as the switch to a TH2 cytokine profile. The understanding of the immune microenvironment of cutaneous malignancies is crucial, as it affords many targets for therapeutic options. There is still much more to explore and understand about the immune microenvironment of each cutaneous malignancy and as more is discovered, there are more opportunities to efficiently treat and cure these cancers.

Questions

1. What effect do each cutaneous form of dendritic cells (DCs) have on the immune microenvironment of SCC?
   A. DC are unable to stimulate Th1 lymphocytes to produce Interferon-gamma
   B. All DC subsets hyper-activated
   C. Langerhans cells isolated from squamous cell carcinomas are more impaired than dermal DC from the same tumor
   D. DC derived from tumors respond normally to maturational signals

Correct answer: (A) DC derived from tumors are unable to activate Th1 lymphocytes, a key cell type in tumor surveillance

2. What statement best describes the role of Tregs in both BCC's and melanoma's tumor milieu?
   A. Tregs directly stimulate tumor growth by secreting cytokines and prostaglandins
   B. Tregs render tumor cells resistant to cell death by causing them to downregulate FAS
   C. Tregs impair DC maturation, promoting tumor tolerance by the immune system
   D. Tregs increase tumor cell mobility, by destroying extra cellular matrix

Correct answer: (C) Tregs impair the maturation of DC

3. Which statements best describe melanoma's immune evasion mechanisms?
   A. Impaired antigen-presenting cell maturation
   B. T cell anergy
   C. Induction and recruitment of Tregs
   D. Induction of myeloid-derived suppressor cells
   E. All of the above
   F. None of the above

Correct answer: (E) All of the above mechanisms are active in melanoma immune evasion

4. What T-cell receptor(s) plays an important role in melanoma's immune evasion (more than one response may be correct)?
   A. CD28
   B. CD40 ligand
   C. Chemokine receptors
   D. Adenosine receptor subtype A2a
   E. PD-1
   F. CTLA-4

Correct answers: (D, E, F) Adenosine receptors, PD-1 and CTLA-4 are all inhibitory receptors that melanomas engage that promotes immune evasion from T cell responses

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Biologic Therapies for Psoriasis

43

Lauren Guggina and Kenneth B. Gordon

Abstract

It is possible to suggest that the past two decades have led to greater advances in the treatment of extensive psoriasis than almost any other disease. The number of treatments available has grown tremendously and our treatment goals have changed radically. While at the turn of the century it was thought that complete clearance of psoriasis in patients who had extensive disease was not feasible in almost any patient, we now have medications that can attain complete clearance in up to 40 % of patients. With achievement of higher levels of response, a new understanding of the benefit of these responses to patients has identified a need for greater responses.

The development of targeted biologic immunotherapy for psoriasis has historically relied on the understanding of the pathophysiology of disease. However, the targeted nature of these medications, in concert with fortuitous clinical observation, has furthered the understanding of the pathological mechanisms in psoriasis. In turn, new insight has led to even more effective treatments. Thus, the use and study of biologics improves disease treatment in two ways. Their targeted nature can promote individual health by helping to treat the patient's disease while their study leads to global benefit by directing the creation of newer treatments. In this chapter, we will examine how biologics have changed our understanding of psoriasis and has led to the development of a multitude of new and exciting treatments.

Keywords

Psoriasis • Biologic therapy • Immunotherapy • Cytokines • Th17 • IL23 • IL17 • T cells

Pre-biologic Understanding of Psoriasis and the Early Biologic Immunotherapies

Today, we take the concept of psoriasis being an immune mediated disease for granted. However, prior to the 1990s, this perspective was not universally held. Treatment with medications that impacted immune activity, like cyclosporin A, implied that immune mechanisms were potent targets for treatment. Immunohistochemical evaluation of tissue from psoriatic plaques for known cytokines and subpopulations of T cells along with human/mouse skin explant models led to a model of psoriasis pathophysiology that mimicked contact dermatitis. Psoriasis was considered to be a disease driven by CD4+ T cells with Th1 cytokines, including IFN-γ, inducing the changes in the skin. These T cells would be stimulated by antigen presenting cells using known pathways of stimulation through the T cell receptor and co-stimulation through the CD80 and 86/CD28 pathways. These cells would traffic in and out of the skin through specific interactions with the vasculature and keratinocytes.

Initial trials with targeted therapy were focused on interacting with these pathways, either by elimination of activated

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CD4+ T-cells or interference with cell surface molecules governing co-stimulation and cell migration. Early studies with anti-CD4 monoclonal antibodies and denileukin diftitox demonstrated that reducing the number of activated T cells could, in fact, treat some patients [1, 2]. Likewise, blockade of co-stimulation the use of CTLA-4Ig, that interfered with CD28/CD80-86 interactions showed limited benefit for patients in very early clinical trials [3].

These observations resulted in the development of the first two biologic therapies approved for the treatment of psoriasis, alefacept and efalizumab. Alefacept was a fusion protein that was designed to block the co-stimulatory action of the CD2/LFA-3 interactions. In fact, it had the additional effect of eliminating T cells that expressed high levels of CD2, primarily previously activated CD4+ T cells [4]. According to the prevailing model of psoriasis at the time, this medication should have had significant efficacy. However, despite a proportion of patients having excellent long lasting improvement in disease, PASI 75 responses were only seen in 28–33 % of patients in the phase III trials [5, 6]. Despite being the first biologic therapy approved by the FDA for the treatment of psoriasis, due to this limited efficacy, alefacept was never adopted significantly by clinicians.

Efalizumab was the second biologic immunotherapy approved for psoriasis by the FDA. Efalizumab, too, was designed according to the CD4/Th1 model of psoriasis. This medication blocked CD11a, a sub-unit of LFA-1 that interacts with ICAM-1 on blood vessels, T cells, and keratinocytes to impact T cell activation and trafficking to the skin. While marginally more effective that alefacept, PASI 75 rates still were only 25–39 % [7–11]. Moreover, paradoxical psoriasis rebound was seen in patients who were withdrawn from the medication and even some who remained on treatment [12, 13]. Eventually, efalizumab was removed from the market due to cases of progressive multifocal leukencephalopathy (PML) [14]. However, the limited benefit of medications that targeted Th1 pathways demonstrated that new models of psoriasis were needed.

New Clinical Observations and a New Model for Psoriasis

Around the same time as alefacept and efalizumab were being developed, anti-tumor necrosis factor alpha (anti-TNF) therapies were being approved for Crohn's disease and rheumatoid arthritis. While TNF did not play a clear role in the early models of psoriasis pathophysiology, clinical observers were noting that treatment in patients treated for other conditions who had coincidental psoriasis were improving. This observation was made as early as 1996 in patients being treated in clinical trials of infliximab for Crohn's disease (Dafna Gordon, M.D., personal communication) and was reported in 2001 by Gottlieb and her co-workers [15]. Clinical trials with the anti-TNF agents, etanercept, infliximab, and adalimumab, discussed below, were significantly more successful than the anti-T cell agents and thus, demanded a re-thinking of the psoriasis model.

A number of observations helped to lead to a new understanding of psoriasis. Most information suggested a predominant role for innate immune responses in psoriasis. The role of cells and cytokines that play a critical role in local innate immune responses has become increasingly clear. Genetic analysis suggested a role for interleukin 23, a molecule that is produced by local mononuclear cells in response to tissue infection and injury [16, 17]. Similarly, Krueger and his associates identified that almost all the p40 protein in the skin was part of interleukin 23, not IL-12, as would be predicted by the Th1 model [18]. Likewise, high levels of IL-20 family cytokines along with IL-17, molecules that can induce keratinocyte changes seen in psoriasis, including more rapid proliferation and poor maturation, were present in high levels in psoriatic plaques [19, 20]. These findings were consistent with the observation that certain signal transduction pathways, including STAT-3 activation were a central finding in psoriatic keratinocytes [21, 22]. A number of experiments showed that all of these elements were necessary to see changes of psoriasis.

From these observations, and others, a new model for psoriasis has been developed that has governed our understanding of this disease and has potentially revolutionized therapy. As seen in Fig. 43.1, in patients who are genetically susceptible, some initiating step, local or systemic inflammation or injury, for example, initiates local, resident cells, including keratinocytes and mononuclear blood derived cells by cytokines like IFN-alpha. In turn, these cells producing innate activating factors, including TNF-α, lead to activation of specific dendritic cells, which, in turn, produce IL-23. IL-23 seems to be the critical switch that impacts local immune cells to produce the cytokines, including IL-17, that can have a direct impact on keratinocytes. At the moment, there is some controversy as to what cells are the primary producers of IL-17 in psoriasis. Specific T cells that produce IL-17, termed IL-17 T cells, presumably are central to IL-17 production in psoriasis [23]. However, immunofluorescence staining of IL-17 in psoriatic plaques has suggested that both neutrophils and mast cells may also be important in IL-17 release [24].

IL-17 is the direct connection between the immune system and keratinocytes. When bound to IL-17 receptor, expressed on keratinocytes, these skin cells begin to express anti-microbial peptides, secrete chemokines, and alter cell growth and differentiation in ways that are found in psoriasis [20, 25, 26]. Thus, a complete pathway for psoriasis, from initiating factors to keratinocyte alterations is evident in this

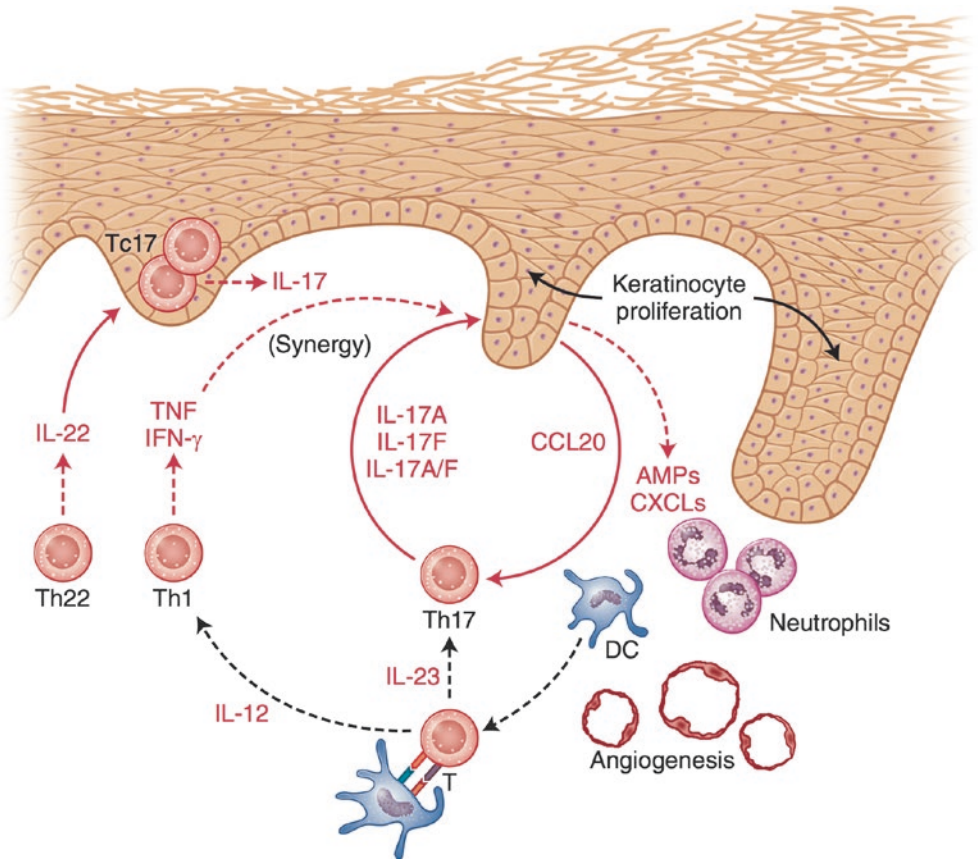

Fig. 43.1 Cytokine cascade resulting in clinical lesions of psoriasis. Interleukin 23 leads to production of IL-17 and IL-22 resulting in keratinocyte hyperproliferation and abnormal maturation. Additionally, the altered keratinocytes produce chemokines and other immune reactive proteins that promote increased inflammation, angiogenesis, and continued disease activity

model. However, it is critical to remember that any depiction of psoriasis immunopathogenesis as strictly linear is misleading. IL-17 and keratinocyte derived chemokines have important effects on the maintenance of the immune response by increasing cell migration into the skin [26–28]. Thus, the model can be visualized as having a feedback loop where psoriatic activation leads to perpetuation of the plaques.

The Psoriasis Model and Biologic Therapy

The model outlined above has significant implications for both older and newly designed biologic immunotherapy. In the schematized model in Fig. 43.2, it becomes clear that a number of places in the immune response could be excellent targets for treatment. These targets include the activation of IL-23 producing dendritic cells, the activation of Th17 cells by IL-23, and the induction of keratinocyte changes by IL-17. In fact, all of these breaks on the psoriasis pathways are in use for the treatment of psoriasis today. Anti-TNF agents have a significant role in inhibiting dendritic cell activation, ustekinumab binds and inactivates IL-12 and 23, while developing agents guselkumab and tildrikizumab block IL-23 alone, the newly approved secukinumab, ixekizumab and brodalumab block IL-17 interactions with keratinocytes. All these agents have a significant role in modern treatment of psoriasis and can lead to marked improvement in patients and better quality of life for those who suffer from psoriasis. These classes of biologic agents will be reviewed from the perspective of where they have the greatest impact on the immunopathogenesis of psoriasis.

Inhibiting Inflammation and Dendritic Cell Activation: The Anti-TNF Agents

One of the most important observations in the validation of the new psoriasis model was the understanding of how anti-TNF therapy could fit into this. As mentioned above, the efficacy of anti-TNF agents was identified prior to the elucidation of the current. Since these agents can be highly effective, it was critical to see how they may work. Krueger and his co-workers used gene expression signatures to investigate the earliest mechanisms by which the anti-TNF agent etanercept impacted local immunity by comparing Th1 expression signatures and Th17 signatures. They found that Th17 gene expression was inhibited earlier than Th1 responses and that the correlation of inhibition of Th17 genes to response was much greater than that of Th1 responses [29]. Moreover, by use of immunohistochemistry,

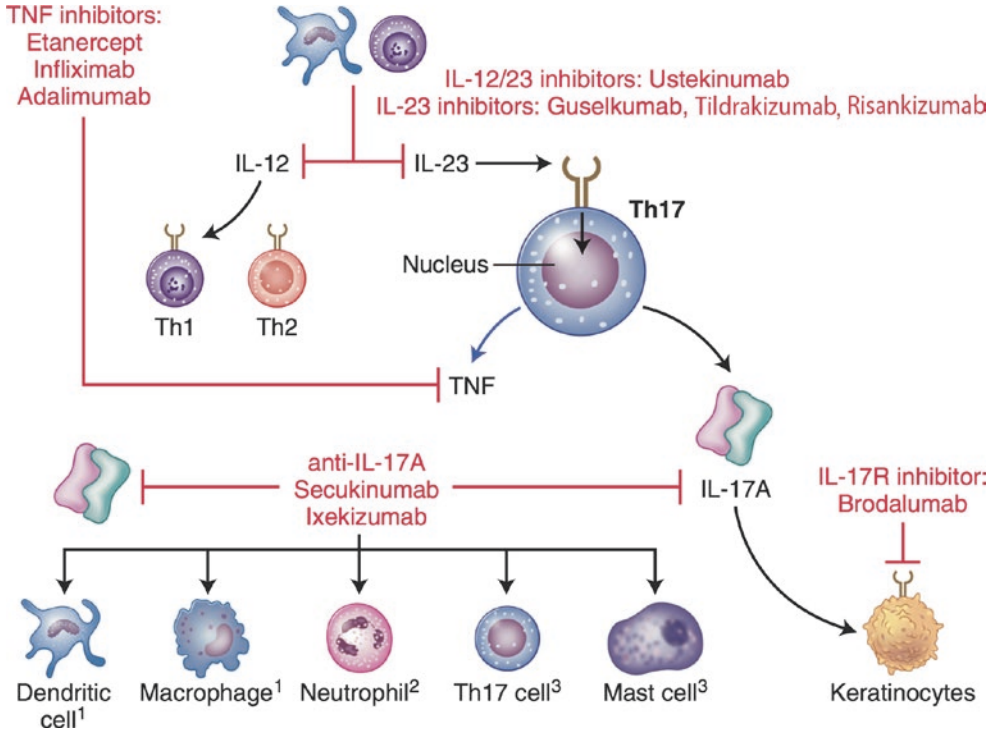

Fig. 43.2 Potential targets of immunotherapy for psoriasis including TNF-a, IL-17, and IL-23. Agents approved or in final stages of trials are listed with their targets

they identified that etanercept inhibited production of IL-23 and TNF by dendritic cells and proposed that this was the primary effect of anti-TNF agents [23].

Anti-TNF agents are, at the time of this writing, the most widely used family of biologic agents for psoriasis. There are three agents in this class that bind and deactivate their TNF target, etanercept, infliximab, and adalimumab that are presently approved for the treatment of psoriasis.

Etanercept

Etanercept is a fusion protein of the p55 receptor for TNF bound linked to a constant region, antibody backbone. It was the first anti-TNF agent approved for the treatment of psoriasis. Etanercept is dosed as a 50 mg subcutaneous injection given twice weekly for the first 3 months of treatment followed by weekly dosing. Etanecept is also approved for the treatment of psoriatic arthritis.

The primary endpoint of the pivotal phase III clinical trials with etanercept was after 12 weeks of therapy, when 49 % of subjects reached a PASI 75 at that time point [30]. Higher rates of response, including PASI 90, and complete clearance were not reported. At 6 months, with the continued 50 mg twice weekly schedule, the PASI 75 rate increased to 59 % [30]. However, with the indicated dosing schedule and dose reduction after 3 months, PASI 75 rates only reach 54 % [31]. Additional studies with 25 mg twice weekly resulted in only 51 % of patients reaching a PASI 75 [32]. Longer-term analysis has not been done in these clinical trials. Additional studies have shown that etanercept can be successful in treating scalp psoriasis [33].

Infliximab

Infliximab was the second medication in this class to be approved for psoriasis and is also approved for psoriatic arthritis. It is dosed as an IV infusion, usually over 2 h starting at a dose of 5 mg/kg given weeks 0, 2, 6 and then every 8 weeks. Infliximab is a chimeric, monoclonal antibody that maintains a primarily murine binding site.

Since its approval to very recently, infliximab has had the highest short term efficacy of any biologic immunotherapy. At the 10 week primary endpoint of the phase III clinical trials, infliximab had a PASI 75 of 80 % and a PASI 90 of 57 %, and complete clearance of 26 % [34]. However, due to a number of potential factors including a rapid decrease in blood levels due to the decrease in the frequency of dosing in maintenance therapy and/or immunogenicity of the medication, the clinical efficacy of infliximab decreases significantly over the first year. At 1 year, the efficacy decreases to only 61 % of patients achieving a PASI 75 from the original highs [34].

Adalimumab

Adalimumab was the most recent anti-TNF agent to be approved for the treatment of psoriasis. This medication is a human monoclonal antibody that is dosed as a 40 mg subcutaneous injection given as 80 mg week 1, 40 mg week 2, then 40 mg every other week. Like the other anti-TNF agents, it is approved for the treatment of psoriatic arthritis, as well.

The primary endpoint of the phase III clinical trials of adalimumab were 16 weeks. Inclusive in these trials was the Champion trial, the first biologic therapy comparator trial, comparing adalimumab with methotrexate. The PASI 75 in these trials was 79.6 % with a reported PASI 90 of 51.3 % and complete clearance of 16.7 %. In the Champion trial, adalimumab was markedly superior to methotrexate. Long term efficacy of adalimumab is, unfortunately, difficult to ascertain as the primary trials were done with a withdrawal and retreatment structure [35]. However, it is clear that there is some loss of effect of adalimumab over time [36]. Studies of adalimumab in palmar-plantar psoriasis have also shown benefit [37].

Anti-TNF Side Effects

While there are variances in safety outcomes between the anti-TNF agents, the general issues associated with them are present across the class. In understanding anti-TNF safety, it is critical to remember that this is the only class of psoriasis biologics that was initially developed for other indications, Crohn's disease and rheumatoid arthritis. Most of the reports of safety concerns with these medications stem from use in these other indications. An analysis by Burmester and colleagues showed that the rates of medication associated side effects for adalimumab were markedly lower for psoriasis in clinical trials than for other indications [38]. This finding may be due to a healthier clinical trial population, younger age, and/or the presence of other immune suppressive medication. Yet it makes comparison of the side effect profiles of anti-TNF agents with other agents approved only for psoriasis extremely difficult.

The primary risk associated with anti-TNF agents is infection. Anti-TNF agents have been associated with slight but increased risk of serious infections, opportunistic infections, and reactivation of tuberculosis and hepatitis B in the general populations studied. However, when psoriasis is studied exclusively, no increased risk of infection can be identified in short-term treatment when compared to placebo [39]. Nonetheless, it is likely that there is an infectious risk associated with anti-TNF agents for psoriasis, especially for non-serious infections [40]. Screening for latent tuberculosis should be done regularly and prior to starting patients should be screened for exposure to hepatitis B [41, 42].

Cancer risk of anti-TNF agents is more controversial. Concerns of an increased risk of lymphoma and certain solid tumors are common. No good information on these long-term risks exist in psoriasis patients treated with anti-TNF agents. However, in meta-analysis analysis of rheumatoid arthritis patients treated with TNF alpha inhibitors, lymphoma risk does not seem to be any higher in anti-TNF treated populations than in comparably severe patients treated with other modalities [43]. Likewise, the incidence of solid tumors as well as recurrence or cancer in patients who are treated with anti-TNF's does not seem to be increased [40]. In a recent prospective long term study of etanercept users, the rates of malignancies excluding NMSC and lymphoma were not higher than the rates of the general psoriasis population [44]. The possible exception to these findings are non-melanoma skin cancer and, possibly, melanoma, that seem to be a bit higher in anti-TNF treated patients [40].

Other risks associated with anti-TNF therapy, including worsening of demyelinating disease and congestive heart failure come from attempts to treat these conditions with this type of therapy [39, 41]. Anti-TNF agents did not seem to show benefit in these conditions and treatment seemed to worsen some patients. Thus, anti-TNF therapy should be avoided in patients who suffer from or are at high risk for these conditions.

Inhibiting Th17 Activation: Ustekinumab and the Anti-IL-23 Agents

Ustekinumab

The next step in the simplified sequential model of psoriasis pathogenesis is the activation of Th17 cells after stimulation with IL-23. Ustekinumab is a human monoclonal antibody directed against the p40 sub-unit protein shared by IL-12 and 23. Interestingly, it was initially postulated to be of use in psoriasis based on the older Th1 model through its impact on IL-12. Subsequently, the belief is that it is the impact on IL-23 that is most important [18, 45–47]. Ustekinumab has been shown to markedly down regulate gene expression in the Th17 pathway emphasizing its mechanism consistent with the sequential model [46].

Ustekinumab is given as either a 45 or 90 mg dose depending whether the patient is greater than or less than 100 kg. The primary endpoint of the initial phase III clinical trials with ustekinumab was PASI 75 at 12 weeks of therapy. Rates of response were around 67 % for those receiving 45 mg and 66–76 % for those receiving 90 mg dosing [48, 49]. In long-term extension trials, response was maintained with no evidence of significant safety signals [50]. One concern with blocking IL-12 was that this would induce immune deviation and increase the risk of infection. However, 5 year follow up

in clinical trials does not seem to lend credence to this theory with no increase in infection rates seen [51]. However, trials with another IL-12/23 inhibition, briakinumab suggested increased rates of infectious risk, skin cancers, and major adverse cardiac events suggesting that continued attention needs to be paid to safety outcomes [52, 53].

P19 Blockade: Inhibiting IL-23 Without IL-12

Despite the evidence suggesting the safety of ustekinumab, the data on briakinumab as well as theoretical concerns has led to the development of biologic medications that bind only to the p19 subunit of IL-23. This mechanism would leave IL-12 intact. Moreover, trials with these agents would answer a fundamental question, whether the IL-12/Th1 pathway that was the focus of the initial immunological models of psoriasis, has a therapeutic role at all in the treatment of this disease. At the time of this writing, phase II data on two medications, guselkumab and tildrakizumab, have been published. Both of these agents have high-level responses [54, 55]. In fact, in a comparator trial with adalimumab, guselkumab was more efficacious for both short and year-long therapy from a phase II study (Gordon, *NEJM*, in press). Peak efficacy was seen with PASI 75 scores at week 16 of 81 % compared to 71 % for those patients receiving adalimumab. Tildrikizumab also showed high level responses in phase II trials with PASI 75 results reaching 72 % (in press *British Journal Of Dermatology*). This high level efficacy continued through week 52. While safety is impossible to evaluate in small, phase II trials, it is difficult to imagine that the safety record will be inferior to that seen with ustekinumab. Thus, these trials validate the present model of psoriasis with a minimal to no role for the Th1 pathway and suggest more specific pathways for the treatment of psoriasis.

Inhibiting IL-17 Effector Function: Anti-IL-17 Monoclonal Antibodies

Upon identification of IL-17 as the critical connection between the immune system and the keratinocyte reaction in psoriasis, a number of biologic immunotherapies have been developed to inhibit this key cytokine. There are a number of different ways this IL-17 can be inhibited [56]. There are multiple isoforms of IL-17 found in psoriatic skin. There is upregulation of IL-17A and IL-17 F, which can form 2 sub-unit homodimers or form an A/F heterodimer, as well as IL-17C [25, 57]. Additionally, IL-17C is produced by keratinocytes and may have an autocrine function in the skin [58]. Interestingly, all of these isoforms bind to the IL-17 receptor A to have their effector function. Thus, it is possible to inhibit this system either by making a biologic molecule that would bind the cytokines themselves or more generally block the pathway by inhibiting binding to the receptor.

There are three new biologic medications that are designed to specifically inhibit IL-17. The first of these to be approved by regulatory authorities is secukinumab, a molecule that binds IL-17A specifically. Similarly, ixekizumab acts specifically on IL-17A not binding IL-17 F or IL-17C. In contrast, brodalumab blocks this pathway at the receptor level, binding and blocking activity of the IL-17RA receptor and inhibiting all three isoforms. Therefore, two theoretical questions arise. First, would blockade of the entire system through the receptor give higher levels of efficacy and second, is more specific blockade of IL-17A alone a safer strategy? These questions can only be answered by clinical data.

Phase III data on all of these drugs has recent become available. At the time of this writing, secukinumab has the most extensive available data set. In a recent publication of two phase III studies, this medication shows PASI 75 responses at week 12 of 77–82 % with 300 mg dosing and 67–72 % with 150 mg dosing [59]. In phase III trials, ixekizumab, shows PASI 75 results of 87–89 % and PASI 100 results of 35–41 % (*Lancet,* in press and presented at World Congress of Dermatology (WCD) 2015). Brodalumab has extremely high responses, as well, with PASI 75 responses of up to 85 % and PASI 100 of up to 42 % (presented at WCD 2015). What is fascinating is that the responses to all these medications is extraordinarily fast. Theoretically, this rapid response is related to the blockade of IL-17 itself which, along with inhibition of continued immune responses, stops the binding of the cytokine that directly impacts keratinocyte responses. Thus, as we are measuring the severity of psoriasis through clinical changes associated with keratinocyte reactions to inflammation, directly modifying this response could lead to greater improvement in disease, faster.

As secukinumab and ixekizumab has only recently been approved and brodalumab is not yet in general clinical use, it is impossible to definitively judge long-term safety. However, the clinical trials for all these medications have very large data sets and thinking of them collectively gives a sense of any safety issues of the class. By considering the role of IL-17 in health, the production of local responses to invading organisms, one would predict that these medications would increase the risk for local staphylococcal infections and candida infections. In fact, all three of these medications show some increase in very mild candida infections in the clinical trials population [59–61]. However, other infectious or other safety risks do not seem to be present. One additional completely unpredicted safety concern is the possibility of worsening established Crohn's disease. From a purely immunological point of view, blockade of IL-17 should

induce a great improvement in Crohn's. However, in clinical trials of patients with active Crohn's disease, a number of subjects worsened. At present, this difference between the very high level responses seen in psoriasis and the worsening of Crohn's remains a mystery though it has been ascribed to differences in the microbiome of the skin and the gut. Luckily, the effect on Crohn's seems to have little impact on patients with psoriasis though caution should be taken in patients who have both conditions.

One final safety concern has recently been identified with brodalumab among this class of medications, specifically. Though the data have not been fully analyzed, there have been a small number of completed suicides in the clinical trials of brodalumab. To date, there have been no completed suicides in either the secukinumab nor the ixekizumab clinical development programs, leading to a concern that this finding may be specific for blockade of IL-17 receptor rather than the cytokine IL-17A itself. While this remains speculation, the lack of events in both secukinumab and ixekizumab are comforting for the clinical use of these agents.

Conclusion

Psoriasis has a tremendous impact on patients who suffer from the condition and our society. Traditional treatment has been insufficient to ease the suffering associated with this disease. In the last two decades, however, the revolution of biological immunotherapy has completely changed how we think of psoriasis. From clinical observations and an understanding of pathophysiology, a new era of treatment has evolved. Rather than a disease that is possible to improve in some patients, we are approaching near clearance in a great majority of patients. This difference cannot be overstated in terms of the benefit it will provide for many years to come.

Questions and Answers

1. Which of the following drugs blocks the interleukin (IL) 17 receptor?
   A. adalimumab
   B. brodalumab
   C. guselkumab
   D. Ixekizumab
   E. Secukinumab

2. Which of the following drugs blocks both IL12 and IL23?
   A. brodalumab
   B. guselkumab
   C. risenkizumab
   D. tildrakizumab
   E. ustekinumab

3. Which isoform of interleukin (IL) 17 is blocked by ixekizumab?
   A. A
   B. B
   C. C
   D. D
   E. E

4. A surprising (from an immunological viewpoint) adverse event reported in some patients receiving anti-IL17 therapy, was worsening of which condition?
   A. Cardiac disease
   B. Crohn's disease
   C. diabetes
   D. Psoriasis
   E. Psoriatic arthritis

5. Which protein subunit is shared between IL12 and IL23?
   A. P12
   B. P19
   C. P23
   D. P35
   E. P40

Answer key

1. B
2. E
3. A
4. B
5. E

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Therapy of Immunobullous Disorders

44

Kyle Amber and Michael Hertl

Abstract

Immunobullous disorders are chronic-relapsing diseases of the skin and mucous membranes with high morbidity and mortality which are linked to IgG autoantibodies that target adhesion molecules of the skin. In most instances, therapeutic approaches comprise the application of general immunosuppressive drugs. Treatment with high-dose systemic glucocorticoids and adjuvant immunosuppressive drugs is effective, but also bears the risk of considerable side-effects. In light of recent advances in the pathogenic understanding of immunobullous disorders, additional therapeutic targets including pro-inflammatory cytokines (TNF- α) and peripheral B cells have been identified. Herein we review the role of biologic inhibitors of these two processes.

Keywords

Immunobullous disorders • Pemphigus • Pemphigoid • Rituximab • Infliximab • Adalimumab • Etanercept

Introduction (Therapeutic Targets in Immunobullous Disorders)

Immunobullous disorders are chronic-relapsing diseases of the skin and mucous membranes with high morbidity and mortality [1, 2]. They are caused by a loss of intercellular adhesion of epidermal keratinocytes or dermal epidermal basement membrane, respectively, as a result of the binding of IgG or IgA autoantibodies against components of desmosomes, hemidesmosomes or components of the dermal-epidermal junction and dermal anchoring fibrils, respectively. Despite remarkable progress in understanding the immune pathogenesis of these disorders, the thorough characterization of their autoantigens and a general understanding of the immunological network which eventually leads to the formation of pathogenic autoantibodies is rather limited [3, 4].

In most instances, therapeutic approaches comprise the application of general immunosuppressive drugs [3, 4]. Treatment with high-dose systemic glucocorticoids and adjuvant immunosuppressive drugs is effective but also bears the risk of considerable side-effects. Due to their rarity, only few prospective controlled clinical trials are available in pemphigus and bullous pemphigoid (BP) which are limited by the low numbers of patients studied and the lack of statistically significant differences in many studies [5, 6]. A few studies compared different doses of prednisolone, i.v. corticosteroid pulses versus placebo, azathioprine versus mycophenolate mofetil, and the use of adjuvant treatment with methotrexate, cyclosporine, cyclosphosphamide, and high-dose intravenous immunoglobulins [7–9]. The combination of systemic corticosteroids (prednisolone, 1.0–1.5 mg/kg/d) and corticosteroid-sparing immunosuppressive drugs, mostly azathioprine and mycophenolate mofetil, is regarded as standard first-line therapy by most dermatologists. Derived from an advanced pathogenetic understanding, additional therapeutic procedures such as the depletion of peripheral B

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cells with rituximab or the removal of pathogenic IgG autoantibodies by immunoadsorption has proved to be efficacious in some of these disorders, such as pemphigus, but have not yet been fully validated [10–12]. We here focus on two specific therapeutic approaches, i.e. the blockade of pro-inflammatory cytokines, i.e. tumor necrosis factor-alpha (TNF-α) and therapeutic depletion of autoaggressive B cells with the anti-CD20 monoclonal antibody, rituximab, which has shown major promise in achieving long-term remissions in pemphigus [13–15].

Blockers of Pro-inflammatory Cytokines

Tumor necrosis factor alpha (TNF-α) is a cytokine involved in systemic inflammation that primarily acts on NK-kB pathway and the MAPK pathways. TNF- α levels have been shown to be elevated in numerous autoimmune diseases, providing an avenue for treatment by decreasing these levels [16]. The finding that TNF- α levels are increased in the sera of pemphigus and pemphigoid patients [17, 18] has led to research in the area of TNF- α inhibition in immunobullous diseases. In pemphigus, TNF- α serum levels correlate with both the number of lesions as well as the IgG autoantibody titers [19, 20]. In patients with bullous pemphigoid, TNF- α serum levels are likewise elevated compared to healthy controls and correlate with the number of lesions [21, 22]. Studies of blister fluid in both PV and BP have demonstrated increased levels of TNF- α compared to serum [22–24].

Despite the clear increase of TNF- α in immunobullous disease, its functional role in the disease process is not entirely clear. In vitro studies have demonstrated that TNF- α increases the IgG autoantibody-mediated acantholysis of pemphigus vulgaris (PV). This was likewise demonstrated in a murine model [25, 26]. This increased acantholysis was demonstrated with TNF- α dependent increases in complement activation [25] which is a common finding histologically in PV patients. Yet, these data only demonstrate TNF- α's synergy with pathogenic IgG autoantibodies.

Initial reports of treatment with biologic TNF- α inhibitors appeared promising for the treatment of PV, pemphigus foliaceus (PF) and pemphigoid with success reported using infliximab [27–29], adalimumab [30] and etanercept [31–36]. Formal studies, though limited in size, have not been quite as promising. A randomized controlled trial of standard therapy with adjuvant etanercept failed to demonstrate any improved efficacy compared to standard therapy plus placebo in PV patients [37]. Likewise, a randomized study of pemphigus patients treated with infliximab with prednisone failed to demonstrate any clinical advantage over prednisone alone, though those patients treated with infliximab did IgG experience a significant decrease in anti-Dsg3 and anti-Dsg1 titers [38]. Oddly, studies using adjuvant sulfasalazine and pentoxifylline, a non-biologic and less specific TNF- α inhibitor, demonstrated improved patient outcomes compared to the standard therapy plus placebo group [39]. Likewise, in a randomized controlled trial of ocular cicatricial pemphigoid patients, adjuvant pentoxyfylline was associated with improved clinical and histopathologic outcomes [40]. The efficacy of non-biologic and less specific TNF- α inhibitors may suggest a therapeutic target that involves TNF- α, but does not solely deplete it.

While the efficacy of biologic anti-TNF- α appears limited, there have been additional reported cases of paradoxical development of immunobullous disease secondary to beginning anti-TNF- α biologic agents [41–44]. The paradoxical development of certain diseases following the initiation of TNF- α inhibitors has been well documented, with psoriasiform dermatoses, granulomatous eruptions, and uveitis being most frequently reported [45]. The paradoxical development of pemphigus may be explained by the observation that in one study, TNF- α negatively correlated with anti-desmoglein IgG autoantibodies in pemphigus patients in remission [46]. Thus, certain TNF- α levels may be required to keep the disease in check.

Rituximab in Pemphigus and the Pemphigoids

Rituximab is a chimeric monoclonal IgG that targets CD20, a glycosylated phosphoprotein expressed on the surface of all B-cells starting at the pro-B-cell phase. As B-cells mature, their expression of CD20 increases. Once B-cells mature into plasma cells, however, expression of CD20, as well as many other common B-cell surface antigens ceases. Rituximab therefore destroys B-cell progenitors, but not antibody secreting plasma cells or stem cells. Short-lived plasma cells are more dependent on CD20 memory B-cells for replenishment than long-lived plasma cells. Thus, rituximab has a greater effect on depletion of short-lived plasma cells. This can be demonstrated by the relative decrease in serum IgG autoantibodies to total immunoglobulin levels seen clinically, as IgG autoantibodies appear to be produced more frequently by short-lived plasma cells [47–49]. Likewise, IgG against common pathogens does not decrease following treatment with rituximab, indicating a failure to deplete long-lived plasma cells [47, 50]. Rituximab additionally exerts an effect on autoreactive T-cells in a less direct manner [51, 52].

The process of rituximab mediated B-cell depletion takes approximately 2–4 weeks, while B-cell repopulation occurs 5–6 months after infusion [14, 52–54]. With the destruction of the late pro-B-cells, new generations of immature B-cells replace the old, undergoing VDJ heavy chain arrangement and VJ light chain arrangement, which results in a novel antibody repertoire [53].

The loss of autoreactivity following treatment with rituximab is likely second to the changes in IgG reactivity against particular subdomains within Dsg3. Thus clinically, patients may maintain IgG antibodies against the ectodomain of Dsg3 despite treatment, yet these antibodies will not be pathogenic. As such, anti-Dsg1 IgG titers may be a more reliable marker of clinical status than anti-Dsg3 IgG [55].

Clinical Application

The efficacy of rituximab in the treatment of PV has been well documented through numerous large sized studies [13, 14, 56–60], with approximately 60–80 % of PV and PF patients experiencing complete remission [61–63]. Likewise, the use of rituximab in mucous membrane pemphigoid has additionally been well documented. Le Roux-Villet et al. demonstrated complete response in all affected sites in 68 % (17/25) of patients while Heelan et al. reported 75 % (6/8) patients to have a complete remission following a single cycle of rituximab [64, 65]. Fewer studies have been conducted in the other immunobullous disorders. In a review of 16 BP patients treated with rituximab, Shetty et al. demonstrated that 69 % of patients experienced complete remission, which remains comparable to that seen in PV and PF [66]. Likewise, numerous case reports have demonstrated successful clinical outcomes in patients with epidermolysis bullosa acquisita (EBA) treated with rituximab [67–75]. As EBA remains extremely rare with an estimated incidence of 0.2 per million per year [76], it is unlikely that larger studies will be possible. Despite the paucity of reported clinical outcomes in EBA patients treated with rituximab, efficacy appears comparable to that of other immunobullous disorders.

In contrast to the other immunobullous disorders, paraneoplastic pemphigus does not exhibit as consistent of a response to treatment, with most studies demonstrating only a marginal clinical improvement [77]. This is curious, as paraneoplastic pemphigus, like PF and PV, is an IgG mediated disease with numerous autoantibodies present. Additionally, paraneoplastic pemphigus most commonly occurs secondary to lymphoproliferative disorders, particularly non-Hodgkin lymphoma which is often responsive to rituximab on its own [77]. Thus, the effect of rituximab could be twofold by targeting the lymphoproliferative disease process while also leading to the destruction of B-cells before they develop into IgG autoantibody secreting plasma cells. Schadlow et al., however, presented a case that demonstrates that this may not be the case. They described a patient with long standing B-cell lymphoma who did not experience clinical improvement with rituximab [78]. It is thus possible that the length of time with the primary malignancy may affect the response to rituximab in paraneoplastic pemphigus. Nevertheless, paraneoplastic pemphigus is a complex disease that does not entirely follow the pathogenic steps seen in other immunobullous disorders.

Treatment Protocols

Standard treatment protocols for immunobullous disorders include the lymphoma protocol (375 mg/m^2 × 4 weeks) and the rheumatology protocol (1000 mg weekly × 2 weeks), with some less common protocols halving the dosage or duration of treatment. In one review by Zakka et al., patients treated with the lymphoma protocol demonstrated a slightly lower response rate with a higher mortality rate, yet a low rate of infection and relapse protocol than those patients receiving the rheumatology protocol [62]. Our analysis, however, demonstrated that patients responding to a single cycle of rituximab had a greater disease free period when treated with the lymphoma protocol rather than the rheumatology protocol. We additionally found the half rheumatology protocol (500 mg weekly × 2 weeks), to be the least efficacious of protocols, with a shorter time until relapse and fewer patients experiencing complete response [79]. Kanwal et al. likewise demonstrated this in a prospective blinded study comparing the full rheumatology protocol to the half rheumatology protocol [80]. Heelan et al. demonstrated success with a modified rheumatology protocol whereby patients receive 1 g on day 1 and 15, with 500 mg given at 6 month intervals when clinically necessary [60]. With this protocol, they achieved 89 % remission with or without adjuvant, and 28 % remission that did not necessitate adjuvant therapy. Nevertheless, treatment preferences vary widely between physicians and there still remains significant controversy regarding protocol selection [81].

Adjuvant therapies may additionally be used with these rituximab protocols, both during the initial cycle of rituximab and as maintenance. While more traditional immunosuppressants such as corticosteroids, azathioprine, mycophenolate and cyclophosphamide have been used, newer adjuvants such as immunoadsorption or IVIG have proven to be efficacious as well [13, 15].

Safety

The most common adverse reaction to rituximab is a mild transfusion reaction [82, 83], with more severe complications including cardiac toxicity and pulmonary toxicity [84, 85]. The most common associated adverse event remains infection. The risks of rituximab in the treatment of immunobullous disorders must, however, be compared to those associated with chronic steroid suppression and non-biologic

immunosuppressive medications. In a review of 153 pemphigus patients treated with rituximab, Feldman et al. demonstrated that only 7 % developed serious infections with 1.3 % fatalities [61]. Similarly, a large study of rituximab treatment for systemic lupus erythematosus (SLE) demonstrated a 9.5 % risk of serious infection [86]. However, in a large scale review of patients treated with rituximab for varying autoimmune disease, those with autoimmune blistering disease had a significantly greater mortality rate (10.4 % vs. 2.4 %) than those with other autoimmune diseases [84]. In contrast, an alternative study demonstrated the highest incidence of opportunistic infection in patients treated for SLE [87]. While the incidence of adverse events is reasonably a cause for concern, these values must be weighed against the risks of alternate therapies

Patients treated with corticosteroids demonstrated an 8 % incidence of mild to severe infections and patients treated with corticosteroids plus mycophenolate mofetil demonstrated a 21 % incidence of infection [88]. Interestingly, of the mucous membrane pemphigoid patients treated with rituximab, only those on concomitant immunosuppressants and high-dose corticosteroids experienced severe infectious complications [64]. As the classification of infection severity varies between individual studies, it remains challenging to truly compare the risks of infection in rituximab to traditional, non-biologic therapies.

Hepatitis B reactivation additionally remains a concern when starting a patient on rituximab. It is thus recommended to screen patients for hepatitis B before beginning therapy [89]. Chronic or high dosed systemic steroid therapy, however, also increases the risk of hepatitis B reactivation, though routine screening is not considered equally essential [90].

While IVIG has demonstrated efficacy in treating immunobullous disorders, it has also been suggested as a useful adjuvant to rituximab in decreasing the incidence of infections [13, 91]. While IVIG in theory repletes serum IgG at the time of B cell depletion, it is unclear how the two medications interact with each other, complement and the Fc receptor. Additionally, the use of IVIG in itself comes with certain risks ranging in severity from mild infusion reactions to aseptic meningitis [92].

Treatment Resistance

Resistance to rituximab can occur through the formation of anti-chimeric antibodies, as the murine sequences of the chimeric IgG_1 may contain immunogenic sequences. These anti-drug antibodies were more often observed in patients treated for autoimmune diseases rather than lymphoma. These anti-drug antibodies interfere with the ability of rituximab to bind to B-cells in vitro [93], leading to a decreased clinical response to treatment [94]. Additionally, these anti-drug antibodies are associated with the development of serum-like sickness and infusion reactions.

Fc receptor polymorphisms may additionally lead to treatment resistance by decreasing the affinity of receptor binding to IgG. For example, the FcγRIIIa polymorphism and FcγRIIa polymorphism are associated with a decreased response to rituximab in patients treated for lymphoma [95]. Similar findings were seen in SLE, where the FcγRIIIa polymorphism was predictive of decreased treatment efficacy, with a tenfold increase in rituximab serum level necessary to achieve comparable B-cell depletion. While these polymorphisms have known effects in other disease processes such as lymphoma and SLE, it is unclear what effect they have in immunobullous disorders. For example, while the spliced mRNA transcript of CD20 (D393-CD20) has been associated with treatment resistance in lymphoma patients, this transcript was not associated with treatment failure in patients with pemphigus [96].

The presence of central memory cells occupying the bone marrow compartment as well as long-lived B memory may lead to a decreased response to treatment, as these are not viable targets for rituximab [97]. This is consistent with our finding that an increase in the duration of disease was associated with a decrease in clinical response to treatment in patients with PV and PF treated with rituximab [79]. Lunardon et al. likewise found that patients treated with rituximab earlier in the course of the disease had better outcomes [58].

Relapse

Despite its immediate effectiveness in a majority of patients, rituximab does not necessarily appear to alter the long-term relapse rate [55]. In fact, in one large retrospective study of 92 pemphigus patients, 61 % of patients relapsed following a single cycle of the rheumatology protocol, with a mean duration of 15 months [60]. Interestingly, patients who required adjuvant immunosuppression experienced relapse sooner than those only receiving rituximab. We did not, however, find a correlation between adjuvants used and time to relapse [79]. Studies in mucous membrane pemphigoid have additionally demonstrated significant relapse rates necessitating further cycles in a fairly short period of time [65, 98].

Relapse rates may, however, be tied to the underlying genetic aberrations leading to clinical disease. For example, following multiple treatments with rituximab over an extended course of time, patients no longer express anti-Dsg3 B-cell response. Once this lineage of B-cells is successfully removed from the patient's immune repertoire, clinical disease ceases [99]. Likewise, there is an increase in the expression of the VH1-46 mutation in pemphigus patients, a

mutation of a gene involved in heavy chain VDJ recombination. This mutation leads to Dsg3 autoreactivity with few to no mutations necessary [100]. Thus in these patients, rituximab may not effectively prevent relapse, as new anti-Dsg-3 clones will simply reform following B-cell depletion.

Certain therapeutic options may exist to increase the length of time until relapse such as immunoadsorption [79] or minimal maintenance therapy. The use of intermittent rituximab following an initial cycle has proven controversial. Gregoriou et al. showed that purely prophylactic rituximab given 6 months following the initial rituximab cycle was ineffective in preventing relapse in pemphigus patients [101]. In cases of impending relapse however, Cianchini et al. demonstrated that repeated cycles of rituximab sufficiently mitigated clinical relapses without necessitating the use of concomitant immunosuppression [59].

Questions

1. What is the most common adverse reaction occurring with rituximab administration?
   A. Mild transfusion reactions

2. Does rituximab directly deplete plasma cells?
   A. No. It destroys pre-B-cells and mature B cells which eventually develop into plasma cells, but it has no direct effect on plasma cells or stem cells

3. What are the two most common dosing protocols for rituximab in the treatment of immunobullous disease?
   A. The lymphoma protocol (375 mg/m^2×4 weeks) and the rheumatology protocol (1000 mg weekly×2 weeks)

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Topical Immune Response Modifiers: Adjuvants

45

Annemarie Uliasz and Mark G. Lebwohl

Abstract

Imiquimod and ingenol mebutate are topical treatments widely utilized for their anti-tumor effects on actinic keratosis (AK). In addition to their anti-tumor effects, both topical medications are reported to be effective as off-label alternatives to treat various viral, inflammatory, and malignant skin conditions.

Imiquimod is a topical immune modulator approved for the treatment of actinic keratoses, anogenital warts, and superficial basal cell carcinomas. Imiquimod exhibits anti-viral and anti-tumor effects by stimulating both the innate and adaptive immune responses via toll-like receptors (TLR). TLRs on antigen presenting cells induce the production of T-helper-1 cytokines leading to malignant cell death. The dosage, application, and duration depend on the condition being treated. The medication is largely well-tolerated, with application site reactions expected.

Ingenol mebutate is derived from the sap of the Euphorbia peplus plant. It has a dual mechanism of action comprised of initial rapid cell death followed by an acute inflammatory response. It is available as a topical gel and is administered over 2–3 days and has a favorable safety profile, making it an appealing alternative to other spot and field therapies.

Keywords

Imiquimod • Ingenol mebutate • Actinic keratosis • Toll-like receptors • Protein kinase C Field therapy

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Key Points

- The topical immune response modifier imiquimod is a low molecular weight heterocycline imidazoquinoline amine
- Imiquimod stimulates both the innate and the acquired arms of the immune system via induction of T-helper-1 cytokines
- Imiquimod is Food and Drug Administration approved for the treatment of anogenital warts, as well as actinic keratoses and superficial basal cell carcinomas

Introduction

Imiquimod (Aldara™, 3M Pharmaceuticals; Zyclara™, Valeant), a low-molecular-weight heterocyclic imidazoquinoline amine, is a topical immune modulator that stimulates both the innate and the acquired arms of the immune system. Initially discovered when screening medications for anti-herpes virus activity [1], imiquimod is FDA-approved for the treatment of external anogenital warts, superficial basal cell carcinoma, and actinic keratoses. Various case reports and preliminary studies have also suggested imiquimod may be effective in the treatment of a wide range of other infectious, inflammatory, and malignant skin conditions.

Imiquimod offers a non-invasive and tissue-sparing alternative to treatments commonly used for warts or cutaneous tumors such as cryotherapy, electrocautery, surgical excision, laser ablation, trichloroacetic acid, and podofilox. For those individuals who are poor surgical candidates, refuse surgery, or whose anatomic site is not amenable, imiquimod is an effective option. As compared to destructive techniques, imiquimod offers an improved safety profile as local cytokine production decreases potential for systemic adverse events. Additionally, imiquimod is less damaging to tissue, resulting in a superior cosmetic outcome. This is especially desirable in the treatment of lesions situated in cosmetically sensitive sites including the face, as well as in patients in which healing from surgical sites is of particular concern. Furthermore, the ease of topical application allows patients to self-treat which may result in decreased cost and avoidance of multiple clinic visits.

Mechanism of Action

Imiquimod enhances the patient's immune response, stimulating both the innate immune response and the cellular arm of acquired immunity, with resultant anti-viral and anti-tumoral effects.

The innate immune response is stimulated via activation of antigen presenting cells (APCs) including monocytes, macrophages, and dendritic cells, and the subsequent release of cytokines and chemokines. Imiquimod produces an innate immune response via its action as a toll-like receptor (TLR) agonist. Toll-like receptors are a family of pattern recognition receptors found on the cell surface of APCs. Specifically, imiquimod binds to TLR-7 [2] and TLR-8 [3].

TLR-7 activation by imiquimod triggers a MyD88-dependent signaling cascade [2]. MyD88, a protein that associates with TLRs, acts to recruit protein kinases and transcription factors, resulting in activation of nuclear factor-kβ (NF-kβ). NF-kβ is a transcription factor that, upon activation, migrates to the nucleus and up-regulates the production of local pro-inflammatory cytokines, particularly interferon (IFN)-α, IFN-β, IFN-γ, tumor necrosis factor (TNF)-α, and interleukin-12 [4].

The IFN-α produced by APCs induces CD4+ T-cells to produce the IL-12β2 receptor. The binding of IL-12 to the IL-12β2 receptor induces the secretion of IFN-γ from naïve T cells, resulting in a Th1 immune response. Conversely, imiquimod suppresses a T helper cell type 2 (T_h2) immune response by inhibiting IL-4 and IL-5 [5].

Studies have described the effects of imiquimod on Langerhans cells, the major antigen presenting cells of the skin [6]. Imiquimod enhances the migration of Langerhans cells from the skin to regional lymph nodes, potentially enhancing viral and tumoral antigen presentation to naïve CD4+ T-cells. This results in differentiation of naïve T-cells into memory and activated T-cells. Upon return to the dermis, the activated T-cells produce the T_h1 cytokines IFN-α, IFN-γ, and TNF-α. These cytokines are responsible for imiquimod's anti-viral and anti-tumoral effects.

Additionally, imiquimod has been shown to produce apoptosis via circumnavigating mechanisms developed by malignant cells to resist apoptosis signals. One way imiquimod activates apoptosis is via activation of membrane-bound death receptors. For example, imiquimod induces Fas (CD95) receptor (FasR)-mediated apoptosis in basal cell carcinoma cells [7]. Fas, a member of the tumor necrosis receptor family, is a death receptor that mediates apoptosis via CD95 receptor-CD95 ligand (Fas-L) interaction. FasR expression is normally absent on BCC cells, allowing tumors to avoid apoptotic signaling. The IFN-α produced by imiquimod induces BCC cells to express FasR.

Another way in which imiquimod induces tumor-selective apoptosis is via the mitochondrial pathway of apoptosis. Imiquimod induces a Bcl-2-dependent translocation of cytochrome c from the mitochondria to the cytosol [8, 9]. This, in turn, leads to activation of caspase-9 and caspase-3 and a subsequent proteolytic cascade resulting in cell death.

Finally, it has been postulated that imiquimod may exert a therapeutic effect at distant sites located between the

application site and the regional lymph nodes via travel of immune cells through the lymphatics [10].

Dosage and Administration

Imiquimod, an off-white, fine crystalline solid, is chemically known as 1-(2-methylpropyl)-1*H*-imidazo[4,5-c]quinolin-4-amine ($C_{14}H_{16}N_4$). The molecular weight of imiquimod is 240.3. Each gram of cream contains 50.0 mg, 37.5 mg, or 25 mg of imiquimod in an off-white, oil-in-water vanishing base consisting of isostearic acid, cetyl alcohol, stearyl alcohol, white petrolatum, polysorbate 60, sorbitan monostearate, glycerin, xantham gum, purified water, benzyl alcohol, methylparaben, and propylparaben.

Imiquimod cream 5 % may be applied to an area of 25 cm^2 while the 3.75 % and 2.5 % formulations may be applied to a larger surface area, 200 cm^2. Imiquimod is supplied in individual 250 mg sachets as well as in pump bottles that dispense a similar amount. Although package inserts specify that a new sachet be opened for each application, one sachet may evenly cover an area of skin up to 386 cm^2 [11]. Imiquimod should be applied in a thin layer extending 1 cm beyond the affected area. Areas treated with imiquimod should not be occluded. Imiquimod should be left on the affected area for approximately 8 h. The frequency of application and duration of therapy depends on the condition being treated.

Safety

Imiquimod is contraindicated in those individuals with hypersensitivity to any of its ingredients. Additionally, it should not be applied in areas of dermatitis as it has been known to exacerbate inflammatory conditions such as pemphigus, psoriasis, and aphthous ulcers.

Despite case reports of imiquimod use during pregnancy without adverse effects on the fetus, at this time, there is insufficient data on the safety of imiquimod in pregnancy to make definitive conclusions [12]. Imiquimod is pregnancy category C. It is unknown if imiquimod is excreted in the breast milk of lactating women.

Application sight reactions (erythema, dryness, edema, crusting, weeping, erosion, ulceration, burning, pruritus, and pain) may occur after application of imiquimod cream. Local skin reactions, well-tolerated by most patients, are considered a normal and expected part of treatment with imiquimod, and are a good predictor of therapeutic efficacy. The intensity of these reactions tends to increase as dosing frequency increases, and rest periods may be required by some patients. Cool compresses, emollients, and topical antibacterials may provide symptomatic relief. Topical corticosteroids, however, should be avoided as they may blunt the therapeutic effect by impairing the immunologic reaction [13]. Systemic signs and symptoms such as malaise, nausea, myalgias, fever, and rigors may accompany local inflammatory reactions.

Approved Clinical Uses

External Genital Warts

Anogenital warts, or condyloma accuminata, are a clinical manifestation of a human papilloma virus (HPV) infection. The human papilloma virus, a non-enveloped double-stranded DNA virus, is classified into more than 100 types reflecting different oncogenic properties as well as tissue tropism. Anogenital warts are often difficult to eradicate, and most therapies involve lesional destruction. Although lesional destruction results in immediate elimination, these procedures are painful and recurrence is common, necessitating repeated treatment. Imiquimod, a non-destructive, patient-applied alternative, is unique in that it may be applied in the privacy of the patient's home, decreasing the number of office visits. More importantly, imiquimod reduces the viral load, thereby decreasing the rate of recurrence.

Imiquimod, in concentrations of 5 % and 3.75 %, is FDA-approved for the treatment of external anogenital warts in immunocompetent patients over the age of 12 years old. Imiquimod 5 % is applied once a day on alternating days three times per week until resolution of warts or for up to 16 weeks. Imiquimod 3.75 % is applied once a day until clearance or for up to 8 weeks. Frequency of application may be adjusted according to the local irritation experienced by patients [14]. Those experiencing minimal local irritation, may increase the efficacy by increasing the frequency of application, while those experiencing uncomfortable irritation may decrease the frequency of application.

In the treatment of warts, the efficacy of imiquimod appears to result from the reduction of HPV itself. In a randomized, controlled molecular study in which tissues were analyzed for HPV DNA and mRNA of several cytokines, both a local increase in interferon and a significant reduction in viral load were observed in skin biopsies taken from patients during and following imiquimod treatment [4]. The increased cell-mediated immunity provided by imiquimod results not only in control or reduction of HPV infection, but also long-term protection from recurrence and reinfection [4].

Several studies have demonstrated the safety and effectiveness of imiquimod 5 % cream for the treatment of external anogenital warts. Randomized, double-blind, placebo-controlled trials examining imiquimod 5 % used three times weekly for 16 weeks resulted in complete clearance in approximately 50 % of subjects [15, 16]. Females were noted to have significantly higher response rates. This

difference was attributed to the semi-occlusive effect of the foreskin as well as a higher degree of keratinization of penile skin compared to vulvar skin, the most common sites for genital warts in men and women.

Imiquimod 3.75 %, while demonstrating efficacy in the treatment of anogenital warts, offers an enhanced tolerability profile and shorter treatment course in comparison to imiquimod 5 %. The combined results from two randomized, placebo-controlled studies involving 534 women demonstrated that imiquimod 3.75 % cream applied once daily until clearance or for up to 8 weeks resulted in complete clearance of all warts in 36.6 % of subjects as compared to 28.3 % in imiquimod 2.5 % and 14.2 % in placebo at 16 weeks from baseline [17].

Actinic Keratoses

Actinic keratoses (AK) are precancerous skin lesions frequently occurring on sun-exposed areas of fair-skinned individuals, becoming more prevalent with advanced age. They are precursors to squamous cell carcinoma. Topical therapies are useful alternatives to cryotherapy and excisional surgery for treating areas of diffuse actinic keratoses. Furthermore, unlike surgical or ablative treatments, imiquimod possesses the unique capacity to uncover and treat sub-clinical lesions. Imiquimod, in concentrations of 5 %, 3.75 %, and 2.5 %, is FDA-approved for the treatment of facial and scalp actinic keratoses in immunocompetent adults. Imiquimod 5 % cream is FDA approved for twice weekly application for 16 weeks, but many variations from this schedule also have been shown to be effective. Imiquimod at concentrations of 3.75 % and 2.5 % is applied once daily for two 2 week treatment cycles separated by a 2 week no-treatment period.

Several clinical studies have been performed evaluating the safety and efficacy of imiquimod 5 % cream for the treatment of AKs. Persaud et al. initially reported the use of topical imiquimod 5 % cream for the treatment of actinic keratoses on the scalp of three individuals [18]. One subject was treated three times weekly for 4 weeks with nearly complete resolution of actinic keratoses accompanied by a marked inflammatory response. Two other subjects treated one half of their scalp with imiquimod, using the other half as comparison. After 8 weeks of two to three times weekly application with frequent rest periods to avoid inflammation, marked reductions in actinic keratoses were noted. These patients then continued to use imiquimod cream on both sides of the scalp two times per week for an additional 9 months. Treatment was well-tolerated and subsequent reduction in lesions was noted.

Persaud et al. conducted another study in which 22 subjects with at least six bilateral actinic keratoses applied imiquimod or placebo three times a week for 8 weeks [19]. If necessary, a 3 week rest period was allowed, followed by a subsequent reduction in dose frequency. Upon evaluation 8 weeks following treatment, average AK counts were significantly decreased in subjects treated with imiquimod compared to placebo-treated subjects. Of note, 53 % of the subjects required rest periods with 18 % of the subjects requiring two rest periods.

A meta-analysis including five placebo-controlled randomized studies was performed examining the treatment of actinic keratoses of the head and scalp with twice weekly imiquimod 5 % application for 12–16 weeks [20]. Complete clearance was observed in 50 % of subjects receiving imiquimod as compared to 5 % in the vehicle groups. Of note, twice weekly dosing versus three times weekly dosing was associated with less local skin reactions, less rest periods, and less subjects discontinuing treatment due to local skin reactions [21].

Trials have been performed to identify treatment regimens that optimize efficacy while reducing local skin reactions. Several studies have examined imiquimod used in a cyclical regimen for the treatment of actinic keratoses. An open-label pilot study by Salasche et al. examined imiquimod used in a cycle regimen for the treatment of actinic keratoses [22]. Discrete areas containing 5–20 AKs were selected for treatment. Twenty-five patients with 33 treatment areas participated. Imiquimod was applied to the entire treatment area three times a week for 4 weeks followed by a rest period of 4 weeks. If AKs in the treatment area were still present, this cycle was repeated up to three times. Of the 22 patients with 30 treatment areas that completed the study, total clearance of AKs was seen in 46 % of the treatment areas after the first cycle and in 82 % of the treatment areas after the second cycle. Four patients required rest periods prior to the scheduled 4-week rest period.

Several subsequent randomized, double-blind, placebo controlled studies have examined the safety and efficacy of this cyclical regimen which is currently approved for use in Europe [23–25]. Complete clearance rates ranged from 26.8 to 40.5 % after one 4-week treatment course, while a second course resulted in rates ranging from 53.9 to 68.9 %. One of the studies found that recurrence rates at a 1-year follow-up visit for imiquimod and placebo were 39 % and 57 % respectively [25].

Further exploration of the optimal dosing schedule lead to a study by Zeichner et al. which evaluated the safety and efficacy of once weekly imiquimod 5 % for 6 months (24 weeks) for the treatment of actinic keratoses on the face and scalp in 20 patients [26]. Although the treatment regimen was tolerable with minimal side effects and, in turn, enhanced compliance, the complete clearance rate was 6.7 %, significantly lower than currently approved treatment regimens.

Long-term efficacy of imiquimod 5 % has been evaluated in a study by Lee et al. [27]. One and a half years following the completion of four phase III studies in which patients applied imiquimod either two or three times weekly for 16

weeks, recurrence rates of approximately 25 % (twice weekly application) and 43 % (three times weekly application) were noted.

A long-term follow-up study by Stockfleth et al., in which the initial clearance rate of actinic keratoses treated with imiquimod 5 % three times weekly for 12 weeks was 84 %, demonstrated that 2 years later, the clearance rate was 80 % [28].

Seeking to enhance the tolerablilty and simplify the treatment regimen of imiquimod, two randomized, double-blind, placebo controlled studies involving 490 subjects with 9–10 AKs were conducted to evaluate the efficacy and safety of imiquimod 2.5 % and 3.75 % creams [29]. Subjects were randomized to receive either imiquimod (2.5 % or 3.75 %) or placebo applied daily to the entire face or balding scalp for two 3 week treatment courses, separated by one 3 week no-treatment interval. Complete and partial (greater than 75 % lesion reduction) rates were 5.5 % and 12.8 % (placebo), 25.0 % and 42.7 % (imiquimod 2.5 %), and 34.0 % and 53.7 % (imiquimod 3.75 %).

In a companion study (479 subjects) evaluating 2 week treatment cycles, complete and partial clearance (greater than 75 % lesion reduction) rates were 6.3 % and 22.6 % (placebo), 30.6 % and 48.1 % (imiquimod 2.5 %), and 35.6 % and 59.4 % (imiquimod 3.75 %) [30]. While these results were comparable to those reported in the previous study evaluating 3 week treatment courses, the 2 week treatment course regimen demonstrated enhanced tolerability with more than 90 % of subjects complying to their treatment regimens.

In contrast to other topical treatments for AKs, imiquimod has been shown to be more effective and produce a less severe local skin reaction than 5-fluorouracil (5-FU). A meta analysis comparing the efficacy of 5-FU and imiquimod in the treatment of actinic keratosis was performed involving ten studies [31]. Results demonstrated average efficacy rates of 52 % for 5-FU and 70 % for imiquimod.

Superficial Basal Cell Carcinoma

Arising from the basal layer of the epidermis, basal cell carcinoma (BCC) is the most common malignancy among Caucasians worldwide [32]. The three most common subtypes are nodular, superficial (sBCC), and morpheaform. Although metastases are rare, BCCs are locally invasive, aggressive, and destructive to the skin and surrounding structures. Treatment is predominantly surgical, consisting of excision, cryosurgery, curettage and electrodessication, and Mohs micrographic surgery. Although surgery offers a high cure rate, imiquimod is an effective alternative and should be considered in cases where patients are poor surgical candidates or cosmetic outcome is a concern.

Imiquimod 5 % is FDA-approved for the treatment of biopsy-confirmed superficial basal cell carcinoma in immunocompetent adults when surgical methods are less appropriate and when patients may be reliably monitored. Imiquimod 5 % is applied five times per week for 6 weeks. Studies have shown that occlusion does not yield a statistically significant effect on the efficacy of imiquimod [33].

Numerous trials evaluating the safety and efficacy of imiquimod 5 % cream for the treatment of sBCCs have been performed. A randomized, double-blind pilot study by Beutner et al. involving 35 patients demonstrated the safety and efficacy of imiquimod for the treatment of BCC [34]. The study examined five different treatment regimens, each lasting up to 16 weeks. Outcomes were evaluated clinically and histologically. Of the subjects receiving imiquimod, 83 % experienced complete clearing of their lesions. Complete resolution of nodular and superficial BCCs was seen in all subjects receiving twice daily, once daily, or three times weekly treatment with imiquimod. Of note, once-daily dosing was more effective than less frequent dosing. Resolution was seen in 60 % of those treated twice weekly, in 50 % of those treated once weekly, and in 9 % of those treated with placebo.

A phase II, open-label, randomized trial involving 99 patients reported a clinical and histological clearance rate of superficial basal cell carcinomas in 100 % in subjects treated with twice daily imiquimod for 6 weeks and 88 % in those treated with once daily imiquimod for 6 weeks [35]. Clearance rates for twice daily treatment three times per week and once daily three times per week treatment for a duration of 6 weeks were 73 % and 70 %, respectively. Although patients in a twice daily regimen arm achieved 100 % resolution, the local skin reactions were unacceptable.

Another phase II randomized, double-blind, vehicle-controlled study involving 128 subjects with sBCC examined longer treatment regimens [36]. Subjects received imiquimod or placebo in one of four dosing regimens lasting 12 weeks: twice daily, once daily, five times per week, or three times per week. Clearance rates of 87.1 % and 80.8 % were observed in those subjects who used imiquimod once daily and five times per week, respectively. Those who treated their tumors three times a week experienced 51.7 % clearance while those in the placebo group displayed a clearance rate of 18.8 %. Due to severe local skin reactions in those who used imiquimod twice daily, the safety profile of this regimen was not considered acceptable. Histological clearance rates for 12 weeks of treatment compared to the clearance rates seen following 6 weeks of treatment in previous studies proved to be similar, suggesting that an additional 6 weeks of treatment may be unnecessary.

Two phase III double-blinded, placebo-controlled studies involving 724 patients with primary superficial basal cell carcinoma were performed [37]. Subjects with one sBCC were treated with placebo or imiquimod 5 % cream once daily either five or seven times per week for 6 weeks. Upon evaluation 12 weeks post-treatment, 75 % of those using imiquimod five times weekly and 73 % of those using imiquimod seven times weekly subjects experienced both

histological and clinical clearance compared to 2–3 % of subjects treated with placebo. As the difference of clearance rates between the two imiquimod dosing regimens was not clinically significant, the authors recommended the five times per week dosing regimen.

A study by Marks et al., examined the safety and efficacy of imiquimod 5 % for the treatment of multiple sBCC's [38]. Sixty-seven adults (208 histologically confirmed tumors total) with 2–6 sBCCs each located on the neck, trunk, extremities were treated with imiquimod 5 % either five or seven times per week for 6 weeks. Histologic clearance was noted in 77 % of tumors with a lower rate of clearance observed in lesions located on the lower limbs as compared to the rest of the body.

A long-term open-label study evaluating the clinical recurrence of sBCC after treatment with imiquimod daily seven times per week for 6 weeks demonstrated an initial clearance rate of 94 % [39]. Five years following treatment, the estimated sustained clearance was 85.4 % [40].

In a similar long-term study performed in Europe, subjects used imiquimod once daily five times per week for 6 weeks for the treatment of sBCC. Initial clearance rates were reported to be 90 % at 12 weeks post-treatment. Five years following treatment, 86.9 % demonstrated an overall clearance success rate with histologic clearance in 81 % [41].

Imiquimod has also been found to be beneficial as an adjunct to the surgical removal of BCCs. Use of imiquimod 5 days per week for 2–6 weeks before Mohs excision of BCC has been reported to significantly reduce the size of the tumor, thereby resulting in a smaller cosmetic defect from the surgery [42].

Off Label Uses

Infectious Conditions

Molluscum Contagiosum

Molluscum contagiosum is a common cutaneous tumor caused by the double-stranded DNA pox virus. Although this infection is often self-limited in immunocompetent patients, patients commonly chose to treat this condition as lesions may be numerous. Current treatment modalities include cryotherapy, curettage, electrodessication, and application of trichloroacetic acid or cantharidin. Imiquimod's anti-viral and anti-tumoral properties may offer an effective alternative.

Several case reports have been published demonstrating eradication of molluscum contagiosum in both pediatric and adult populations [43]. In an open-label study involving 15 subjects, imiquimod 5 % cream was applied once daily five times per week for 16 weeks. Results demonstrated complete clearance in 80 % of the subjects [44]. Furthermore, there are reports of immunosuppressed individuals with molluscum contagiosum responding to imiquimod treatment [45]. Although several case reports and a small double-blind, randomized, vehicle-controlled pilot study [46] have suggested that imiquimod is effective in the treatment of molluscum contagiosum, two unpublished randomized, double-blind, vehicle-controlled trials involving 702 children (ages 2–12 years) in which imiquimod 5 % or vehicle was applied three times weekly for up to 16 weeks failed to demonstrate efficacy [47].

Herpes Simplex Virus 2

Genital herpes is a chronic sexually transmitted infection of the herpes simplex virus 2 (HSV-2). Imiquimod has been reported to be a successful alternative treatment in resistant cases.

A case of a 34 year old, HIV positive man with herpes simplex 2 virus infection resistant to acyclovir, famiciclovir, and valacyclovir has been reported in which imiquimod 5 % cream was used three times in 1 week [48]. Following imiquimod application, the lesions improved with no recurrence at 1 month post-treatment.

Martinez reported the case of a 37 year old, HIV positive man with a recurrent anogenital HSV-2 infection despite daily suppressive therapy with acyclovir or valacyclovir [49]. He was treated with imiquimod 5 % cream three times the first week and then two times the following week. Two weeks later, the skin lesions improved and HSV-2 detection by culture and PCR remained negative. Twelve months post-treatment, during which time the patient did not use suppressive therapy, no recurrence was observed.

In addition, there are case reports of successful treatment with imiquimod 5 % cream of HSV-2 resistant to both foscarnet and acyclovir [50].

In regard to recurrence rates of HSV-2, however, imiquimod has not demonstrated benefit in controlled studies. A double-blind, randomized, placebo-controlled trial examining the effect of imiquimod 5 % on recurrence rates of HSV-2 was performed [51]. Subjects with six or more HSV-2 outbreaks per year were randomized to apply placebo or imiquimod to the affected area once, twice, or three times weekly for 16 weeks for each recurrence. No significant difference in recurrence rates between placebo and imiquimod was found.

Cutaneous Warts

Common warts (caused by HPV types 2, 4, and 7) as well as flat warts (caused by HPV types 3 and 10) also appear to respond to imiquimod treatment. An open-label study using imiquimod 5 % cream once daily 5 days per week for up to 16 weeks for the treatment of common warts

resulted in complete clearance in 56 % of the subjects [44]. After approximately 9 weeks of treatment, wart size was found to be reduced greater than 50 %. At a 32-week follow-up evaluation, no recurrence of treated warts was observed.

An open-label trial evaluating the efficacy of imiquimod in the treatment of recalcitrant subungual and periungual warts has been conducted [52]. Salicylic acid was initially applied to the lesions to reduce hyperkeratosis and optimize imiquimod penetration. Imiquimod 5 % cream was then applied once per day five times per week for 16 weeks. Results showed that 80 % (12/15) of the subjects experienced complete resolution after an average of 3 weeks of treatment.

Another open-label study in which patients with recalcitrant plantar warts or periungual warts were treated with imiquimod 5 % cream daily under occlusion for 4 weeks resulted in complete clearance in 8/10 subjects [53].

Case reports have also detailed efficacy in the treatment of flat warts [54, 55]. A case report of a 21 year old, healthy woman with numerous flat warts of the forehead recalcitrant to topical retinoids, 5-fluorouracil, cryotherapy, and oral cimetidine documented subsequent complete clearance after 3 weeks of imiquimod 5 % cream applied three times a week [56].

Imiquimod has also proven beneficial in the treatment of recalcitrant warts in individuals with HIV [57–60].

Leishmaniasis

Leishmaniasis is a parasitic infection seen in developing countries. It is transmitted to humans through the bite of infected sandflies. Cutaneous leishmaniasis, the most common form of the disease, manifests as skin lesions at the infection site which may last for months to years. In animal studies, imiquimod was observed to stimulate leishmanicidal activity in macrophages [61].

Although not sufficiently effective as a monotherapy, imiquimod may offer benefit in the treatment of cutaneous leishmaniasis when used in conjunction with meglumine antimonate [62], and may provide enhanced cosmesis of the healing lesions [63].

Oncologic Conditions

Nodular Basal Cell Carcinoma

Compared to superficial basal cell carcinoma, nodular basal cell carcinoma tends to extend deeper into the dermis. Studies evaluating the efficacy of imiquimod on this subtype of basal cell carcinoma have shown varying results.

Two multi-center, randomized, dose response studies were performed evaluating four different dosing regimens for either 6 or 12 weeks [64]. Subjects in the 6 week open-label study were randomized to apply imiquimod either once or twice daily for 3 or 7 days per week. Those in the 12 week placebo-controlled study were randomized to apply imiquimod or vehicle once daily for 3, 5, or 7 days per week or twice daily for 7 days per week. Results demonstrated that dosing once daily 7 days per week resulted in the highest clearance rates: 71 % in the 6 week study and 76 % in the 12 week study. These results, although statistically significant do not approach the clearance rates seen in the treatment of superficial basal cell carcinoma. The difference may be attributable to the fact that nodular tumors tend to be denser and extend deeper into the dermis as compared to superficial tumors.

Another open-label study in which 15 subjects with nBCC were treated with imiquimod three times per week for 12 weeks yielded complete clearance in 100 % of the subjects [65].

A phase III, randomized, open label study involving 90 patients in which imiquimod 5 % was applied three times a week for 8 and 12 weeks showed only modest improvement in small nBCC's (<1 cm) with residual tumor present in more than one third (36 %) of treated lesions. There was no significant difference noted between the two treatment arms [66].

Although not as effective as the treatment of sBCC's, imiquimod may offer benefit in the treatment of nBCC when paired with other treatment modalities. A preliminary study in which imiquimod cream was used once daily for 1 month following curettage and electrodessication (C&D) of BCC resulted in a reduced frequency of residual tumor as well as an improved cosmetic outcome when compared to C&D alone [67]. Another study examined the use of imiquimod 5 % for 4 weeks prior to Mohs surgery for nBCC's located on the face [68]. The use of imiquimod significantly reduced the tumor size and subsequent surgical defect.

Basal Cell Nevus Syndrome

Basal cell nevus syndrome is an autosomal dominant disorder that, in addition to various systemic abnormalities, manifests with multiple BCCs. The successful treatment of both superficial and nodular basal cell carcinomas in the setting of basal cell nevus syndrome with imiquimod has been documented with nodular BCCs requiring more frequent dosing and a longer duration of treatment [69–74]. Imiquimod appears to offer an appealing alternative to multiple surgical excisions for people with BCNS.

Lentigo Maligna and Malignant Melanoma

Lentigo maligna is the in situ form of malignant melanoma. If left untreated, it may progress to invasive melanoma. Imiquimod may offer benefit in lentigo maligna or metastatic melanoma as an adjuvant therapy or when

surgery or radiotherapy is not a viable option. Numerous case reports and uncontrolled studies have detailed efficacy of imiquimod using various treatment regimens and with limited follow-up periods [75–90]. Close follow-up is warranted with multiple post-treatment biopsies given the possibility of recurrence or progression to invasive disease [91–94].

An open-label study involving 30 patients with lentigo maligna was conducted in which imiquimod was applied once daily for 3 months. One month following treatment, 93 % of subjects experienced complete histological and clinical clearance [95].

Another study examined the efficacy of imiquimod 5 % cream in the treatment of biopsy-proven lentigo maligna of the face in 12 subjects [96]. Imiquimod was applied three times a week for 6 weeks. If no inflammatory response occurred, the frequency of use was increased to daily application. Ten of the patients demonstrated histologic clearance with no relapse at a 6 month follow-up visit.

Cases of disseminated metastatic melanoma successfully treated with imiquimod 5 % cream have also been reported [97–105], and although the prognosis of metastatic melanoma is poor, imiquimod may offer benefit as an adjunctive or palliative treatment. A subject with history of melanoma involving the right knee presented with metastasis to the right lower leg [100]. After the metastases were treated with carbon dioxide laser ablation, new lesions appeared and were subsequently treated with imiquimod 5 % cream three times per week. Clinical and histological clearance was seen after 4 months, and no recurrence was observed after 15 months.

Xeroderma Pigmentosa

Xeroderma pigmentosa is an autosomal recessive disease characterized by defects in DNA repair. Exposure to sunlight results in a 1000-fold increase in the development of skin cancers. Several case reports have found benefit in the use of imiquimod for the treatment of active cutaneous neoplasms in these individuals while reducing the development of new lesions [106–110].

Squamous Cell Carcinoma In Situ and Invasive Squamous Cell Carcinoma

Several case reports have shown promise in the treatment of both in situ and invasive squamous cell carcinomas with imiquimod 5 % cream [111–119]. In the case of involvement of anatomic areas such as the genitalia that prove challenging for surgical treatment, imiquimod may offer an appealing alternative to surgery.

In a phase II, open-label study, imiquimod was applied once daily for 16 weeks for the treatment of Bowen's disease on the legs and shoulders of 15 subjects [120]. Sixteen weeks post-treatment, clearance rate of 93 % were observed.

A randomized, double-blind, placebo-controlled trial involving 31 subjects, revealed that those receiving imiquimod 5 % cream daily for 16 weeks resulted in 73 % resolution of cutaneous SCC in situ with no relapse during a 9 month follow-up period [121].

An open-label study examined the use of imiquimod 5 % cream used once daily five times per week for a maximum of 16 weeks in subjects who were unsuitable surgical candidates [122]. Following 8–12 weeks of treatment, complete clinical and histological resolution was observed in 4/5 Bowen's disease lesions and 5/7 invasive SCCs.

Although benefit has been observed, further studies are necessary to fully establish the efficacy and safety of imiquimod for the treatment of in situ and invasive squamous cell carcinoma.

Cutaneous T-Cell Lymphoma

Cutaneous T-cell lymphoma manifests as patches or plaques in early stages to tumors and erythroderma in advanced stages. Several case reports document successful treatment of limited patch and plaque stage mycosis fungoides with imiquimod 5 % cream [123–125].

A preliminary open-label pilot study involving six subjects was performed to evaluate the use of imiquimod in the treatment of patch and plaque stage mycosis fungoides when applied three times per week for 12 weeks [126]. Results demonstrated a histological and clinical response rate of 50 %.

Kaposi's Sarcoma

A case report describes resolution of HIV-related Kaposi's sarcoma following daily application of imiquimod 5 % cream for 4 months [127].

Imiquimod has also been examined in the treatment of classic or endemic Kaposi's sacroma in HIV-negative individuals [128]. A phase II, open-label trial evaluated the efficacy and safety of imiquimod 5 % cream applied under occlusion three times a week for 24 weeks in 17 subjects. Two subjects experienced complete resolution and six subjects had partial clearance [129].

Other Dermatologic Conditions

Keloids and Hypertrophic Scars

Keloids are hypertrophic scars that grow outside of the original borders of an injury. They represent an exaggerated, proliferative healing response. Current treatment includes intralesional corticosteroids, laser therapy, and cryosurgery.

Case series have found imiquimod 5 % cream to be effective in prevention of keloid recurrence on the earlobe when applied following surgical excision via tangential shave technique [130–132].

One pilot study demonstrated that 24 weeks following the application of postoperative imiquimod 5 % cream nightly for 8 weeks to 13 keloids excised surgically from the earlobes and back of 12 patients, none recurred at 24 weeks post-surgery [133].

Another study evaluated the use of imiquimod 5 % cream in the prevention of hypertrophic scarring following breast surgery [134]. In this double-blind, randomized, placebo-controlled trial involving 15 subjects, imiquimod was applied over the scar once every 3–4 days for 8 weeks. Twenty-four weeks after the surgery, the imiquimod treated scars were noted to have improved color and elevation when compared to placebo.

Subsequent studies, however, failed to demonstrate significant efficacy of imiquimod in prevention of recurrence of keloids on the trunk [135–137].

Porokeratosis of Mibelli

Porokeratosis of Mibelli is a disorder of epidermal keratinization with potential for malignant transformation. Imiquimod has been shown to be effective in the treatment of this condition in several case reports [138, 139].

A case of a 77 year old woman with porokeratosis of Mibelli involving the left shin has been reported [140]. Following application of imiquimod 5 % cream to the lesion once a day three times per week for 6 weeks, clinical resolution was observed at a 6 week post-treatment follow-up visit and no recurrence was seen at a 2-year follow-up visit.

In another case report, a 12 year-old girl with porokeratosis of Mibelli involving her left axilla was treated with imiquimod 5 % cream three times per week for 6 weeks [141]. Treatment was well-tolerated and no recurrence was observed 2 years following cessation of treatment.

Infantile Hemangioma

Infantile hemangiomas are benign vascular tumors that present within the first year of life and spontaneously regress over a period of years. Although the majority of these lesions resolve spontaneously, many leave residual fibro-fatty change, atrophy, or pigmentary alteration. Complications including ulceration, visceral involvement, or underlying structural impairment may occur in lesions of larger size or specific locations. Imiquimod has been found to offer benefit in the treatment of superficial infantile hemangiomas via increased tumor apoptosis and inhibition of vascular tumor enlargement [142].

Several case reports have illustrated success using imiquimod for uncomplicated infantile hemangiomas with resolution of lesions after 3–5 months [143–147].

A phase II open-label study evaluating imiquimod 5 % cream applied three to seven times per week for 16 weeks to non-ulcerated proliferating superficial or mixed infantile hemangiomas demonstrated that benefit was limited to superficial hemangiomas [148].

Imiquimod has been found to be well tolerated in the treatment of infantile hemangiomas with the majority of patients experiencing local skin reactions. Reports of fever have been documented in cases of imiquimod applied to ulcerated hemangiomas. Epistaxis was observed when imiquimod was used for a lesion located on the nasal side wall [147].

Granuloma Annulare

Granuloma annulare is a self-limited dermatosis manifesting as confluent papules in an annular configuration often involving the extremities. A case of a 12 year old girl with granuloma annulare involving the right foot has been reported [149]. After failing to respond to topical superpotent steroids, the lesion was treated with imiquimod 5 % cream nightly for 6 weeks. Following 6 weeks of treatment with imiquimod, the lesion had clinically resolved.

The successful use of imiquimod 5 % cream in the treatment of granuloma annulare was also documented by Badavanis et al. [150]. Four subjects were treated with imiquimod 5 % cream once daily from three to seven times per week for up to 12 weeks of treatment. One subject experienced relapse and was treated for an additional 6 weeks with subsequent resolution. All patients remained free of recurrence at 18-months post-treatment.

Conclusion

Imiquimod cream, an immune response modifier, offers a safe and effective alternative to ablative and surgical treatments for external genital warts, actinic keratoses, and superficial basal cell carcinomas. Although surgical excision may be more efficacious in the treatment of skin cancers, imiquimod may serve as a tissue-sparing, cosmetically appealing, and cost-effective option to those patients who are poor surgical candidates, who refuse surgery, or whose anatomical site is not amenable to surgery. As a stimulator of both innate and acquired immune responses with resultant anti-viral and anti-tumoral effects, imiquimod demonstrates potential in the treatment of several other virus-associated and oncological cutaneous conditions.

Key Points

- Ingenol mebutate is derived from the sap of the *Euphorbia peplus* plant.
- Ingenol mebutate has a unique dual mechanism of action inducing rapid and direct cell death within hours of application followed by an acute inflammatory response that eliminates residual tumor cells.
- Ingenol mebutate is Food and Drug Administration approved for the treatment of actinic keratoses

Ingenol Mebutate

Ingenol mebutate (Picato™, Leo Pharma), a macrocyclic diterpene ester, is the active constituent in the sap of *Euphorbia peplus*. Commonly known as radium weed, petty spurge, or milkweed, *E. peplus* has been used for centuries as a traditional remedy for cutaneous conditions including skin cancer [151–153].

Owing to a substantially shorter course of treatment (2–3 days) and duration of local cutaneous reaction, ingenol mebutate offers an appealing alternative to other topical agents used for the treatment of actinic keratoses.

Mechanism of Action

Ingenol mebutate has a dual mechanism of action. Ingenol mebutate first induces rapid and direct cell death within hours of application followed by an acute inflammatory response that eliminates residual tumor cells.

In vitro and in vivo studies on B16 mouse melanoma cells exposed to ingenol mebutate revealed disruption of the cell membrane, loss of mitochondrial membrane potential, and mitochondrial swelling resulting in rapid primary necrosis. As a result of the rapid destruction of tumor cells, a treatment duration of 2–3 days is sufficient. Unlike other anti-cancer agents, ingenol mebutate does not trigger apoptosis, and thus the development of apoptosis resistance in tumor cells is unlikely to compromise its activity [154].

Following the initial cell necrosis, an acute inflammatory response occurs [155]. Ingenol mebutate activates protein kinase c resulting in the release of pro-inflammatory cytokines (IL-1B, IL-8, TNF-alpha), the production of tumor-specific antibodies, an enhanced endothelial adhesion molecule expression, and a substantial infiltration of neutrophils which generate tumoricidal reactive oxygen intermediates [156–158]. The end-result is eradication of residual tumor cells via neutrophil mediated antibody dependent cellular cytotoxicity (ADCC). The elimination of residual tumor cells is thought to be essential in preventing relapse. Furthermore, the inflammatory response appears to confer a favorable cosmetic outcome via expedited healing and a rapid regeneration of the normal cutaneous architecture.

Dosage and Administration

Ingenol mebutate (chemical structure $C_{25}H_{34}O_6$) has been formulated as a topical gel. Each gram contains 150 or 500 mcg of ingenol mebutate in a base of isopropyl alcohol, hydroxyethyl cellulose, citric acid monohydrate, sodium citrate, benzyl alcohol, and purified water.

For the indication of actinic keratoses. The 0.015 % gel is applied once daily for three consecutive days (face or scalp) while the 0.05 % gel is applied once daily for two consecutive days (trunk or extremities) [159].

Safety

The most common side effects of ingenol mebutate were application-site reactions including erythema, scale, crusting, edema, vesiculation, erosions, ulceration, pruritus, pain, and irritation. Skin reactions typically occurred within 1 day of treatment initiation, peaking in intensity 1 week following application, and resolving within 2 weeks on the face/scalp and 4 weeks on the trunk/extremities.

Reports of periocular pain, edema, ptosis have been documented. Prompt hand washing after application and avoidance of contact with the eyes is recommended.

No evidence of skin sensitization, photoirritation, or photoallergic potential has been demonstrated [160].

Ingenol mebutate is pregnancy category C. It is unknown if ingenol mebutate is secreted in the breast milk of lactating women.

Approved Clinical Uses

Actinic Keratoses

The long history of community use of the sap of *Euphorbia peplus* for skin cancers without significant adverse effects prompted the initiation of a phase I/II study on the effect of E. peplus on non-melanoma skin cancer [161]. Participating subjects had one or more basal cell carcinomas or squamous cell carcinomas (in situ or invasive) that had failed previous treatments or were considered unsuitable for surgical intervention (due to size, location, multiplicity of lesions, or medical comorbidity). The sap of *E. peplus* was applied once daily for three consecutive days to a total of 48 skin cancer lesions amongst 36 patients. Histologic clearance at 1 month was 82 % (BCC), 94 % (SCCIS), and 75 % (SCC). At a 15

month follow-up, rates were 57 % (BCC), 75 % (SCCIS), and 50 % (SCC).

Several subsequent studies have demonstrated the safety and efficacy of ingenol gel for the treatment of actinic keratoses. A randomized, double blind, vehicle-controlled, parallel group phase II study by Siller et al. evaluated the safety and efficacy of two applications of various concentrations of ingenol mebutate gel (0.0025 %, 0.01 %, and 0.05 %) [162]. Fifty-eight subjects participated. Five AKs on each subject were selected to be treated by the investigators. Lesions were located on the face, scalp, chest, and arms. The treatment was found to be safe and well tolerated. The most common skin responses were dose-related erythema, flaking, scaling, xerosis, scabbing and crusting which resolved rapidly returning to baseline 1 month after treatment cessation. Efficacy was greatest for the 0.05 % gel, and by the end of the study on day 85, complete clinical clearance was achieved in 71 % of those who were treated with the 0.05 % gel. Clinical clearance of 4 out of 5 AK's was achieved in 67 % of those treated with the 0.05 % gel compared to 17 % of those who received the vehicle only. Histological clearance was observed in 42 % of treated lesions irrespective of the concentration of gel.

Another randomized, double blind, double dummy, vehicle-controlled phase II study by Anderson et al. involving 222 subjects evaluated the safety, tolerability, and efficacy of ingenol mebutate gel applied to non-facial actinic keratoses (0.025 % for 3 days and 0.05 % for 2 or 3 days) [163]. Subjects had 4–8 actinic keratoses within a 25 cm^2 area. Efficacy was dose dependent with 56.0–75.4 % of those receiving ingenol mebutate achieving the primary efficacy end point of partial clearance (≥75 % of baseline lesions cleared) at day 57 compared to 21.7 % for vehicle. Local skin reactions were experienced by up to 96 % of subjects in the active treatment arms with resolution noted 2–4 weeks later. No evidence of scarring or serious adverse events were observed.

The results of four randomized, double-blind, placebo-controlled phase III studies involving a total of 1005 subjects were combined to evaluate the safety and efficacy of ingenol mebutate 0.015 % (once daily for three consecutive days on the face and scalp) and 0.05 % (once daily for two consecutive days on the trunk and extremities) applied to a 25 cm^2 area [164]. Complete clearance at day 57 was higher in those treated with ingenol mebutate versus placebo: 42.2 % vs 3.7 % for lesions of the face and scalp, and 34.1 % vs 4.7 % for lesions on the trunk and extremities. Ingenol mebutate gel was well tolerated with local skin reactions peaking at day 4 (face/scalp) and between days 3 and 8 (trunk/extremities). Local skin reactions approached baseline for all groups receiving active medication by day 29. Minimal pigmentary changes and scarring were observed in subjects receiving the study medication. Of note, over 98 % of the patients assigned to the ingenol mebutate group applied the study medication on all days.

Those who had achieved complete clearance at day 57 were enrolled in a subsequent 12 month observational follow-up study evaluating subsequent recurrence [165]. Compared to baseline, the sustained clearance rate in the face/scalp group was 87.2 %. The sustained clearance rate in the trunk/extremity group was 86.8 %. The median time to recurrence was estimated to be 365 days (face/scalp) and 274 days (trunk/extremity).

The safety and efficacy of ingenol mebutate 0.015 % gel following cryosurgery for the treatment of actinic keratoses on the face and scalp was assessed in a randomized, double-blind, placebo-controlled phase III trial [166]. Subjects (n = 329) were randomized to receive either ingenol mebutate 0.015 % gel or vehicle 3 weeks after cryosurgery. Complete clearance rate was greater in those treated with ingenol mebutate compared to placebo (60/5 % vs 49.4 %).

Off Label Uses

Nonmelanoma Skin Cancers

A phase II study involving 60 patients with biopsy proven basal cell carcinomas was performed evaluating the safety of topical ingenol mebutate gel at three different concentrations (0.0025 %, 0.01 %, and 0.05 %) [167]. The gel was applied once daily for two (0.0025 %, 0.01 % gels) or three (0.05 % gel) consecutive days to a total of 60 BCCs. The safety profile was tolerable with local skin responses reported. Efficacy was dose-dependent with 5/8 patients (63 %) in the 0.05 % group achieving histologic clearance.

Recurrent Malignant Melanoma In Situ

A case report details the treatment of recurrent malignant melanoma in situ of a 91 year old woman [168]. The patient had initially presented with a nodular melanoma that was subsequently surgically excised. Recurrence was noted at a follow-up visit and histologically determined to be a malignant melanoma in situ. As the patient refused further surgical intervention, ingenol mebutate gel 0.015 % gel was applied once daily for three consecutive days. At a 1 month follow-up visit, the lesion was both clinically and histologically resolved.

Molluscum Contagiosum

The treatment of molluscum contagiosum with ingenol mebutate is described in a case report of a 4 year old girl with lesions on the abdomen and extremities [169]. She had failed prior treatment with imiquimod 1 % for 1 month and was subsequently treated with ingenol mebutate 0.015 % gel once daily

for three consecutive days. The patient noted a mild local skin reaction 3 days after initial application. Treatment was continued for an additional 3 days resulting in clearance of lesions with no signs of recurrence at a 1 month follow-up visit.

Conclusion

Ingenol mebutate is a valuable new option for the treatment of actinic keratoses. Its unique dual mechanism of action provides significant efficacy while a brief 2 or 3 day dosing regimen and highly favorable tolerability profile ensure considerable patient adherence.

Questions

1. Which of the following best describes the mechanism of action of imiquimod?
   A. blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication
   B. competitively inhibits and inactivates HSV-specified DNA polymerases preventing further viral DNA synthesis without affecting the normal cellular processes
   C. it produces an innate immune response via its action as a toll-like receptor (TLR) agonist
   D. Following an initial cell necrosis, protein kinase c is activated resulting in the release of pro-inflammatory cytokines, tumor-specific antibodies, an endothelial adhesion molecule expression, and a substantial infiltration of neutrophils which generate tumoricidal reactive oxygen intermediates

2. Which of the following statements is false?
   A. imiquimod has been shown to be more effective and produce a less severe local skin reaction than 5-fluorouracil (5-FU)
   B. imiquimod is FDA approved to treat molluscum contagiousum
   C. The most common side effect of ingenol mebutate are application-site reactions
   D. Imiquimod possesses the unique capacity to uncover and treat sub-clinical actinic kersatoses

3. Which of the following best describes the mechanism of action of ingenol mebutate?
   A. Following an initial cell necrosis, protein kinase c is activated resulting in the release of pro-inflammatory cytokines, tumor-specific antibodies, an endothelial adhesion molecule expression, and a substantial infiltration of neutrophils which generate tumoricidal reactive oxygen intermediates
   B. a MyD88-dependent signaling cascade2 acts to recruit protein kinases and transcription factors, resulting in activation of nuclear factor-kβ (NF-kβ) that, upon activation, migrates to the nucleus and up-regulates the production of local pro-inflammatory cytokines
   C. blocks synthesis of the pyrimidine thymidine, which is a nucleoside required for DNA replication
   D. choices A+B
   E. none of the above

Answers

1. C
2. B
3. A

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Topical Immune Response Modifiers: Antiinflammatories

46

Thomas A. Luger, Ian McDonald, and Martin Steinhoff

Abstract

In recent years, major findings such as blockade of the calcineurin pathway in T lymphocytes led to the identification of novel targets for the treatment of inflammatory skin diseases. The first systemic specific calcineurin inhibitor (CI) for the treatment of inflammatory skin diseases was cyclosporin A (CsA), which demonstrated efficacy both in psoriasis and atopic dermatitis (AD). Because of its systemic adverse effects and the inability to generate a topical CsA compound, there still exists a need for better immunomodulatory agents. Later, the calcineurin inhibitor tacrolimus (FK506) was successfully approved as an efficient topical drug. Subsequently a second calcineurin inhibitor (pimecrolimus, ASM 981) was developed and approved for the topical treatment of atopic dermatitis. Both CIs have been shown to function as effective inhibitors of inflammatory responses in the skin. In addition to T cells, they appear to target other inflammatory cells including mast cells, eosinophils and basophils, blocking cytokine production and reducing associated pruritus. This chapter focuses on topical CIs and briefly discusses recent promising developments of topical antiinflammatory agents.

Keywords

Antiinflammatories • Topical calcineurin inhibitors • Tacrolimus • Pimecrolimus • CI • TH1 and TH2 cytokines • Pharmacokinetic Studies • Atopic-induced pruritus • Pruritus

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Key Points

- New antiinflammatory therapies against atopic dermatitis and other inflammatory skin diseases have become available in recent years.
- Topical calcineurin inhibitors include tacrolimus and pimecrolimus.
- Topical calcineurin inhibitors exert a potent antiinflammatory activity with a low immunosuppressive potential and no induction of skin atrophy.

In recent years, major findings such as blockade of the calcineurin pathway in T lymphocytes led to the identification of novel targets for the treatment of inflammatory skin diseases. The first systemic specific calcineurin inhibitor (CI) for the treatment of inflammatory skin diseases was cyclosporin A (CsA), which demonstrated efficacy both in psoriasis and atopic dermatitis (AD). Because of its systemic adverse effects and the inability to generate a topical CsA compound, there still exists a need for better immunomodulatory agents. Later, the calcineurin inhibitor tacrolimus (FK506) was successfully approved as an efficient topical drug [1]. Subsequently a second calcineurin inhibitor (pimecrolimus, ASM 981) was developed and approved for the topical treatment of atopic dermatitis. Both CIs have been shown to function as effective inhibitors of inflammatory responses in the skin. In addition to T cells they appear to target other inflammatory cells including mast cells, eosinophils and basophils, blocking cytokine production and reducing associated pruritus [2, 125]. This chapter focuses on topical CIs and briefly discusses recent promising developments of topical antiinflammatory agents.

Besides CIs, glucocorticoids (GCs) are widely used topical antiinflammatory agents in dermatology. However in addition to their therapeutic benefits their adverse effects have also become apparent [13–17].The use of glucocorticosteroids is discussed in Chapter 48 and so this chapter focuses on the impact of CIs as antiinflammatory agents in dermatology.

Mechanism of Calcineurin Inhibition by Tacrolimus and Pimecrolimus

Tacrolimus and pimecrolimus are ascomycin macrolactam derivatives produced by bacteria. While tacrolimus is a product of *Streptomyces tsukubaensis*, pimecrolimus was generated from *Streptomyces hygroscopicus var. ascomycetus*. Both bind, albeit with different affinity, to a cytosolic immunophilin receptor, defined as FK-binding protein-12 (macrophilin-12) [9]. After binding, the macrophilin complex associated with either tacrolimus or pimecrolimus inhibits a calcium-dependent serine-threonine phosphatase, defined as calcineurin. Thereby, dephosphorylation and nuclear translocation of a cytosolic transcription factor, the nuclear factor of activated T-cell protein (NF-ATp) is inhibited [6, 18]. Therefore, both tacrolimus and pimecrolimus can be defined as CIs.

In Vitro Effects

Tacrolimus and pimecrolimus inhibit the production of TH1 and TH2 cytokines such as interleukin-2 (IL-2), IL-4, IL-8, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) in vitro. The generation of granulocyte-macrophage colony-stimulating factor (GM-CSF) can also be blocked by these compounds. Pimecrolimus is also capable of supporting antiinflammatory effects on T-helper-2 (Th2) cells by downregulating IL-5 and IL-13 in $CD4^+$ as well as in $CD8^+$ T cells [19], diminishing the number of $CD1^+$ inflammatory dendritic cells from the epidermis [19, 20], and stimulating apoptosis in skin T cells but not in Langerhans cells [20]. In mast cells, pimecrolimus inhibits the release of mast cell mediators such as histamine [1, 18, 21]. In contrast to GC, the topical application of pimecrolimus does not affect the density of epidermal Langerhans cells [22], and does not alter the function of dendritic cells with respect to costimulatory molecule expression or T-cell proliferation [23]. In keratinocytes or endothelial cells, pimecrolimus does not affect cell adhesion molecule expression.

In contrast to pimecrolimus, tacrolimus modulates certain effects of inflammatory dendritic epidermal cells (IDECs) such as the expression of the high-affinity receptor for IgE (FcεRI) [21, 24, 25].

Tacrolimus also inhibits apoptosis of keratinocytes and T cells, thereby suppressing chemokine secretion by eosinophils and release of inflammatory mediators from mast cells [20]. In contrast to corticosteroids, neither tacrolimus nor pimecrolimus affects fibroblast functions such as collagen synthesis and therefore do not cause skin atrophy. In contrast to GC, pimecrolimus does not impair epidermal barrier function. This may explain why tachyphylaxis has not been observed upon treatment with topical CI.

In Vivo Effects

The in vivo antiinflammatory capacities of tacrolimus and pimecrolimus have been investigated in several animal models of contact dermatitis. Both compounds block the elicitation phase of contact dermatitis, thereby diminishing the inflammatory activity. The in vitro evidence of a significantly lower immunosuppressive potential of pimecrolimus

in comparison to tacrolimus has been supported by in vivo animal studies. Here, pimecrolimus, in contrast to tacrolimus, had no effect on the sensitization phase of allergic contact dermatitis and thus apparently does not impair the primary immune response. This has been further supported by a variety of other animal models of immune-mediated diseases. Using a localized rat model of graft-versus-host reaction, pimecrolimus was significantly less effective than tacrolimus. In another rat model of kidney transplantation, pimecrolimus again was less effective in preventing graft rejection when compared to tacrolimus or cyclosporin A. Moreover, upon investigation of the effect on T-helper-cell–assisted B-cell activation in rats, pimecrolimus turned out to be significantly weaker when compared to that of tacrolimus [24, 26, 27].

Pharmacokinetic Studies

The question of potential systemic exposure is one of major concern in the development of a novel compound for topical application. The intact skin barrier restricts the passage of substances greater than 500 Da. As a result the penetration and permeation of topical cacineurin inhibitors whose molecular weight is greater than this (tacrolimus 822 Da, pimecrolimus 810 Da) is impaired. In addition, their lipophilic properties showed higher affinity for skin and lower potential for systemic absorption compared with other agents [125]. The capacity of pimecrolimus to penetrate into and permeate through the skin was investigated in vitro using human cadaver skin. This was compared to corticosteroids and tacrolimus. Accordingly, the amount of pimecrolimus penetrating into the skin was similar to that of corticosteroids or tacrolimus. However, pimecrolimus was observed to permeate significantly less through skin in comparison to corticosteroids or tacrolimus [28]. Therefore, one may suggest that following the topical application of pimecrolimus, the risk of systemic exposure is low and the ultimate possibility of systemic side effects is most unlikely [27]. This has been supported by several pharmacokinetic studies, which proved that after topical use of pimecrolimus in patients with atopic dermatitis, serum concentrations were equally low regardless of the age, severity of disease, and body area treated. In 99 % of the samples tested, concentrations were below 2 ng/mL, which is far below the level of 10–15 ng/mL, which is required for a systemic antiinflammatory effect [29].In contrast to tacrolimus, serum concentrations of pimecrolimus in this range did not cause systemic adverse events as has been shown in several clinical trials [30, 31].

Although the metabolism of pimecrolimus in the skin has not yet been carefully investigated, it might be assumed that it is removed by desquamation. In contrast, serum tacrolimus levels were detected more frequently following topical application in patients with atopic dermatitis. However, usually these levels were low and transient because circulating tacrolimus was no longer detectable upon improvement of skin barrier function after short-term treatment [32]. The reason for the observed differences between tacrolimus and pimecrolimus, however, is not completely understood. One possible explanation may be the different structure, lipophilicity, as well as content of lipophilic groups of these compounds. Moreover, pimecrolimus in contrast to tacrolimus has a high affinity for epithelial structures such as the skin but a low affinity for lymphoid organs [27].

Pharmacokinetic long-term studies over 1 year have further demonstrated [33] that the blood concentrations of pimecrolimus cream were rather low; moreover, no accumulation was observed and only a minimal increase was detected with increasing body surface area (BSA) during treatment [33, 34].

These results suggest that pimecrolimus does not cause any detectable systemic effects during long-term studies [35, 36].

Clinical Studies on Efficacy and Safety of Calcineurin Inhibitors

Both CIs have been developed for the topical treatment of atopic dermatitis and are approved for this indication in many countries around the world. Tacrolimus can be obtained as 0.03 % and 0.1 % ointment (Protopic®), whereas pimecrolimus is available as a 1 % cream (Elidel®). While tacrolimus is approved for the treatment of moderate and severe atopic dermatitis in adults and children ≥2 years old, pimecrolimus cream (1 %) is available and approved for mild and moderate cases of atopic dermatitis in adults and children ≥2 years old. In some countries, pimecrolimus has been approved for the therapy of atopic dermatitis regardless of age and severity of the disease [37–39]. For both CIs, several clinical trials verified both compounds to be highly effective for the treatment of atopic dermatitis [9, 24, 40].

In January 2006 the US Food and Drug Administration (FDA) issued a boxed warning based on a theoretical risk of malignancy including lymphoma with topical CI use. In September 2010 the FDA released a review of TCI safety based on five peer reviewed studies. At that time they concluded that there may be a possibility of an association between tacrolimus and an increased risk of lymphoma. However epidemiological and clinical data since then have failed to demonstrate a causal link. Indeed in post marketing registries the observed number of malignancies and lymphomas has been very low and comparable or less than the number observed in the general population. In addition a number of published reviews have concluded that no conclusive proof has emerged to link TCI with malignancy in the 9 years since

the boxed warning [131–133]. Meanwhile, topical CIs have been established or reported as alternative therapeutic strategies for skin diseases other than atopic dermatitis. Both tacrolimus ointment and pimecrolimus cream have been documented as successful treatment modalities for rosacea, lichen planus, psoriasis, lichen sclerosus et atrophicans, lupus erythematosus, and many others (reviewed elsewhere [39]).

From an economic point of view, in the long run CIs have been shown to be cost-effective [41], although at the moment they are more expensive than glucocorticoids [42]. However, as already mentioned, one has to consider age (infants, children, elderly people with skin atrophy), localization (face, groin, inframammary area), and severity (large proportions of the skin), for in these cases CIs are superior and safer therapeutic modalities. Thus, a fair calculation between CIs and other drugs such as glucocorticoids (GCs) may be difficult [41, 43, 44]. More studies are needed to further calculate costs of GC versus CI therapy.

In the first clinical trial of adults with atopic dermatitis, tacrolimus ointment significantly reduced skin lesions and pruritus within 3 weeks of treatment [45]. Subsequently, randomized double-blind controlled studies further defined the efficacy, tolerability, and safety of tacrolimus [44, 46]. In adults, 0.1 % tacrolimus ointment was as effective as hydrocortisone butyrate 0.1 % ointment [44, 47]. In children (2–15 years), 0.03 % ointment was more effective as compared to 1 % hydrocortisone acetate ointment [48], and more effective than a mild topical glucocorticoid ointment [44]. Subsequently, tacrolimus was compared to various topical glucocorticoids. In a meta-analysis of 25 randomized controlled trials, tacrolimus 0.1 % ointment was superior to hydrocortisone acetate (1 %), hydrocortisone valerate, and hydrocortisone butyrate (0.1 %), whereas tacrolimus (0.03 %) ointment was as effective as hydrocortisone acetate (1 %), but less effective than hydrocortisone butyrate.

After these successful short-term studies revealing efficacy of topical tacrolimus, a multi-center, open-label, non-comparative trial was performed [49]. Within the first week of treatment, most of these patients experienced a significant amelioration of eczema and pruritus. Of note, an increasing improvement was observed until month 3 after treatment. After 12 months, an excellent improvement (≥90 %) or clearance of the symptoms was reported in 68.2 % of patients. An improvement (≥50 %) was noted in 90.9 % of the cases [50]. Laboratory parameters did not change significantly during the study period. A burning sensation (47 %) usually terminated after initiation of treatment, and burning or itching was only occasionally reported [50]. Importantly, no tachyphylaxis was observed in these patients. The excellent long-term efficacy of tacrolimus ointment for the treatment of atopic dermatitis in children as well as adults was verified in several clinical studies [51]. By measuring tacrolimus plasma levels in patients treated with tacrolimus ointment it was revealed that in 67.1 % of patients tacrolimus plasma levels remained below the level of detection. High levels were found only in 0.4 % (≥5 ng/mL) of patients [5]. In patients with Netherton syndrome, blood concentrations over 20 ng/ml could be detected [53]. Despite these rare cases, systemic exposure after topical application of tacrolimus is very low. In summary, there is no evidence for systemic accumulation resulting in adverse side effects following the long-term treatment with tacrolimus ointment [54].

The efficacy of pimecrolimus 1 % cream for the treatment of atopic dermatitis in adults, children, and infants was verified in several clinical trials [4, 24, 39, 55–58]. Importantly, no significant drug-related adverse events were observed in these studies when applied twice daily. In comparison to both corticosteroids and tacrolimus, the capacity of pimecrolimus to permeate through the skin was significantly lower, indicating a very low risk of systemic exposure following the topical application of pimecrolimus cream [27]. This is also supported by studies with patients suffering from Netherton syndrome [59, 60]. In one recent study by Yan et al., three patients with Netherton syndrome received twice daily application of pimecrolimus 1 % cream over 18 months. In addition to the significant clinical improvements noted, blood levels recorded ranged from 0.625 to 7.08 ng/ml, revealing levels much lower than expected or required to cause systemic immunosuppression. These results were seen even when pimecrolimus was applied to 50 % of the body surface area [130] Therefore, long-term studies indicate a very low potential of systemic toxicity, immunosuppression, and local or systemic infections for pimecrolimus 1 % cream [61–63].

In children (2–17 years) and infants (3–23 months) with mild, moderate, or even severe atopic dermatitis, several multicenter clinical trials have further demonstrated [64] that pimecrolimus 1 % cream is highly effective within 8 days for treating both the eczema and the pruritus. No side effects including viral or bacterial infections were reported [56, 65, 66]. Thus pimecrolimus 1 % cream is a safe and effective therapeutic option in children and infants with atopic dermatitis. Of note, significantly more patients in the pimecrolimus group were maintained without glucocorticoid therapy [65, 67]. Due to its efficiency and low profile of adverse events, it is recommended to begin a topical CI therapy at an early stage of atopic dermatitis and probably for other inflammatory skin diseases [40, 68]. Under these circumstances, when the disease develops during CI therapy, intermittent use of other antiinflammatory compounds such as GC will be beneficial [69]. Tacrolimus ointment and pimecrolimus cream may even have a prophylactic effect when used intermittently after an episode of atopic dermatitis when patients still suffer from pruritus (every second to third day). In two clinical trials the efficiency of pimecrolimus was compared to that of glucocorticoids. In a short-term study pimecrolimus was less effective after 3 weeks than betamethasone valerate,

although the maximal efficacy of pimecrolimus was not studied in detail. Moreover, in a long-term, double-blind, randomized multicenter clinical trial, the efficacy of pimecrolimus was compared to that of triamcinolone-acetonide 1 % cream or 1 % hydrocortisone. Although a significant improvement was observed in both groups, less severe side effects were observed in the pimecrolimus group [70–73].

When comparing the effect of topical CIs with topical GC on the epidermal barrier it has been shown that both tacrolimus and pimecrolimus result in a significant increase in the number of lipid lamellae in the intracellular space. This increase is greater than that observed in the GC treated group. As the synthesis and storage of lipids in the lamellar bodies is essential for an effective barrier, this highlights an important therapeutic effect that TCIs have on repairing the epidermis and in reducing epidermal water loss [125, 126]. This may suggest a superior effect of TCIs in repairing skin barrier architecture, preventing penetration of allergens and subsequent relapse.

Effects of Calcineurin Inhibitors on Innate Immunity and Host Defense

The safety and tolerability of tacrolimus ointment has been demonstrated in children and adults with atopic dermatitis. The most common local adverse event was a sensation of burning (in 29.9 % of children and 46.8 % of adults). Transient itching was noted in some children (23.1 %) and adults (25.8 %), which was most likely due not to infection but to neuronal activation [74]. The local adverse events were only noted during the first few days of treatment and were mild to moderate [51]. Concerning side effects on skin appendages, the risk of developing folliculitis or acne was increased in a few young adults [50].

An increased rate of bacterial skin infections could not be observed [50], and a decreased colonization with *Staphylococcus aureus* in the eczematous skin lesions was observed [75], which may be due to a normalization of cutaneous innate immunity after restoration of skin integrity. Atopic dermatitis patients exert an impaired capacity to produce antimicrobial peptides such as defensins and cathelicidins [76–82]. This effect may be due to a predominant TH2 immune response in atopic individuals. Moreover, IL-4 and IL-13, which are increased in atopic dermatitis, inhibit the production of antimicrobial peptides [82].

A slight, nonstatistically significant, increase of local viral infections such as herpes simplex was reported [11]. However, none of these cases caused a therapeutic problem [83]. The idea that pimecrolimus exerts long-term preventive effects in atopic dermatitis was verified by studies showing no relation between pimecrolimus treatment and the occurrence of eczema herpeticum [84, 85]. Moreover, clinical investigations verified that pimecrolimus 1 % cream was not associated with a significantly increased risk of the development of fungal, bacterial, or viral skin infections [61–63].

The effect of pimecrolimus on innate immunity in patients with AD was investigated in a double-blind, randomized, vehicle-controlled study which looked at its effect on IL-13 and antimicrobial peptides (AMPs) cathelicidin and human β defensin (HBD)-3. After 3 weeks of application a statistically significant reduction in IL-13 (which plays a pivotal role in the development of AD lesions) was observed when compared with the vehicle-treated group. In addition there was no significant reduction in catelicidin expression observed. While there was no increase in AMPs overall, its use was not associated with further suppression of the innate immune response [127]. This is in contrast to topical corticosteroids which have a more pronounced inhibition of AMP protein and mRNA levels suggesting a greater suppression of the innate immune system [126].

By using recall antigen tests, no impaired cellular immune response was observed in the skin even after long-term application of tacrolimus 0.1 % ointment [50]. Thus, tacrolimus-associated infections may not be regarded as a major risk factor in atopic dermatitis. Moreover, there is no evidence that the capacity to respond to vaccination with an appropriate antibody production is affected after topical pimecrolimus therapy. It does not alter the migratory capacity of antigen-presenting dendritic cells and does not impair the primary immune response. Despite these studies and reports, cessation of topical application with CI is recommended until total clearance of a viral infection. Surprisingly, the incidence of bacterial infections was found to be decreased during the application of pimecrolimus or tacrolimus. This was most likely due to a normalization of innate defense mechanisms [82].

Effects of Calcineurin Inhibitors on Pruritus

Calcineurin inhibitors play an important role in the treatment of atopic-induced pruritus [86]. Of note, a significant improvement of pruritus was observed within a few days of treatment using pimecrolimus cream [63], which has a beneficial effect on the quality of life in patients with atopic dermatitis [62]. Within 1 week of treatment pruritus was significantly decreased in these patients [87]. Thus, pimecrolimus 1 % cream is effective for the treatment of mild, moderate, and severe atopic dermatitis in adults as well as children and infants. In randomized multicenter, double-blind studies it was further demonstrated that significantly fewer infants treated with pimecrolimus developed severe flares as compared to controls [61, 63].

Itchy lesions that are often resistant to therapy, such as on the face and neck, also responded well to pimecrolimus

therapy [65]. The same adverse events were also observed with tacrolimus in patients with atopic dermatitis, namely burning and a feeling of warmth. These sensations were regarded as mild and transient, lasting only 1–3 days [61–63]. This seems to be dependent on a transient release of preformed neuromediators such as substance P (SP) and calcitonin gene–related peptide (CGRP) from primary afferent nerve endings [74, 86, 88]. Siepmann et al. also demonstrated a significant improvement of pruritus in patients with prurigo nodularis (PN) when treated with topical pimecrolimus, including those patients with PN who did not have an atopic background. This randomized doubleblind phase II trial, demonstrated antipruritic effects within 10 days of starting treatment. It also demonstrated improvement of the prurigo nodules and dermatological quality of life [128].

Effects of Calcineurin Inhibitors on Atrophy

The effect of tacrolimus on fibroblast collagen formation was also determined in a double-blind study. Tacrolimus (0.03 % and 0.1 %), betamethasone valerate, and a vehicle control were compared after 1 week of application for skin thickness and procollagen peptide concentration in suction blister fluids. In contrast to betamethasone valerate, tacrolimus had no effect on procollagen propeptide production and caused no reduction of skin thickness [89]. Thus, the absence of skin atrophy is a major advantage in the treatment with CI [1, 7]. In summary, it is well documented that pimecrolimus does not affect collagen synthesis and therefore does not cause skin atrophy in mice or humans [90].

Risk of Pimecrolimus by Ultraviolet Exposure and in Skin Cancer

The use of systemic immunosuppressants such as cyclosporin A is well known to be associated with an increased risk for the development of ultraviolet (UV)-induced skin cancer such as basal cell carcinoma and squamous cell carcinoma, as well as of the development of actinic keratosis [91, 92]. The long-term experience with topical corticosteroids indicates that they might not be applicable for local treatment with immunomodulators. Therefore, it was necessary to analyze the incidence of developing skin cancer after treatment with tacrolimus. The incidence of skin cancer following the use of tacrolimus ointment has remained very low [93].

In contrast, it is well documented from animal studies that tacrolimus inhibits the development of phorbol ester-(TPA)-induced skin tumors [94].Tacrolimus also suppresses transforming growth factor-β1 receptor (TGFβ1R) activation [95], and prevents keratinocyte apoptosis [96]. Importantly, various animal studies have demonstrated that the topical application of pimecrolimus cream and additional UV-irradiation were not associated with an increased incidence of epidermal or melanocytic skin tumors [10]. Moreover, topical treatment with tacrolimus and pimecrolimus prevents the UV-mediated formation of dimethylthymidine dimers, suggesting a protective effect of these compounds against UV exposure [97]. However, future controlled studies are required to further elucidate the role of CI in UV-mediated skin damage. Meanwhile, a preventive strategy using appropriate sunscreens with topical CI treatment is recommended [10, 11, 98]. The question of tumor formation following long-term treatment with topical tacrolimus cannot be definitively answered at present. A recently published literature review looking at the extent to which topical CI use is associated with melanoma and non melanoma skin cancers found no evidence to date to support such an association. However limitations of the studies have meant that existing data to date is inadequate to give conclusive recommendations [129].Therefore, concomitant UV therapy should be avoided and the patients should be instructed to use UV-protective measures [10, 99].

Comparison of Topical Calcineurin Inhibitors

In a multicenter, randomized study, it was shown that tacrolimus 1 % ointment was more effective than pimecrolimus 1 % cream in adults and children with moderate/severe atopic dermatitis (AD) and at week 1 with mild AD. Tacrolimus was also superior with respect to itch scores and onset of action while no differences were observed concerning adverse side effects [100]. In summary, the first clinical trials already provided evidence for tacrolimus and pimecrolimus as safe and effective topical compounds for the treatment of AD in adults and children, with improvement both in pruritus as well as eczematous lesions [46]. A recent clinical investigator-blinded trial compared pimecrolimus 1 % cream and tacrolimus 0.03 % ointment in children. The efficacy of pimecrolimus 1 % cream was comparable to that of tacrolimus 0.03 % ointment. Pimecrolimus cream was better tolerated, and lesions in the face and neck healed faster after treatment with pimecrolimus cream [101–103]. However, one has to consider that the vehicle of tacrolimus and pimecrolimus is different: while tacrolimus is approved as an ointment, pimecrolimus is a cream. Therefore, patients with dry skin show a better tolerability of the ointment (tacrolimus), while the cream is predominantly preferred by patients with acute, erosive lesions.

Future Topical Anti Inflammatory Treatments

As a result of ongoing, successful research, exploring the pathophysiology of atopic dermatitis and its inflammatory pathways, potential therapeutic targets have been identified. This has resulted in the development of some novel topical anti inflammatory agents, many of which are currently in clinical trials.

Phosphodiesterase inhibitors (PDEs) inhibit the degradation of cyclic adenosine monophosphate (cAMP) and have emerged as potential topical treatments for AD. Blocking PDE4 reduces TNF-α gene expression in macrophages and dendritic cells and also reduces T cell proliferation and production of proinflammatory cytokines IL-2, IL-4 and IL5. They also enhance the expression of IL-10 [135]. The topical application of E6005, a novel PDE4 inhibitor has been demonstrated to have significant antipruritic activity in mice with chronic atopic dermatitis [136]. As a result a phase 1 study looking at the therapeutic action of a topical PDE4 inhibitor is currently underway in patients with atopic dermatitis.

NF-kB decoy is a double-stranded deoxyribonucleic acid (DNA) oligodeoxynucleotide that mimics the NF-kB binding sequence on chromosomal DNA, thereby inhibiting the production of the inflammatory response triggered by NF-kB. The efficacy of topical NF-kB decoy in AD has been demonstrated in a number of mouse models [136, 137]. It reduces the expression of inflammatory cytokines such as IL-1β, TNF-α, ICAM-1 and macrophage inflammatory protein 2-α precurser, thereby reducing inflammation and restoring skin barrier function [137]. A phase 2 study was subsequently performed to evaluate the safety and tolerability of twice daily application of NF-kB decoy to adults and results are pending.

WBI-1001 is a novel synthetic compound demonstrating nonsteroidal antiinflammatory activity. It was originally derived from metabolites of a unique group of bacterial symbionts of entomopathogenic nematodes. It has been shown to inhibit proinflammatory cytokines including IL-2, IL-13, IL-17A and TNF-α [140]. Bissonnette et al., in a 12 week, multicenter, randomized, placebo-controlled double-blind trial demonstrated that topical WBI-1001 at concentrations of 0.5 % and 1.0 % was an efficacious and safe topical treatment for patients with mild to severe AD. However the study did not match the effectiveness to an active comparator and so further studies are required [141].

Mapracorat is a selective glucocorticoid receptor agonist (SERGA) which is currently in two phase 2 clinical trials evaluating its safety and efficacy in patients with AD. It is a highly selective glucocorticoid receptor ligand with immunomodulatory and antiinflammatory effects but with a more favourable side effect profile than topical GCs [139]. Its effect has been demonstrated at a cell signalling level by the inhibition of p38 mitogen-activated protein kinase (MAPK), C-Jun N-terminal kinase (c-JNK), activator protein 1 (AP-1) and NK-kB transcriptional activity.

Conclusion

Topical CIs have established a broad, effective, and safe treatment modality for mild to severe subtypes of atopic dermatitis and other inflammatory skin diseases. During the usage of topical tacrolimus or pimecrolimus, respectively, adverse side effects are rare. Topical CIs are the first antiinflammatory compounds that are suitable for effective, long-term treatment of inflammatory skin diseases. Moreover, they also may be used as an early local therapy when the first signs of itching and eczema appear. Perhaps early and effective local therapy using these novel compounds in infants and children may even have a preventive effect [5, 11]. Therefore, early therapeutic intervention is recommended when lesions or pruritus occur. However, clinical studies are still required to investigate the course of the chronic skin disease treated with CIs with respect to the frequency and severity of the skin lesion. There is evidence that the quality of life in these patients and their relatives has significantly improved [5, 11].

The availability of tacrolimus ointment and pimecrolimus cream as two different formulations is useful because the different vehicle formulations vary regarding skin dryness, patient age, and severity of the disease. Because of its profile and vehicle, pimecrolimus 1 % cream can be recommended in infants and children [27, 104].

Thus far, successful treatment with topical CI has been described in atopic dermatitis [7, 10, 68, 69, 72, 105, 106], seborrheic eczema [107], steroid-induced perioral dermatitis, steroid-induced rosacea [108], erythrotelangiectatic as well as papulopustular and edematous rosacea [109], perianal dermatitis, chronic actinic dermatitis [110], disseminated granuloma annulare [111, 112], lichen planus [113], hand eczema, mucous lesions of lichen planus [114], lichen sclerosus et atrophicans, pyoderma gangrenosum, lupus erythematosus, dermatomyositis, bullous autoimmune diseases, lichen amyloidosus, lichen aureus, chronic actinic dermatitis [5, 11, 104, 111, 115–118], chronic graft-versus-host disease [119, 120], asteatotic eczema [121], and vitiligo [122], although UV light may be additionally mandatory [123, 124]. In contrast, treatment of alopecia areata in humans with CI was not effective [123].

The introduction of topical CI as antiinflammatory agents to combat inflammatory skin diseases has already changed our position about the optimal treatment of inflammatory and autoimmune skin diseases. However, the future position of topical CI for the treatment of atopic dermatitis and other inflammatory skin diseases depends on further well-controlled clinical trials.

In the last few years, a huge improvement has been observed for the development of new anti-inflammatory therapies against atopic dermatitis and other inflammatory skin diseases by immunomodulatory agents. Topical CIs such as tacrolimus or pimecrolimus exert a potent antiinflammatory activity with a low immunosuppressive potential. In many controlled clinical trials, tacrolimus ointment as well as pimecrolimus cream have been shown to be highly effective and safe. They are also well tolerated and do not induce skin atrophy in long-term studies. One of the major adverse events observed with CIs is a transient sensation of burning, which ceases within days. UV-protective modalities are recommended during the treatment with topical CI, although side effects such as skin cancer and systemic immunosuppression have not been observed as of yet in controlled clinical studies. In addition to atopic dermatitis topical CI are effective agents for the treatment of many inflammatory skin diseases including perioral dermatitis, seborrheic eczema, and lichen sclerosus et atrophicus. Future studies will have to determine whether early and perhaps prophylactic application of topical CI may prolong or prevent the onset of inflammatory responses in various skin diseases. In recent years, our knowledge about potent topical GCs with fewer side effects has also greatly improved. Long-term studies revealed a low potential of topical GC to induce atrophy as compared to classic GC. Thus, modern topical therapies with topical CI and GC have established an improvement for the treatment of atopic dermatitis. However, other novel specific antiinflammatory therapies are still needed for rapid and safe long-term treatments for atopic dermatitis and other inflammatory or autoimmune skin diseases. It is possible that the new agents discussed within this chapter may some day become part of the treatment algorithm for various inflammatory dermatoses.

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Questions

1. **The following statements describe the actions of calcineurin inhibitors except for?**
   A. Calcineurin inhibitors inhibit the effect of T1 and T2 cytokines in addition to the generation of granulocyte-macrophage colony-stimulating factor
   B. In contrast to topical glucocorticosteroids, the use of Calcineurin inhibitors is not associated with tachyphylaxis
   C. Calcineurin inhibitors have a direct affect on fibroblast function including collagen synthesis resulting in skin atrophy
   D. Unlike glucocorticoids pimecrolimus does not affect the density of epidermal Langerhans cells or affect the keratinocyte or endothelial cell expression of cell adhesion molecules

2. **Regarding systemic absorption of calcineurin inhibitors which of the following is NOT true?**
   A. Penetration and permeation of CIs is limited by their molecular weight
   B. In vitro and pharmacokinetic studies demonstrate that topical application of pimecrolimus results in significant risk of systemic exposure and side effects
   C. Their lipophilic properties show higher affinity for skin and less potential for systemic absorption compared with other agents
   D. The concentration of CIs required for a systemic anti-inflammatory affect is 10–15 ng/ml

3. **Local adverse affects to TCIs include all of the following except?**
   A. Stinging sensation
   B. Burning sensation
   C. Transient itching
   D. Increased risk of bacterial skin infections
   E. Onset in the first few days of treatment

4. **Which of the following is NOT true regarding the effect of TCIs on itch?**
   A. They are effective in significantly reducing atopic dermatitis associated pruritus within 1 week
   B. They are effective in treating pruritus associated with nodular prurigo
   C. Tacrolimus has shown superior efficacy over pimecrolimus with regard to itch scores and onset of action
   D. Itchy lesions that are often treatment resistant on the face and neck have been shown to respond well to pimecrolimus
   E. While they have effective antipruritic effects in nodular prurigo they have no effect on the prurigo nodules

5. **Which of the following statements regarding the safety of TCIs is NOT true?**
   A. The FDA issued a black box warning regarding a theoretical risk of malignancy including lymphoma with topical CI use in 2006
   B. There has been no conclusive proof to date to link TCI with malignancy
   C. Patients should be advised to use UV-protective measures while using TCIs
   D. Concomitant UV therapy should be avoided
   E. Animal studies have demonstrated an increased incidence of epidermal and melanocytic skin tumors when topical application of Pimecrolimus was combined with UV-irradiation

6. **Which of the following statements is true regarding TCIs?**
   A. Pimecrolimus is available as a 1 % ointment
   B. Both tacrolimus and pimecrolimus have no effect on rosacea, lichen planus, psoriasis or lichen sclerosis et atrophicans
   C. Tacrolimus has demonstrated superior and comparable efficacy to topical GCs in the treatment of atopic dermatitis
   D. Tacrolimus is approved for the treatment of moderate and severe atopic dermatitis in adults only
   E. Treatment should be stopped once symptomatic control has been achieved

Answers

1. C
2. B
3. D
4. E
5. E
6. C

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Traditional Immune-Modulating Drugs

47

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Abstract

The subject of traditional immune modulating drugs is potentially vast. However, only a small number of these drugs are commonly used by dermatologists. This chapter addresses the key mechanisms of how the majority of inflammatory skin diseases are treated, and discusses six systemic drugs from four drugs groups: (1) calcineurin inhibitors: cyclosporine; (2) antimetabolites/purine analogues: azathioprine and mycophenolate mofetil; (3) antimetabolites/folate antagonists: methotrexate and dapsone; (4) alkylating agents: cyclophosphamide; and (5) lysosomotropic agents: hydroxychloroquine. This classification scheme is a reasonable way to categorize the drugs, although it should be noted that several of these drugs have additional mechanisms of action that differ from the above categories. Furthermore, it is not realistic to discuss all available immune-modulating drugs; several notable drug groups not covered in this chapter include retinoids and interferons.

Keywords

Immune-Modulating Drugs • Calcineurin inhibitors • Antimetabolites • Alkylating agents • Systemic calcineurin • Cyclosporine • Alkylating agent • Cyclophosphamide • Psoriasis • Atopic dermatitis • Azathioprine Mechanisms • Methotrexate

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Key Points

- Traditional immune modulating drugs include calcineurin inhibitors, antimetabolites, and alkylating agents.
- Antimetabolites include purine analogues (e.g., azathioprine and mycophenolate mofetil), and folate antagonists (e.g., methotrexate and dapsone).
- The most widely used systemic calcineurin inhibitor is cyclosporine.
- The most commonly used alkylating agent is cyclophosphamide.

The subject of traditional immune modulating drugs is potentially vast. However, only a small number of these drugs are commonly used by dermatologists. This chapter addresses the key mechanisms of how the majority of inflammatory skin diseases are treated, and discusses six systemic drugs from four drugs groups: (1) calcineurin inhibitors: cyclosporine; (2) antimetabolites/purine analogues: azathioprine and mycophenolate mofetil; (3) antimetabolites/folate antagonists: methotrexate and dapsone; (4) alkylating agents: cyclophosphamide; and (5) lysosomotropic agents: hydroxychloroquine (Table 47.1). This classification scheme is a reasonable way to categorize the drugs, although it should be noted that several of these drugs have additional mechanisms of action that differ from the above categories. Furthermore, it is not realistic to discuss all available immune-modulating drugs; several notable drug groups not covered in this chapter include retinoids and interferons.

The emphasis here is on the primary mechanisms of action, particularly as these mechanisms relate to common indications, significant adverse effects, and drug interactions. The discussion of these indications, adverse effects, and drug interactions is brief, emphasizing those with the greatest clinical relevance for the practicing dermatologist. Chapters and reviews that provide greater detail of potential interest are cited.

The drugs above can be divided into two broad groups: immunosuppressive and anti-inflammatory. But many drugs overlap the categories; methotrexate, for example, has both immunosuppressive and anti-inflammatory mechanisms. Thus, considering the drugs discussed as "immune modulating" in a broad sense is a very reasonable approach.

Cyclosporine

Mechanisms of Action [1]

The most established role of cyclosporine (CsA) in psoriasis and other immune-mediated dermatoses is its effect on T lymphocytes [2]. Calcineurin is a calcium- and calmodulin-dependent enzyme that is of central importance to the T-cell amplification of the immune response, in particular inducing increased levels of interleukin-2 (IL-2) (Fig. 47.1). Cyclosporine inhibits calcineurin, which leads to reduced activity of the transcription factor, nuclear factor of activated T cells 1 (NFAT-1) [3]. This transcription factor is important in regulating transcription of a number of cytokine genes, the most significant being IL-2. Because IL-2 causes the proliferation of activated helper T cells (CD4) and cytotoxic T cells (CD8), impaired IL-2 production leads to a decline in the number of activated CD4 and CD8 cells in the epidermis and dermis.

In addition, CsA inhibits the production of interferon-γ, which in turn downregulates intercellular adhesion molecule 1 (ICAM-1) production. ICAM-1 is expressed on the surface of various cells such as keratinocytes and dermal capillary endothelium, playing an important role in the immune process by affecting trafficking of various inflammatory cells. Finally it is important to note that cyclosporine is both a cytochrome P-450 (CYP) 3A4 substrate and inhibitor, explaining many of the numerous potential drug interactions involving cyclosporine.

Table 47.1 Traditional immune-modulating drugs

Category	Drug name	Specific enzyme(s) inhibited
Calcineurin	Cyclosporine	Calcineurin inhibitors
Antimetabolites/purine analogues	Azathioprine	None
	Mycophenolate mofetil	Inosine monophosphate dehydrogenase
Antimetabolites/folate antagonists	Methotrexate	Dihydrofolate reductase, Thymidylate synthetase
	Dapsone	Dihydropteroate synthetase, Myeloperoxidase
Alkylating agents	Cyclophosphamide	None
Antimalarials	Hydroxychloroquine	None

Clinical Applications of Cyclosporine Mechanisms [1]

Common Indications

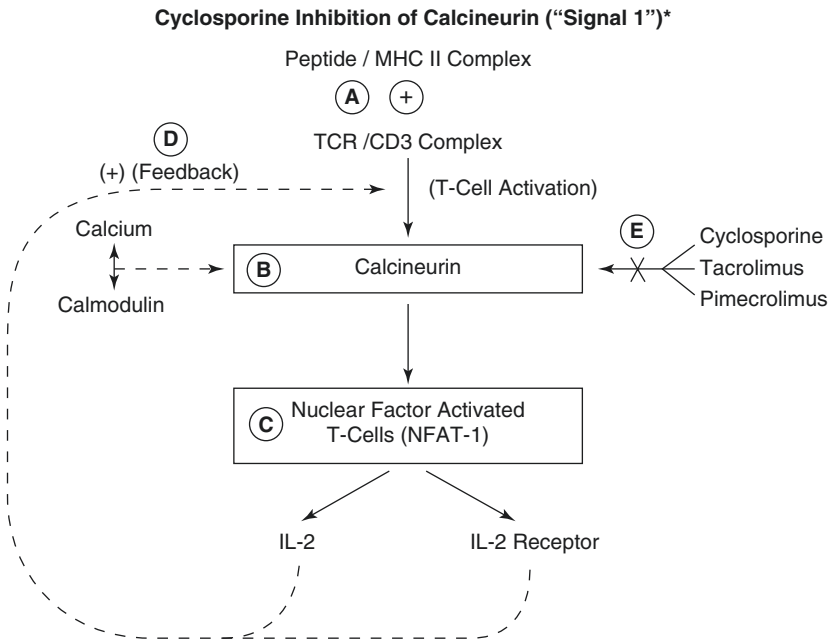
1. Psoriasis, atopic dermatitis, refractory urticaria; T-cell inhibition through cyclosporine inhibition of calcineurin and resultant reduced NFAT-1 production.
2. Pyoderma gangrenosum, immunobullous dermatoses (pemphigus and pemphigoid), autoimmune connective tissue diseases (dermatomyositis), and many others; additional dermatoses in which the T cell has a key role in the pathogenesis.

Significant Adverse Effects

1. Renal disease and resultant hypertension; kidney has relatively high levels of calcineurin.
2. Neurologic adverse effects (such as tremors, headache, paresthesias); various neurologic cell types likewise with relatively high levels of calcineurin.

Drug Interactions

1. Macrolides (erythromycin > clarithromycin), azole antifungals (ketoconazole > itraconazole); cyclosporine toxicity due to these CYP3A4 inhibitors.
2. Rifampin (and other "enzyme inducers"); loss of cyclosporine efficacy due to CYP3A4 inducers.
3. Statins such as simvastatin, atorvastatin, lovastatin > rosuvastatin, fluvastatin. CYP3A4 inhibition increasing the risk of rhabdomyolysis from these statins. Pravastatin has no CYP metabolism and is the best choice to use with cyclosporine.
4. Numerous other CYP-based drug interactions (see pertinent table in Lee and Koo [1]).



* This calcineurin/"signal 1" system creates a highly efficient immunologic responce to various antigenic (or superantigen) stimuli.

A The peptide/MHC II complex on the antigen presenting cell interacts with the T-cell receptor (TCR)/CD3 complex and results in T-cell activation; ↑calcineurin activity is one result of this T-cell activation.

B With calcium as a cofactor, and through interaction with the calcium binding protein calmodulin, calcineurin ↑ activity of the transcription factor NFAT-1.

C NFAT-1 ↑ formation of both the cytokine IL-2 and the IL-2 receptor.

D Through subsequent binding of IL-2 to IL-2 receptor, the T-cell activation is further amplified.

E **Cyclosporine** (as well as **tacrolimus** and **pimecrolimus**) inhibits the key enzyme, calcineurin, in this system with ↓ IL-2 and ↓ IL-2 receptor production, with the net effect of inhibiting "signal 1".

Fig. 47.1 Cyclosporine inhibition of calcineurin*. *IL* interleukin, *MHC* major histocompatibility complex

Azathioprine

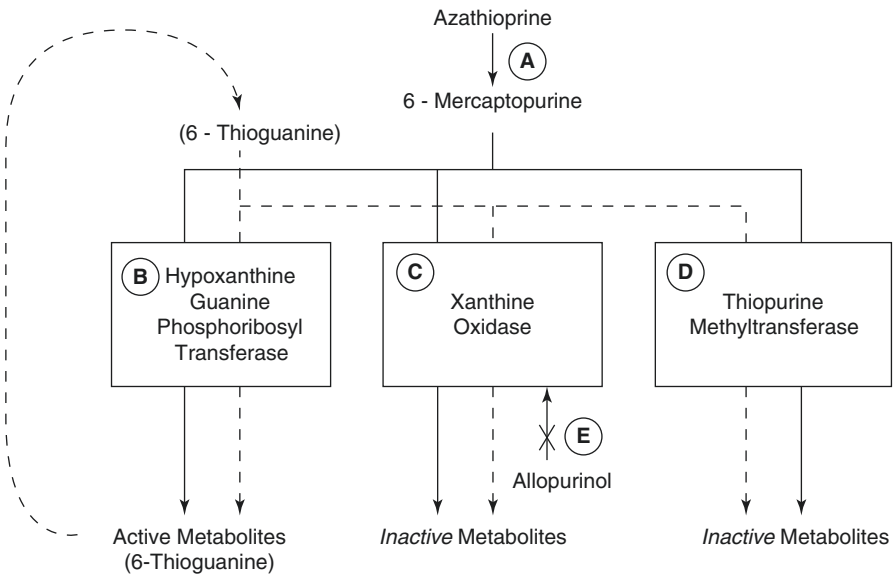
Mechanisms of Action [4]

Azathioprine's active metabolites are 6-thioguanine (6-TG) monophosphate and other 6-TG metabolites; these metabolites are structurally very similar to the endogenous purines adenine and guanine. This structural similarity to the endogenous purines allows these 6-TG metabolites to be incorporated into DNA and RNA, inhibiting purine metabolism and cell division [5, 6]. T-cell–mediated function is depressed, and antibody production is diminished in the B cell [7]. Azathioprine also decreases the number of Langerhans cells (and the ability to present antigens) and other antigen-presenting cells in the skin [8].

Azathioprine is a prodrug which is rapidly converted to 6-mercaptopurine (6-MP) upon absorption. There are three metabolic pathways which subsequently metabolize 6-MP: (1) hypoxanthine- guanine phosphoribosyltransferase (HGPRT), which leads to formation of the active 6-TG metabolites; (2) thiopurine methyltransferase (TPMT), which leads to inactive metabolites; and (3) xanthine oxidase (XO), which also leads to inactive metabolites (Fig. 47.2).

The degradative pathways TPMT and XO may indirectly alter the levels of 6-TG metabolites in different ways. The TPMT activity is reduced or absent in certain patients with a genetic polymorphism, while XO can be inhibited by drug interactions with azathioprine involving allopurinol and febuxostat [9, 10]. The net effect of these clinical scenarios is the risk of significant myelosuppression due to increased 6-TG metabolites. In contrast, patients with high levels of TPMT have relatively low levels of the active 6-TG metabolites and may be therapeutically underdosed with azathioprine [11, 12].

It is important to note that significant variation of TPMT activity is present when comparing different ethnic



A **Azathioprine** is initially converted to active form **6-mercaptopurine** (6-MP).

B Hypoxanthine guanine phosphoribosyl transferase (HGPRT) converts 6-MP to active metabolites **(6-thioguanine)**.

C Xanthine oxidase converts 6-MP to *inactive* metabolites.

D TPMT converts 6-MP to *inactive* metabolites (with genetic TPMT deficiency, shunting 6-MP to the HGPRT pathway occurs, with ↑↑ 6-thioguanine metabolites and subsequent *toxicity*; with genetically high TPMT levels, the result is ↓ 6-thioguanine metabolites, with *loss* of *efficacy*.

E **Allopurinol** inhibits xanthine oxidase, shunting 6-MP to HGPRT pathway, with ↑ toxicity.

Fig. 47.2 Azathioprine metabolic pathways

groups. Genetic testing (genotype) for TPMT is readily available, and can generally at least verify that the patient is a homozygote for high activity (TPMT 1*/1*) or a heterozygote for high activity (TPMT 1*/other allele). Functional assays of thiopurine methyltransferase red blood cell (RBC) activity are also available and widely utilized [11].

Clinical Applications of Azathioprine Mechanisms [4]

Common Indications

1. Pemphigus and pemphigoid spectrums; azathioprine inhibition of antibody production.
2. Cutaneous vasculitis (refractory), pyoderma gangrenosum, severe atopic dermatitis, chronic actinic dermatitis, sarcoidosis; inhibition of T-cell function.

Significant Adverse Effects

1. Carcinogenicity including non-Hodgkin's B- cell lymphomas (this does not appear to be a significant risk with dermatologic conditions with immunologic etiologies; no doubt is a risk with organ transplantation patients), due to altered immune surveillance resulting from azathioprine immunosuppressive properties.
2. Myelosuppression, especially with genetically decreased TPMT levels, shunting 6-MP increasingly to HGPRT pathway, resulting in increased 6-TG metabolites.
3. Opportunistic infections (theoretically; in reality opportunistic infections are very uncommon with azathioprine use for dermatologic indications), due to altered immune surveillance resulting from T-cell and B-cell effects of azathioprine.
4. Gastrointestinal (GI) adverse effects, such as rapidly dividing cells given that azathioprine a cell-cycle–specific antimetabolite.

Drug Interactions

Allopurinol; XO inhibition by allopurinol shunts increased amounts of 6-MP through the HGPRT pathway, leading to increased 6-TG metabolites.

Mycophenolate Mofetil

Mechanisms of Action [13]

Mycophenolate mofetil is rapidly converted to mycophenolic acid (MPA). On systemic absorption, MPA is inactivated by glucuronidation in the liver, and subsequently converted back to its active form by β-glucuronidase within the epidermis and gastrointestinal tract.

Mycophenolic acid has a key role in immune-mediated skin diseases by inhibiting de novo purine synthesis. It is a noncompetitive inhibitor of inosine monophosphate dehydrogenase (Fig. 47.3). Cells relying on de novo purine synthesis, rather than the purine salvage pathway, are preferentially affected. Therefore, the proliferative responses of T lymphocytes and B lymphocytes, which lack the purine salvage pathway, are blocked [14, 15]. Virtually all other cell lines in the body can utilize the purine salvage pathway, which lessens the inhibitory effects of this drug on nonimmunologic cells. Mycophenolic acid also leads to decreased levels of immunoglobulins and decreased delayed-type hypersensitivity responses [16].

Clinical Applications of Mycophenolate Mofetil Mechanisms [13]

Common Indications

1. Pyoderma gangrenosum, psoriasis; relatively selective T-cell inhibition by mycophenolate mofetil.
2. Immunobullous dermatoses (including cicatricial pemphigoid, pemphigus vulgaris, others); relatively selective B-cell inhibition by this drug.

Significant Adverse Effects

1. Gastrointestinal adverse effects; antimetabolite, cell-cycle specific effects on rapidly dividing cells theoretically; however, these cells in the GI tract largely have salvage pathway for purine metabolism.
2. Relatively small number of serious adverse effects; probably the result of the selectivity for the mechanism, with effects primarily on T- and B-lymphocyte subsets.

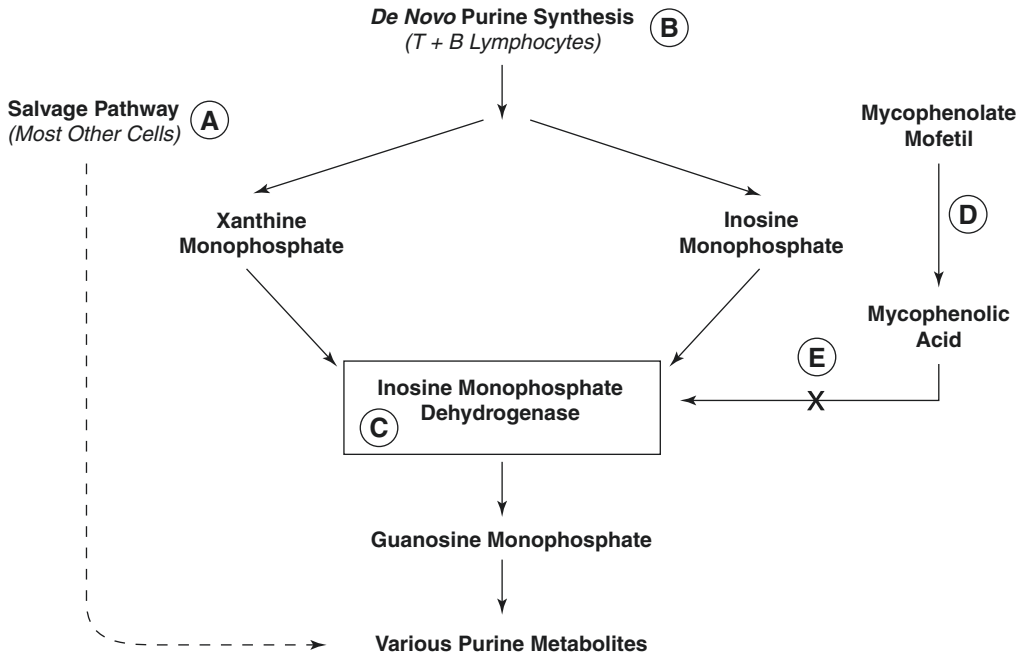
Drug Interactions

Azathioprine, methotrexate, tumor necrosis factor (TNF) inhibitors; pharmacodynamic interaction with potential for increased myelosuppression or opportunistic infections.

Methotrexate

Mechanisms of Action [17]

Methotrexate competitively and reversibly binds to dihydrofolate reductase, which prevents the conversion of dihydrofolate to tetrahydrofolate (Fig. 47.4). Tetrahydrofolate is a necessary cofactor in the synthesis of thymidylate and purine nucleotides needed for DNA and



A Most nucleated cells in the body are able to produce various purine metabolites via the "salvage pathway" (not shown in detail).

B "De novo" purine synthesis is the major route of purine synthesis for T-lymphocytes and B-lymphocytes.

C Inosine monophosphate dehydrogenase converts two different substrates – xanthine monophosphate and inosine monophosphate – into guanosine monophosphate.

D **Mycophenolate mofetil** is a prodrug which first must be converted to the active drug form mycophenolic acid.

E **Mycophenolic acid** inhibits inosine monophosphate dehydrogenase, thus depriving the T- and B-lymphocytes of purine metabolites necessary for growth and replication; the net result is a relatively selective immunosuppression.

Fig. 47.3 Mycophenolate mofetil inhibition of de novo purine synthesis

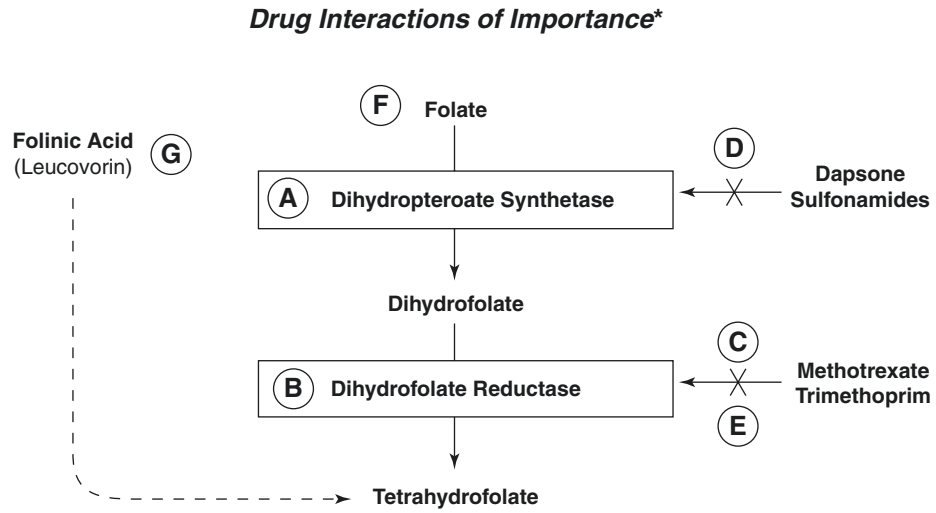
RNA synthesis. A partially reversible, competitive inhibition of thymidylate synthetase also occurs within 24 h after administration of methotrexate. Methotrexate is an antimetabolite specific for the S phase (synthesis, including DNA synthesis) of cell division, with the greatest impact on rapidly dividing cells. Cells of the GI tract and various hematologic cells are rapidly dividing groups of cells that are particularly sensitive to methotrexate inhibition of cell division.

Immunosuppression probably occurs because of inhibition of DNA synthesis in immunologically competent cells. The drug can suppress primary and secondary antibody responses as well [18, 19]. There is no significant effect on delayed-type hypersensitivity. An additional effect of MTX is to block migration of activated T cells into various tissues through alteration of various adhesion molecules [20]. The drug's anti-inflammatory effects are likely predominantly mediated by local increases in adenosine concentration, which has inherent anti-inflammatory properties. This increased adenosine production is the result of complex interactions with aminoimidazole carboxamide ribonucleotide (AICAR) transformylase and ecto-5′-nucleotidase [21].

Clinical Applications of Methotrexate Mechanisms [17]

Common Indications

1. Psoriasis, related to methotrexate T-cell inhibitory effects.
2. Atopic dermatitis; perhaps T-cell and anti-inflammatory effects as well, at least in part due to locally increased adenosine levels.
3. Bullous dermatoses (pemphigus and pemphigoid spectrums), autoimmune connective tissue diseases



* In general, this metabolic pathway is more important to the adverse effects of methotrexate (including drug interactions) than it is for drug efficacy. The fully reduced tetrahydrofolate is important for subsequent pyrimidine nucleotide synthesis.

A Folate (folic acid) is initially reduced to dihydrofolate by dihydropteroate synthetase.

B Dihydrofolate if further reduced to tetrahydrofolate by dihydrofolate reductase.

C **Methotrexate** inhibits this pathway through competitive inhibition of dihydrofolate reductase (DHFR).

D **Dapsone** and various **sulfonamides** inhibit dihydropteroate synthetase, and thus, can amplify the inhibition of DHFR by methotrexate.

E **Trimethoprim** (including fixed combinations with sulfamethoxazole) also inhibits DHFR, and thus can amplify the inhibition of this pathway by methotrexate.

F **Folic acid** given in therapeutic doses essentially competes with methotrexate for DHFR, reducing the adverse effects of methotrexate by ↑ tetrahydrofolate production.

G **Folinic acid**, in a sense does an "end run" around the methotrexate inhibition of folate, serving as a fully reduced substrate for pyrimidine synthesis.

Fig. 47.4 Methotrexate and folate metabolism

(dermatomyositis, morphea), sarcoidosis; methotrexate has a backup role as steroid-sparing agent due to the drug's immunosuppressive effects.

Significant Adverse Effects

1. Hepatotoxicity; nonimmunologic etiology in the great majority of cases, with methotrexate inducing fatty liver changes (risk further increases with conditions that induce fatty liver such as obesity, diabetes mellitus, excess alcohol).
2. Carcinogenicity; theoretically related to methotrexate immunosuppressive properties (this risk is primarily an issue in rheumatoid arthritis patients; minimal risk if any in psoriasis patients).
3. Gastrointestinal adverse effects such as nausea, related to cell-cycle specific properties as an antimetabolite; this risk is largely reduced by folic acid (folate) supplementation as a competitive antagonist of dihydrofolate reductase (DHFR).
4. Cytopenias such as pancytopenia, agranulocytosis, related to cell-cycle specific properties as an antimetabolite; also largely reduced risk by folic acid (folate) supplementation.

Drug Interactions

1. Trimethoprim, like methotrexate, is a DHFR inhibitor, thus amplifying the effect on this important folate metabolism enzyme.
2. Sulfonamides, dapsone (a sulfone); these drugs are dihydropteroate synthetase inhibitors, magnifying the folate pathway effects of the DHFR inhibitor methotrexate.

3. Alcohol and systemic retinoids, the pharmacodynamic effect being drugs with a risk of liver toxicity as well.

Dapsone

Mechanisms of Action [22]

The antimicrobial activity of dapsone in the treatment of leprosy is the result of inhibition of the folate metabolic pathway, specifically by the inhibition of dihydropteroate synthetase (Fig. 47.4) [23].

In contrast, dapsone inhibits the myeloperoxidase-peroxide-halide–mediated cytotoxic system as a central component of neutrophil respiratory burst (Fig. 47.5). This inhibition likely plays a key role in controlling the degree of neutrophil-induced destruction in cutaneous lesions [24]. The lack of neutrophils in the skin of patients being treated with dapsone suggests that this drug may also affect the chemotaxis of neutrophils. Dapsone inhibits chemotaxis to the chemoattractant N-formyl-methionyl-leucyl-phenylalanine (F-met-leu-phe) [25]. The net result of these two effects on neutrophils is the decreased presence of neutrophils (inhibition of chemotaxis) and decreased destructive capacity of neutrophils (inhibition of myeloperoxidase and the resultant respiratory burst) in a wide variety of dermatologic conditions.

The enzyme inhibited by dapsone, myeloperoxidase, is also present in eosinophils and monocytes, with a probable role in respiratory burst-mediated microbial destruction in these cells as well.

Clinical Applications of Dapsone Mechanisms [22]

Common Indications

1. Dermatitis herpetiformis and related conditions such as linear immunoglobulin A (IgA) bullous dermatosis; dapsone effects on neutrophil chemotaxis and respiratory burst mechanism due to myeloperoxidase inhibition.
2. Bullous lupus erythematosus, pyoderma gangrenosum, pemphigoid (bullous, cicatricial), urticarial vasculitis, aphthous stomatitis; also dermatoses with a central role of neutrophils in the disease process; chemotaxis and respiratory burst effects of dapsone.
3. Eosinophilic cellulitis, granuloma faciale; dermatoses with a central role of eosinophils; dapsone myeloperoxidase inhibition relevant in eosinophils as well.
4. Granuloma annulare, granulomatous rosacea; dermatoses with a central role of monocytes and granuloma formation; dapsone myeloperoxidase inhibition relevant in monocytes as well.
5. Infectious diseases such as leprosy, malaria; largely due to dihydropteroate synthetase inhibition.

Significant Adverse Effects

1. Agranulocytosis, mechanism uncertain; however, selectivity for neutrophils (over platelets, red blood cells [RBCs]) likely due at least in part to neutrophil myeloperoxidase inhibition.
2. Hemolysis, not immunologically mediated; instead is related to dapsone-induced RBC oxidative stress.
3. Dapsone hypersensitivity syndrome, immunologically mediated, but not directly related to the two primary enzymes dapsone inhibits (myeloperoxidase and dihydropteroate synthetase).

Drug Interactions

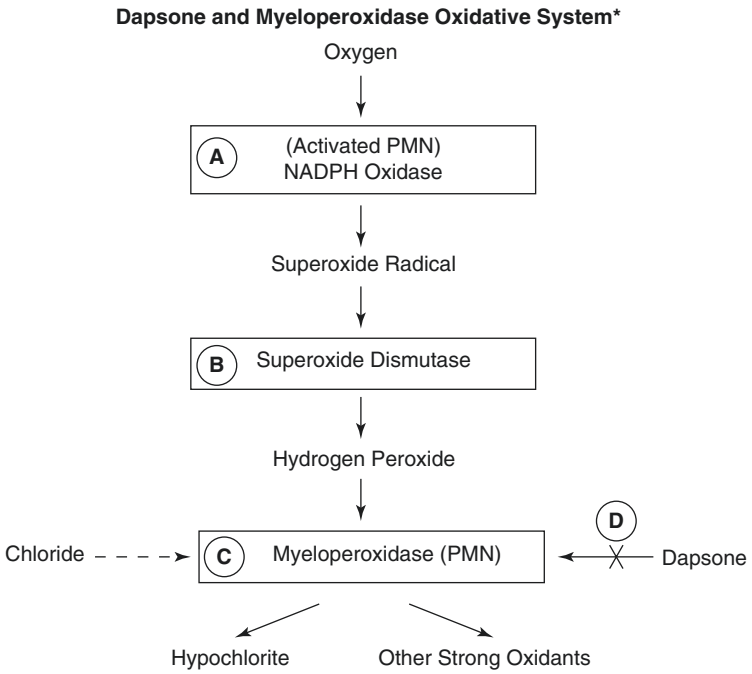
1. Trimethoprim, methotrexate; inhibition of dihydrofolate reductase, thus amplifying folate metabolism inhibition at two different steps (given dapsone dihydropteroate synthetase inhibition).
2. Sulfonamides; inhibition of dihydropteroate synthetase, theoretically amplifying the effects of dapsone on this enzyme.

Cyclophosphamide

Mechanisms of Action [13]

Systemic cytotoxic agents from the alkylating agents group include cyclophosphamide and chlorambucil. This section discusses the more potent immunosuppressive agent cyclophosphamide. In contrast with the antimetabolites previously discussed, cyclophosphamide is cell-cycle nonspecific. Thus, all cell types can be susceptible to the benefits and adverse effects of this drug. Alkylating agents alter the chemical properties and structure of DNA, regardless of timing in the cell cycle. The highly reactive ethyleneimine intermediate for cyclophosphamide is formed and covalently binds with various nucleophilic centers within DNA. Through their effect on protein synthesis, the alkylating drugs interfere with the production of cytokines, growth factors, adhesion molecules, and other substances required for cell growth and differentiation. As a result, these drugs are often mutagenic [26].

Cyclophosphamide depresses B-cell function more than T-cell function. The effect on T-cell activity is variable, with greater activity when the drug is given before antigen



* This enzyme system is present in neutrophils (PMN), eosinophils, and monocytes as a mechanism of microbial destruction through its oxidative properties.

A Oxygen is metabolized to the superoxide radical by the enzyme NADPH oxidase, which is produced by activated PMN.

B Subsequently, superoxide dismutase converts the superoxide radical to hydrogen peroxide.

C With hydrogen peroxide and chloride ions as substrates, myeloperoxidase produces hypochlorite and other strong oxidants leading to microbial destruction; however, in many neutrophilic dermatoses this system may damage various normal tissues.

D Through inhibition of myeloperoxidase, **dapsone** reduces the oxidative damage to normal tissues in various neutrophilic dermatoses, as well as dermatoses in which eosinophils and monocytes (granulomatous dermatoses) play a pathogenic role.

Fig. 47.5 Dapsone and myeloperoxidase oxidative system*. *NADPH* reduced nicotinamide adenine dinucleotide phosphate, *PMN* polymorphonuclear lymphocytes

presentation. In addition, suppressor T cells (CD8) appear to be significantly more affected than helper T cells (CD4) [27].

Three broad primary effects result from alkylation: (1) DNA may cross-link with another nucleophilic residue, (2) there is an abnormal base pairing with thymine, and (3) depurination may occur with resultant chain scission by several different mechanisms. If these mutations overwhelm the DNA repair system, the result is either cell death or mutagenesis and carcinogenesis [27, 28].

Hemorrhagic cystitis and resultant increased risk of bladder carcinoma due to cyclophosphamide are believed to be largely due to local increased concentrations of acrolein metabolites of cyclophosphamide [29].

Clinical Applications of Cyclophosphamide Drug Mechanisms [13]

Common Indications

1. Cicatricial pemphigoid (sight-threatening), severe pemphigus vulgaris; of all the drugs discussed in this chapter, cyclophosphamide is probably the most potent immunosuppressant and thus can be a definitive treatment of the most serious dermatoses.
2. Systemic vasculitis syndromes (such as Wegener's granulomatosis); similar reasoning as above, although these vasculitis subsets are seldom managed by dermatologists alone.

Significant Adverse Effects

1. Myelogenous leukemias; not an issue with other immunosuppressive agents; probably an issue with cyclophosphamide and other alkylating agents due to the structural alterations of DNA.
2. Bladder carcinoma (typically preceded by hemorrhagic cystitis), due largely to the acrolein metabolites of cyclophosphamide.
3. Myelosuppression; cyclophosphamide is a cell- cycle nonspecific drug, yet still has its greatest effect on rapidly dividing cells.

Drug Interactions

Chlorambucil, methotrexate, azathioprine-induced myelosuppression; pharmacodynamic effect with negatively synergistic impact on myelogenous cell precursors.

Hydroxychloroquine

Mechanisms of Action

The systemic antimalarial agents used in dermatology include hydroxychloroquine, chloroquine, and quinacrine. Although the exact mechanism of action is not fully understood, it is postulated that antimalarial agents are lysosomotropic agents that act on a wide range of pathways which provide an immunomodulatory, anti-inflammatory, anti-proliferative and photo-protective effects [30, 31].

Immunomodulatory effects occurs due to the accumulation of antimalarials in lysosomes and endosomes, increasing the pH and thereby disrupting proper endosomal maturation. This results in disruption of Toll-like receptor interactions and can lead to a decrease in the ability of antigen presenting cells in processing and expressing major histocompatibility complexes. Additionally, this disruption of endosomal maturation can lead to a decrease in release of interleukin 2 from CD4+ T-cells, reducing lymphocyte responsiveness and proliferation. Furthermore, the reduction of proper release of cytokines from these lysosomes would lead to an impaired chemotaxis of various inflammatory cells. Finally, antimalarials protect against ultraviolet induced damage on cells through effects of prostaglandin metabolism and inhibition of superoxide production or possibly by enhancing UVB-induced factors involved to protect against UV damage. This would result in a decrease in inflammation within the keratinocytes and possible release of self-antigens as a result of apoptosis that can be induced by UV radiation.

Clinical Applications of Hydroxychloroquine Drug Mechanisms [30]

Common Indications

1. Lupus erythematosus and other photosensitive dermatosis such as porphyria cutanea tarda, polymorphous light eruption, solar urticaria and dermatomyositis (including sine myositis); antimalarials effects on enhancing UVB-induced factors and inhibition of superoxide production;
2. Granulomatous dermatoses which includes cutaneous sarcoidosis and generalized granuloma annulare;
3. Panniculitis such as chronic erythema nodosum and lupus panniculitis;
4. Oral lichen planus.

Significant Adverse Effects

1. Ocular adverse effects include corneal deposition, loss of accommodation and premaculopathy which are all reversible. True retinopathy is also possible with prolonged use and is irreversible.
2. Hematological: rarely agranulocytosis or pancytopenia. Hemolysis in patients with G6PD deficiency.

Drug Interactions

1. Most significant is between antimalarials as usage of both would have an additive effect on causing retinopathy.
2. Antimalarials have been known to increase digoxin levels.
3. There may be an increased risk of hemolysis with the concurrent use of other agents such as dapsone in individuals with reduced G6PD activity (this effect is much greater with 8-aminoinolines such as primaquine).

Conclusion

Most traditional immune-modulating drugs used by dermatologists fall into four groups: calcineurin inhibitors, antimetabolites/purine analogues, antimetabolites/folate antagonists, and alkylating agents. The systemic calcineurin inhibitor most commonly used by dermatologists is cyclosporine. Purine analogues are represented by azathioprine and mycophenolate mofetil. Methotrexate and dapsone are commonly used folate antagonists. Alkylating agents are represented by cyclophosphamide. Although the antimalarial mechanisms are hardest to define, the lysosomotropic effects may explain much of the efficacy of the 4-aminoquinolines such as hydroxychloroquine. All of these drugs have uses in dermatology, but they also have significant adverse effects and the potential for drug interactions.

Questions and Answers

1. Which of the following statements is false?
   A. Calcineurin is a calcium-dependent enzyme
   B. Calcineurin is a calmodulin-dependent enzyme
   C. Cyclosporine inhibits calcineurin
   D. Cyclosporine stimulates production of interferon gamma

2. Which of the following statements is true?
   A. One of azathioprine's active metabolites is 6-thioguanine (6-TG) monophosphate
   B. Azathioprine increases the number of Langerhans cells
   C. 6-mercaptopurine is metabolized by hypoxanthine-guanine phosphoribosyltransferase (HGPRT) leading to formation of the inactive 6-thioguanine metabolites
   D. 6-mercaptopurine is metabolized by thiopurine methyltransferase (TPMT) leading to active 6-thioguanine metabolites

3. Which of the following statements is true regarding mycophenolate mofetil?
   A. It is a competitive inhibitor of inosine monophosphate dehydrogenase
   B. It is activated by glucuronidation in the liver
   C. It is rapidly converted to mycophenolic acid (MPA)
   D. Cells relying on the purine salvage pathway, are preferentially affected by mycophenolic acid

Answers

1. D
2. A
3. C

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Topical Corticosteroids

48

Ulrich R. Hengge

Abstract

Since Marion Sulzberger introduced glucocorticosteroids (GCSs) in 1951, they have revolutionized clinical medicine. This chapter provides an updated overview of their mode of action, their use in dermatology, and their adverse-effect profile. While systemic GCSs have a long list of indications, topical corticosteroids represent the mainstay for treating inflammatory diseases of the skin. Adverse effects depend on the dose, the duration of treatment, and the preexisting medical conditions. For topical application, the nature of the drug, the vehicle, and the site of application determine the side-effect profile. The most frequent cutaneous adverse effects include atrophy, striae, rosacea, perioral dermatitis, acne, and purpura. With lower frequency, hypertrichosis, pigmentation changes, delayed wound healing, and skin infections as well as contact sensitization are observed. Important systemic adverse effects include musculoskeletal, ophthalmologic, nervous system, metabolic, and cardiovascular manifestations. The main characteristics of GCSs are potent anti-inflammatory, antiproliferative, and immunosuppressive effects, which give them a long list of potential indications in medicine. In particular, GCSs are extremely effective in the treatment of many autoimmune and inflammatory diseases.

Keywords

Topical Corticosteroids • Glucocorticosteroids • GCSs • Hypothalamicpituitary-adrenal (HPA) • Corticotropin-releasing hormone (CRH) • Adrenocorticotropic hormone (ACTH) • Musculoskeletal Osteoporosis • Osteonecrosis • Axis Suppression • Skin • Skin disease

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Key Points

- Glucocorticosteroids (GCSs) have anti-inflammatory, antiproliferative, and immunosuppressive effects.
- Glucocorticosteroids exert their effects by binding to glucocorticoid receptors (GR) and by modification of transcription of corticosteroid-responsive genes.
- Glucocorticosteroids induce neutrophilia, lymphopenia, eosinopenia, and monocytopenia as well as reduce access of inflammatory cells at the site of active infection.
- To improve the benefit-risk ratio of GCSs, new interventions have been developed, including liposomal GCS, GR agonists, and nitroso-glucocorticoids.
- Drugs that induce the hepatic cytochrome P-450 system accelerate the clearance of GCSs, while other drugs inhibit this system.
- The side effects of GCSs are strictly dose dependent.
- The vehicle in which the topical steroid is formulated influences the absorption and potency of the drug.

Since Marion Sulzberger introduced glucocorticosteroids (GCSs) in 1951, they have revolutionized clinical medicine [1]. This chapter provides an updated overview of their mode of action, their use in dermatology, and their adverse-effect profile. While systemic GCSs have a long list of indications, topical corticosteroids represent the mainstay for treating inflammatory diseases of the skin. Adverse effects depend on the dose, the duration of treatment, and the preexisting medical conditions. For topical application, the nature of the drug, the vehicle, and the site of application determine the side-effect profile. The most frequent cutaneous adverse effects include atrophy, striae, rosacea, perioral dermatitis, acne, and purpura. With lower frequency, hypertrichosis, pigmentation changes, delayed wound healing, and skin infections as well as contact sensitization are observed. Important systemic adverse effects include musculoskeletal, ophthalmologic, nervous system, metabolic, and cardiovascular manifestations. The main characteristics of GCSs are potent antiinflammatory, antiproliferative, and immunosuppressive effects, which give them a long list of potential indications in medicine. In particular, GCSs are extremely effective in the treatment of many autoimmune and inflammatory diseases.

Synthesis of Glucocorticosteroids

Glucocorticosteroids are produced in the adrenal cortex, which secretes cortisol as well as the weak androgens androstenedione and dehydroepiandrosterone. Normally, cortisol secretion is regulated by hormonal interactions within the hypothalamic pituitary-adrenal (HPA) axis upon pulse secretion of the hypothalamic corticotropin-releasing hormone (CRH). This prompts the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which then causes the secretion of cortisol from the adrenal cortex at a daily dose of 20 mg; however, cortisol output may increase by ten times upon stress.

Pharmacology

The family of steroids, including GCSs, is based on the four-ring structure of cholesterol, with 3-hexane rings and one pentane ring (Fig. 48.1) [2].

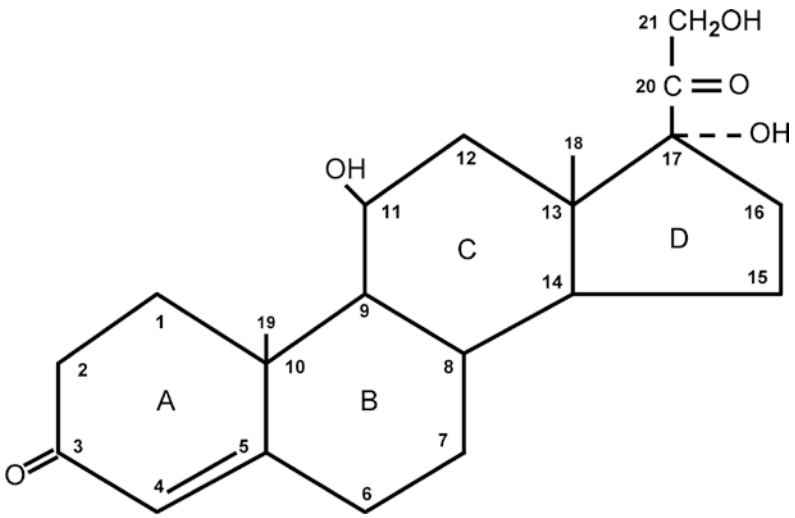

Fig. 48.1 Chemical structure of cortisol. Note the four-ring structure and the hydroxyl group at position 11

Modifications of the basic GCS structure result in agents with variant potency, mineralocorticoid activity, metabolism, and duration of action (Table 48.1). Important for the therapy of patients with hepatic insufficiency is the fact that the ketone group at the 11 position of cortisone must undergo hepatic conversion to a hydroxyl group to produce the active agent hydrocortisone (cortisol). Likewise, prednisone must undergo the same activation by 11-hydroxylation to become the active prednisolone. Therefore, for patients with liver problems, the use of prednisolone is recommended instead of prednisone. Glucocorticosteroids are absorbed in the jejunum with peak plasma levels occurring within one hour. Of note, administration with food does not decrease peak plasma concentrations but may delay its absorption.

Molecular Mechanism of Action

Glucocorticosteroids exert their effect by binding to glucocorticoid receptors (GRs) and by modification of transcription of corticosteroid-responsive genes. Free GCSs readily diffuse through the plasma membrane to bind to GRs in the cytoplasm, which leads to release of the heat-shock protein (hsp) 90. Upon release of hsp90, two nuclear localization signals are exposed, which allow the nuclear accumulation of the GR complex.

The GR forms a dimer that binds to glucocorticoid response elements within the promoter region of steroid-responsive genes. From this interaction, transrepression or transactivation of regulatory proteins may occur. It has become increasingly clear that many adverse effects of GCS are predominantly caused by transactivation (e.g., diabetes and glaucoma, osteoporosis, skin atrophy, growth retardation, and Cushing syndrome) [3]. By contrast, anti-inflammatory effects are mostly mediated by transrepression of proinflammatory cytokines or cyclooxygenase-2 (COX-2) [3, 4].

The discovery that activation and repression of the GR are genetically separable has fueled intense research on more selective receptor ligands [5]. The precise confirmation that the receptor assumes after ligand binding is determined by the structure of the given binding partner. Upon binding, structural alterations occur that allow interactions of the DNA-binding surface with specific glucocorticoid response elements such as the formation of homodimers and the binding of different co-activators and co-repressors at the ligand-binding domain that induce either activation or repression of gene transcription [6]. In particular, transcriptional repression activity is sensitive to the glucocorticoid-mediated anti-inflammatory and antiproliferative effects [7]. Several mechanisms of transcriptional repression such as interactions of the GR with DNA or with nonreceptor protein– protein complexes have been described [6]. Recently, a number of more selective GR ligands have been discovered; they are called selective GR agonists (SEGRAs) or dissociating GCSs [6]. The SEGRAs predominantly induce the desired transrepression effects, whereas transactivation properties are significantly reduced [3]. While some of these compounds have an encouraging side-effect profile, equivalent anti-inflammatory efficacy as compared with prednisone and dexamethasone still has to be demonstrated for compounds such as deflazacort [8].

Effects on Inflammatory Cells

Glucocorticosteroids induce neutrophilia, lymphopenia, eosinopenia, and monocytopenia. They also reduce access of inflammatory cells at the sites of active inflammation. However, important neutrophil functions such as phagocytosis and bactericidal activity remain largely unaffected by pharmacologic doses of GCSs. Transient lymphopenia occurs through redistribution of T lymphocytes to other lymphoid compartments, possibly through a change in adhesion molecule expression.

Table 48.1 Glucocorticosteroid agents

Compound	Equivalent glucocortico steroid dose (mg)	Mineralocorticoid potency (relative)	Duration of activity (h)
Short acting			
Cortisone	25	2 +	8–12
Hydrocortisone	20	2 +	8–12
Intermediate acting			
Prednisone	5	1 +	24–36
Prednisolone	5	1 +	24–36
Methylprednisolone	4	0	24–36
Triamcinolone	4	0	24–36
Long acting			
Dexamethasone	0.60	0	36–54
Betamethasone	0.75	0	36–54

Table 48.2 Major indications for systemic GCS use in dermatology

Inflammatory dermatoses and allergies
Contact dermatitis (various)
Atopic dermatitis
Photodermatitis
Exfoliative dermatitis
Erythrodermas
Urticaria
Erythema exudativum multiforme
Stevens-Johnson syndrome
Erythema nodosum
Sweet syndrome
Bullous dermatoses
Pemphigus (all forms)
Bullous pemphigoid
Cicatricial pemphigoid
Linear immunoglobulin A bullous dermatosis
Epidermolysis bullosa acquisita
Herpes gestationis
Erythema multiforme (major/minor)
Toxic epidermal necrosis
Vasculitis
Cutaneous (various types)
Systemic (various types)
Collagen vascular diseases
Lupus erythematosus (all subsets)
Dermatomyositis
Systemic sclerosis
Mixed connective tissue disease syndrome
Eosinophilic fasciitis
Relapsing polychondritis
Neutrophilic dermatoses
Pyoderma gangrenosum
Acute febrile neutrophilic dermatosis
Behçet's disease
Miscellaneous dermatoses
Sarcoidosis Lichen
Planus Polyarteriitis
Nodosa Panniculitis
Urticaria/angioedema
Arthropod bites/stings
Hemangiomas

Potential Indications and Contraindications

The list of potential indications for use of GCS in dermatology is long (Table 48.2). Primary indications are severe forms of eczema and autoimmune diseases, including bullous and collagen vascular diseases. In addition, GCSs are extremely useful drugs in the treatment of graft-versus-host disease [9].

Moreover, a number of cancers such as certain lymphomas and leukemias (e.g., multiple myeloma) respond well to combination therapy that includes GCSs. In these diseases, cancer cells are killed through GCS-mediated induction of apoptosis.

Contraindications include herpesvirus keratitis, active viral infections, invasive mycosis, and allergy to GCSs as well as administration following vaccination. Relative contraindications include hypertension, cardiac insufficiency,

peptic ulcers, psychosis, tuberculosis, diabetes, osteoporosis, glaucoma, cataracts, and pregnancy.

Dosing and Administration

Dermatologists most often prescribe GCSs for short periods of time to treat acute dermatoses such as contact dermatitis or different kinds of disorders. The therapeutic principle is to start at a high dose and to reduce the dose upon effect to maintenance dosing below the Cushing equivalent. Many corticoid-sensitive conditions are treated by oral burst therapy followed by a 2- to 3-week tapering course with a drug of intermediate duration of action such as prednisone; typically initial doses are in the range of 40–60 mg per day. From a pharmacoeconomic standpoint, GCSs have a very high cost effectiveness ratio; however, this is hampered by the costs of management of side effects. For example, prednisone is convenient as it is inexpensive and available in many dosages. The drug is usually given as a single dose in the morning rather than dosing being divided over the course of the day in order to minimize HPA axis suppression.

Severe and life-threatening dermatoses such as pemphigus vulgaris or severe drug reaction require higher doses of GCSs to suppress and control the disease. Especially for high-dose treatment, the doses can be separated by 4 to 6 hours to achieve better early control. The next step is to consolidate the drug to a single morning dose, prior to tapering. Alternatively, every-other-day administration may also be used as it has been shown to minimize suppression of the HPA axis. The addition of a steroid-sparing agent (e.g., mycophenolate mofetil or azathioprine) is often necessary for long-term GCS treatment such as for pemphigus vulgaris prior to tapering. Typical tapering is accomplished by 20-mg steps when the initial dose was more than 60 mg per day. Smaller tapers are used for lower initial doses until the physiologic dose range of 7.5 mg per day of prednisone is reached.

Intravenous pulse therapy is used for lifethreatening dermatoses using methylprednisolone in doses of 0.5–1 g per day for 5 days with a subsequent change to oral therapy. Cardiac conditions due to acute electrolyte shifts and arrhythmias are the most significant complications of a high-dose regimen. Due to the mineralocorticoid activity, potassium substitution may also be necessary.

To minimize corticosteroid side effects associated with GCS use, local application (e.g., inhalation) or fine-tuned dosing regimens have been developed to improve the benefit-risk ratio. One additional progress report includes the development of liposomal GCSs, which selectively accumulate at the site of inflammation [10]. Another current approach to optimize therapy with conventional GCS is to change the timing of glucocorticoid delivery (timed-release capsules) and the combination with 11β-hydroxysteroid dehydrogenase that increases the level and action of endogenous GCSs. For an additional improvement, new drugs such as selective GR agonists or nitroso-glucocorticoids (nitrosteroids) are in development. The nitrosteroids are characterized by an aliphatic or aromatic molecule, which links a conventional GCS derivative with nitric oxide (NO). Representatives of this class are NO-prednisolone and NO-hydrocortisone, which slowly release NO, exerting antiinflammatory effects [11]. The NO effect is synergistic to the effect of prednisolone, leading to an up to tenfold more potent antiinflammatory response than those of prednisolone alone [12].

Modes of Application

Glucocorticosteroids can be applied as topical and systemic treatment, but may also be administered locally to the nasal mucosa or be inhaled for the treatment of asthma. Recent studies suggest that inhaled steroids may also exhibit comparable side effects, including growth retardation in children [13] and reduction of bone markers in adolescents [14].

Local injections of triamcinolone are frequently being used to treat keloids. In addition, intraarticular injection represents a local application against rheumatic diseases.

Drug Interactions

As several drugs, such as rifampin, phenytoin, and phenobarbital, induce the hepatic cytochrome P-450 system, the clearance of GCSs may be accelerated in patients taking these medications. Dose-lowering adjustments may be necessary with enzyme inhibitors such as ketoconazole. Estrogens also potentiate the effect of GCS, because the two agents are metabolized similarly and have similar protein-binding characteristics [15].

The dose of GCS needs to be adjusted in patients with chronic active hepatitis and reduced renal function [16]. Conversely, in patients with hyperthyroidism, the biologic effect of prednisolone is reduced and may require higher doses.

Side Effects

The side effects of GCS are strictly dose-dependent. In addition, some side effects are known to depend on age and sex. In general, the side effects of GCS therapy show different degrees of severity. Table 48.3 contains a list of relevant side effects of GCS.

Table 48.3 Important side effects of glucocorticosteroid therapy

Musculoskeletal effects
Osteoporosis
Osteonecrosis
Growth retardation
Myopathy
Ophthalmologic effects
Cataract
Glaucoma
Ocular bacterial, fungal, and viral infections
Nervous system effects
Euphoria
Psychosis
Neuropsychiatric changes (anxiety, insomnia, and emotional lability)
Pseudotumor cerebri
Metabolic effects
Hyperglycemia
Hyperlipidemia
Weight gain
Cardiovascular effects
Hypertension
Atherosclerosis
Infection
Obstetric and gynecologic effects
Pregnancy and lactation
Amenorrhea
Gastrointestinal effects
Nausea and vomiting
Peptic ulcer disease
Cutaneous effects
Striae, purpura, telangiectasias, and atrophy
Cushing syndrome
Impaired wound healing
Hypothalamic-pituitary-adrenal axis suppression

Musculoskeletal

Osteoporosis

Osteoporosis is the most prevalent of the extremely important and severe musculoskeletal effects of long-term GCS therapy, but can be reduced or prevented with early physician intervention. Osteoporosis develops in 30–50 % of patients treated with long-term GCS therapy [17]. The typical patients suffer from chronic diseases such as rheumatoid arthritis, chronic destructive pulmonary disease, and asthma, or have had an organ transplantation. Postmenopausal women are especially at a significantly increased risk of fractures [18].

Importantly, the rate of bone loss is highest in the first 6 months of therapy; thereafter, the rate of bone loss is diminished. Upon discontinuation of steroid therapy, patients partly regain bone tissue. Glucocorticosteroid-induced bone loss affects trabecular bone and the cortical rim of vertebrae to a significantly higher degree than cortical bones ("long bones"). This is due to the much higher metabolic turnover rate of trabecular bone. Glucocorticosteroids cause this side effect by reducing intestinal absorption and renal tubular resorption of calcium. The reduced calcium serum concentration causes increased parathormone release that further promotes bone loss (secondary hyperparathyroidism). In addition, GCS can induce decreased gonadal function in both sexes. The most sensitive technique to diagnose osteoporosis is dual-energy x-ray absorptiometry (DEXA) [19].

Bone density studies should be performed every 12 months in patients with long-term corticosteroid therapy. Besides the use of bisphosphonates (alendronate and risedronate) as effective drugs preventing and treating GCS-induced osteoporosis [20], progress in treating GCS-associated side effects has been limited. There are additional data to support the effectiveness of calcium and vitamin D supplementation in preserving bone mass in patients receiving long-term GCS therapy [21].

Osteonecrosis

Osteonecrosis (aseptic necrosis) can also result from GCS therapy. It most commonly occurs on the proximal femur or the humeral head. In contrast to osteoporosis, osteonecrosis more frequently affects active patients and is particularly common in men [22, 23].

Growth Retardation

Growth suppression from GCS usually occurs with systemic therapy and may only occasionally be a consequence of extensive treatment with topical or inhaled potent GCS. When GCSs are given under the age of 2 or at puberty [24], the risk of growth retardation is especially significant. The studied patients were significantly shorter in height, had a significantly greater body mass index, and a higher prevalence of obesity than did the controls [24]. On average, they had received 23 grams of GCS for the treatment of nephrotic syndrome. The causes are multifaceted: interference with nitrogen and mineral retention, bone formation, inhibition of mitosis, and collagen synthesis [25]. Treatment of GCS-mediated growth inhibition with growth hormone shows some promise [26].

Myopathy

There are two forms of myopathy induced by GCS. One form is an acute myopathy seen almost exclusively in patients treated with high-dose intravenous GCS for status asthmaticus. The second form of myopathy is relevant to dermatology and is characterized by progressive symmetric proximal muscle weakness, which is usually painless and begins on the lower extremities after several months of therapy [27]. While the particular mechanism of GCS's effect on muscle mass has not been determined, hypogonadism (e.g., estrogen and testosterone) is likely to be involved, as it is present in many GCS patients. Diagnosis of GCS myopathy may be difficult in some patients, as muscle biopsies usually show nonspecific findings, and electromyographic studies are usually normal in this condition.

Ophthalmologic Effects

Cataract formation upon extended periods of GCS treatment has been described [28]. To detect initial changes, routine eye examinations twice yearly are recommended for all patients treated with longterm systemic GCS.

Open-angle glaucoma may also occur upon GCS treatment, especially in patients with a history of rheumatoid arthritis, type 1 diabetes, or a positive family history for glaucoma [29]. The detection of elevated intraocular pressure is important, as this condition is usually reversible within 1–4 weeks, when detected early.

Nervous System Effects

Affective disorders (anxiety, insomnia, euphoria, and emotional lability) are more frequent than confusional or psychotic states. The onset of symptoms is usually within 1 or 2 weeks after starting therapy, especially in patients with a prior history of psychiatric diseases [27]. Importantly, patients with systemic lupus erythematosus, who may suffer from lupus and encephalopathy may be difficult to differentiate from patients with steroid-induced psychosis. Discontinuation of GCS is usually the treatment of choice, rather than starting neuroleptic or antidepressive therapy.

Pseudotumor cerebri, presenting with headache, nausea, vomiting, and papillary edema, is a rare complication of long-term GCS administration and occurs predominantly in boys. It usually occurs when steroids are rapidly tapered or stopped [27].

Metabolic Effects

The manifestation of hyperglycemia and secondary diabetes is a typical complication of GCS therapy [30]. Relative insulin resistance is produced by decreasing the insulin affinity of cellular receptors and possibly by diminishing postreceptor effects of insulin [31, 32]. In addition, GCSs effect insulin-mediated increases in blood flow to muscles and increases in glucose output by increasing the rate-limiting enzyme of gluconeogenesis (i.e., phosphoenolpyruvate carboxy kinase). Therefore, following patients' regular blood glucose levels during continued GCS therapy is important [33]. If needed, insulin is the therapy of choice, as oral antidiabetics often take weeks before the onset of effects.

Hyperlipidemia may be worsened by GCS, especially in patients with diabetes mellitus, obesity, hypothyroidism, or family history of lipid disorders.

Weight gain may occur as a consequence of increased appetite and fluid retention with GCS therapy. Facial, supraclavicular, and posterior cervical fat depots are particularly sensitive to GCS, resulting in the moon face and buffalo humps that are characteristic of long-term GCS treatment. These symptoms severely impact on patients' well-being by negatively affecting their appearance and by predisposing them to obesity-related health issues.

Cardiovascular Effects

Excess GCSs can lead to increased blood pressure as they affect several points of blood pressure regulation. Hypertension may develop as a consequence of the mineralocorticoid activity of exogenous GCS. Usually, the kidney

is protected from the effects of excess cortisol through the oxidizing effect of 11β-hydroxysteroid dehydrogenase-2, a tissue-specific enzyme capable of converting cortisol to cortisone. However, aldosterone as well as synthetic steroids with modifications and different positions are not susceptible to this activity and exert a major effect directly on the kidney through both mineralocorticoid receptors and GR, leading to transepithelial sodium transport and sodium reabsorption in the proximal tubule [34]. A similar mode of action may be present in brain, where the 11β-hydroxysteroid dehydrogenase-2 is expressed along with mineralocorticoid receptors in selected areas that are involved in central regulation of salt, water balance, and blood pressure [35].

These processes trigger intravascular and extracellular volume expansion. In addition, a decrease in vasodilatory prostaglandins and prostacyclins has been detected upon GCS therapy [36], which contributes to hypertension.

Infection

Patients on GCS therapy are at an increased risk for infections, including bacterial, fungal, viral, and protozoal infectious agents. Making the diagnosis is sometimes difficult, as fever and many other signs of inflammation may be masked. Although neutrophilia is induced by GCS, the presence of greater than 6 % band forms suggests a coexistent infection [37]. Opportunistic infections such as *Pneumocystis jirovecii* and *Toxoplasma gondii* may occur in patients on chronic GCS therapy who are not HIV-positive. In addition, reactivation of tuberculosis remains a concern in this patient population [38]. Concomitant isoniazid has been advocated in patients with a positive tuberculin skin test undergoing long-term GCS therapy.

Inhibition of Wound Repair

The inhibition of wound repair results from inhibiting the natural and critical process of inflammation as part of the normal wound-healing process to remove debris and bacteria [39]. Moreover, GCSs inhibit collagen synthesis and collagen cross-linking, and thereby affect structural components of a healing wound [40].

Obstetric and Gynecologic Effects

Clinical experience in several trials has shown minimal adverse effects in pregnant and lactating women during GCS therapy [41, 42]. The American Academy of Pediatrics has determined prednisone to be compatible with breast-feeding, even though there is an excretion in breast milk [43].

Despite the fact that systemic GCS can cross the placenta to various degrees, there is no proof of the detrimental effect on the developing human brain or on vascular disease such as hypertension and atherosclerosis [44, 45]. However, pregnant or lactating women should be monitored for complications such as osteoporosis and glucose intolerance (gestational diabetes).

Gastrointestinal Effects

Nausea and vomiting may occur with oral GCS therapy. These side effects can be minimized if the GCS is taken with food. The association of GCS therapy and peptic ulcer disease remains controversial [46]. In particular, the simultaneous intake of GCSs with nonsteroidal anti-inflammatory drugs as well as smoking and alcohol use have to be considered. Concurrent therapy with proton pump blockers is advised in patients with a history of peptic ulcer disease.

Cutaneous Effects

Cutaneous side effects consist of atrophy of the dermis and subcutaneous tissue, striae, rarefaction of elastic tissue (frequently causing corticoid purpura and steroid acne), telangiectasias, and hirsutism. These and other cutaneous side effects are discussed in the section on topical GCS below.

Hypothalamic-Pituitary-Adrenal

Axis Suppression

The onset of HPA axis suppression is usually evident within 5 days of high-dose prednisolone therapy, suppressing ACTH and cortisol secretion. With longer treatment duration, clinically important adrenal suppression becomes a concern. Full recovery of adrenals may require up to 1 year in certain cases [38]. In addition, patients with chronic adrenal suppression, including HIV patients, may need GCS supplementation in critical conditions such as perioperative stress or sepsis.

A more common clinical problem than adrenal crisis is the steroid withdrawal syndrome. Patients' symptoms generally include arthralgias, mood swings, headache, lethargy, nausea, and vomiting, and are most frequently noted on rapidly tapering of GCS after extended periods of treatment [47].

Topical Corticosteroids

Topical corticosteroids (TCSs) have a particular adverse-effect profile, primarily directed at the treated skin area, that occurs regularly with prolonged treatment. The severity

Table 48.4 Potency of selected topical corticosteroid preparations

Class 1 (superpotent)	Betamethasone dipropionate ointment, cream 0.05 % (Diprolene, Diprosone)
	Clobetasol propionate ointment, cream 0.05 % (Temovate, Dermoxin)
	Diflorasone diacetate ointment 0.05 % (Florone, Psorcon)
	Halobetasol propionate ointment, cream 0.05 % (Ultravate)
Class 2 (potent)	Amcinonide ointment 0.1 % (Cyclocort)
	Desoximetasone ointment, cream, gel 0.25 % (Topicort, Ibaril)
	Diflorasone diacetate ointment 0.05 % (Florone, Maxiflor)
	Fluocinonide ointment, cream, gel 0.05 % (Lidex)
	Halcinonide cream 0.1 % (Halog)
	Mometasone furoate ointment 0.1 % (Elocon, Ecural)
	Triamcinolone acetonide ointment 0.5 % (Kenalog)
Class 3 (less potent)	Amcinonide cream, lotion 0.1 % (Cyclocort)
	Betamethasone valerate ointment 0.01 % (Valisone)
	Diflorasone diacetate cream 0.05 % (Florone, Maxiflor)
	Fluticasone propionate ointment 0.005 % (Cutivate)
	Fluocortolone 0.25 % cream (Ultralan)
	Fluocinonide cream 0.05 % (Lidex E cream, Topsyn)
	Halcinonide ointment 0.1 % (Halog)
	Triamcinolone acetonide ointment 0.1 % (Aristocort A)
	Triamcinolone acetonide cream 0.5 % (Aristocort-HP)
Class 4 (mid-strength)	Betamethasone valerate lotion 0.01 % (Valisone, Luxiq)
	Desoximetasone cream, gel 0.05 % (Topicort-LP)
	Fluocinolone acetonide cream 0.2 % (Synalar-HP)
	Fluocinolone acetonide ointment 0.025 % (Synalar)
	Flurandrenolide ointment 0.05 % (Cordran)
	Halcinonide cream 0.025 % (Halog)
	Hydrocortisone valerate ointment 0.2 % (Westcort)
	Mometasone furoate cream 0.1 % (Elocon, Ecural)
	Triamcinolone acetonide ointment 0.1 % (Kenalog)
Class 5 (less mid-strength)	Betamethasone dipropionate lotion 0.05 % (Diprosone)
	Betamethasone valerate cream 0.01 % (Valisone)
	Fluocinolone acetonide cream 0.025 % (Synalar)
	Fluocinolone acetonide oil 0.01 % (Derma-Smoothe/FS)
	Flurandrenolide cream 0.05 % (Cordran)
	Fluticasone propionate cream 0.05 % (Cutivate)
	Hydrocortisone butyrate cream 0.1 % (Locoid)
	Hydrocortisone valerate cream 0.2 % (Westcort)
	Triamcinolone acetonide lotion 0.1 % (Kenalog)
Class 6 (mild)	Alclometasone dipropionate ointment, cream 0.05 % (Aclovate)
	Betamethasone valerate lotion 0.05 % (Valisone)
	Desonide cream 0.05 % (Desowen, Tridesilon)
	Fluocinolone acetonide cream, solution 0.01 % (Synalar)
	Prednicarbate 0.1 % cream (Dermatop)
	Triamcinolone acetonide cream 0.1 % (Aristocort)
Class 7 (least potent)	Dexamethasone cream 0.1 % (Decadron phosphate)
	Hydrocortisone 0.5, 1, 2.5 % (Hytone, others)
	Methylprednisolone 1 % (Medrol)
	Topical preparations with flumethasone, prednisolone

depends on the potency of the drug, the vehicle, and the location of its application (Table 48.4). The most frequent adverse effects include atrophy, striae, rosacea, perioral dermatitis, acne, and purpura (Table 48.5). With lower frequency, hypertrichosis, pigmentation changes, delayed wound healing, and exacerbation of skin infections are observed. Of particular

Table 48.5 Adverse effects of topical corticosteroids

Most frequent cutaneous changes
Steroid atrophy
Teleangiectasia
Striae
Purpura
Stellate pseudoscars
Ulceration
Easy bruising
Less frequent cutaneous changes
Steroid acne
Perioral dermatitis
Steroid rosacea
Hirsutism
Hyperpigmentation
Hypopigmentation
Masked microbial infections (tinea incognito)
Aggravation of cutaneous candidiasis, herpes, or Demodex
Reactivation of Kaposi's sarcoma
Granuloma gluteale infantum
Miscellaneous
Steroid rebound, Steroid addiction Tachyphylaxis

interest is the rate of contact sensitization against corticosteroids, which is considerably higher than generally believed. Systemic reactions following topical application such as hyperglycemia, glaucoma, and adrenal insufficiency have also been reported, especially in children.

Selection and Characteristics of Topical Glucocorticosteroids

Low- to medium-potency agents are generally used for treating thin, acute, inflammatory skin lesions (e.g., face, intertriginous areas), whereas highly or superpotent agents are often required for treating chronic, hyperkeratotic, or lichenified lesions (e.g., palms and soles) (Table 48.4). Most preparations are applied once or twice daily. More frequent application may be necessary on the palms or soles due to the thick stratum corneum. Every-other-day or weekend-only application may be effective for treating several chronic conditions. Low-potency agents are preferentially used in infants and the elderly. Infants have a high body surface to weight ratio; elderly patients have thin, fragile skin.

The vehicle in which the topical corticosteroid is formulated influences the absorption and potency of the drug [48]. Ointment bases enhance penetration of the drug by their occlusive effect and increase the hydration of the stratum corneum. Creams are preferred for acute and subacute dermatoses; they may be used on moist skin or intertriginous areas.

Marked regional variation in the extent of transcutaneous penetration has been documented [49]. While absorption on the forearm (1 %) is poor, the scalp absorbs around 4 % and the scrotum up to 35 % of applied drug. Consequently, the groin, axillae, neck, and face are more likely to develop local side effects [50, 51]. The reasons for this difference in absorption are due to the thickness of the stratum corneum and its lipid composition [49]. For these reasons, the skin of delicate sites such as the eyelids is much more likely to develop side effects of TCS therapy.

Pharmacologic Characteristics of Topical Glucocorticosteroids

Chemical substitution at certain key positions is able to modify the potency of GCS (see Tables 48.1 and 48.4). For example, halogenation at the 9-α position enhances the potency by improving activity within the target cell and decreasing breakdown into inactive metabolites [52]. Along the same lines, masking or removing the hydrophilic 17-dihydroxyacetone side chain or the 16-α- hydroxy group increases the molecule's lipophilicity, thus enhancing penetration through the stratum corneum [52].

The strength of TCS has been classified according to the vasoconstrictor assay, which is based on the extent to which the compound induces cutaneous vasoconstriction ("blanching effect") in normal human subjects (Table 48.4) [53]. The vasoconstriction test has been established in 1962 as a rough estimate of the efficacy of TCS [54, 55].

It represents a nonspecific and simple in vivo test, since the phenomenon of vasoconstriction is not linked to the

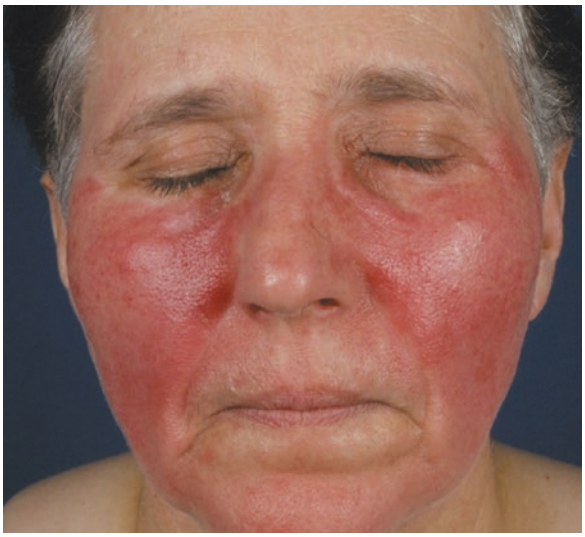

Fig. 48.2 Thickened lichenified skin, severe epidermal atrophy, and erythema following inappropriate use of high-potency corticosteroids on the face and eyelids. Telangiectasias were also present

receptor-mediated activity of steroids. However, the exact cause of this vasoconstriction remains unknown [56]. Alternatively, the ultraviolet (UV) erythema test measures the inhibitory effects of TCS on an experimentally elicited erythema [57]. The atrophy test is an important addition to the antiinflammatory tests, since it can be used to determine those TCSs that have only a slight antiproliferative effect (atrophogenic potential). Using the Duhring chamber, the corticosteroid to be tested is applied to the same skin area for 3 weeks under occlusion [58]. At this point, the resulting atrophy and the extent of telangiectasias are evaluated by a defined score.

Under normal conditions, up to 99 % of TCSs applied to human skin are removed by rubbing, washing off, and exfoliation, and only about 1 % is therapeutically active [59]. However, only this 1 % of percutaneously absorbed TCSs can exert systemic adverse effects, while cutaneous adverse effects may also result from the transient presence of TCSs [60].

Adverse Effects of Topical Glucocorticosteroids

A number of possible adverse effects of TCSs have been reported (see Table 48.5). Principally, systemic GCS can cause the same cutaneous manifestation as TCS.

Atrophy

All TCSs have been shown to cause skin atrophy, albeit to a variable degree [61, 62]. Signs of atrophy include telangiectasias (an abnormal dilatation of capillary vessels and arterioles), increased transparency and shininess of the skin, as well as the appearance of striae [50, 63]. The factors that influence the degree of skin atrophy include age, body site, potency of TCS, and the presence of occlusion. Atrophy has now been recognized as the most common adverse effect of topical corticosteroid therapy (Fig. 48.2) [64]. Topical application of corticosteroids can cause atrophy, not only because of the suppressive action on cell proliferation, but also due to inhibition of collagen synthesis [65]. In addition to epidermal and dermal thinning, corticosteroids stimulate human dermal microvascular endothelial cells, leading to the occurrence of telangiectasias [66].

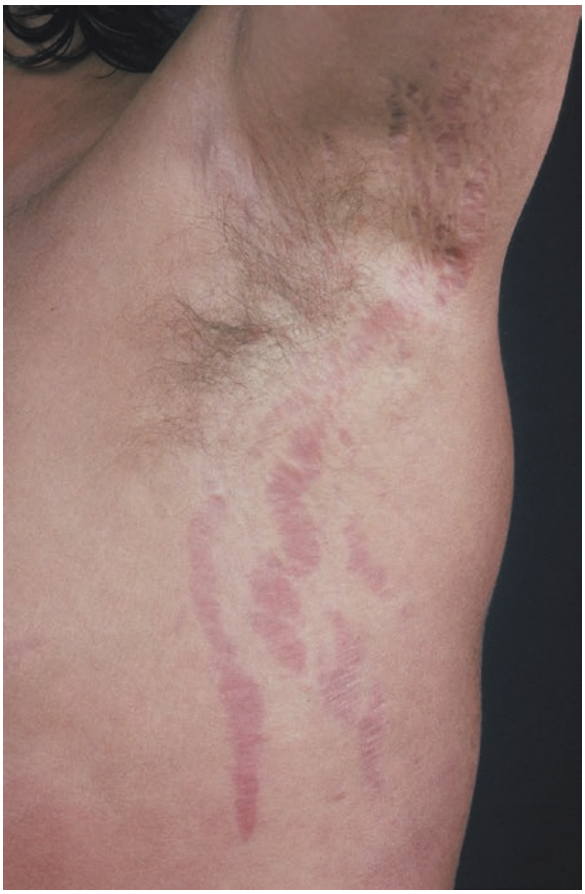

Fig. 48.3 Striae distensae rubrae as a sign of topical corticosteroid (TCS) abuse from treating eczema on the left axilla in a nonobese 27-year-old man

Striae

Striae (rubrae distensae) are visible linear scars that form in areas of dermal damage, presumably upon mechanical stress (Fig. 48.3) [67]. They develop as an initial inflammation and edema in the dermis, followed by the deposition of dermal collagen along the lines of mechanical stress.

Steroid Rosacea

Facial dermatoses are usually steroid-sensitive and do not require potent formulations. The classical history of steroid

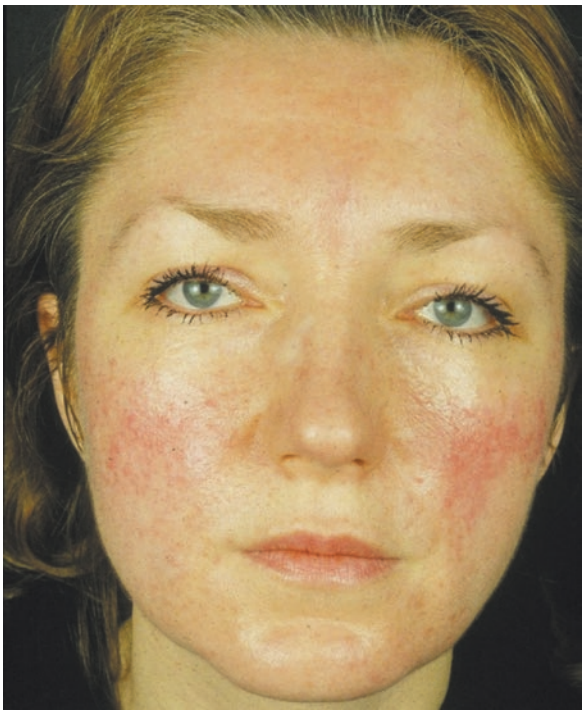

Fig. 48.4 Long-term inadvertent use of TCS for treatment of perioral and cheek dermatitis. Note the prominent telangiectasias

rosacea begins in a middle-aged woman with intermittent papules and pustules that are initially controlled by steroids of low potency (Fig. 48.4). Subsequently, the lesions reappear, leading to tachyphylaxis and steroid addiction [68].

Acne

Topical steroids can rapidly induce acneiform eruptions [69–71]. These studies attributed the acnegenic effect to the degradation of the follicular epithelium, resulting in extrusion of the follicular content (Fig. 48.5).

Perioral Dermatitis

Steroid-induced perioral dermatitis was described as a facial eruption in females that was composed of follicular papules and pustules on an erythematous background beginning in a perioral distribution with prominent sparing of the skin adjacent to the vermilion border [72].

Steroid Addiction and Tachyphylaxis

Corticosteroid addiction has been described as an ongoing inadvertent use of potent topical corticosteroids used mostly for inflammatory diseases of the face [68].

Tachyphylaxis is characterized by decreasing effects of corticosteroids upon continued treatment as revealed by the vasoconstrictor and proliferation inhibition assays. Due to the tissue becoming less sensitive (tachyphylaxis), more potent preparations are frequently being used to achieve comparable effects [73], yielding more severe side effects.

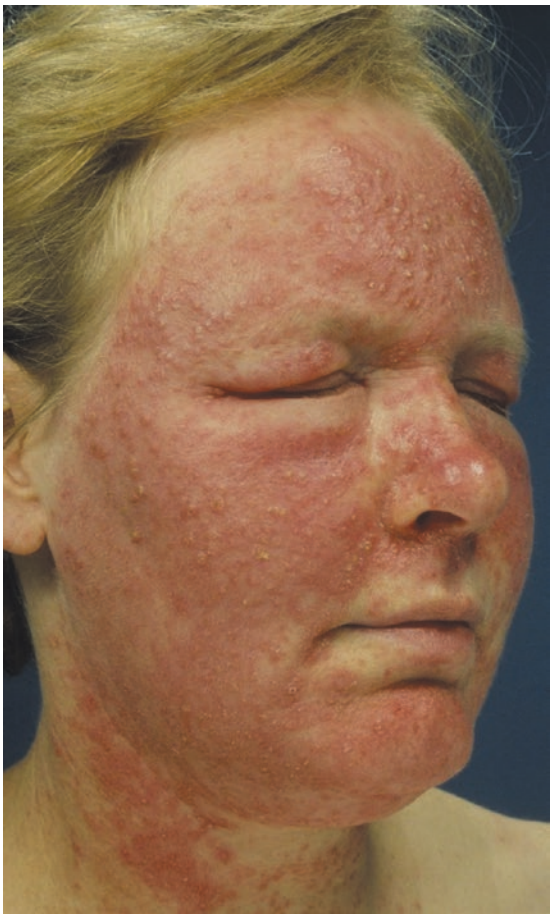

Fig. 48.5 Steroid acne in the face characterized by pustules, erythema, and several open and closed comedones on the forehead. Note the free margins around the vermilion border

Hypertrichosis

Steroids promote the growth of vellus hair by an unknown mechanism [74]. Variable degrees of hypertrichosis remain a more common manifestation of systemic corticosteroid use. The darker hair may persist for months after withdrawal of steroids.

Hypopigmentation

Decreased pigmentation after topical use is quite common, though frequently unnoticed. Americans of sub-Saharan African lineage are particularly affected. Most likely, steroids interfere with the synthesis of melanin, leading to patchy areas of hypopigmentation. The lesions are generally reversible upon discontinuation of steroid therapy.

Purpura and Stellate Pseudoscars

Purpura occurs due to severe dermal atrophy and loss of intercellular substance, and blood vessels lose their support and rupture. The resulting fragility of dermal tissue leads to hypopigmented, depressed scars [75]. These stellate pseudoscars develop most frequently over the extremities mostly on severely atrophic, telangiectatic purpuric skin (see Fig. 48.2).

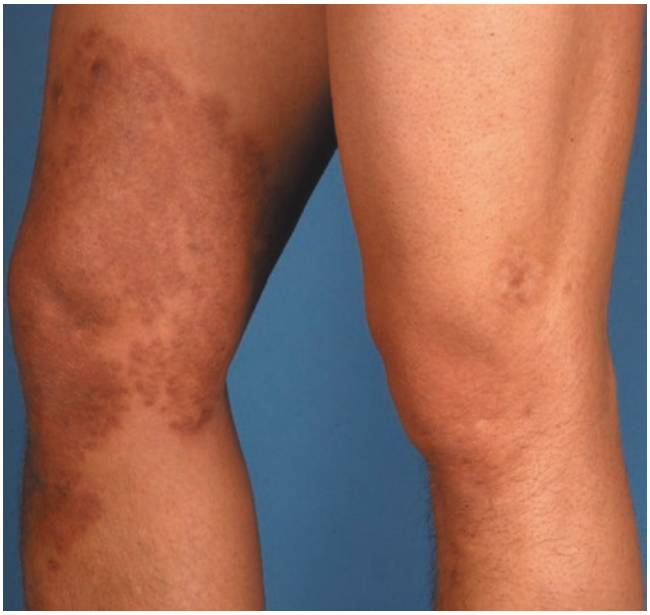

Fig. 48.6 Tinea incognito of the leg. This patient was treated with corticosteroids masking the diagnosis of tinea

Aggravation of Cutaneous Infections

Mucocutaneous (e.g., skin, nails, mucous membranes) fungal infections are common during treatment with corticosteroids and often occur early in therapy [76, 77]. The incidence of skin infections varies, but is probably between 16 and 43 % [76]. Infections include tinea versicolor, onychomycosis due to *Trichophyton* and *Candida* species, and dermatophytosis (Fig. 48.6) [76]. The term *tinea incognito* serves to describe dermatophyte infections that became unrecognizable because of suppression of inflammation and fungal proliferation [78].

Granuloma gluteale is a persistent reddish-purple, granulomatous papulonodular eruption occurring on the buttocks, thighs, or inguinal folds in children. It is a well-known consequence of diaper dermatitis that is being treated with corticosteroids [79].

Delayed Wound Healing

The effects of TCS and systemic GCS on wound healing include keratinocytes (epidermal atrophy, delayed reepithelization), fibroblasts (reduced collagen and ground substance resulting in dermal atrophy and striae), vascular connective tissue support (telangiectasias, purpura, easy bruising), and impaired angiogenesis (delayed granulation tissue formation) [38].

Contact Sensitization

Several multicenter studies have been performed addressing contact hypersensitivity to TCS, yielding a prevalence of 0.2–6 % [80–83]. It seems that nonfluorinated corticosteroids (e.g., hydrocortisone, hydrocortisone-17-butyrate, and budesonide) result in a higher prevalence of corticosteroid allergy in comparison with fluorinated compounds [84].

Systemic Adverse Effects with Topical Administration

Systemic adverse effects from TCS are relatively infrequent, but may occur. In particular, glaucoma following the use of TCS around the eye has been recognized as a rare but serious problem [85, 86]. This is not surprising, if one considers that the penetration of TCS is up to 300 times greater through the eyelid than on other body sites [49]. Topical corticosteroid and systemic GCS therapy have been associated with cataract formation, but systemic GCSs are most often responsible for this complication [87]. In addition, short-term enhancement of plasma cortisol levels has been detected upon topical application of hydrocortisone [88]. All effective TCSs possess the potential to suppress the HPA axis [89].

Increasing steroid penetration has been shown to increase the potential for HPA suppression especially in children with atopic dermatitis [90–92]. The advent of superpotent derivatives such as clobetasol propionate, betamethasone dipropionate, and diflorasone diacetate have an increased ability to suppress adrenal function. As little as 2 grams per day of clobetasol propionate 0.05 % cream can cause a decreased morning cortisol after only a few days [93, 94]. A recent open-label trial has addressed the potential suppression of the HPA axis using 0.1 % fluocinonide cream in pediatric patients with atopic dermatitis with regard to adrenal suppression [95].

In this multicenter, multidosing, open-label trial, 0.1 % fluocinonide cream was applied to patients in four different age cohorts, from 3 months to 18 years of age, who suffered from moderate to severe atopic dermatitis in excess of 20 % body surface area involvement. No suppression of the HPA axis was observed in any patient treated once daily or in patients younger than 6 years of age. One of 15 (7 %) and two of 16 (13 %) patients from 3 months to 2 years and 2–6 years, respectively, developed HPA axis suppression when receiving twice daily 0.1 % fluocinonide treatment to more than 20 % of body surface area [95].

With the majority of patients with HPA suppression demonstrating exclusive laboratory test abnormalities, several cases of severely impaired stress responses have been reported, especially in children following treatment with high potency TCS [96–98]. Recovery from steroid-induced adrenal insufficiency is time-dependent and occurs spontaneously. The administration of TCS has also led to iatrogenic Cushing's syndrome [99, 100]. In addition, there is a possibility of retarded growth in children exposed to long-term potent and superpotent topical TCS formulations [96]. For prevention, it has been suggested that less than 50 grams per week of potent corticosteroids should be used [101].

Table 48.6 Optimal use of topical glucocorticosteroids

1. Appropriately potent compound to achieve disease control
2. Continuation with a less potent preparation after sufficient response
3. Reduction of frequency of application
4. Continuation of daily application with the weakest effective preparation
5. "Tapering off" treatment upon complete healing
6. Particular care in treating children and the elderly, in particular at certain locations (e.g., scrotum, face and flexures, and around the eyes)

Prevention of Adverse Effects

Guidelines regarding the use of TCS and suggestions were provided to prevent their misuse [62, 102, 103].

Possible measures to prevent side effects include the use of lower potency steroids, the application only in the morning, and alternate-day treatment (reducing tachyphylaxis and avoidance of occlusion) (Table 48.6) [104].

Conclusion

Glucocorticosteroids have anti-inflammatory, antiproliferative, and immunosuppressive effects. These effects are exerted by GCS binding to glucocorticoid receptors (GRs) and by modification of transcription of corticosteroid-responsive genes. Neutrophilia, lymphopenia, and eosinopenia are induced by GCSs, which also reduce access of inflammatory cells at the site of active infection. To improve the benefit-risk ratio of GCSs, new interventions have been developed, such as liposomal GCSs, GR agonists, and nitroso-glucocorticosteroids. Some drugs inhibit the P-450 system, reducing clearance of GCSs, while other drugs induce the system, accelerating clearance. The side effects of GCSs are strictly dose-dependent, but the vehicle in which topical steroids are formulated influence the absorption and potency of the drug.

Questions

1. Which of the following drugs inhibit the hepatic cytochrome P-450 system, requiring dose-lowering adjustments in glucocorticosteroids?
   (a) Rifampin
   (b) Ketoconazole*
   (c) Phenytoin
   (d) Phenobarbital

2. Which of the following modifications of glucocorticosteroids enhances the potency by improving activity within the target cell and decreasing breakdown into inactive metabolites?
   (a) Removing the 17-dihydroxyacetone side chain
   (b) Removing the 16-alpha hydroxyl group
   (c) Halogenation at the 9-alpha position*
   (d) Halogenation at the 16-alpha position

3. Glucocorticosteroids can induce acneiform eruptions by which mechanism?
   (a) Increased growth of *P. acnes*
   (b) Degradation of the follicular epithelium*
   (c) Inhibition of collagen synthesis
   (d) Inhibition of elastin synthesis

Answers

1. b
2. c
3. b

* indicates the correct answers to the questions

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Vaccines

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Michael Lee, Christopher Downing, Ramya Kollipara, Jacqueline Guidry, and Stephen K. Tyring

Abstract

Vaccines are one of the leading tools used for maintenance of public health, and have made a tremendous contribution to the goal of reducing the incidence of numerous diseases since their inception. The prevention of diseases such as smallpox, polio, measles, and hepatitis B have had an enormous impact on world health over the last century. Research has led to the development of different types of vaccines, including live-attenuated, inactivated, conjugate, and recombinant vaccines. Herpes zoster and diseases caused by human papillomavirus represent important dermatologic conditions which have been the target of vaccines. Recent advances in the development of new vaccines for HIV, herpes simplex, and certain malignancies represent the next horizon for disease prevention and treatment with vaccines. Despite the challenges among implementing vaccination programs and public education regarding immunization, the available data from approximately 200 years of research shows that manipulation of the immune system with vaccines has been of great benefit.

Keywords

Vaccine • Immunity • Live-attenuated • Virus • Conjugate • Adjuvant • Recombinant • Gardasil • Therapeutic

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Key Points

- Vaccination is ranked as the greatest public health achievement in the last century.
- Universal childhood immunization in the United States has led to a significant decrease in the incidence of several diseases.
- Vaccines may cause adverse reactions but most are mild and well tolerated.
- Many more vaccines are currently in development, and continuing vaccine research provides a promising outlook for prevention of more diseases in the near future
- The future of vaccines includes live-agent vaccines as vectors of other antigens, sequential immunization, immunization with DNA, and therapeutic vaccines.

Four criteria are needed for the success of a vaccine-based eradication program: (1) the infection must be limited to humans (no animal reservoir); (2) with viral infections there must be only one or a few strains of the virus, and these strains must have constant antigenic properties; (3) the virus must not persist in the infected host; and (4) the vaccine should be effective [1]. Vaccination as the route to prevention is the best option for controlling the spread of infectious diseases. Immunization can prevent or modify the severity of illness in the individual and interrupt or reduce the transmission of the pathogens to other susceptible people. Through these mechanisms, vaccines against smallpox, polio, measles, and hepatitis B have had an enormous impact on world health over the last 50 years. Vaccination is ranked as the greatest public health achievement in the last century, contributing the most to decreased global morbidity and mortality [2, 3].

There is a still great need for vaccines for many organisms since an inadequate number of antimicrobial drugs are available for the multiple microbes that exist. There are many reasons why it is difficult to produce the many necessary vaccines, including that all microorganisms deploy evasion methods that interfere with effective immune responses; further, for many organisms, it is still unclear which immune responses provide effective protection, particularly in developing vaccines against agents that cause persistent or chronic infections. Such infections, which notably include the human immunodeficiency virus (HIV), are subject to significant antigenic variation. Most current vaccines are directed against acute infections, when a sublethal dose of the agent is controlled and rapidly cleared by the immune system (Table 49.1).

Control of Infection

Extracellular Infection

Cytokines secreted by $CD4^+$ T-helper-1 (Th1) T cells aid in activating phagocytic cells such as macrophages and thus help in the destruction of the foreign agent in this fashion or complexed with an antibody.

Intracellular Infection

With intracellular infections with viruses, cytotoxic T lymphocytes (CTLs) play a dominant role in controlling and clearing the infection. In addition to its active role in killing infected cells, cytotoxic T cells secrete potent cytokines with antiviral and macrophage-activating capabilities, such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) [1]. With intracellular bacterial infections, the immune response is not as straightforward. Both $CD4^+$ Th1 and $CD8^+$ T cells have an important role in reducing, controlling, and clearing the infection [4–7].

Regional Immunity

The mucosal surfaces of the human body are far greater in area in comparison to the skin and are well endowed with draining lymphoid tissues, with the exception of the vagina. The main routes of infection are the gut, the rectum, the genitourinary tract, the respiratory tract, and the eye. There is a common mucosal system, so that immunization at one site can result in protection at that and other mucosal areas. For instance, the adenovirus vaccine is administered orally but offers protection against a respiratory infection [8]. In addition, since the female genital tract is devoid of lymph tissue, systemic immunization is necessary to confer protection. Specifically, immunization through the respiratory tract was shown to be superior to other routes in inducing protection in the female genital tract [7, 9–11].

The formation and secretion of secretory immunoglobulin A (sIgA), is the first line of defense in the mucosal immune system; sIgA binds to antigens and their toxins and subsequently aids in prevention of attachment and penetration of the mucosal surface. Cytotoxic T cells have also been shown in the cytobrush specimens from the cervix of HIV-infected women [12]. Thus, protection in mucosal immunity is likely mediated through humoral and cell-mediated mechanisms.

Table 49.1 Current U.S. vaccines

Vaccine	Name	Manufacturer	Type	Route
Anthrax	BioThrax	BioPort	Inactivated bacterial	SC
DTaP	Daptacel	Sanofi	Inactivated bacterial	IM
	Infanrix	GSK	Inactivated bacterial	IM
	Tripedia	Sanofi	Inactivated bacterial	IM
DT	Generic	Sanofi	Inactivated bacterial toxoids	IM
DTaP/HiB	TriHIBit	Sanofi	Inactivated bacterial	IM
DTaP/IPV/HiB	Pediarix	GSK	Inactivated bacterial and viral	IM
Haemophilus influenzae type b (Hib)	HibTITER	Wyeth	Inactivated bacterial	IM
	PedvaxHIB	Merck	Inactivated bacterial	IM
	ActHIB	Sanofi	Inactivated bacterial	IM
Hepatitis A	Havrix	GSK	Inactivated viral	IM
	Vaqta	Merck	Inactivated viral	IM
Hepatitis B	Engerix-B	GSK	Inactivated viral (recombinant)	IM
	Recombivax HB	Merck	Inactivated viral (recombinant)	IM
HepA/HepB	Twinrix	GSK	Inactivated viral	IM
HPV	Gardasil	Merck	Inactivated viral (recombinant)	IM
HepB/Hib	Comvax	Merck	Inactivated bacterial and viral	IM
Influenza	Fluarix	GSK	Inactivated viral	IM
	Fluvirin	Chiron	Inactivated viral	IM
	Fluzone	Sanofi	Inactivated viral	IM
	FluMist	Medimmune	Live attenuated viral	Intranasal
Japanese encephalitis	JE-Vax	Sanofi	Inactivated viral	SC
MMR	M-M-R II	Merck	Live attenuated viral	SC
MMRV	ProQuad	Merck	Live attenuated viral	SC
Measles	Attenuvax	Merck	Live attenuated viral	SC
Mumps	Mumpsvax	Merck	Live attenuated viral	SC
Rubella	Meruvax II	Merck	Live attenuated viral	SC
Meningococcal	Menomune	Sanofi	Inactivated bacterial	SC
	Menactra	Sanofi	Inactivated bacterial	IM
Pneumococcal	Pneumovax 23	Merck	Inactivated bacterial	SC or IM
	Prevnar	Wyeth	Inactivated bacterial	IM
Polio	Ipol	Sanofi	Inactivated viral	SC or IM
Rabies	BioRab	BioPort	Inactivated Viral	IM
	Imovax Rabies	Sanofi	Inactivated Viral	IM
	RabAvert	Chiron	Inactivated Viral	IM
Rotavirus	RotaTeq	Merck	Live viral	Oral
Td (tetanus & diphtheria)	Decavac	Sanofi	Inactivated bacterial toxoids	IM
	(Generic)	Massachusetts Biological Labs	Inactivated bacterial toxoids	IM
Tdap (tetanus & diphtheria toxoids/pertussis vaccine	Boostrix	GSK	Inactivated bacterial	IM
	Adacel	Sanofi	Inactivated bacterial	IM
TT (tetanus toxoid)	(Generic)	Sanofi	Inactivated bacterial toxoid	IM

(continued)

Table 49.1 (continued)

Vaccine	Name	Manufacturer	Type	Route
Typhoid	Typhim Vi	Sanofi	Inactivated bacterial	IM
	Vivotif Berna	Sanofi	Live bacterial	IM
Varicella	Varivax	Merck	Live viral	SC
Vaccinia (smallpox)	Dryvax	Wyeth	Live viral	Percutaneous
Yellow fever	YF-Vax	Sanofi	Live viral	SC
Zoster (shingles)	Zostavax	Merck	Live viral	SC

Immunologic Memory

Most organisms are detected and destroyed within hours by defense mechanisms that are not antigen-specific and do not require any prolonged period of induction. This illustrates the mechanism of innate immunity. Only when a pathogen or antigen is able to break this early line of defense will an adaptive immune response ensue, including the production of antigen-specific effector cells, secretion of antibodies by B cells, direct cytotoxic activity (CTLs), or via the secretion of immunologic mediators and effector molecules such as cytokines and chemokines. Most of the effector cells will die within 10–14 days after infection, but some cells survive as highly reactive plasma cells (B cells) or memory cells (B and T cells) to combat subsequent infection. The goal in preventative vaccination is to stimulate the adaptive response in the body with formation of long-lasting antibodies against a specific pathogen and induction of memory cells. The mechanism of how it should be achieved through immunization is less certain, but significant information has been elucidated about immunologic memory thus far [13–15].

B-Cell Memory

A memory B-cell is one that has undergone immunoglobulin isotype switching and somatic hypermutation [16]. This differentiation process starts late in the primary immune response and takes place in the germinal centers of lymph nodes. Upon reencountering the same pathogen (or antigen in a booster vaccine), the memory cells start dividing at a high rate and differentiate into antibody-secreting plasma cells. Further, memory cells produce antibody of higher average affinity than unprimed B cells; the affinity of that antibody persists to increase during the ongoing secondary and subsequent antibody responses.

T-Cell Memory

After immunization, the number of T cells reactive to a given antigen increases markedly as the majority become effector T cells; then the number falls back to continue at a level significantly (100- to 1000-fold) above the initial frequency. These cells carry cell-surface proteins more characteristic of effector cells than of naive T cells. Both $CD4^+$ and $CD8^+$ T cells can differentiate into two types of memory cells. One type is called effector memory cells because they can quickly mature into effector T cells and secrete large amounts of cytokines such as IFN-γ, interleukin-4 (IL-4), and IL-5 early after re-stimulation. They probably develop from effector T cells and are relatively short-lived [17]. The other type is called central memory cells, and they are long-lived in the absence of antigen and are able to deal with systemic pathogenic infections. Recently, a new type of T cell, the T memory stem cell, has been recognized in the $CD8^+$ which has the ability to reproduce itself as well as give rise to effector memory and central memory cells. These cells have been shown to play a significant role in immune homeostasis and have been a target in recent therapies targeting intracellular pathogens and cancer [18].

Routes of Vaccination

Most vaccines are currently given by injection, which has two main disadvantages. The first is that injections are painful and expensive, requiring needles, syringes, and a trained injector. Mass vaccination can be arduous through this route. The disadvantage from an immunologic standpoint is that the injection may not be the most effective way of stimulating an appropriate immune response since it is not imitating the route of the majority of the pathogens.

Although the presentation of soluble protein antigens by the oral route often results in tolerance, which is necessary with the large load of food-borne and airborne antigens presented to the gut and respiratory tract, many pathogens that cause significant morbidity and mortality across the world infect mucosal surfaces or enter the body through mucous surfaces. For this reason, several vaccines targeting the mucosal immune response have been and are currently being studied. For instance, there is the Sabin trivalent oral poliovirus vaccine (OPV). Unfortunately due to one case of paralysis, the Centers for Disease Control (CDC) and the American Academy of Pediatrics have recommended that only inactivated polio vaccine (IPV) be used after January 1, 2000 [19]. Oral polio vaccines are still being used in

areas outside the United States where polio is endemic [20]. In addition, a safe and effective intranasal influenza vaccine has been available for several years. Buccal, sublingual, vaginal, and eye mucosa methods of vaccination have recently been studied in preclinical investigations and have shown promising results [21, 22].

Due to the significant advances in the technology of immunization, other routes of delivery are being considered. For instance, viral and bacterial antigens have been produced in transgenic plants [23, 24]. Plant-based vaccines offer a means to deliver large quantities of a designated antigen in an encapsulated form. This encapsulation appears to protect against rapid and complete degradation of orally administered recombinant proteins. Thus, there is the potential for antigen to be gradually released into the gastrointestinal tract as host tissue is digested. This should theoretically permit an increased proportion of orally administered antigens to reach the effector sites, such as Peyer's patches lining the gastrointestinal tract [25]. A few human vaccine candidates have entered or completed phase I clinical trials. These include diarrheal vaccine candidates targeting enterotoxigenic *Escherichia coli* [26, 27] and Norwalk virus [28] and candidates against hepatitis B, rabies, smallpox, RSV, diphtheria, influenza, and anthrax [29–31].

Another route being considered is epicutaneous immunization. In the past few years, it has been elucidated that application of antigen onto bare skin induces potent systemic and mucosal immunity in an antigen-specific manner via Langerhans and dendritic cells [32–34]. Coadministration of nonspecific adjuvants, such as cholera toxin, is necessary to induce good immune responses. It has recently been demonstrated that a natural adjuvant effect can be achieved by disrupting the stratum corneum prior to topical antigen application; this act stimulates the Langerhans cells [32]. Epicutaneous immunization also generates active antigen-specific immunity in the gut and particularly augments Th2 responses subsequent to oral antigen [35] and inhalation of antigen [36]. The search for methods of vaccine delivery not requiring a needle and syringe has been influenced by concerns of pandemic disease, bioterrorism, and disease eradication campaigns [37]. Early testing of skin-patch tests is beginning, and past data with epicutaneous influenza vaccine showed that the vaccine was well tolerated by human volunteers [38].

Vaccine Development

Live-Attenuated Vaccines

Viruses

Live-attenuated viral vaccines are composed of viruses that are traditionally grown in cultured cells. Viruses are typically selected for preferential growth in nonhuman cells, and, in the course of selection, become less capable of growth in human cells. Since these attenuated strains do not replicate well in human hosts, they induce immunity but not disease when given to individuals. There may be a slight possibility that the pathogenic virus can reemerge by a further series of mutations even though attenuated virus strains contain multiple mutations in their genome. Further, attenuated viral vaccines can cause significant damage by inducing viral opportunistic infections in those that are immunodeficient [39]. Live-attenuated viral vaccines are currently in use for smallpox, measles, mumps, rubella, varicella, herpes zoster, polio, influenza (intranasal), yellow fever, and rotavirus. The vaccines targeting diseases that cause cutaneous manifestations are discussed in this section and throughout the chapter.

Smallpox

Edward Jenner, in 1796, was the first to demonstrate that inoculation of cowpox virus into human skin could lead to protection from subsequent smallpox infection [40]. Jenner named the inoculation substance *vaccine*, based on the Latin word *vacca*, meaning "cow." The vaccines used for smallpox vaccination are derived from vaccinia virus, a species similar to cowpox. The virus that causes smallpox is the variola virus, which belongs to the Poxviridae family and *Orthopoxvirus* genus, which also includes the vaccinia, cowpox, and monkeypox viruses. The smallpox vaccine, consisting of several types of strains of the live attenuated vaccinia virus, has served as the prototype of success of a viral vaccine; it was employed in the eradication of the disease. Prior to immunization, smallpox infection killed hundreds of millions of people unremittingly. The eradication of smallpox has been viewed as one of the greatest achievements in medicine.

Approximately two decades ago, after the complete elimination of smallpox, vaccine production ceased [41]. However, due to the recent concerns about biologic warfare and the use of smallpox as an agent, the danger of this disease has not been eliminated. Thus, renewed interest has been developed in production of the smallpox vaccines. ACAM2000 (Acambis) is the currently licensed vaccine used for immunization against smallpox in public health, health-care-response teams, and laboratory workers involved with vaccinia virus research. It is derived from the formerly used vaccine Dryvax, with the main difference being vaccine preparation; ACAM2000 is prepared in cell culture rather than calf lymph, which gives it a lesser risk of causing rare but serious complications [42].

Contraindications to the use of this vaccine in the absence of circulating smallpox include allergies to polymyxin B sulfate, streptomycin sulfate, chlortetracycline hydrochloride, and neomycin sulfate. Patients in an immunocompromised state or women who are pregnant or

Table 49.2 Childhood immunization schedule for diseases with cutaneous manifestations

Dose Vaccine	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Boostert
Hepatitis B	Birth	1–2 months of age	6–18 months	X	X	X
Hib	2 months	4 months	12–15 months	X	X	X
MMR	12–15 months	4–6 years	X	X	X	X
Pneumococcal (PCV)	2 months	4 months	6 months	12–15 months	24–59 months[a]	X
Varicella	12–15 months	4–6 years	X	X	X	X
Meningococcal	Single dose[b]	X	X	X	X	X
Hepatitis A	2 doses between 12 and 23 months[c]		X	X	X	X
Human papillomavirus	Total of 3 doses[d]					

Source: Adapted from Department of Health and Human Services Centers for Disease Control and Prevention: 2007 Recommended Immunization Schedules for Children in the United States

Hib Haemophilus influenzae type B; *MMR* measles, mumps, rubella

[a]This last dose is only needed in high-risk groups. Pneumococcal polysaccharide vaccine (PPV) can be administered to children >24 months of age who are in the high-risk groups

[b]MPSV4 should be given to patients ages 2–10 who have terminal complement deficiency, asplenia, and to other certain high-risk groups. Patients over age 10 can receive either MPSV4 or MCV4. Strongly suggest meningococcal vaccine administration before start of high school or before freshman year of college

[c]Two doses between 12 and 23 months of age should be given 6 months apart

[d]In females, a three dose regimen can be initiated starting at age 9. The second dose should occur 2 months after the first dose with the third dose to follow 6 months later

intend to become pregnant or who are breastfeeding should also not receive the vaccine. The vaccine should be administered concurrently with antihistamines or glucocorticoids to individuals who have allergic symptoms to the above compounds and have contact with individuals with smallpox. Further, the use of the smallpox vaccine is cautioned in persons with a history of eczema or atopic dermatitis; in persons who have acute, chronic, or exfoliative skin conditions; in persons who are using topical ocular steroid medications; and in persons who are younger than 18 years of age [43].

Measles, Mumps, and Rubella

Vaccination for the three classic childhood diseases of measles, mumps, and rubella (MMR) includes live attenuated viruses, and was introduced in the 1960s. The annual reported cases of these infections have declined by more than 98 % in the United States and Europe [44]. This decrease in incidence is largely due to the recommendation that all states require a two-dose MMR vaccination prior to children entering school. The two-dose MMR vaccine is advocated to induce immunity in the small percentage of individuals who do not respond to one or more components of the first dose. The most updated recommendations from the CDC are vaccination with the first MMR dose at 12–15 months and the second dose at 4–6 years of age [45]. Table 49.2 summarizes the childhood immunization schedule for MMR, as well as other vaccines described in this chapter [46]. Immunization evokes a mild subclinical infection that is noncommunicable.

Since the vaccines are composed of live attenuated viruses, they are not recommended for pregnant women or those planning to become pregnant within the next 3 months. The primary concern with pregnant women has been the risk of eliciting the congenital rubella syndrome (CRS). However, a study of 321 women who received the vaccine 3 months prior to or after conception demonstrated no congenital malformations compatible with congenital rubella infection [47]. Another contraindication is a history of anaphylactic hypersensitivity to neomycin. Persons with asymptomatic HIV infection or with mild immunosuppression can get vaccinated with MMR. Further, healthy persons with minor illnesses with or without fever, and those with an allergy to eggs can receive the vaccine as well. The risk of severe anaphylactic shock reaction is exceedingly low in individuals with a history of allergy to eggs [45].

The recommendation is to observe these patients for up to 90 minutes after immunization [48]. Also, despite suggestions that the MMR vaccine has a reported association to autism, studies have proved strong evidence against that hypothesis [49–53].

Measles

The measles virus is very efficient in transmission, and has been noted to be the most infectious disease of humankind in regard to the minimal number of virions required to elicit infection [54]. Since humans are the only reservoirs for the measles virus, global eradication is a realistic goal. In the U.S., measles is no longer considered an indigenous disease due to universal childhood

immunization programs [55], and the reported incidence of measles has decreased by >99 % since the measles vaccine has become available until a recent surge in reported cases since 2013 owing largely to travelers from abroad and outbreaks among children whose parents refused immunization. Data from the CDC regarding 2014 demonstrated 644 confirmed cases, which exceeded the previous highest annual total number of cases since 2000 [56]. Unfortunately, however, the current rapid spread of measles among unvaccinated persons in the U.S. resulted in another large outbreak in 2015.

Subsequent to receiving two doses of the vaccine, 95–99 % of individuals develop serologic evidence of immunity to measles [57, 58]. Immunity is viewed as lifelong, and comparable to an acquired infection with the wild-type virus [59]. Rare cases of measles infection have been reported in patients with previously documented postimmunization seroconversion [60, 61].

Adverse effects after measles vaccination are usually mild, and include fever (5–15 %) [62], transient viral exanthems [44], and less commonly encephalitis or encephalopathy (less than one per one million vaccines) [63]. Further, a small number of cases have been described of the occurrence of subacute sclerosing panencephalitis (SSPE) in individuals with a history of vaccination but no known history of infection [64–66]. A more detailed review of these cases demonstrated that some cases had unrecognized natural measles infection prior to vaccination, and the SSPE was directly correlated to the infection [45]. Widespread measles immunization has practically eliminated SSPE in the U.S., and the live measles vaccine does not increase the risk of this adverse event [45].

Mumps

A live attenuated mumps vaccine (Jeryl-Lynn strain) was introduced in 1967 and is prepared in a chick embryo cell culture. Immunization evokes a mild subclinical infection that is noncommunicable. Early clinical efficacy studies have shown that 97 % of children and 93 % of adults develop serologic evidence of immunity after vaccination [67–69] and serologic and epidemiologic evidence suggests that immunity persists for at least 30 years after immunization [70–73].

Adverse reactions are usually mild and rare after vaccination, and include low-grade fever, mild parotitis, and viral exanthem. Serious adverse reactions, namely neurologic events are extremely rare and have not been causally associated with the mumps vaccine [74, 75].

Rubella

The RA 27/3 (rubella abortus 27, explant 3) rubella vaccine is grown in human diploid fibroblast cell culture. It induces a response in more than 97 % of recipients [54, 68]. Immunity in vaccinated individuals is believed to be lifelong and has been demonstrated to persist for at least 16 years [76, 77].

Adverse events following rubella vaccination include fever, lymphadenopathy, and viral exanthemas, usually between 5 and 12 days after vaccination [74, 78]. Arthralgias and arthritis occur more frequently with adult vaccinees, especially women, ranging from 25 to 40 % in this population [79–81].Although no longer common, failure to develop 50–60 % of immunity to rubella by vaccination leaves women of childbearing age susceptible to developing rubella infection during pregnancy. This causes congenital rubella in children born of mothers who contract rubella during early pregnancy.

Varicella-Zoster Virus

Prior to the prevalent use of varicella vaccine, annual U.S. figures for varicella infection included an estimate of 4 million cases, 11,000 hospitalizations, and 100 deaths [82]. The annual incidence of varicella has decreased significantly since the use of this vaccine. The vaccine, Varivax, developed by Takahashi in 1974 and approved in 1995, is a live attenuated Oka strain vaccine that has been shown to be very safe and effective [83–85]. In May 2006 the Food and Drug Administration (FDA) approved a vaccine, Zostavax (Merck) which is 14 times more concentrated than the varicella vaccine, against herpes zoster in individuals 60 years age and older [86]. After further studies showed its efficacy in a younger cohort, the vaccine was subsequently approved by the FDA for use in individuals aged 50–59 in 2011. Cell-mediated immunity is increased following vaccination in immunocompetent older adults, and the incidence and severity of herpes zoster [87–91] is reduced. The vaccine lessened the overall incidence of herpes zoster by 51.3 % and notably reduced the pain and discomfort by 66.5 % among subjects in whom herpes zoster developed. The zoster vaccine had low rates of serious adverse events, which were similar to the placebo vaccine.

Long-term follow-up data of the varicella vaccine depicted protection against chickenpox for at least 17–19 years, and, furthermore, all of the subjects vaccinated continue to have persistent antibodies and delayed-type skin reaction to the varicella-zoster antigen [92]. In a double-blind, placebo-controlled study of the Oka vaccine in 914

U.S. children, the varicella vaccine demonstrated an efficacy of 100 % at 9 months [93, 94]. Following 7 years of vaccination, 95 % of the subjects remained without chickenpox clinically [81]. Additional studies in the U.S. have demonstrated that the Oka vaccine induces humoral and cell-mediated immunity in healthy children [94–97], with protection for at least 8 years, while other data have suggested effectiveness decreases markedly after 1 year post-vaccination [98]. Further, case series depict that vaccinated individuals have less severe varicella (<50 lesions, no fever, and shorter duration of illness) than those who are unvaccinated [99, 100].

The vaccine is recommended as part of the childhood immunization schedule, and all susceptible children from 12 months of age to 18 years of age should receive the varicella vaccine. The first dose of the Oka vaccine should be administered at 12–15 months and the second dose should be given at 4–6 years of age [86]. Those over the age of 13 years should receive two doses, 4–8 weeks apart, to generate seroconversion rates and antibody responses comparable to those attained in healthy children [83–85]. In the US, childhood vaccination with a single dose continues to provide up to 95 % protection 10 years after immunization; children with two doses can see up to 98 % protection [101]. The vaccine is also recommended for susceptible adults, notably those in high-risk situations (e.g., health care personnel); children who have no prior history of chickenpox and are required to be present in school; and immunosuppressed subjects, particularly those with acute lymphocytic leukemia (ALL) [102–104]. The varicella-zoster virus (VZV) vaccination is safe for all patients with lymphocyte counts >700/mm [3]. Further, recent data suggest that varicella vaccine is >95 % effective for prevention of disease and 100 % for prevention of moderate or severe disease in susceptible contacts when given within 36 h of exposure [105].

A combination vaccine with the live attenuated viruses of MMR and VARIVAX is also available (ProQuad; Merck) and it is administered to individuals 12 months to 12 years of age. Post-licensing studies have shown the combination vaccine to be as effective as separate injections of MMR and Varivax [106].

Varicella transmission is approximately one- fourth the rate of natural varicella (20–25 % vs. 87 %) [107] if a rash develops in the immunized individual. In children, the incidence of primary varicella is between 18 and 77 per 1 million person years of follow-up [108]. Herpes zoster can later develop from this vaccine type virus or from natural wild-type varicella-zoster virus [109, 110]. Although there are reports of herpes zoster in healthy children vaccinees, the incidence is less than that seen in children with prior chickenpox, suggesting that vaccinated children may have a decreased risk for herpes zoster [111].

The most common side effects from the vaccine include mild tenderness, erythema, induration at the injection site (19.3–24.4 %), fever (10.2–14.7 %), and a localized or generalized varicella- like rash (3.8–5.5 %). The transmission rate of the varicella vaccine virus from a healthy vaccinated individual is low, but may be more likely if a rash appears subsequent to vaccination, particularly in those who are immunocompromised. Vaccinated individuals should avoid close contact with susceptible high-risk individuals for up to 6 weeks. The vaccine is contraindicated in pregnant women or any woman planning to become pregnant within 3 months since the vaccine uses a live attenuated virus and natural varicella can cause fetal harm [111].

A separate adjuvant subunit vaccine has recently been shown in Phase 3 clinical studies to be advantageous in reducing the risk of herpes zoster in adults over 50 years of age by up to 97 % [112]. The efficacy of this vaccine was shown to be similar across age group stratifications, making it more effective than Zostavax which has waning efficacy as patients increase in age. While still currently under investigation, this new adjuvant subunit vaccine represents a significant improvement in providing protection to those at highest risk for developing herpes zoster.

Yellow Fever Virus

A live attenuated vaccine produced from the 17D strain of yellow fever virus is available for subcutaneous administration. It is recommended for travelers to areas where yellow fever is endemic or enzootic, such as tropical parts of South America and Africa. Booster immunizations are required every 10 years. This vaccine is contraindicated in persons allergic to eggs, in pregnant women, in immunocompromised persons, and in children younger than 4 months of age.

Bacteria

The only currently used live-attenuated bacterial vaccine in the U.S. is against the causative agent of typhoid fever, *Salmonella typhi*. This vaccine, given orally, was first developed through chemical mutagenesis in vitro. Protection is conferred by mucosal and serum antibodies as well as cell-mediated immunity [113]. The induction of mucosal antibodies offers protection against infection and disease [114].

Inactivated Vaccines

Both inactivated viral and bacterial vaccines consist of viruses and bacteria, respectively, treated so that they are unable to replicate. They are less potent in the effector response than the live-attenuated vaccines, and consist of vaccines currently in use against hepatitis A, *Haemophilus influenzae* type b (Hib), influenza (intramuscular), Japanese encephalitis, meningococcus, pneumococcus, polio (subcutaneous or intramuscular), rabies, tetanus/diphtheria toxoids, and typhoid fever (intramuscular). Again, in this section, the focus is on the vaccines targeting diseases that cause cutaneous manifestations.

Viruses

Hepatitis A

Both the inactivated and attenuated forms of hepatitis A vaccines have been developed and studied, although only the inactivated vaccine is licensed and available in the U.S.

(Havrix and Vaqta). These vaccines are propagated in human diploid fibroblast culture and inactivated by formalin. Immunization typically involves two doses given 6 –12 months apart in adults and in children aged 1 year and older. Studies of both available vaccines depict excellent safety profiles in addition to comparable immunogenicity and efficacy rates. Overall, 97–99 % of those vaccinated develop protective levels of antibodies 1 month after the first dose, and 99–100 % are protected 1 month after second dose [115–120]. Long-term data are limited, but one recent study showed that protection following primary hepatitis A vaccination persists for more than 10 years [121].

The hepatitis A vaccine is recommended for individuals at least 1 year of age living in or traveling to high endemic areas for hepatitis A. Further, individuals with chronic liver disease due to causes other than hepatitis A, persons engaging in high-risk sexual activity, residents of a community experiencing an outbreak of hepatitis A, and users of illicit injectable drugs are strongly advised to receive hepatitis A vaccination. In addition, in some states and regions, the hepatitis A vaccine is recommended for routine pediatric use.

Adverse effects with hepatitis A vaccination are typically mild, and no serious adverse side effects have been credited to the vaccine in clinical trials [122]. Side effects include soreness at the injection site, headache (14 %) and malaise (7 %) in adults, and feeding problems (8 %) and headache (4 %) in children.

Bacteria

Anthrax

Pasteur in 1881 demonstrated the protective efficacy of the first anthrax vaccine when he injected sheep with heat-attenuated *Bacillus anthracis*. In the 1930s, wide use of vaccine composed of attenuated strains markedly decreased the incidence of anthrax in domesticated animals in industrialized countries. The licensed human vaccine, anthrax vaccine adsorbed (AVA), also known as BioThrax since 2002, is a culture supernatant of a toxigenic, avirulent, nonencapsulated *B. anthracis* strain, V770–NP1–R, derived from the Sterne strain [123]. A vaccine similar to AVA is used in the United Kingdom, produced from culture supernatant-derived human vaccine (PL 1511/0058) [124].

Anthrax vaccinations are recommended for individuals working in the production of *B. anthracis* cultures, those engaged in activities with a high risk of aerosol exposure, individuals in the military, and other groups of persons with a calculable risk [125]. Preexposure vaccinations are currently not recommended for emergency first responders, medical practitioners, and civilians. However, due to the bioterrorist attacks in 2001 and limited supply of anthrax vaccine, supplemental recommendations of the Advisory Committee on Immunization Practices endorse the anthrax vaccine in combination with antimicrobial postexposure prophylaxis (PEP) for unvaccinated persons at risk for inhalational anthrax [126].

Anthrax vaccine adsorbed has numerous limitations despite being relatively efficacious and safe [127]. The schedule of AVA administration, which consists of subcutaneous injections at 0, 2, and 4 weeks and boosters at 6, 12, and 18 months (with recommended annual boosters to maintain immunity), is probably suboptimal. The enduring protective efficacy in humans is unknown. AVA contains other cellular elements that aid in the fairly high rate of local and systemic adverse reactions [127], including injection-site hypersensitivity, edema, pain, headache, arthralgia, asthenia, and pruritus. Approximately 20 % of vaccinees develop mild cutaneous reactions such as erythema, edema, and induration. In <1 % of recipients, systemic side effects of fever, chills, nausea, and body aches occurred [128]. Due to these limitations, newer anthrax vaccines that involve a purified form of the anthrax toxin are currently being developed, some of which have passed Phase 1 trials [129].

Conjugate Vaccines (Fig. 49.1) [1, 130]

Many bacteria, including *Neisseria meningitidis* and *Haemophilus* species, have an outer capsule composed of polysaccharides that are species and type specific for particular strains of the bacterium. The goal of vaccination is to generate antibodies against the polysaccharide capsule of the bacteria since the most effective defense against these organisms is opsonization of the polysaccharide coat with antibody. However, children under 2 years of age do not mount a good response against capsular polysaccharide, which is a T-cell–independent antigen. An effective way to overcome this problem is to conjugate bacterial polysaccharide (chemically) to protein carriers, which offer peptides that can be recognized by antigen-specific T cells. Thus, a T-independent response is converted into a T-cell–dependent antipolysaccharide response [1, 39].

Haemophilus Influenzae

In the second generation of Hib vaccines, the polyribosylribitol phosphate (PRP) vaccine has been covalently conjugated with various protein molecules to form the Hib conjugate vaccine. This has resulted in a vaccine that is able to stimulate the immunologic system to produce a T-dependent response, for use in infants [131]. Four different types of the Hib conjugate vaccine have been licensed for use: PRP conjugated to the diphtheria toxoid (PRP-D), PRP conjugated to the outer membrane protein of *Neisseria*

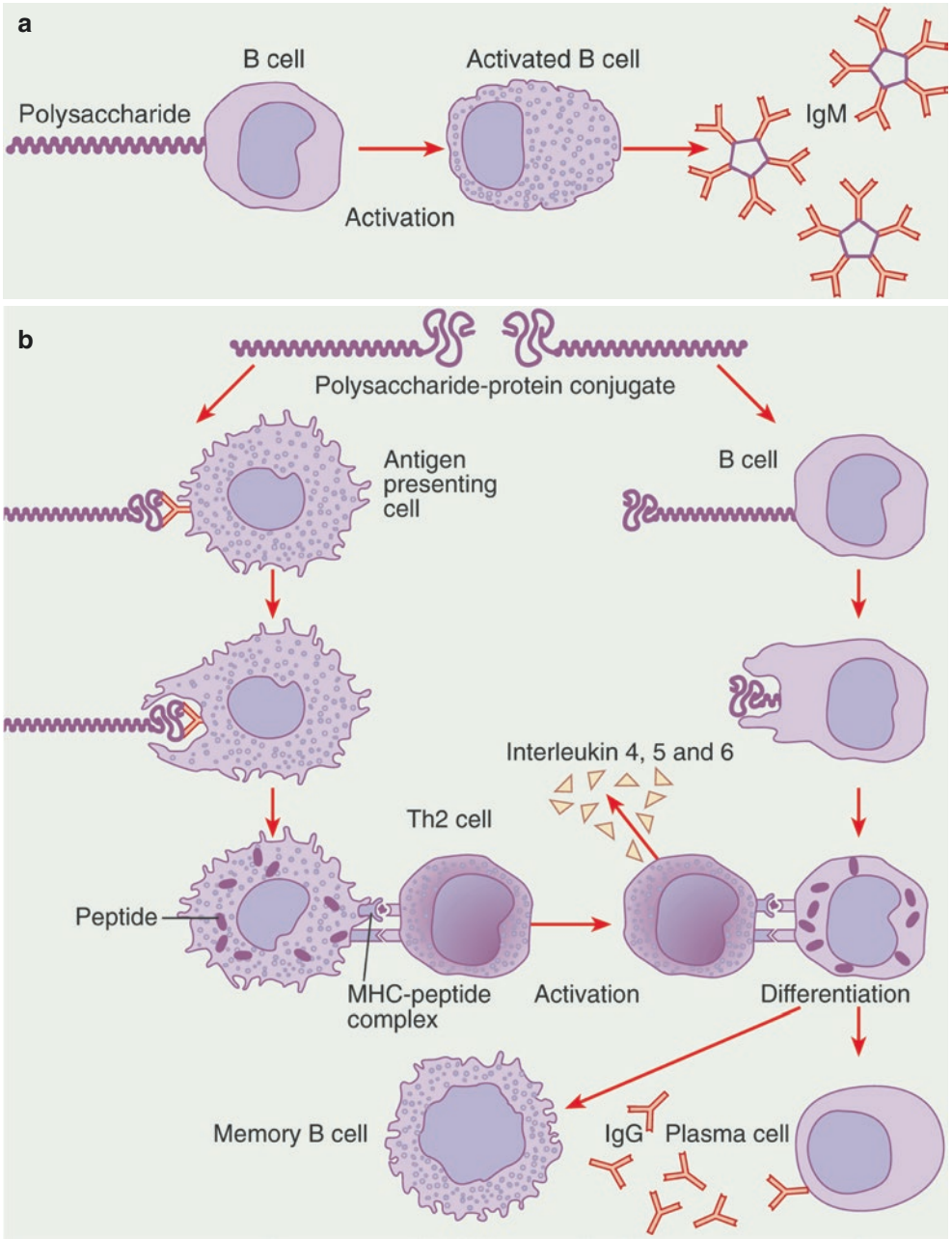

Fig. 49.1 Antibody responses to polysaccharide antigens and polysaccharide–protein conjugates. (**a**) A polysaccharide antigen binds to an immunoglobulin M (IgM) receptor on the surface of a B cell in lymphoid tissues. Once B cells are activated, they produce and then secrete IgM antibody molecules. The individual Fab segments of the IgM molecule have only a moderate affinity, but because there are 10 such segments, an IgM molecule has a high avidity. (**b**) In contrast, some polysaccharide–protein conjugates are taken up by dendritic cells, which present peptides from the protein portion of the conjugate to type 2 helper T (Th2) cells. Other conjugate molecules bind to B cells that have IgM receptors specific for the carbohydrate moiety and will undergo endocytosis and be processed by the B cell; the resulting peptides will be expressed with major histocompatibility complex (MHC) class II molecules on the surface of the B cell

meningitidis group B (PRP- OMP), PRP conjugated to tetanus toxoid (PRP-T), and PRP oligosaccharides conjugated to mutant diphtheria toxin CRM (HbOC) [132]. Recommended immunization involves three doses of the Hib vaccine given at 2, 4, and 12 months of age [46].

Neisseria Meningitidis

Bacterial meningitis remains a severe threat to global health, with an estimated 500,000 cases worldwide with at least 50,000 deaths and as many cases of neurological damage [133]. *Neisseria meningitidis* is responsible for 60–65 % of

cases of bacterial meningitis, and it is the only bacterium that is able to generate epidemics of meningitis [134].

It is also associated with a petechial eruption or more severe hemorrhagic lesions on the trunk and lower extremities.

The significant determinant of virulence in *N. meningitidis* is the polysaccharide capsule. Among the 13 distinct *N. meningitidis* serogroups that have been defined on the immunohistochemistry of their polysaccharide group, groups A, B, C, W135, and Y account for over 90 % of severe meningitis and septicemia. Two meningococcal vaccines, each containing antigens to serogroups A, C, Y, and W-135, are licensed in the U.S. In 2015 the FDA approved the first vaccine that provides immunity against serogroup B, which accounts for an estimated one third of cases of meningococcal disease. This recombinant serogroup B vaccine, Bexero (Novartis) has been licensed for use in Europe, Canada, and Australia; before being licensed for use in the US, it was used to control two separate outbreaks of meningococcal disease within the US and had been allowed by the FDA to treat certain outbreak situations [135, 136].

Over 30 years ago, the first meningococcal polysaccharide vaccine (MPSV4 or Menomune, Sanofi Pasteur), was developed against serogroups A and C among U.S. military recruits [137–139]. It is approved for all ages in which meningococcal vaccine is currently recommended. Further, the vaccine is safe and bestows protection to 90–95 % of people. However, there are limitations to the vaccine. First, the vaccine offers a short duration of immunity: 1–3 years in children younger than 5 years of age [134, 140] and 3–5 years in adolescents and adults. Second, like other polysaccharide vaccines, this vaccine could not elicit memory T cells, and even after repeated injections, booster responses are low [141, 142]. In addition, like other polysaccharide vaccines, this vaccine does not prevent mucosal colonization and hence does not offer herd immunity by breaking up the transmission of *N. meningitidis* [143, 144]. This vaccine is a logical option for individuals requiring protection for a limited amount of time.

The second vaccine, meningococcal conjugate vaccine A, C, Y, and W135 (MCV4 or Menactra, Sanofi Pasteur), was approved in January 2005 for use in individuals 11–55 years of age. This conjugate vaccine contains the same antigen as that found in the meningococcal polysaccharide vaccine, but it is conjugated to 48 μg of diphtheria toxoid [145]. What is different regarding this vaccine is that immunity is more durable, and revaccination can generate a rise in antibody level. Although not yet proved, it is suggested that, like other conjugate vaccines, it is likely that this vaccine will offer more durable protection than the polysaccharide vaccine alone and will further reduce nasopharyngeal carriage, allowing herd immunity to occur.

The serogroups A and C vaccines have demonstrated estimated clinical efficacies of >85 % among school-aged children and adults and are useful in controlling outbreaks. Serogroups Y and W-135 polysaccharides are safe and immunogenic among adults and children aged >2 years. The advantages of the meningococcal conjugate vaccine have led the Advisory Committee on Immunization Practices (ACIP) of the CDC to extensively widen the recommendations for immunization, with routine vaccination of all persons aged 11 through 18 years old, composed of a single dose at age 11–12 years and a booster dose at age 16 [146].

Both vaccines are currently recommended for use in control of outbreaks as a result of *N. meningitidis* serogroups A, C, Y, and W-135 [145]. However, due to its enhanced booster effect, the conjugate vaccine is favored when revaccination is indicated for individuals who have received the meningococcal polysaccharide vaccine before and who remain at higher risk. Long-term follow-up studies are still needed for those that have been immunized with the meningococcal conjugate vaccine to establish if boosters are needed.

Adjuvants

Purified antigens are not typically immunogenic on their own, and thus most acellular vaccines require adjuvants to enhance their immunogenicity. Alum, the adjuvant most often used, delays the release of an antigen and enhances the generation of antibodies. Most adjuvants are thought to act on antigen-presenting cells, particularly on dendritic cells. Adjuvants manipulate the immune system into reacting as though there were an active infection, and just as different classes of infectious agents elicit different types of immune response, different adjuvants may stimulate different types of response.

Recombinant Subunit Vaccines

Another route to vaccine development is the identification of the T-cell peptide epitopes that stimulate protective T-cell–mediated immunity. T cells recognize their target antigens as small protein fragments or peptides presented by major histocompatibility complex (MHC) molecules at the cell surface, and thus these peptide epitopes, once identified, can then be tried as vaccines. Using peptides has many advantages and some drawbacks [1]. Advantages include that the peptide is chemically defined, stable, and safe and contains only important B-cell and T-cell epitopes. Drawbacks include the complexity in mimicking the conformation of antigen polymers found with many viruses, that B-cell epitopes recognized by neutralizing antibodies are

sometimes discontinuous sequences, and the vulnerability of peptides to proteolysis. Several administrations, likely with an adjuvant, may be required. Since 1990, over 100 chemically synthesized short peptide vaccines have been initiated into phase I clinical trials, less than 20 have advanced into phase II, and none has entered phase III clinical trials [147].

Subunit vaccines have often made use of recombinant-DNA technology. Immunogenicity can be improved and the immune response aimed at induction of both cell-mediated and humoral responses through the formation of aggregates such as immunostimulating complexes, virus-like particles, antigen-coated beads, or lipid-encapsulated antigens under many conditions. Linked peptides are also being tested in the field of subunit vaccines; for instance, aggregates of linked peptides from group A streptococcus as a vaccine against rheumatic fever have demonstrated promising results in animal models [148, 149].

Viruses

Human Papillomavirus

According to the WHO, cervical cancer is the fourth most common cause of death in women globally from cancer, and it is the cause of death of approximately 265,000 women per year [150]. Almost half a million new cases of invasive cervical cancer develop each year, and in the U.S. it was estimated to afflict over 11,800 women in 2010 [151]. Epidemiologic studies have proved that persistent HPV infection is the main cause of almost all cervical carcinogenesis [152–154].

Approximately 40 of the 100 HPV genotypes have an affinity for infecting the mucosal epithelium and subsequently causing genital infection. The major types associated with malignancy (16, 18, 31, 33, 45, 52, and 58) and condyloma (6 and 11) are relatively few in number, which has allowed for more focused strategies for immunization against these specific types [111].

Papillomaviruses are nonenveloped double-stranded DNA viruses with the genome comprised of three regions: the long control region (LCR), the early region (E), and the late region (L). The early region consists of genes *E1* to *E8*, which encode nonstructural proteins. The late regions code for the major (L1) and minor (L2) proteins that compose the viral capsid. The advancement in producing the prophylactic HPV vaccine came from findings that L1 self-assembles into empty capsids, named virus-like particles (VLPs), when it is expressed from eukaryotic vectors such as recombinant vaccinia, baculovirus, and yeast [155, 156]. The VLPs do not contain viral DNA and are noninfectious; their similarity to virions offers a neutralizing epitope that subsequently produces an antibody response.

Gardasil® (Merck) is a quadrivalent vaccine that targets HPV types 16, 18, 6, and 11. It is a yeast-expressed vaccine, which was approved by the FDA in 2006 for use in girls and young women of ages 9–26, and is marketed as a vaccine for prevention of cervical cancer, precancerous genital lesions, and genital warts due to HPV types 6, 11, 16 and 18. Per the ACIP, the recommended age of vaccination for females is 11–12; catch-up vaccination is also recommended for females aged 13–26. It is given in three doses; provisional recommendations from ACIP include giving the second and third dose of the vaccine at months 2 and 6, respectively [157].

In one study involving greater than 12,167 adolescents and women of ages 16–23, Gardasil prevented 100 % ($n = 5301$) of cases of cervical intraepithelial neoplasia (CIN) grades 2 and 3 linked with HPV types 16 and 18 compared to 21 cases in the placebo group ($n = 5258$). All subjects elicited 100 % antibody responses to the HPV strains 6, 11, and 16, and 99.1 % developed strain 18 antibodies. Protection has been observed for at least 6 years following vaccination. Males were also studied in the Gardasil trials; one study analysis involving 4,065 males aged 16–26 showed 85–90 % efficacy in preventing external genital lesions from HPV strains 6, 11, 16, and 18 [158–160]. This subsequently led to the FDA approval of Gardasil for males aged 9–26 years in October 2009 for protection against genital warts, anal cancer, and some potentially precancerous lesions such as high-grade penile-perianal/perineal intraepithelial neoplasia.

Gardasil has been shown to be generally safe, and well tolerated, with pain, swelling, and erythema at the site of injection being the primary side effects. Females who have an equivocal or abnormal Pap test can receive the quadrivalent vaccine, but data from clinical trials do not suggest that the vaccine will have a therapeutic effect on existing Pap test abnormalities; it would only provide protection against other HPV types not already acquired. Females who are immunocompromised can be vaccinated; however, the immune response may be less than in those who are immunocompetent. Further, those with moderate or severe illness should not be vaccinated until after the illness improves. Provisional contraindications to Gardasil include those with immediate hypersensitivity to yeast or another vaccine component. Gardasil is not recommended for use in pregnancy since data for vaccination during pregnancy is limited, and lactating women cannot receive the vaccine either [157].

In an effort to address the need for a vaccine effective across other oncogenic HPV types, Merck began clinical testing in 2007 of a nine-valent vaccine targeting HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58. An international randomized double-blinded phase III trial involving 14,840 female subjects ages 16–26 compared the nine-valent vaccine to Gardasil in regards to incidence of HPV-type disease, mean antibody tiers, and safety profiles [161]. Results of this study demonstrated the nine-valent vaccine to be 97% effective in the prevention of cervical, vaginal, and vulvar

neoplasms caused by HPV types 31, 33, 45, 52, and 58, as well as having non-inferior protection to Gardasil in the prevention of diseases caused by HPV types 6, 11, 16, and 18. Mean antibody titers to HPV types 6, 11, 16, and 18 as well as safety profiles were similar between Gardasil and the nine-valent vaccine. A separate study which enrolled 2,604 male and female children ages 9–15 compared mean antibody titers after nine-valent vaccine administration to those seen in subjects aged 16–26 and showed higher titers in the younger cohort. Another large-scale study involving 14,215 women comparing the nine-valent vaccine to the quadrivalent vaccine also demonstrated prevention of high-grade cervical, vulvar, and vaginal disease related to HPV types 31, 33, 45, 52, and 58 among those receiving the nine-valent vaccine and similar protection against HPV types 6, 11, 16, and 18 with both vaccines [162]. These positive results led to the FDA approval of the nine-valent vaccine in December 2014, marketed as Gardasil 9® [163].

Cervarix® (GlaxoSmithKline) is a bivalent VLP vaccine that was FDA-approved in 2009 for the prevention of infections from HPV types 16 and 18. While similar to Gardasil, Cervarix differs in its preparation, coverage, and dosing schedule. It is produced within insect cells that are infected with L1 recombinant insect virus vectors and carries the same age recommendations as Gardasil, per the ACIP [164].

The PATRICIA trial, a large Phase III clinical trial involving Cervarix, demonstrated up to 100 % efficacy compared to controls against CIN grade 3+ caused by HPV types 16/18 among women who initially were HPV negative ($n = 11644$). Additionally, the vaccine demonstrated 93.2 % efficacy within this cohort against CIN grade 3+ regardless of HPV type in the lesion. Cervarix was also shown to be safe, with pain, swelling, and erythema being the most common side effects and vaccine-related serious adverse events seen in less than 0.1 % of subjects [165]. Unlike Gardasil, Cervarix is not indicated for the prevention of genital warts in both sexes or genital lesions caused by HPV in males.

Contraindications to Cervarix include history of severe allergic reactions to any of the vaccine components. Caution is exercised in individuals with latex allergies, as the tip caps of the prefilled syringes contain dry natural rubber latex. Similarly to Gardasil, use in pregnant subjects is not recommended due to lack of safety data and the immune response may be decreased in immunocompromised individuals.

Hepatitis B

The annual incidence of individuals infected with hepatitis B virus (HBV) in the U.S. was estimated to be 200,000–300,000 prior to the development of the hepatitis B vaccine [166]. In 1981, a plasma-derived hepatitis B vaccine was licensed in the U.S.; the vaccine was highly effective in inducing immunity, but was associated with several limitations. Large-scale production was not feasible because of the insufficient supply of suitable carrier plasma. Further, despite the chemical treatment of plasma products for safety, there was a small concern regarding the risk of HIV transmission. Both of these issues were resolved with the licensure of the recombinant yeast hepatitis B vaccine (Merck) [167].

This vaccine was the first licensed recombinant viral vaccine prototype as well as the first effective viral vaccine for a sexually transmitted disease. Clinical studies in high-risk homosexual men illustrated three-dose vaccine efficacy of 82–93 % in preventing acute hepatitis B [168, 169]. Approximately 95 % of immunocompetent adults develop significant antibody titers following a three-dose hepatitis B vaccination. An estimated 99 % of children [170] respond to the vaccination, while only 50–70 % of those over age 60 develop immunity [171, 172]. Factors linked with lower likelihood of seroconversion include immunosuppression, renal failure, prematurity with low birth weight, age older than 40 years, obesity, and smoking [173–175]. In these individuals, annual antibody testing should be completed, and a booster dose administered for those with antibody levels <10 mIU/mL.

Long-term efficacy is expected from the duration of immunity afforded by the hepatitis B vaccine, although few studies are available on this topic [176]. Antibodies levels decline rapidly in the first year following vaccination, and then decline at a slower pace in the following years [176]. A study involving high risk individuals (1st year medical students) showed that only approximately 60 % of students had protective levels of antibodies 20 years after immunization [177]. However, the loss of detectable antibodies years after hepatitis B vaccination does not necessarily signify a lack of immunity. Most of the persons vaccinated are protected by immunologic memory in B lymphocytes, which mount an anamnestic response to natural infection [178]. It should be noted, however, that rare cases of hepatitis B infection have been reported in previously vaccinated patients [179, 180]. These individuals usually have subclinical disease, and none has developed chronic infection or serious complications [175].

The immunization regimen includes three doses, given at months 0, 1, and 6. Hepatitis B vaccination is recommended for individuals living in or traveling to areas of high endemicity for hepatitis B, health care personnel, morticians, persons engaging in high-risk sexual activity, persons with chronic liver disease due to causes other than hepatitis B, prisoners, users of illicit injectable drugs, police and fire department personnel who participate in first aid, and all children 0–18 years of age. Due to the widespread use of the vaccine in children, a thimerosal-free vaccine was approved in 1999 by the FDA (Merck and GlaxoSmithKline). Thimerosal is a preservative that contains mercury, which had driven the limitation of its use in children [181].

Adverse effects after hepatitis B vaccination are usually mild and well tolerated. The most common side effects include fatigue (15 %), headache (9 %), and fever (1–9 %) [182, 183]. A postmarketing clinical surveillance of 4.5 million doses of hepatitis B vaccine over 5 years depicted no serious or severe reactions attributable to the vaccine. Reports of a causal relationship between hepatitis B vaccine and a variety of autoimmune diseases have been disproven, and furthermore this vaccine does not increase the risk of multiple sclerosis [184], nor does it cause a relapse of underlying multiple sclerosis [185].

In 2001 the FDA licensed a new combination vaccine that protects individuals at least 18 years of age against hepatitis A virus and hepatitis B virus. This vaccine, Twinrix (GlaxoSmithKline), combines two already approved vaccines, Havrix, and Engerix-B, in order for persons at high risk for exposure to both viruses to be immunized against both at the same time and reduce the number of injections needed. This vaccine is administered at months 0, 1, and 6. Data from 11 clinical trials indicate that 99.9 % of vaccinees develop seroconversion against hepatitis A virus and 98.5 % against hepatitis B surface antigen, with persistence up to 4 years (GlaxoSmithKline Biologics, unpublished data, 2001).

Lyme Disease

Lyme disease is the most common vector-borne human disease in the U.S., with 22,014 cases reported to the CDC in 2012 [186]. Since 1982, the annual incidence of Lyme disease has increased more than 25-fold [187].

Although a recombinant vaccine, Lymerix, for prevention of Lyme disease was approved in 1998, for many reasons including cost, need for frequent revaccinations, and a highly publicized but theoretical risk of precipitating autoimmune arthritis, sales of Lymerix declined rapidly after its initial introduction, and its manufacturer removed the vaccine from the market in 2002 [188, 189]. As a result, there has been renewed interest in developing new strategies for the reduction of Lyme disease, for instance, the use of oral delivery of an OspA vaccine to reduce carriage of *Borrelia burgdorferi* in its reservoir hosts [15]. Newer injectable OspA vaccines have entered Phase 1 trials and have shown promising results, inducing high antibody titers while causing no vaccine-related serious adverse events [190].

Investigational Vaccines

Live-Agent Vaccines as Vectors of Other

Vaccine Antigens

Wide interest exists in the use of vaccines composed of attenuated viruses or bacteria as carriers (vectors) of other antigens. More than 20 different RNA and DNA viruses as well as bacteria are used experimentally as vectors, including poxviruses. All of these strains infect but do not replicate in human cells [191, 192]. Since approximately 10 % of the large poxvirus genome can be replaced by foreign DNA, genes encoding protective antigens from several different organisms could be placed in a single vaccine strain. This approach allows immunization against several different pathogens at one time, but such a vaccine could not be used twice since the vaccinia vector itself elicits long-lasting immunity that would neutralize its effectiveness on a second dose. Further, live-attenuated strains of *Salmonella* have been used as vectors of tetanus toxoid, *Listeria monocytogenes*, *Bacillus anthracis*, *Leishmania major*, *Yersinia pestis*, and *Schistosoma mansoni* to provide mucosal response due to its oral administration [1, 39].

Immunization with DNA

Vaccination with DNA is one of the most promising novel immunization techniques against pathogens in which conventional vaccination regimens have failed. A DNA plasmid encoding a desired protein is injected into the muscle or skin of an animal, where it subsequently enters host cells and directs the synthesis of its polypeptide antigen. Once the plasmid antigen is processed and presented by transected host cells, a cellular and humoral immune response against the antigen is elicited. The DNA vector is bacterial-derived and outfitted with eukaryotic or viral promoter/enhancer transcription elements that direct the high-efficiency transcription of the plasmid-antigen within the nucleus of the host cell. The most common way of administering DNA vaccines has been parenterally, but noninvasive routes of delivery involving the topical application of pure DNA plasmid to skin or mucosa have been illustrated (Fig. 49.2) [1, 193].

This approach has many potential advantages, including low cost, stability, and inability to return into virulence. A possible disadvantage would be integration of the DNA into the genome of the host cell, leading to transformation or tumorigenic events, but such occurrences have not yet been observed [1].

Sequential Immunization

There are also a group of pathogens that do not respond to the vaccine approaches described thus far. These pathogens include HIV, *Mycobacterium tuberculosis*, and the malaria parasite, all which traditionally evade the humoral response elicited by traditional vaccines [194]. Thus, over the past few years there has been a drive to generate vaccines targeting the cell-mediated immune system for these and related pathogens. With the advent of vaccines, repeated administration with the same vaccine (homologous boosting) has proven to be very effective for boosting humoral response. However, this approach is not effective at boosting cellular immunity since prior immunity to the vector appears to impair robust antigen presentation and the generation of appropriate inflammatory signals. One approach to evade this problem has been to sequentially administrate vaccine,

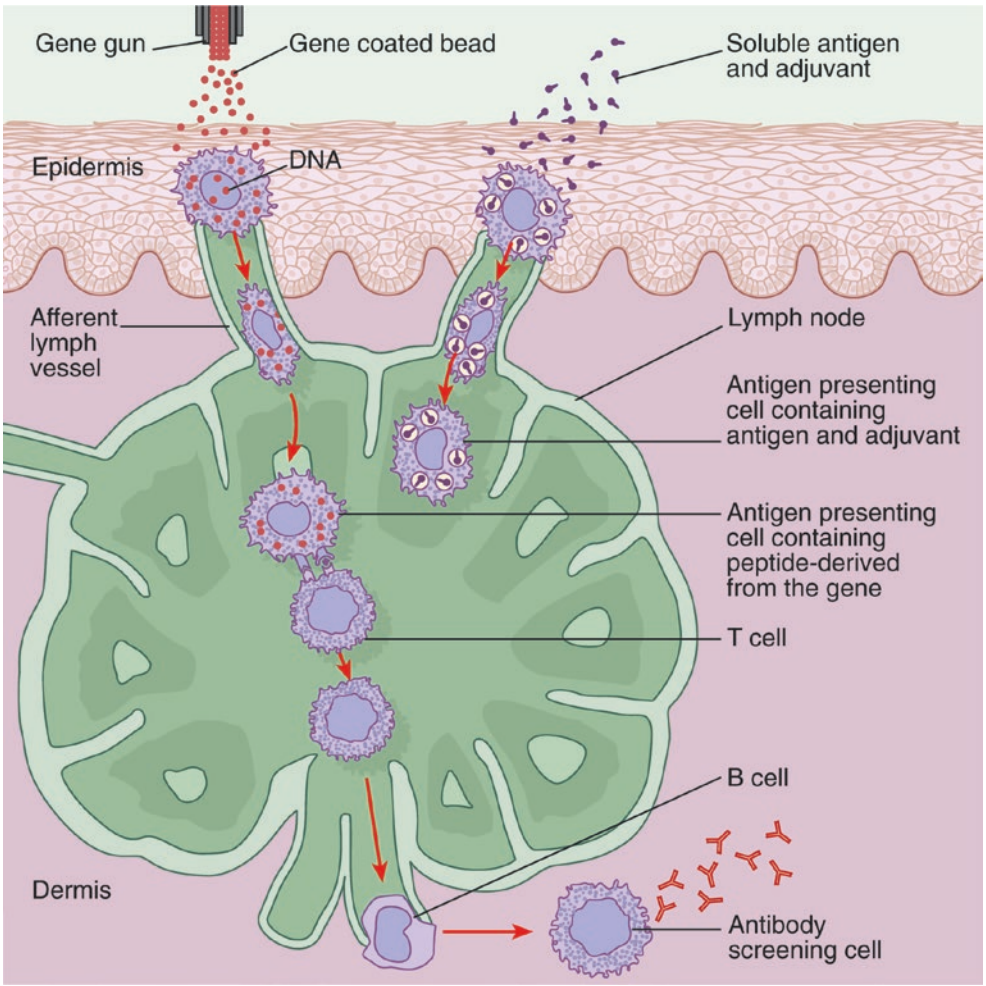

Fig. 49.2 Activation of helper T cells after the application of antigen-coated beads with the aid of a "gene gun" or the application of soluble antigen to the skin as an alternative to vaccination with a needle. A gene gun is used ballistically to accelerate the transdermal passage of microscopic gold beads coated with DNA plasmids (about 600 copies per bead) through the stratum corneum into the epidermis, where some are taken up by resident dendritic (Langerhans') cells. Alternatively, a soluble antigen together with an adjuvant, usually cholera toxin, is applied to the skin (transcutaneous immunization). Some antigen reaches the epidermis and also undergoes endocytosis by Langerhans' cells. During migration to the draining lymph node through the afferent lymphatics, these cells mature and express receptors for chemokines. The foreign DNA is expressed, and the antigens are degraded, some of which bind to MHC antigens. These activated T cells can interact with an activated B cell to induce a humoral response (Copyright © 2001 Massachusetts Medical Society. All rights reserved)

usually weeks apart, using different antigen-delivery systems (heterologous boosting). This method is referred to as prime-boosting, and is very effective at generating high levels of T-cell memory (Fig. 49.3) [195]. Although many of the initial studies were to develop a vaccine to control malaria, this method of vaccine development was applied to a variety of other pathogens [196].

Much advancement has been made in vector design and progress, and several vectors have proven to be effective, which include replication-defective adenoviruses, fowlpox viruses, vaccinia virus, influenza virus, Sendai virus, and naked DNA [195, 197–201]. Vaccination strategies where a DNA prime is boosted with a poxvirus vector are particularly effective and have surfaced as the major approach for generating protective $CD8^+$ T-cell immunity. DNA, for unclear reasons, appears to be more effective at priming immune responses versus as a boosting agent [202]. The general efficacy of prime-boost vaccination in humans is still not yet determined; the initial results of clinical trials in progress have been promising [203, 204].

AIDS/HIV

Currently over 35 million people suffer from HIV/AIDS, which is the primary cause of death in sub- Saharan Africa and ranks as the fourth major cause of death in the world. Approximately 6,300 people/day (2.3 million persons/year, including 600,000 children less than 15 years of age) become infected with HIV, with more than 95% of them residing in nonindustrialized countries [205]. The production of a safe, effective, easily administered and affordable HIV/AIDS vaccine is greatly

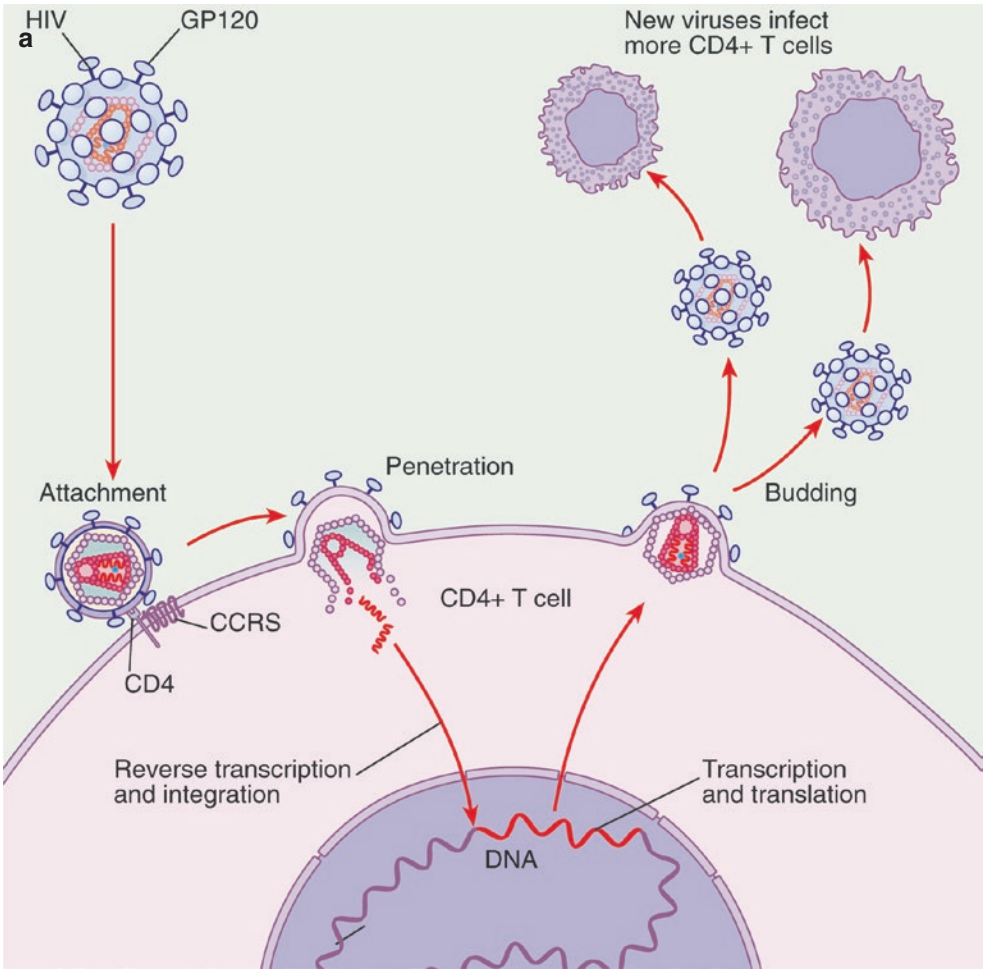

Fig. 49.3 Prime–boost vaccination strategies synergistically amplify T-cell immunity to specific antigens. Priming with the first vaccine results in the presentation of both the target antigen (*red triangles*) and vector antigens (*blue triangles*) on antigen-presenting cells (APCs). The APCs then stimulate naive T cells in the lymph nodes and drive the expansion of both target-specific T cells (*red cells*, high-avidity cells are indicated by the darker red) and vectorspecific T cells (*blue cells*). Subsequent boosting with a second vaccine results in the re-presentation of the target antigen (*red triangles*) and antigens from the second vector (*green triangles*) on APCs. These APCs then drive the expansion of target-specific memory T cells (*red cells*) and vector-specific naive T cells (*green cells*). This results in both a synergistic expansion of the T cell specific for the target antigen and selection of T cells that have greater avidity for the antigen. The situation with priming and boosting vectors that induce strong T-cell responses to themselves, as well as the target antigens, is shown. However, it should be noted that many vectors, such as DNA and some of the popular replication-defective viral vectors, induce little or no response to the vectors themselves. This is probably a key issue underlying their efficacy (From Woodland [202]. Copyright 2004, with permission from Elsevier)

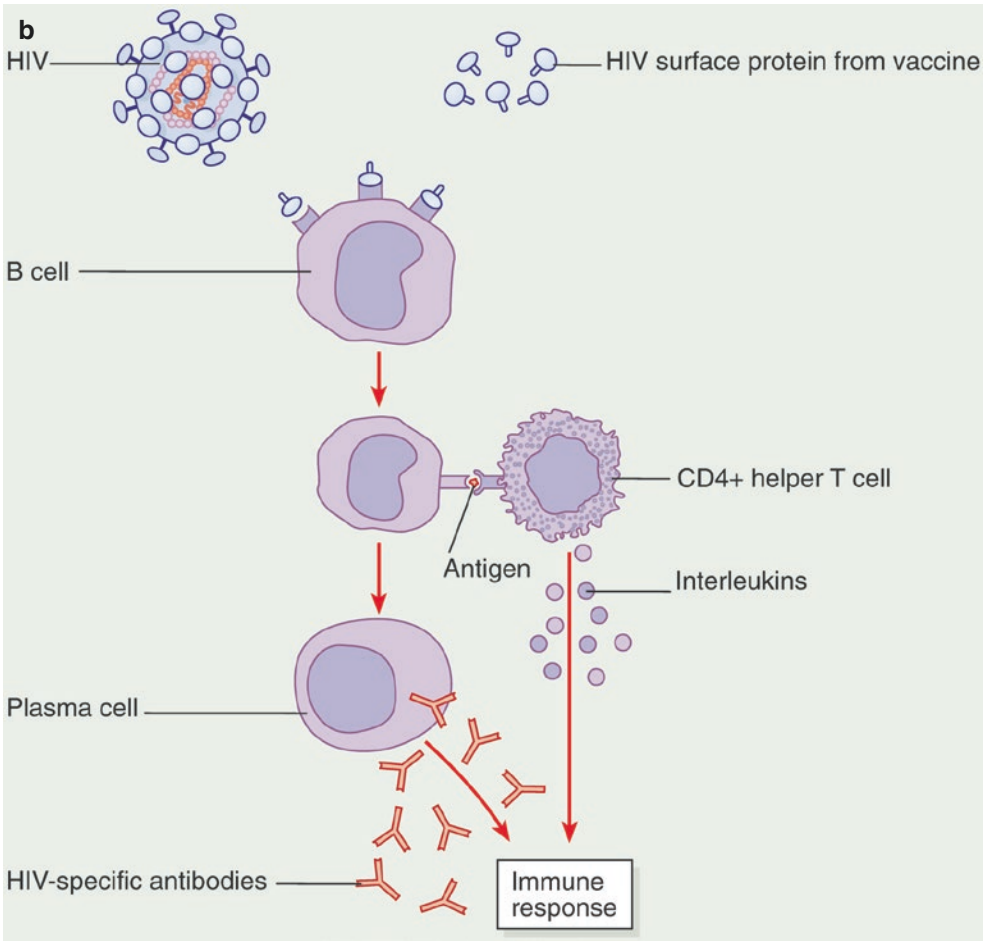

Fig. 49.3 (continued)

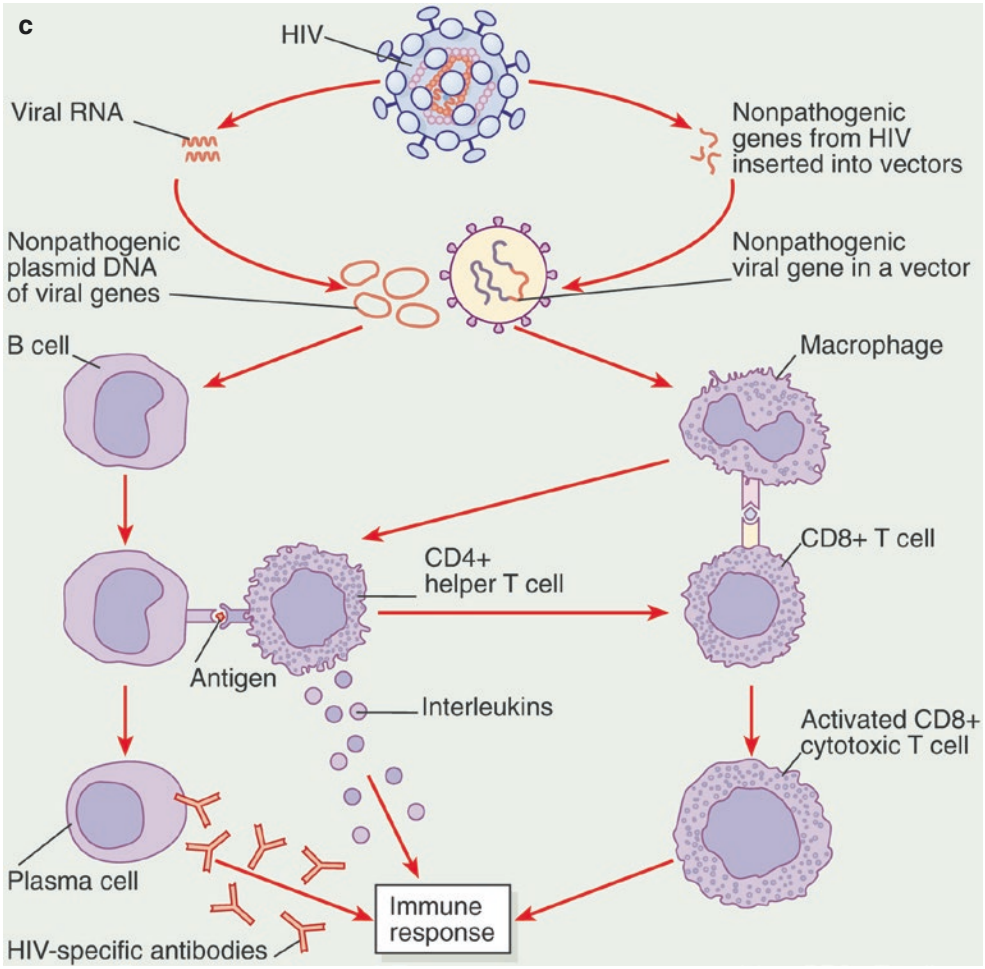

Fig. 49.3 (continued)

needed. Figure 49.4 [206] illustrates three approaches currently being studied in the development of an AIDS vaccine.

Nanoparticle Vaccines

Nanotechnology has gained increasing popularity within the health sciences within the past decade. The small size of nanoparticles allows them to reach virtually anywhere within the body at a subcellular level and have opened the door to a plethora of potential medical uses. One such field is in vaccinology, where nanoparticles have been developed to act as delivery systems or adjuvant immunostimulants. Nanoparticles can be synthesized to carry a variety of antigens such as *Plasmodium vivax*, tetanus toxoid, *Bacillus anthracis*, and hepatitis B virus [207–210]. Advantages of nanoparticles include sustained antigen release, protection of antigen, and the ability for targeted uptake by the lymphatic system to increase efficiency of antigen response [211]. The promise held by nanotechnology has spurred further research on how nanoparticles interact with the body at micro- and macrocellular levels and formulating consistent synthetic methods for nanoparticles.

Therapeutic Vaccines

All of the vaccines used up to the 21st century were used primarily for prevention. In recent years, several new candidate vaccines have undergone trials and are being developed for infections that are already acquired. One such vaccine, GEN-003 (Genocea), has been developed for herpes simplex-2 (HSV-2) infections. It is a recombinant vaccine composed of T- and B-cell antigens adjuvanted with Matrix-M2 and administered sequentially. During a phase I/IIa clinical trial ($n = 143$), subjects receiving the vaccine achieved up to 65 % reduction in genital lesions rate and 40 % reduction in viral shedding from baseline compared to placebo; reduction persisted through a 6-month follow-up period. Safety data indicated a benign side effect profile and tolerability at all doses. Further studies are currently being conducted to determine

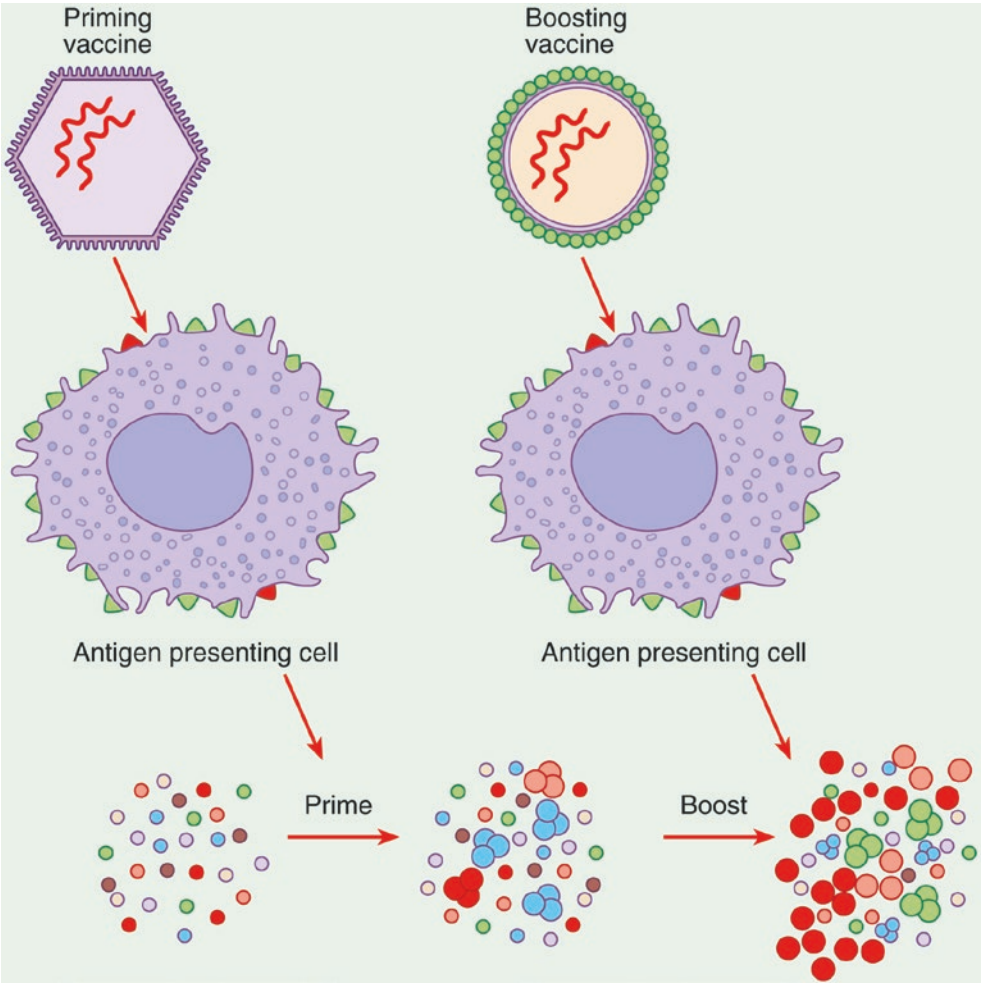

Fig. 49.4 Approaches to HIV-vaccine development. The mechanism of normal HIV infection is shown (**a**), along with the mechanisms of three types of potential vaccines: a subunit vaccine containing a synthetic protein from the CD4–binding site on the envelope of the virus (**b**), a naked DNA vaccine, and a recombinant vaccine with a bacterial or viral vector (**c**) [185] (Copyright © 2005 Massachusetts Medical Society. All rights reserved)

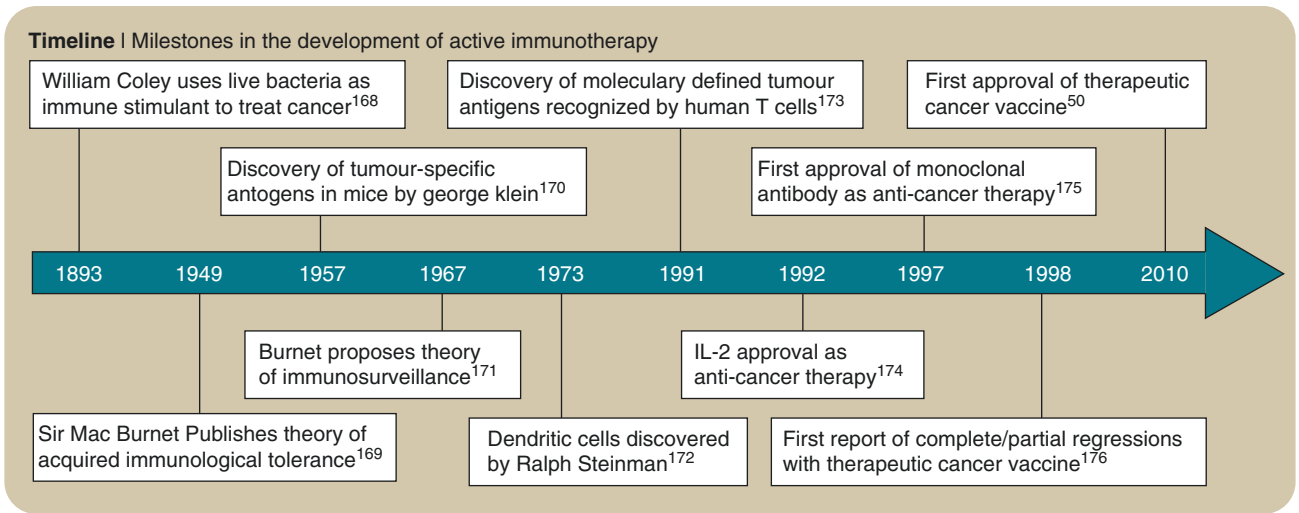

Fig. 49.5 Timeline of milestones in the development of active immunotherapy (Reprinted by permission from Macmillan Publishers Ltd: Melero et al. [219], copyright 2014)

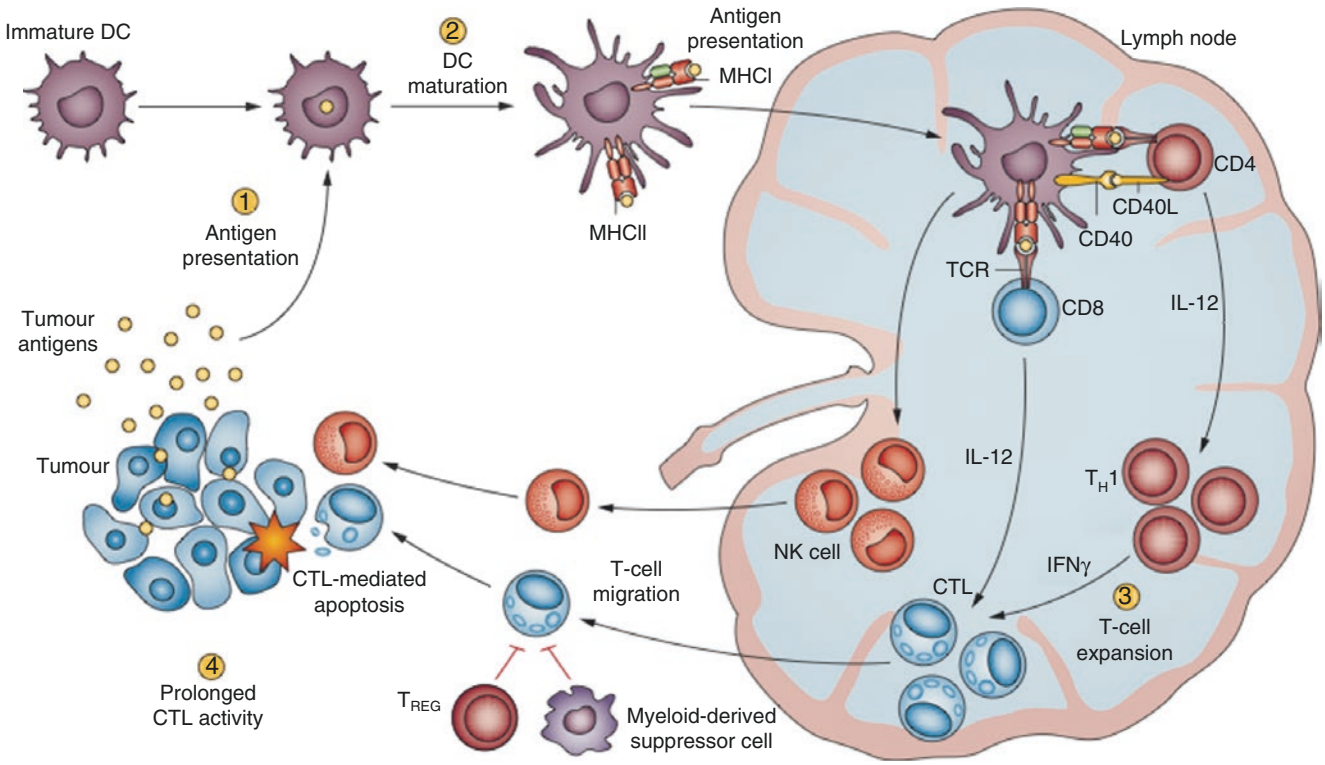

Fig. 49.6 Steps in the development of a cellular immune response against tumor-associated antigen. Multiple steps and processes are involved in the generation of an immune response directed against tumor antigens, offering multiple opportunities for therapeutic enhancement. For example, immunization can be used to present tumor-associated antigens to DCs (*1*). Tumors can deploy a number of immunosuppressive factors, including TGF-β and activators of STAT3 phosphorylation, which inhibit DC maturation (*2*). Small-molecule inhibitors of these factors can be used to promote DC maturation and enhance antitumor activity. T-cell expansion (*3*) can be supplemented by adoptive transfer of activated antitumor T cells, expanded or genetically modified in culture to recognize tumor antigens. Immunostimulatory monoclonal antibodies (such as agonists of CD40, CD137, or OX40) and cytokines (such as IL-12, IL-15, IL-21) can also enhance the performance of active immunotherapies or the action of adoptively transferred T cells. Finally, new studies have demonstrated the clinical potential of checkpoint modifiers that interfere with key immunosuppressive mechanisms (such as CTLA-4 and PD-1) and prolong CTL activity (*4*). *Abbreviations*: *CD40L* CD40 ligand, *CTL* cytotoxic T cell, *DC* dendritic cell, *IFN-γ* interferon-γ, *MDSC* myeloid-derived suppressor cell, *MHC* major histocompatibility complex, *MHCI* MHC class I, *MHCII* MHC class II, *NK* natural killer cell, *PD-1* programmed cell death protein 1, *TCR* T-cell receptor, *TGF-β* transforming growth factor β, *TH1* type 1 T helper cells, *TREG* regulatory T cells (Reprinted by permission from Macmillan Publishers Ltd: Melero et al. [219], copyright 2014)

optimal dosing of the vaccine prior to large scale clinical trials [212].

Modern uses of vaccines are not limited to only infections. Cancer is an area that has gained popularity as a therapeutic target for vaccines (Fig. 49.5). Dysregulated cell growth in malignancy produces proteins, or tumor-associated antigens, which are not expressed to a great degree in healthy individuals. Vaccines targeting these tumor-associated antigens have shown efficacy in producing an immune response in humans, and epidemiologic data suggests these responses may reduce the risk of developing cancer as well as improve survival rates [213]. Figure 49.6 demonstrates several ways how tumor-associated antigens elicit a cellular immune response. Prophage (Agenus) is an autologous vaccine for glioblastoma multiforme prepared from protein isolates from the patient's own cancer cells. Phase II trials since 2011 have reported as much as doubled life expectancy and a median progression-free survival of nearly 2–3 times longer compared with traditional therapies alone [214, 215]. A separate vaccine for melanoma, Allovectin ® (Vical), was designed to be injected directly into lesions to restore an MHC class 1 complex into tumor cells, creating a target for the patient's immune responses [216]. A multicenter phase II study ($n = 133$) demonstrated complete or partial responses greater than expected compared to spontaneous regression; however a multinational phase III trial completed in 2013 failed to demonstrate a statistically significant improvement in response and survival rate compared to first-line chemotherapy [217, 218]. Despite the results of Allovectin, therapeutic tumor vaccines continue to offer promise of new options for cancer treatment and phase III trials involving prostate, breast, lung, pancreatic, colorectal, and lymphoma are currently being studied [219].

Conclusion

Vaccines are one of the leading tools for maintenance of public health, and they have made a tremendous contribution to reducing the incidence of numerous diseases. While there has been much success with immunization, there are still many diseases uncontrolled by vaccination, and there are still challenges regarding implementing vaccine programs and dispelling fears about immunization side effects. Most associations between vaccines and adverse events are not demonstrated to be causal. Nevertheless, suspected relationships between vaccines and adverse events need to be reported to the Vaccine Adverse Event Reporting System (telephone 800–822–7967) in order to maintain an excellent safety record of vaccines.

Approximately 200 years of research have allowed us to direct the immune system for the gain of the people, and to have a clearer comprehension of microbial pathogenesis and host responses. From the continual presence of experimental and clinical trials, we can expect to see safe and effective vaccines for numerous infectious and non-infectious diseases in the future.

Questions and Answers

1. Which of these statements best describes the purpose of a vaccine conjugate?
   A. **Offer recognizable peptides for antigen-specific T-cells to aid in creating an adaptive immune response**
   B. Carries vaccine antigen across cellular membranes
   C. Provides protection against degradation of vaccine components

2. Which of the following is NOT a live-attenuated vaccine?
   A. MMR
   B. Small pox vaccine
   C. Varicella-Zoster vaccine
   D. **Hepatitis A**
   E. Salmonella typhi

3. Which of these statements is true regarding the nine-valent HPV vaccine?
   A. The nine-valent vaccine was shown to be approximately 5 % less efficacious in the prevention of cervical, vaginal, and vulvar diseases in HPV types 6, 11, 16, and 18 when compared with Gardasil
   B. **The nine-valent vaccine was shown to be of similar efficacy in the prevention of cervical, vaginal, and vulvar diseases in HPV types 6, 11, 16, and 18 when compared with Gardasil, in addition to providing prevention of cervical, vaginal, and vulvar disease in HPV types 31, 33, 45, 52, and 58**
   C. The nine-valent vaccine was shown to be of similar efficacy in the prevention of cervical, vaginal, and vulvar diseases in HPV types 6, 11, 16, and 18 when compared with Gardasil, and only 50 % efficacious in providing prevention of cervical, vaginal, and vulvar disease in HPV types 31, 33, 45, 52, and 58
   D. The nine-valent vaccine was shown to be of similar efficacy in the prevention of cervical, vaginal, and vulvar diseases in HPV types 6, 11, 16, and 18 when compared with Gardasil, and not efficacious in providing prevention of cervical, vaginal, and vulvar disease in HPV types 31, 33, 45, 52, and 58

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Intravenous Immunoglobulin: Dermatologic Uses and Mechanisms of Action

50

Irene K. Mannering, Yang Yu, and Sergei A. Grando

Abstract

Intravenous immunoglobulin (IVIg) is a fractioned blood product consisting of pooled, polyvalent, IgG antibody. It is being increasingly utilized as off-label therapy of a variety of dermatological conditions including autoimmune mucocutaneous blistering diseases, autoimmune connective tissue diseases, Stevens–Johnson syndrome, toxic epidermal necrolysis, cutaneous vasculitides, skin infections and several other dermatoses, such as chronic urticaria, atopic dermatitis, pyoderma gangrenosum, scleromyxedema. Randomized trails are generally lacking. IVIg is a relatively safe and well-tolerated therapy inducing and maintaining a prolonged clinical remission, and has a corticosteroid-sparing effect. The mechanism of action of IVIg is multifactorial and includes: (1) reduction of autoantibody concentration, (2) modulation of cytokine production and (3) prevention of keratinocyte apoptosis. Normal degradation and removal from the body of all kinds of IgG antibodies after IVIg infusion results in a selective decrease of relative titer of pathogenic antibodies, because the level of normal antibodies is maintained by those present in the IVIg preparation. To prevent a "rebound" effect resulting from the negative feedback stimulation of B cells, IVIg should be combined with a cytotoxic immunosuppressive drug.

Keywords

Intravenous immunoglobulin • Polyvalent IgG antibody • Corticosteroid-sparing effect • Treatment • Autoimmune mucocutaneous blistering diseases • Autoimmune connective tissue diseases • Stevens–Johnson syndrome • Toxic epidermal necrolysis • Cutaneous vasculitides • Skin infections • FcRn • Cytokines

Introduction

Intravenous immunoglobulin (IVIg) is a fractioned blood product consisting of pooled, polyvalent, IgG antibody extracted from the plasma of over 10,000 blood donors per batch. Historically, it was used to treat primary and secondary immune deficiencies, however, its use has expanded tremendously over the past several decades. Today it is being increasingly utilized as off-label therapy for a variety of autoimmune and inflammatory conditions, especially in dermatology (Table 50.1).

In contrast to standard immunotherapy for autoimmune and inflammatory conditions, IVIg is not immunosuppressive. It is also safe to use in pregnancy and is not associated with reproductive organ suppression, nor is it carcinogenic. It is generally accepted that the use of IVIg should be reserved for those cases that: (1) fail conventional therapy; (2) have severe side effects or contraindications to conventional therapy; and/or (3) have rapidly progressive disease.

IVIg exhibits several effects with the most well described being: (1) complement blockade and degradation; (2) neonatal Fcγ receptor saturation; (3) induction of

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Table 50.1 Dermatologic diseases treated with IVIg

Autoimmune connective tissue disorders
Cutaneous lupus erythematosus
Dermatomyositis
Mixed connective tissue disease
Nephrogenic fibrosing dermopathy
Scleroderma (Systemic sclerosis)
Autoimmune mucocutaneous blistering diseases
Bullous pemphigoid
Epidermolysis bullosa acquisita
Lichen planus pemphigoides
Linear IgA bullous disease
Mucous membrane (Cicatricial) pemphigoid
Paraneoplastic autoimmune multiorgan syndrome (a.k.a. Paraneoplastic pemphigus)
Pemphigoid (Herpes) gestationis
Pemphigus foliaceus
Pemphigus vulgaris
Vascular disorders
Anti-neutrophil cytoplasmic autoantibody (ANCA) positive vasculitides:
Microscopic polyangiitis
Wegener's granulomatosis
Behçet's disease
Churg–Strauss syndrome
Cutaneous polyarteritis nodosa
Degos' disease
Leukocytoclastic vasculitis
Livedoid vasculopathy
Drug-induced skin disorders
Drug reaction eruption with eosinophila syndrome/Anticonvulsant hypersensitivity syndrome
Erythema multiforme
Kaposi sarcoma due to immunosuppression
Methotrexate-induced acral erythema
Stevens–Johnson syndrome
Toxic epidermal necrolysis (Lyell's syndrome)
Miscellaneous dermatoses
Alopecia universalis
Atopic dermatitis
Calcinosis cutis
Chronic urticaria:
Angioedema with hypereosinophilia
Autoimmune urticaria
Chronic idiopathic urticaria
Delayed pressure urticaria
Solar urticaria
Graft-versus-host disease
Hyper-IgE syndrome
Kawasaki's syndrome
Necrobiosis lipoidica diabeticorum
POEMS syndrome: *polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes*
Polymorphous light eruption
Pretibial myxedema
Psoriasis
Pyoderma gangrenosum
Scleromyxedema
Wiskott–Aldrich syndrome
Skin infectious and infection-related diseases
Lyme disease
Measles
Necrotizing fasciitis
Rubella
Staphylococcal scalded skin syndrome (SSSS; Ritter's disease)
Streptococcal toxic shock syndrome (STSS)
Varicella

Table 50.2 Standard IVIg infusion protocol

Administer IVIg product at 400–500 mg/kg/day on 4–5 consecutive days up to the total dose of 2 g/kg/month times 6–18 months
Premedicate patient with 25 mg Benadryl (diphenhydramine) and 500 mg Tylenol (acetaminophen) 15–30 min prior to starting the infusion
Place peripheral i.v. and maintain with 0.9 % sodium chloride
Infusion Rate: start at 0.5 ml/kg/h, then increase by 15 ml/h every 15 min until target rate of 150–200 ml/h, as tolerated. Maximum rate is 200 ml/h
Observe vital signs prior to infusion. Blood pressure and pulse every 30 min until stable infusion rate, then every hour
Watch for signs of fluid overload, cardiovascular symptoms, allergic reactions, skin rash, fever, and moderate to severe headache
For adverse events, stop the infusion. Can restart the infusion at the same or lower rate if the symptoms subside

immunomodulatory Fc receptors; (4) inhibition of B cells; and (5) altering T cell function, cytokine production and migration.

IVIg is given intravenously over several hours, gradually increasing the rate of infusion up to 200 ml/h. It is given daily for 2–5 days usually at 400 mg/kg/day up to 2 g/kg per month. The dose can be repeated in 2–4 weeks, depending on the disease and patient. Its half-life is 3–4 weeks. Multiple cycles are usually required with up to 50 or more sometimes needed. Table 50.2 outlines the standard infusion protocol for the administration of IVIg. IVIg can be administered in a hospital if there is extensive disease, concomitant very high steroid doses, or other serious medical problems. More commonly, it is administrated at an infusion center or at a patient's home under medical supervision.

The annual cost of multiple infusions of IVIg in patients with autoimmune bullous diseases is estimated to be between $33,000–$85,000. This may seem high initially however; the cost is significantly less than the cost of conventional therapy when factoring in management complications and adverse events associated with conventional immunosuppressive therapy [1]. The cost nevertheless results in extensive scrutiny by health insurance companies, requiring significant evidence that several other therapies have failed. Even when approved, the length of treatment may still be restricted.

IVIg is a relatively safe and well-tolerated therapy. Severe adverse events associated with the use of IVIg are rare. Most side effects can be minimized by decreasing the rate of infusion and by giving it over 4–5 days. Additional measures to decrease adverse events include modifications in dilution and using preservative free preparations. Not all IVIg preparations are equal with formulations available in the United States differing in immunoglobulin A (IgA) content, need for reconstitution, method of viral inactivation, osmolarity and sugar content.

In a study of 9892 infusions given to 174 patients, the most commonly reported adverse event was headache, which occurred in 8.9 % of infusions and was reported more often in patients with a history of migraines [2]. Acute self-limited cutaneous reactions such as urticaria also occurred in a small subset of patients.

Other immediate and delayed side effects can be seen with IVIg. Immediate effects include flushing, chills, fever, nausea, vomiting, dizziness, sweating, hypertension, chest pain, back pain, and muscle aches. These are related to the infusion rate and can be minimized by decreasing the rate. Headache and acute cutaneous reactions, such as urticaria, are generally best managed with prophylactic pre-medication with acetaminophen and diphenhydramine.

More serious immediate adverse effects include aseptic meningitis, thrombosis and stroke, anaphylaxis and acute renal failure. These events are rarely reported in association with IVIg, however do warrant consideration and close monitoring in high-risk patients. Thromboembolic events have been reported with the use of IVIg. This rare complication is reported to occur when a large dose is administered at a rapid rate, suggesting it might be related to high plasma viscosity [3]. To avoid this complication, high-risk patients should be treated only if necessary and monitored closely. In addition, low dose, low viscosity reconstituted preparations infused at a slow rate can decrease the risk of this serious adverse event. The risk for thromboembolic events is more common in those with a history of cardiac disease, stroke, thrombosis, and advanced age.

Anaphylactic reactions have been reported when IVIg was administered to patients with an IgA deficiency. Current preparations contain less than 2.5 % of IgA. Anti-IgA antibodies produced in patients with IgA deficiency subsequently react with the infused IgA. Evaluation of IgA deficiency prior to administration of IVIg will eliminate this potential complication.

Acute renal failure results from osmotic injury secondary to sucrose. Sucrose accumulates and causes osmotic nephrosis in the renal tubules, leading to reversible renal failure, usually within a week of administration of IVIg. Patients with pre-existing renal insufficiency, diabetes mellitus, older age or those concomitantly taking nephrotoxic agents should have their renal function closely monitored. These patients should receive a sucrose-free preparation administered slowly and at the lowest effective dose.

Delayed side effects include anemia, cardiac insufficiency due to fluid overload, renal insufficiency from immune complex deposition, and viral infections. Anemia is due to anti-ABO antibodies. The risk for infection is decreased with the recent use of detergent treatments and ultrafiltration.

Autoimmune Mucocutaneous Blistering Diseases

Autoimmune mucocutaneous blistering diseases represent a diverse group of rare diseases that involve the mucous membranes and skin. The immunologic basis is well established with each having a unique group of targeted antigens within the epidermis and dermis. The clinical course, presentation, morbidity, and mortality varies greatly within each disease. Conventional treatment for autoimmune blistering diseases is centered around the use of systemic steroids and immunosuppressive agents. These agents can cause serious side effects, some of which may be fatal.

More recently, IVIg has emerged as an important agent in the treamtent of blistering diseases. An expanding number of reports on the efficacy of IVIg in autoimmune cutaneous blistering disorders (pemphigus vulgaris, pemphigus foliaceus, bullous pemphigoid, mucous membrane pemphigoid, linear IgA dermatosis, epidermolysis bullosa acquista; paraneoplstic autoimmune multiorgan syndrome) parallel its increased utilization. More is known about the use of IVIg in pemphigus and pemphigoid than in any other autoimmune cutaneous blistering disease.

Pemphigus antibodies target several antigens on the cell surface of keratinocytes [4, 5]. These antibodies can also penetrate the cell membrane and bind to antigens on the mitochondrial outer membrane, thus triggering the intrinsic apoptosis pathway [6, 7]. Both anti-desmoglein and anti-mitochondrial antibodies are pathogenic because their adsorption abolishes the ability of pemphigus IgG to cause acantholysis in in vitro and in vivo models of pemphigus. Keratinocytes with damaged mitochondria shrink from the lack of energy and because activation of apoptotic cascades leads to collapse of the cytoskeleton. In turn, antibodies to adhesion molecules such as desmogleins prevent cell re-attachment by steric hindrance at the attachment points on nascent desmosomes. The neonatal Fc receptor (FcRn) expressed by keratinocytes is essential for both the disease-causing activities of pemphigus and pemphigoid autoantibodies and the therapeutic action of IVIg [8]. Most recently, we have demonstrated that these receptors can assist pemphigus IgG in reaching mitochondrial targets. Therefore, saturation of these receptors by IVIg may prevent pathogenic antibodies against intracellular antigens to reach their targets.

IVIg is a safe and effective treatment for autoimmune blistering diseases resistant to systemic immunosuppresion [9–11]. Failure of conventional therapy is defined as continued new blister formation, extension of existing lesions or lack of healing while on a moderate dose of prednisone, specifically, up to 1.5 mg/kg/day for at least 3 weeks. However, due to the rarity and severity of these diseases, well designed prospective trails evaluating its use are generally lacking. As a monotherapy, IVIg rapidly controls disease activity in those with pemphigus vulgaris, pemphigus foliaceus, and bullous pemphigoid within a few weeks [12–14]. Reported benefits of IVIg include a improved clinical outcome, decrease in pathogenic autoantibodies, and a steroid sparing effect [9, 12–15, 21]. In cases of pediatric pemphigus, IVIg delays the need for immunosuppression [16].

A recent review indicates treatment for pemphigus ranges from 1 to 2 g/kg/cycle over 2–5 days, at 3–4 week intervals [1]. However, the dose can be lower, and cycles can be extended by several weeks while maintaining effectiveness [17]. The most effective way to use IVIg is unknown though most favor the use of IVIg at a dose of 2 g/kg/cycle. Ahmed and Dahl [17] proposed the use of IVIg at a dose of 2 g/kg divided over 3–5 days every 4 weeks until disease control is obtained. The interval between infusions is then slowly increased to 6, 8, 10, 12, 14 and 16 weeks, and stopped afterwards. If the disease flares, the frequency of infusions is increased until control is obtained and then the tapering regimen is again resumed. A randomized, double-blind, placebo-controlled trial found that patients treated with one cycle of IVIg at 400 mg/kg/day for 5 days stayed on protocol longer without any additional treatment than the placebo group and also had suppression of autoantibodies levels [10]. These findings suggest a single cycle of IVIg maybe effective in the treatment of pemphigus.

Pemphigus and other autoimmune skin blistering diseases appear more likely to respond when IVIg is used concomitantly with additional treatments. When IVIg is used in combination with systemic steroids and/or immunosuppressive agents, a response rate of 91 % was reported, compared to a response of 56 % when it was used as monotherapy [18]. It has been well documented that IVIg causes a rapid decline in pathogenic autoantibody levels. Following this decline there is often a rebound increase. Therefore, the use of an agent that suppresses the rebound increase in pathogenic antibodies should lead to a sustained response to therapy. Indeed, co-administration of a cytotoxic agent improves the ability of IVIg to lower serum levels of pathogenic autoantibodies in pemphigus [19]. The combination of IVIg with rituximab has been shown to induce a sustained remission in the treatment of pemphigus vulgaris [20]. In that study, 11 patients were treated with two cycles of rituximab 375 mg per square meter of body-surface area every 3 weeks followed by once monthly infusions of IVIg at 2 g/kg every fourth week. All had rapid resolution of skin lesions and long-lasting disease remission off all therapy (mean 31 months). This regimen allowed rapid tapering of systemic steroids and immunosuppressive agents within 2 months.

IVIg has also been shown to effectively treat mucous membrane pemphigoid, bullous pemphigoid and epidermolysis

bullosa aquisita. Several studies have demonstrated that in severe mucous membrane pemphigoid, IVIg is more effective at resolving lesions, preventing progression, and producing longer remissions than conventional immunosuppressive therapy [12]. A review of 5 cases of patients with bullous pemphigoid, at one institution, found 4 out of the 5 had either partial or complete response to treatment with IVIg in conjunction with an immunosuppressant [21]. In a single prospective study, the use of IVIg at 2 g/kg/cycle every 4 weeks in 10 patients with bullous pemphigoid led to a significant decline in pemphigoid antibodies at 3 months and complete sustained serologic and clinical remission at 11 months [22]. An additional observation showed that these autoantibodies declined at a greater rate when IVIg was combined with immunosuppressive agents compared to its use as monotherapy [23]. Similar results have been observed in the treatment of recalcitrant epidermolysis bullosa aquisita either alone or in combination with oral corticosteroids at doses of 1–2 g/kg [12, 24].

Drug Hypersensitivity Reactions

IVIg is being increasingly used to treat several drug hypersensitivity reactions such as toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome (SJS) and drug reaction with eosinophilia and systemic symptoms (DRESS). TEN and SJS are acute and life-threatening mucocutaneous reactions, most commonly due to drugs. These conditions are characterized by fever and full thickness necrosis and detachment of the epidermis. The two conditions differ by the extent of skin involvement, management, etiology and prognosis. TEN is more severe with skin involvement being greater than 30 % and has a mortality rate as high as 50 % [25, 26]. Transcutaneous fluid loss can be large causing significant electrolyte abnormalities, renal insufficiency and hypovolemia. The large amount of cutaneous involvement also puts patients at risk for infection, which can ultimately lead to sepsis.

The exact pathogenesis of TEN and SJS is only partially understood with evidence pointing to a T cell mediated process that leads to keratinocyte apoptosis. TEN is initiated either by non-covalent, direct interaction of a drug with a specific MHC I allotype or by covalent binding of a drug metabolite to a cellular peptide, forming an immunogenic molecule [26]. Treatment of TEN/SJS includes discontinuation of the suspected medication, supportive therapy and transfer to a hospital with intensive care or burn units.

Several studies report successful treatment with IVIg, with mortality rates ranging from 0 to 12 % [27–30]. A randomized trial and two retrospective studies, found that patients treated with IVIg in addition to standard therapy had more rapid disease resolution compared to patients not given IVIg [31–33]. A recent meta-analysis with meta-regression of 13 studies found that IVIg at dosages of greater than 2 g/kg significantly decreases mortality in patients with SJS or TEN [34]. The most accepted total dose for the treatment of TEN and SJS is 3–4 g/kg per day, given early in the disease. In children, somewhat lower doses ranging from 0.5 to 2.2 g/kg per cycle have been shown to be safe and effective [35]. In TEN and SJS, IVIg is thought to reduce Fas-mediated keratinocyte apoptosis by blocking the binding of FasL to Fas.

The reports on IVIg use in patients with SJS or TEN are inconsistent. A large retrospective analysis of patients in the European Study of Severe Cutaneous Adverse Reactions (EuroSCAR) evaluated the use of corticosteroids, IVIg, and supportive therapy alone, and found that the group treated with IVIg did not have improved mortality when compared to the supportive therapy group [30]. A retrospective analysis of 64 patients at one center found no benefit from IVIg at any dose in the treatment of TEN or SJS/TEN overlap [36]. There were also no differences with regard to delays in treatment or the duration of IVIg therapy.

DRESS is characterized by a widespread cutaneous eruption, eosinophilia and systemic involvement. Its etiology also is not clear but hypothesized to involve drug detoxification enzyme abnormalities with accumulation of reactive drug metabolites, sequential reactivation of herpes viruses, and genetic predisposition [37]. DRESS has a 10 % mortality rate, most commonly from fulminant hepatitis with hepatic necrosis [37]. Initiation of systemic corticosteriods is the mainstay of treatment. Patients have been effectively treated with adjunctive high dose IVIg [38–42]. IVIg can be used as an alternative steroid-sparing agent or in cases unresponsive to systemic steroids. IVIg provides immune protection, has anti-inflammatory properties and increases immunoglobulin levels in patients with this condition [38]. A case report found monotherapy with IVIg successful [38], but a multicenter prospective open study of 6 patients found no benefit [43]. In DRESS, IVIg may provide benefit via anti-viral antibodies in addition to its more general anti-inflammatory and immunomodulatory effects [38, 44].

Cutaneous Lupus Erythematosus

Lupus erythematosus (LE) is a chronic inflammatory autoimmune disorder that can manifest as a systemic disease or be limited to the skin. Several cutaneous subtypes of LE exist, each of which is partly defined by depth of cutaneous involvement. The three most well described cutaneous variants include acute, subacute, and discoid LE. Its etiology is multifactorial with genetic and environmental factors playing a role. Polymorphisms of the major histocompatibility

complex leading to increased immune response to self-antigens, deficiencies of complement components, gender, and autoantibodies are all thought to play a role [45, 46]. In regards to autoantibodies, anti-Ro, anti-La, anti-dsDNA and anti-nucleosome antibodies are thought to play a role in skin disease [45]. Autoantibodies lead to increased cell apoptosis and reduced immune tolerence [45]. Dysregulation of T cells may also play a role in the pathogenesis of LE [46, 47].

The initial treatment approach in limited cutaneous LE is photoprotection and the use of topical corticosteroids and calcineurin inhibitors. In widespread, scaring or refractory cases systemic therapy is necessary. For years antimalarials, primarily hydroxychloroquine, have been the gold standard. In patients who fail antimalarials, therapeutic options include oral retinoids, thalidomide, immunosuppressive agents such as mycophenolate mofetil or methotrexate, dapsone, rituximab, clofazimine, sulfasalazine and systemic corticosteroids [48]. IVIg is a consideration in these difficult to control cases as well.

IVIg has been used to successfully treat cutaneous lupus, either as monotherapy or in conjunction with an immunosuppressant, however controlled studies are necessary to fully evaluate its efficacy [49–54]. Three cases described by Lampropoulos et al. [53] highlighted the use of IVIg in the treatment of refractory subacute cutaneous LE. In two of the cases, IVIg at 0.4 g/kg/day for 5 days resulted in rapid improvement of the disease. However in the third case, initial treatment with 2 g/kg IVIg repeated every 4 weeks was not effective and so a single high dose was given, which resulted in greater than 80 % improvement [53]. An open prospective study of 12 patients receiving an initial dose of 1 g/kg body weight followed by 400 mg/kg monthly, for at least 6 months, found that at least five patients experienced complete clearing. Two had a partial response and three had a limited response. The benefit however was temporary in these cases with relapse occurring shortly after stopping [49]. IVIg has also been used to successfully manage lupus profundus, a type of cutaneous LE in which there is deep involvement of the dermis and subcutaneous fat. A case report describes clinical improvement with IVIg given monthly for 6 months followed by 3 month pulses [52].

Several reports describe positive results when using IVIg for the treatment of systemic LE [55, 56]. An observational, retrospective, clinical study of 52 patients treated with at least one cycle of 2 g/kg found complete remission in 28 % of patients, and partial remission in an additional 38 % [57]. IVIg is also helpful in ameliorating myocarditis, lupus nephritis, and lupus-induced bone marrow suppression [58–64]. IVIg treatment is also reported to improve cutaneous findings in systemic LE such as ulcers, urticaria, and malar rash [65, 66]. The optimal dose for treatment of cutaneous LE is yet to be determined. Typical IVIg dosages are 0.4–2 g/kg given monthly for several months. Positive effects are usually observed within a few days, but these often do not persist after IVIg therapy is discontinued. One study of patients with systemic LE achieved remission for over 2 years in 11 out of 18 patients that had an initial response to therapy [57]. Overall, IVIg appears to be a useful adjunctive or alternative therapy in LE cases that do not respond to traditional treatments. Systemic LE patients demonstrate a significant decline of anti–double stranded DNA autoantibodies after treatment with IVIg in the majority of case reports and mouse models [67]. The mechanism is thought to be due to inhibition of B cell receptors, the cells producing the pathogenic autoantibodies, or cytokine signaling [68, 69].

Dermatomyositis

Dermatomyositis (DM) is a multisystem autoimmune disease affecting skeletal muscle, skin and other organs. It most commonly presents as a symmetric proximal extensor inflammatory myopathy and a characteristic photo distributed violaceous cutaneous eruption favoring the face, scalp, and extensor surfaces. There is an amyopathic form in which only cutaneous findings are present, with the muscle disease being absent or subclinical. It has a bimodal distribution with both childhood and adult-onset variants. DM is a complement-dependent microangiopathy mediated by early activation of the complement system with deposition of the C5b-9 membrane attack complex on the endomysial capillaries, which ultimately leads to muscle ischemia and inflammation [70].

First-line therapy for DM is high dose corticosteroids. Although the majority of DM patients respond to corticosteroids, up to 20–30 % will not [71]. In cases that are unresponsive to corticosteroids or a serious steroid related side effect occurs, an immunosuppressant, usually methotrexate or azathioprine, should be started. Both corticosteroids and immunosuppressants are effective in controlling the myopathy, however cutaneous involvement can be particularly refractory to these therapies [72].

IVIg is used as a second line therapy in patients with refractory DM despite treatment with corticosteroids, methotrexate and/or azathioprine, or in those with contraindications to cytotoxic drugs [73]. Several reports justify the use of IVIg early in the course of the disease. IVIg can be considered as a first-line treatment, although IVIg monotherapy has not been shown to be effective [74]. IVIg exerts its action by inactivating immune complexes and decreasing complement amplification. This effect of IVIg was highlighted in vivo in repeated muscle biopsy specimens from DM patients. Membrane attack complex deposits decreased and neovascularization and restoration of muscle cytoarchitecture was observed [75]. In DM, IVIg is usually administered at 2 g/kg over 2–5 days monthly, typically for 3–6 months. If no benefit is noted within 6 months discontinuation of treat-

ment should be considered. The positive outcomes seen with IVIg therapy can be realized for several months after discontinuing therapy. Relapses can occur, however the disease is usually less severe and the need for steroids or immunosuppressive agents is usually decreased.

A double-blind placebo-controlled study by Dalakas et. al assessed the efficacy of 2 g/kg IVIg infusions monthly for 3 months in 15 patients with steroid resistant DM. In the IVIg treatment group, there was significant improvement in muscle strength and active skin lesions [76]. A retrospective review evaluating the effect of IVIg on refractory cutaneous DM in patients with classic and amyopathic DM was performed. All 13 patients treated with 2 g/kg IVIg every 4 weeks (given as 1 g/kg/day for 2 consecutive days) demonstrated improvement, with complete clinical response achieved in 8 [77]. The response to IVIg was very rapid, with all patients improving after the first cycle [77]. IVIg has also been shown to be effective when combined with high dose steroids in treating life-threatening DM associated esophageal dysfunction [78]. More recently, subcutaneous administration of IVIg via a pump, at the usual monthly dose fractionated into equal weekly intervals, was shown to be effective in treating DM and polymyositis [79].

Cutaneous Vasculitis

Cutaneous vasculitis represents a group of disorders characterized by inflammation of small, medium, or large vessels. This condition can be a systemic disease with secondary cutaneous manifestations, restricted only to the skin, or be a primary cutaneous disease with secondary systemic involvement. Systemic findings are broad and nonspecific but commonly include arthralgias, fatigue, hypertension, abdominal pain, renal insufficiency, and neurologic dysfunction. Cutaneous findings are dependent upon the size of the affected vessels. The most common cutaneous manifestations include erythema, purpura, ulcerations, and necrosis. Patients with cutaneous vasculitis often show increased sedimentation rate, anemia, and decreased albumin. The pathophysiology is thought to be due to immune complex deposition in the vessels or anti-neutrophil cytoplasmic antibodies (ANCAs). Immune complex deposition activates the complement system, which in turn leads to mast cell degranulation and neutrophil activation. The end result is vessel wall necrosis. In ANCA mediated vasculitis, cytoplasmic proteins from neutrophils become expressed on the cell surface, leading to antibody formation and then neutrophil mediated vessel damage via release of inflammatory cytokines including IL-1, TNF-α, and IFN-γ, reactive oxygen species, and up regulation of adhesion molecules ELAM-1 and ICAM-1 [80, 81].

Treatment of vasculitis depends on the severity of the disease. In more severe and chronic cases, medium to high-dose glucocorticoids and adjuvant immunosuppressants such as cyclophosphamide or azathioprine are the standard therapy. IVIg is shown to be beneficial in the treatment of organ-specific and systemic vasculitis [82–85]. IVIg has been used to treat ANCA-positive Wegener's granulomatosis and microscopic polyangiitis [63, 82, 84, 86–92], Churg–Strauss Syndrome [93–98], polyarteritis nodosa [99–106], antineutrophil cytoplasmic antibody-negative nonleukocytoclastic vasculitis [107], Henoch–Schönlein purpura [108], leukocytoclastic vasculitis [109, 110], urticarial vasculitis [111, 112], Behçet's disease [113], and Kawasaki disease [114, 115].

There is clear evidence that IVIg is beneficial in the treatment of ANCA-positive vasculitides and Kawasaki disease. For the other vasculitidies, there are few studies looking at the efficacy of this treatment. Case reports suggest it may inhibit disease progression [108]. IVIg inhibits TNFα and IL-1 induced proliferation of endothelial cells and reduces expression of adhesion molecules, chemokines, and proinflammatory molecules. Regulatory T cells may also play an important role in the effectiveness of IVIg [116, 117].

IVIg is an alternative or adjunctive treatment for ANCA-associated vasculitis, with significantly less toxicity than standard therapy. A randomized clinical trial investigated the efficacy of IVIg (total dose 2 g/kg) in patients with recalcitrant Wegener's granulomatosis and microscopy polyangiitis. The study found partial or complete remission in 82 % of the IVIg group versus 35 % of the placebo group at 3 months. Despite these results, disease activity, frequency of relapse, and exposure to immunosuppression was the same in both groups, indicating that the benefit of IVIg was not maintained beyond 3 months [82, 98]. A prospective, open-label study of patients with Wegener's granulomatosis and microscopic polyangiitis looked at the efficacy of adjunctive IVIg along with a standard immunosuppressive therapeutic for remission maintenance. At 9 months, 62 % were in complete remission, with 53 % maintaining remission at 24 months [118]. IVIg was used to successfully treat 12 patients with rapidly progressive glomerulonephritis from ANCA-positive vasculitis [91]. In another report, patients with Churg–Strauss syndrome were treated with IVIg in addition to corticosteroids, with or without cyclophosphamide [94]. In that study, motor neuropathy was improved in 13 of 15 patients and cardiac function improved in all five patients with heart failure.

In vitro studies have found IVIg inhibits ANCA induced neutrophil activation and cytokine release [119]. Using IVIg early in the course of ANCA-positive vasculitis may stop apoptotic cell death and reduce damage, particularly in serious forms such as Wegener's granulomatosis [90]. Therefore, the use of IVIg may be justified as a first-line agent in this systemic vasculitis. It also offers an alternative to cytotoxic agents when contraindications exist. IVIg is usually administered for a total dose of 2 g/kg as a single dose or repeated in

4-week intervals. The patient response should be assessed after several intervals [120].

Kawasaki disease, also known as mucocutaneous lymph node syndrome, is an acute vasculitis of the small and medium sized vessels that typically presents in children between 6 months to 5 years of age. IVIg and high-dose aspirin together is considered the most appropriate and effective regimen for patients with this condition [121]. The standard treatment is 2 g/kg IVIg given as a single infusion within 10 days of symptom onset. Administration of IVIg after 10 days may alleviate symptoms, but it is not as effective in preventing coronary artery disease [122, 123]. In one study, 1 g/kg dose was just as effective [124]. Longer intervals may be used as well [125]. Although IVIg is the most effective treatment for reducing acute symptoms, 10–20 % of patients are resistant to initial IVIg treatment, putting them at risk for developing coronary artery disease [126]. Elevated IL-6, TNF-α, percentage of circulating neutrophils, and plasma clusterin are found in cases resistant to IVIg treatment [126–128]. Combination therapies may prove efficacious in resistant cases [129, 130].

Atopic Dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that usually starts in childhood. It is a complex genetically-determined disease often associated with other atopic disorders including asthma and allergic rhinoconjunctivitis [131]. Its most common features are pruritus and a chronic and relapsing dermatitis characterized by erythematous edematous papules/plaques, xerosis, and lichenification. Its exact etiology is unclear but thought to be due to an impaired epidermal barrier and dysfunctional stratum corneum that allows antigens to enter and activate immune cells.

A multi-faceted approach is needed to treat AD with the main focus being on avoiding triggers, a daily moisturizer to repair the skin barrier defect, topical steroids and topical calcineurin inhibitors, and education. Adjunctive modalities are used in severe refractory cases. These include systemic immunosuppressants (methotrexate, cyclosporine, oral corticosteroids, azathioprine, mycophenolate, etc.), phototherapy, IVIg, and targeted therapy with rituximab and omalizumab.

High dose IVIg, either as monotherapy or in conjunction with immunosuppression, has been reported to be effective in treating patients with recalcitrant disease, especially children [132–134]. However, there is a paucity of double-blind, placebo-controlled trials evaluating the use of IVIg for the treatment of AD. A report of 3 cases of adults treated with 2 g/kg monthly demonstrated improvement of skin lesions, reduction of concomitant systemic medications, and a decrease in serum IgE [135]. A randomized, placebo controlled study of 40 children with moderate to severe AD treated with three doses of 2 g/kg IVIg at 1 month intervals found that disease severity improved at 3 months [136]. A retrospective study of 10 children with severe refractory AD found similar results. Patients were treated with IVIg monthly for up to 24 months and found to have significant clinical improvement along with decreases in serum IgE. The clinical benefit varies with results lasting anywhere from 6 months to 4 years or longer [136–139]. On the other hand, a study of 10 patients evaluating the use of IVIg in adults with severe AD found that a single high-dose treatment did not significantly improve symptoms [140]. IVIg may provide benefit in AD patients by downregulating T cell function, particularly IL-4, and IL-5 and through its general immunomodulating effects [135]. Further randomized studies examining the effective dose, dosing intervals for initiation of therapy and maintenance are needed.

Mechanisms of Therapeutic Action of IVIg in Mucocutaneous Autoimmune and Inflammatory Diseases

The mechanism of action of IVIg is not completely understood but thought to be due to: neutralization of bacterial superantigens or other infectious agents, inhibition of TNF-α production, neutralization of pathogenic autoantibodies, regulation and modulation of Fc receptors, downregulation of proinflammatory cytokine production, suppression of the function of B lymphocytes, enhancement of regulatory T cells with inhibition of other T cells via T-cell receptor signaling, antioxidative effects, inhibition of TH_{17} differentiation, inhibition of differentiation of dendritic cells and suppression of the endocytosis of nucleosomes [69, 119, 121, 141, 142].

In immunobullous diseases, several mechanisms are thought to drive the decline in autoantibody levels and disease activity. A dramatic and rapid effect of treatment with IVIg is the selective decrease in autontibodies, leaving normal protective antibodies unaffected [23, 143, 144]. This correlates well with IVIg's fast therapeutic effect. The rapid decrease suggests that this is due to increased catabolism of antibodies rather than decreased production [145]. A clinical study of 12 patients found that IVIg decreased anti-desmoglein 3 by 45 % and anti-desmoglein1 by 32 % from baseline within 2 weeks of the last cycle [146]. The lack of activity against long-term autoantibody production necessitates the use of cytotoxic immunosuppressants to supress production of new pathogenic autoantibodies during the catabolic phase [147].

The FcRn receptor is present on many cell types, inlcuding keratinocytes and endothelial cells. It functions to regulate serum immunoglobulin levels by protecting immunoglobulin from degradation. By increasing the concentration of IgG, these receptors become saturated, and are no longer able to protect IgG from degradation. This leads to increased catabolism of all antibodies. Because pathogenic antibodies are not replenished by IVIg therapy, levels of these

antibodies decline, preventing their activation. In addition, IVIg may increase the expression of the inhibitory FcRIIB receptor on effector cells, resulting in decreased clearance of opsonized platelets [148]. This same mechanism could act to decrease activation of anti-keratinocyte primed phagocytes.

Within the natural autoantibody population, there are antibodies that can bind the variable region of other antibodies. This anti-idiotypic interaction may interfere with autoantibody binding to autoantigen, preventing effector function. It may also cause anti-idiotype cross-linking of surface IgG, leading to apoptosis of autoimmune B cells [149, 150]. There is also evidence that antibodies in pooled IVIg may interact with pathogenic autoantibodies, blocking their function [151].

In addition to reducing autoantibody concentration, IVIg has been shown to alter cytokine profiles [152–154]. IL-2, IL-3, IL-4, IL-5, IL-10, TNF-β, and GM-CSF are downregulated, but not IFN-γ or TNF-α. In some diseases, the major effect of IVIg modulation may be regulation of Th1 and Th2 cytokines [155]. IVIg may increase differentiation of dendritic cells [156]. At the higher levels used in treating autoimmune diseases, however, IVIg blocks maturation of dendritic cells *in vitro*, resulting in increased IL-10 and decreased IL-12 secretion [157].

All of the above mechanisms deal primarily with the effect of IVIg on antibodies, lymphocytes, or antigen-presenting cells. Additionally, IVIg appears to act directly in the skin, preventing autoantibody induced cell death. Notably, antibodies from pemphigus patients induce cell death through apoptosis and oncosis, termed apoptolysis [158]. Apoptolysis develops due to the combined and synergistic effects of autoantibodies and auto/paracrine mediators secreted by autoantibody stimulated keratinocytes, including TNF-α and Fas ligand [159]. Analysis of the activity of enzymes in apoptotic or oncotic pathways from keratinocytes treated with IgG antibodies from patients with pemphigus vulgaris demonstrated that the patients could be grouped according to whether their antibodies activated apoptosis-inducing caspase-3 and caspase-8 or oncosis-inducing calpain [158]. This indicates that the pathogenic antibodies can activate both pathways separately, which may account for the variability in clinical presentation and response to treatment of patients with pemphigus vulgaris.

It has been demonstrated that IVIg protects keratinocytes from pathogenic autoantibodies by preventing the autoantibody-induced apoptolysis [158]. Normal human IgG prevented the pemphigus vulgaris autoantibody induced increase of pro-apoptotic caspases and pro-oncotic calpain, while increasing the expression of the anti-apoptotic FLIP-I and anti-oncotic calpastatin [158]. These changes correlated with decreased induction of acantholysis in vitro, in keratinocyte cultures, and in vivo, in the mouse model of experimental pemphigus. Thus, protection of the target cells through upregulation of the endogenous caspase and calpain inhibitors may be a novel mechanism of therapeutic action of IVIg [15].

In vivo experiments demonstrated that pemphigus vulgaris antibodies cause an unopposed upregulation of mTOR selectively in basal keratinocytes associated with apoptosis, which can be abolished due to pretreatment with the mTOR inhibitor sirolimus [160]. This important observation justified a clinical trial of sirolimus in patients with pemphigus vulgaris. Initial results demonstrated that combination of therapy of 2 mg/day of sirolimus and 2 g/kg/month of IVIg provides for a good therapeutic response allowing complete withdrawal of systemic steroids [161]. This suggests that the combination of these agents is effective for acute pemphigus vulgaris because of the immunosuppressive activity and the protection provided against keratinocyte antibody-induced apoptolysis.

Questions

1. How is IVIg therapy given?
   A. Intravenously over several hours, gradually increasing the rate of infusion up to 200 ml/h
   B. Daily for 2–5 days. Usually at 400 mg/kg/day up to 2 g/kg per month
   C. Cycles can be repeated in 2–4 weeks
   D. Multiple cycles are usually required: from 3–5 to 30–50 and more
   E. All of the above
   F. None of the above

2. IVIg can be used in pregnancy.
   A. True
   B. False

3. What affects the safety of IVIg therapy?
   A. IgA content
   B. Concentration
   C. Sugar content
   D. Frequency and administration rate.
   E. All of the above
   F. None of the above

4. IVIg can be made to work better by combining it with:
   A. systemic corticosteroids
   B. topical corticosteroids
   C. cytotoxic immunosuppressors
   D. antibiotics
   E. multivitamins

Answers

1. E
2. A
3. E
4. C

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Immunobiology and Immune Based Therapies of Melanoma

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Abstract

Despite efforts in preventative care, the incidence and mortality rate of melanoma have continued to rise. However, in the past decade, an increasing understanding of melanoma immunology has resulted in tremendous bench to bedside advancements in melanoma therapies. Many of these new treatment modalities have proven efficacious, leading to seven new melanoma therapies receiving FDA approval within the past 5 years. This chapter explores our current understanding of melanoma immunology, with an emphasis on tumor microenvironment and the ability of melanoma to evade immune surveillance. The remainder of the chapter focuses on how this understanding of melanoma immunology has been translated into clinical therapies. These agents include cytokines such as interleukin-2 and interferons, checkpoint inhibitors such as anti-CTLA4 and anti-PD1 antibodies, and vaccines. For each melanoma therapy, mechanism of action, efficacy, side effects, dosing strategy and future directions will be discussed.

Keywords

Cancer • Melanoma • Skin disease • Immune based therapies • T-lymphocytes • Dendritic Cells • Adhesion Molecules • Chemotherapy • Gangliosides • Biochemotherapy

While melanoma accounts for only a small percentage of all cutaneous malignancies, it remains an important health issue due to its high mortality rate. In 2016, there were 76,380 new cases of malignant melanoma diagnosed, and 10,130 deaths attributed to melanoma [1]. These statistics are particularly concerning, as they represent a greater than 25 % increase from data only 8 years ago [2]. Cutaneous malignant melanoma originates from melanocytes, neural crest derived pigment cells residing in the basal layer of the epidermis. Our current understanding of melanoma development involves a step-wise progression through stages of growth, including cellular atypia, radial growth, vertical growth, and the development of metastases [3]. This model corresponds to the American Joint Commission on Cancer (AJCC) staging classification of melanoma that defines primary cutaneous melanoma as Stage I or Stage II disease, lymph node metastases as Stage III disease, and distant metastases as Stage IV disease [4–6]. This transition of melanocytes into neoplastic melanoma cells that initially grow in the skin and later metastasize to distant sites involves complex host-tumor cell interactions. Our understanding of host-tumor cell interactions has progressed rapidly in recent decades, and translating these basic science developments into clinical treatment of melanoma has yielded promising results. Insights into the host immune response to malignant melanoma have led to the development of new approaches to melanoma therapy.

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Melanoma Immunology

It is widely accepted that melanoma cells and the immune system are intricately connected. For instance, systemic immune suppression from human immunodeficiency virus infection is associated with an increased rate and more aggressive behavior of malignant melanoma [7–9]. Melanoma cells are capable of triggering host immunologic responses—depigmentation and regression of primary melanomas provide supporting evidence that host immune surveillance can occur [10, 11]. Finally, different types of immune cells have been noted to infiltrate tumor sites in malignant melanoma, and an increased number of tumor infiltrating immune cells has been associated with an improved prognosis, even in more advanced disease [12]. Indeed, different components of the immune system are activated in response to melanoma cells, and basic investigations of these immune responses are critical to the development of immune-based therapeutic approaches to melanoma.

T-Lymphocytes and Dendritic Cells

Numerous studies have shown that T-lymphocytes play a major role in melanoma immunology and immunotherapy. T-lymphocytes have been observed to accumulate in melanoma lesions and lyse autologous tumor cells *in vitro*, as well as to cause tumor regression when transferred *in vivo* in 30 % of melanoma patients [13]. Cytotoxic T-lymphocytes (principally CD8+) cells respond primarily to target cells with specific antigens associated with class I major histocompatibility complex (MHC) proteins. Activation of cytotoxic T-lymphocytes results in destruction of target cells that have altered MHC I antigen expression. Helper T-lymphocytes (CD4+ cells) respond primarily to class II MHC proteins and the associated antigens on the antigen presenting cells (APCs). Activated CD8+ cytotoxic T-lymphocytes and CD4+ helper T-lymphocytes produce a variety of cytokines that function to amplify and modify the immune response to the growing tumor [14, 15]. Melanoma cells, however, may thrive and proliferate by evading host immune surveillance and defense, as demonstrated by the fact that brisk immune activation in clinical trials does not always result in successful clinical response [16]. This evasion occurs by a number of mechanisms, including antigen loss, downregulation of MHC expression, and reduction of the T-helper 1 (Th1) arm of the immune response [17]. In addition, a subpopulation of human regulatory T-lymphocytes (Tregs), characterized as $CD4^{+}CD25^{+}$ T-lymphocytes, typically has the function of suppressing self-reactive T-lymphocytes [18]. However, Tregs may suppress anti-tumor immune responses, as seen in animal models of melanoma [19]. Patients with metastatic melanoma have increased numbers of these $CD4^{+}CD25^{+}$ T-lymphocytes that may influence responses to various immune modulating therapies [20]. More recently, T-lymphocytes were demonstrated to express cytotoxic T-lymphocyte antigen 4 (CTLA-4), which when bound to the B7 target on APCs, can lead to T-lymphocyte anergy, a T-lymphocyte poor microenvironment, and immunologic ignorance of tumor, in melanoma and other solid tumors [21]. Even in a T-lymphocyte rich environment, melanoma evasion may occur due to T-lymphocyte exhaustion and dysfunction due to the expression of programmed death-1 (PD-1) receptor on T-lymphocytes and the binding of its ligands, PD ligand (PD-L) 1 and PD-L2 that are expressed on tumor cell surface [21–23].

The role of B cells and humoral immunologic responses to melanoma cells remains undefined. Natural killer cells also appear to play a role in melanoma immune surveillance and the destruction of neoplastic cells with reduced MHC expression [24, 25].

Dendritic cells are active participants in the host immune response to melanoma. Dendritic cells are bone marrow derived leukocytes, which are potent APCs and play a critical role in initiation of T-lymphocyte immune responses [26]. In the skin, APCs are also known as Langerhans cells. Dendritic cells express high levels of both MHC class I and class II molecules, as well as T-lymphocyte costimulatory molecules [i.e., CD40, CD80 (B7-1), CD86 (B7-2)] [27]. Dendritic cells are capable of secreting a variety of cytokines, such as Interleukin (IL)-1 and IL-12, which serve as accessory factors for T-lymphocyte activation. Activated dendritic cells are mobile, monocyte-derived cells that traffic to lymphoid organs to present foreign or tumor cell antigens to specific populations of T-lymphocytes, which results in the activation of antigen specific effector T-lymphocytes [28]. It has been documented that dendritic cells themselves can infiltrate melanomas, although the purpose of this infiltration still needs to be defined [29]. Previous studies have suggested that melanoma-derived factors may convert dendritic cells from potent immune response inducers into tolerance-inducing APCs [26], which may limit the development of dendritic cell melanoma immunotherapies.

Adhesion Molecules

A number of distinct cellular adhesion molecules and cytokines have been implicated in the local growth and metastatic progression of melanoma cells. Tumor progression and the development of metastatic disease are often associated with loss of normal cellular controls. The alteration of tumor cellular adhesion molecule expression and function allows neoplastic cells to separate from the primary tumor mass to

establish metastatic lesions. With separation of the metastatic cells from the primary tumor mass, neoplastic cells are freed from local cell contact inhibitory mechanisms. Altered expression of adhesion molecules in neoplastic cells can also improve their ability to adhere to vascular endothelium facilitating metastases to distant sites. Many different tumor-associated cell adhesion molecules have been implicated in the progression of melanoma from radial growth, to vertical growth and then to metastases. These key adhesion molecules include integrins, cadherins and immunoglobulins such as ICAM-1 [30]. *In vitro* and *in vivo* studies have shown that melanoma cells alter their cell surface adhesion molecules as they progress through the different stages of growth, and that expression of certain cell surface molecules by melanoma can yield prognostic information [30, 31].

One example of a cell adhesion molecule associated with melanoma pathogenesis is MUC18/MCAM/CD146, a cell surface glycoprotein that mediates adhesion to different cell types. MUC18 is expressed on both vascular endothelia and activated T-lymphocytes, facilitating the extravasation and/or homing of activated T-lymphocytes [32, 33]. In contrast, the expression of MUC18 on melanoma cells is associated with tumor progression and increased metastatic potential in both humans and mice [34, 35]. The level of expression of MUC18 on primary melanoma tumors increases as the tumors increase in thickness and develop metastatic potential [34, 36]. The ability of human melanoma cell lines to form metastases in mice has been correlated with the level of expression of MUC18 [37]. In light of these findings, a human monoclonal antibody against MUC18, ABX-MA1, has demonstrated therapeutic promise in mouse models of melanoma [38].

Cytokines

Melanoma cells are capable of producing a variety of cytokines, which not only modulate the growth of tumor cells, but also act to promote or inhibit the host antitumor inflammatory and immune response [39]. These melanoma-derived cytokines have different biologic activities and can act as autocrine growth factors, growth inhibitors, growth promoters, or immunosuppressive factors. Some of these cytokines allow the melanoma cells to grow independently of exogenous growth factors.

Basic fibroblast growth factor (bFGF), IL-6, and chemokines such as IL-8 are capable of acting as melanoma autocrine growth factors. bFGF is expressed by melanoma cells, but not by melanocytes, and appears to provide a growth advantage to tumor cells [40, 41]. Some melanoma cell lines produce IL-6, which is a major immune and inflammatory mediator that appears to have conflicting roles in regulating melanoma growth and progression [42–44]. Previous studies indicate that IL-6 may be both a growth inhibitor and an autocrine growth stimulant for melanoma cells depending on the stage of tumor progression [45]. *In vitro* studies also demonstrate the ability of IL-8 to function as an autocrine growth factor for melanoma cells [46]. A positive correlation between melanoma IL-8 production and tumor thickness in primary cutaneous melanoma has been reported [47]. Additionally, IL-8 production has been shown to correlate with melanoma metastatic potential in nude mice [48] and with the induction of migration by melanoma cells [49].

Tumor derived transforming growth factor-β (TGF-β) and colony stimulating factors (CSFs) may also modulate the growth and progression of melanoma. The TGF-βs are potent regulators of the immune response, cellular proliferation, differentiation, and extracellular matrix synthesis and deposition [50]. The proliferation of most melanoma cell lines is inhibited by TGF-β [40, 51]. However, some melanoma cell lines established from metastatic tumors have been shown to become resistant to the growth inhibitory effects of TGF-β. This cytokine may even act as a growth stimulator in some of these advanced melanoma cell lines [52]. Since TGF-β has potent immunosuppressive activities, the production of this cytokine by melanoma cells may inhibit the host immune responses to the tumor. Some melanoma cells are capable of producing a number of CSFs including granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), stem cell factor/mast cell growth factor, and monocyte-colony stimulating factor [43, 53]. Host anti-melanoma effects have been most clearly demonstrated with GM-CSF. Studies have shown that GM-CSF produced by melanoma cells can induce monocyte Tumor Necrosis Factor α (TNF-α) production, which may contribute to host anti-tumor responses [54]. GM-CSF gene transfection into melanoma cells results in significant inhibition of melanoma progression by activating host immune responses [55, 56]. In human pathological specimens, melanoma tumor thickness inversely correlates with GM-CSF melanoma cell production, suggesting that GM-CSF may have an anti-melanoma role in early primary lesions [47].

IL-10, which typically has a number of immunosuppressive properties, is another cytokine produced by some melanoma cells. Studies have also demonstrated that, in some circumstances, IL-10 appears to act as a growth factor for melanoma cells *in vitro* and can downregulate the expression of HLA-I, HLA-II, as well as ICAM-I [57]. Melanomagenesis is known to be involved in the IL-10 cytokine pathway. Ultraviolet (UV) radiation can decrease epidermal Langerhans (dendritic) cells and stimulate production, by both T-lymphocytes and a small population of melanoma cells, of immunosuppressive and tumor growth promoting IL-10. The result is a milieu that allows the initial

expansion of melanoma cells [58, 59]. Patients with advanced metastatic melanoma have also been found to have elevated serum IL-10 levels, which is rarely observed in healthy patients [60]. Thus, IL-10 predominantly has immunosuppressive and tumor promoting activities in the setting of melanoma [61].

Melanoma Associated Antigens

A number of antigens located on the cell surface of melanomas have been referred to as melanoma associated antigens (MAA). These antigens can be divided into two major categories: HLA-associated antigens that are recognized by T-lymphocytes and cell surface antigens such as gangliosides that do not directly activate T-lymphocytes [17, 62]. Unlike gangliosides, melanoma associated antigens recognized by T-lymphocytes are derived from proteins that do not normally reside on the surface of normal cells, and thus can evoke an immune response in association with particular MHC molecules on the cell surface. These peptide-based antigens can be grouped into three different categories: antigens expressed only on cells of melanocytic lineage (i.e. tyrosinase, TRP-1, TRP-2, Melan-A/MART1, and gp100), antigens expressed in different kinds of tumor cells but not in normal adult tissues outside of the gonads (i.e. MAGE, BAGE, GAGE, NY-ESO-1 and PRAME), and unique antigens found only on some tumor cells [17]. Interest in MAAs focuses around potential immunotherapies for melanoma, particularly vaccine therapy.

Cytotoxic T-lymphocyte clones that have been identified and expanded from melanoma patients may recognize specific antigens expressed on both melanoma cells and normal melanocytes [63]. Melanoma associated cytotoxic T-lymphocyte antigens include tyrosinase, tyrosinase-related protein 2, tyrosinase-related protein 1/gp75, silver/gp100 protein, Melan-A/MART1, and the Lerk proteins (lerk-1/protein B61 and Lerk-5/Eplg5/Elf2/HTK-L). Tyrosinase is an enzyme that converts tyrosine into dihydroxyphenylalanine (DOPA), the precursor of melanin. The gene that codes for tyrosinase is found in all melanoma cells as well as normal melanocytes, but is not expressed in other cell types. The silver/gp100 protein (also referred to as Pmel [64]) is a melanosomal matrix protein that acts as a solid-phase substrate for the accumulation of melanin [65]. The gp100 peptide is a melanocyte differentiation antigen that is recognized by HLA-A2 restricted T-lymphocytes [66]. Another gene that is expressed only in cells of melanocytic origin is Melan-A [67]. The antigen encoded by Melan-A is called MART-1. MART-1 is only found on normal melanocytes, melanoma cells and retinal cells [68]. MART-1 is presented by HLA-A2 and is capable of causing a strong T-lymphocyte immune response [69].

Antigens that are expressed almost exclusively on tumor cells and extensively on melanoma cells include products of the MAGE gene family. MAGE genes are all located on the long arm of the X chromosome and are only found on the testis in normal adult tissue. The MAGE proteins are commonly expressed on melanoma cells and less frequently on the surface of other tumor cells. The genes MAGE-1, 2, 3, 4, 6, and 12 are expressed in different tumors such as breast carcinomas, non-small cell lung carcinomas, head and neck tumors, sarcomas, and ovarian carcinomas [70–73]. The products of MAGE-1 and MAGE-3 have been found to produce proteins that bind the different MHC class I molecules expressed on melanoma tumor cells. Products of MAGE-1 have been reported to be expressed on 48 % of metastatic melanomas, while products of MAGE-3 are expressed in >90 % of metastatic melanomas [74, 75]. Even though some proteins produced from MAGE genes have been characterized, their function on the surface of tumor cells is unknown.

Melanoma Gangliosides

Melanoma cells also express a number of gangliosides on their cell surfaces. Gangliosides are glycolipids that are characteristically found in neural crest-derived tissues [14]. Gangliosides regulate cell growth by altering growth factor signals and are involved in cell adhesion and cell matrix interactions [76]. The monosialoganglioside GM3 makes up 90 % of the gangliosides found on normal melanocyte cell surfaces, while the disialoganglioside GD3 comprises only 5 % [76]. It has been shown that quantitative and qualitative changes occur in the expression of gangliosides during oncogenic transformation [77]. The predominant gangliosides in melanoma are GM3 and GD3. GM3 is found on virtually all melanoma cells. GD3 is found on few normal melanocytes and at lower levels than on melanoma cells. The minor gangliosides are expressed on most melanoma cells but less often in normal tissues. These minor gangliosides include GM2, GD2, GT3, and 9-O-Ac-GD3 [78]. As a result, melanoma gangliosides are a possible target for immunotherapy against melanoma, and work has focused on several of these molecules in vaccine therapy.

Treatment Strategies for Melanoma

When detected early, melanoma responds well to treatment. However, it becomes a difficult cancer to treat once it extends beyond the skin. In 2014, more than 9700 people are estimated to have died from malignant melanoma in the US, and the incidence of the disease continues to increase yearly [2, 79]. Surgical excision of thin primary cutaneous melanoma that is detected early in melanomagenesis

remains the most effective therapy. The 5 year and 10 year survival rate is 97 % and 93 %, respectively, for fully excised cutaneous melanoma that is 1 mm or less in thickness, without ulceration, having less than 1 mitotic figure per millimeter squared, and without nodal involvement or distant metastases [4, 6]. In contrast, patients with melanoma that has metastasized to the viscera have a drastically reduced survival: the 1 year survival rate is 33 % and the 5 year survival is only 10–15 % [4, 6]. Novel therapies for melanoma have begun to improve the poor prognosis associated with thicker, more invasive melanomas, as well as metastatic disease. Recent advances in our understanding of the immunobiology of malignant melanoma offer the potential for new and more effective approaches for disease management. As immunological treatments are being combined with non-immunological therapy clinically, we begin with a brief discussion of non-immunological treatments.

Non-immunological Treatments

Surgical Treatments

As noted above, melanoma has an excellent prognosis when detected early and treated with surgical intervention. The depth of the tumor determines the local surgical margins, and for most thin tumors surgery is considered curative [80]. Tumor depth is reported as Breslow depth, the measurement in millimeters of the tumor invasion from the granular layer of the epidermis. However, the presence of local lymph node metastases (Stage III disease) is a strong predictor of poor clinical outcomes. Lymph node involvement in patients with melanoma has a 5-year survival rate of 46 %, compared to greater than 90 % for patients without lymph node involvement. The technique of sentinel lymph node biopsy (SLNB) has been developed as a way to identify patients with clinically occult nodal involvement, and is a far less morbid surgical intervention than empiric complete lymph node dissection (CLND) [6, 81–86]. The SLNB technique involves the injection of a vital blue dye or radiolabeled agent into the area of the primary tumor. This labeling allows for identification and subsequent sampling of the sentinel node (or nodes) during or prior to wide local excision [87]. Studies have shown that if the sentinel node is negative, there is less than a 1 % chance of positive nodes elsewhere in the lymph node basin [87]. This technique has been primarily studied in melanomas that are at high risk for metastatic disease but still amenable to early intervention – melanomas of intermediate depth of 1–4 mm [81, 85, 88]. Based heavily on the data from the Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1), the clinical practice guidelines from the American Society of Clinical Oncology and the Society of Surgical Oncology recommend that SLNB should be performed in patients with melanoma of intermediate depth, followed by CLND if the SLNB is positive. This trial has demonstrated that SLNB improves relapse free survival and regional disease control in melanoma, as well as providing prognostic and staging information for patient management [83, 85, 88–90]. However, in spite of significant optimism for this technique in the treatment of melanoma, clinical trials have not shown an overall survival benefit from SLNB, and, as a result, the technique remains a subject of much debate [91].

Traditional Chemotherapy

Dacarbazine (DTIC) is the only traditional chemotherapeutic agent approved for the treatment of advanced melanoma by the Food and Drug Administration (FDA). Clinical trials demonstrate that approximately 10–20 % of patients with metastatic melanoma have a measurable clinical response to the drug, but fewer than 5 % achieve complete responses [92]. Innumerable combinations of different chemotherapeutics have been tried in the treatment of metastatic melanoma [93]. In spite of this effort, a recent meta-analysis failed to show a survival benefit in several different multi-drug chemotherapy regimens when compared to DTIC alone [93]. There has also been interest in the drug temozolomide, an oral formulation of DTIC, in the management of melanoma. Temozolamide has not been shown to have improved efficacy over DTIC, but has been used for palliative treatment of melanoma with brain metastases, due to its improved ability to penetrate the central nervous system [94–97]. While traditional chemotherapy agents have not been shown to improve overall survival, they remain useful in select clinical cases as palliative options [96, 97].

Small Molecule Inhibitors

The mitogen-activated protein kinase (MAP-Kinase) pathway is a cellular signal transduction pathway that regulates cell division and differentiation. The majority of melanomas carry an activating mutation in the upstream serine-threonine protein kinase BRAF in this pathway, leading to uncontrolled cell growth and eventually tumor development. Genetic studies have shown that up to 90 % of the BRAF mutations in melanoma result from a substitution of glutamic acid for valine at amino acid position 600 (V600E mutation) [98]. This discovery has led to the development of small molecule inhibitors against the V600E target. The first of these agents, vemurafenib, demonstrated significant improvement in overall survival in patients with metastatic melanoma harboring the mutation as compared to DTIC in Phase III trials, leading to FDA approval for treatment of V600-mutated melanoma in 2011 [99–101]. Dabrafenib, another V600E small molecule inhibitor, was approved by the FDA for similar indications after demonstrating success in its Phase III clinical trial [102]. Vemurafenib is given orally at a dose of 960 mg twice

per day, while dabrafenib is given orally at a dose of 150 mg twice per day.

V600E-mutated melanomas eventually become resistant to BRAF inhibition, due to the development of bypass neuroblastoma RAS oncogene homolog (NRAS) pathway or downstream mitogen activated protein kinase kinase (MEK) mutation [103]. The discovery of this resistance prompted the development of trametinib, a small molecule agent that blocks downstream MEK targets. Trametinib is now approved by the FDA for salvage monotherapy and for use in combination with BRAF inhibitors in the treatment of metastatic melanomas harboring the V600E mutation [104, 105]. Trametinib is prescribed as 2 mg by mouth daily. Cobimetinib is another MEK inhibitor that has been shown to increase overall and progression-free survival when used in conjunction with BRAF inhibitors [106, 107].

C-KIT is a receptor tyrosine kinase that can activate downstream MAP-kinase pathway. Small molecule inhibitors of C-KIT, such as imatinib and nilotinib, were developed for the treatment of malignancies with c-kit mutations, most notably chronic myelogenous leukemia [108]. Recent discoveries of c-kit mutations, found in mucosal and acral melanomas, and melanomas arising from chronically sun-damaged skin, have led to studies of imatinib and nilotinib in melanoma therapy, although these remain in clinical trials at the present [109–111]. An overexpression of vascular endothelial growth factor (VEGF) has been found in some melanomas, and humanized monoclonal immunoglobulin bevacizumab, which targets VEGF-ligand, is also in clinical trials in patients with advanced melanoma [112, 113].

Immunological Therapies

Overview

Immunotherapeutic approaches to melanoma can be classified as those designed as adjuvant therapy after treatment of the primary lesion to prevent subsequent metastatic disease and those designed as immunotherapy for clinically apparent metastatic tumors. Adjuvant therapy is administered to patients at high risk for the development of metastatic disease, but who have no detectable disease at the time of surgical resection of the primary melanoma or after surgical resection of limited metastatic lesions such as to regional lymph nodes. High-risk melanoma patients include those with thick primary lesions in the skin, those with ulcerated melanomas, or those in which tumor cells are detected in regional lymph nodes. Current melanoma adjuvant treatment options are limited and, for the most part, ineffective.

Adjuvant therapeutic approaches for high-risk cutaneous melanoma have included clinical trials with interferons, specific and nonspecific immunotherapy, adjuvant chemotherapy and biochemotherapy, isolated limb perfusion, adjuvant radiation therapy, adjuvant hormonal therapy, and adjuvant retinoid therapy [114–116]. The development of active specific immunotherapy has resulted in melanoma vaccines employing purified gangliosides, shed tumor antigens, specific isolated tumor peptides, mechanical or viral melanoma cell lysates, antigen-primed dendritic cells, and allogeneic or autologous whole tumor cell preparations [117, 118]. More recently, vaccines are being made that target tumor-specific missense mutations, and these have been shown to increase anti-tumor immunity on a personalized level, bringing about the possibility for individualized melanoma immunotherapy that targets private tumor mutations [119].

Research into immune-based management of melanoma has focused not only on adjuvant therapy, but also in the treatment of advanced metastatic melanoma [80, 120]. These patients are a significant therapeutic challenge from an immunologic standpoint, because they are often immunosuppressed and debilitated. Nevertheless, a number of clinical immunotherapeutic trials for metastatic melanoma are currently in progress for patients with Stage IV disease, with some reaching FDA approval. The role of various cytokines as immunotherapy for melanoma treatment remains an active area of investigation [121], as well as the use of tumor vaccines and techniques such as adoptive immunotherapy [49, 118]. Antibodies that inhibit T-lymphocyte surface receptors, which when activated have a downregulatory effect, have also shown promise in melanoma therapy [122]. Whereas prior immunotherapies have shown little survival advantages over surgical therapy alone, novel new immunotherapy agents have shown great promise in demonstrating a survival benefit with an acceptable tolerability profile.

Immunotherapeutic Agents

Cytokines

The role of cytokines in immunotherapeutic approaches to cancer treatment remains an active area of investigation. Most of the data dealing with the role of melanoma-derived cytokines in the immunology of melanoma has been obtained by transfecting immunomodulating cytokine genes into neoplastic cells and then observing the biologic response in murine model systems. This approach has been useful to evaluate the *in vivo* consequences of tumor cell cytokine production in an animal host in a number of different tumor systems [123–127]. The *in vivo* consequences of tumor cell cytokine production have been evaluated for a number of cytokines including IL-2, IL-4, IL-18, IL-12, G-CSF, GM-CSF, M-CSF, IFNγ, IL-6, and IL-10 [55, 56, 123–131]. Although IL-2 and IFN-α are the only FDA approved cytokines for the treatment of melanoma, several other molecules in this family have also entered early clinical trials. IL-12 has shown promise in early studies, although systemic administration resulted in significant toxicities, including arthralgias,

neutropenia, arrhythmias, and hypotension [132]. However, new methods of targeted delivery, including electroporation and transfection of IL-12 plasmids directly into tumor, or the use of a fusion protein of IL-12 and an antibody that recognizes tumor-specific antigens, hold promise in minimizing side effects by limiting the action of IL-12 to the area immediately surrounding the tumor [133–135]. Recombinant human IL-18 had gone through Phase I and II testing and demonstrated only limited efficacy, although there remains interest in its use as an adjuvant agent [136, 137].

Interleukin-2

Currently, one of the few FDA approved treatments for metastatic melanoma is high dose systemic IL-2. High dose systemic intravenous IL-2 has been shown to induce a clinical response in 16 % of patients with metastatic melanoma, with 10 % of patients achieving partial response (reduction of tumor) and 6 % achieving complete response (resolution of tumor) [138]. While response rates to systemic IL-2 are low, recent studies demonstrate that the treatment appears capable of inducing durable responses in a small percentage of patients [139]. Follow-up data at 5 years indicates that, of the patients with metastatic melanoma who responded to IL-2 therapy longer than 30 months, none have had disease progression [121]. In order to determine which patients might benefit most from the treatment, attempts have been made to identify clinical, genetic or immune characteristics of melanoma patients who have a vigorous response to IL-2 [140, 141]. For instance, the majority of patients with metastatic melanoma receiving standard high dose IL-2 had an increase in immunosuppressive $CD4^+CD25^+$ Tregs, but those patients who achieved an objective clinical response had a significant decrease in Tregs [20, 142, 143]. Targeting patients who would be most likely respond to IL-2 would be beneficial, as IL-2 does not have a favorable toxicity and side effect profile. Most significantly, treatment results in severe vasodilation and capillary leak, leading to a septic shock profile with hypotension in 64 % of patients. These toxicities limit both the patients who can receive the medication and the medical centers eligible to administer this therapy [139]. Of note, intralesional IL-2 injection has been used with success and with decreased side effect profiles in case series to induce regression of in-transit metastases [144]. High dose systemic IL-2 is dosed as 600,000 international units (IU)/kg, given intravenously over 15 min every 8 h for a maximum of 14 doses. This is followed by 9 days of rest, with a maximum of 14 more doses. The patient is retreated if tumor shrinkage is observed.

Interferon

The FDA approved high dose interferon α-2b as an adjuvant treatment for thick melanoma lesions or positive nodal disease in 1995. The FDA approval stemmed from a Phase III trial demonstrating an improvement in disease free survival from 1.1 to 1.7 years and overall survival from 2.8 to 3.8 years with adjuvant interferon treatment after surgical excision [145]. However, subsequent trials have not supported these results and a recent meta-analysis concluded that interferon is associated with an increase in disease free survival, but not an improvement in overall survival [146, 147]. Additionally, attempts to modify the dosing of interferon in order to decrease toxicity and improve efficacy have not been successful. Trials with both intermediate and low dose levels of interferon-α have failed to show an improvement in survival [148–150]. In addition, side effects of interferon-α, including fever, chills, myelosuppression, neurologic deficit, and hepatotoxicity, are poorly tolerated, and affect a significant percentage of patients, with less than 40 % of patients able to complete at least 80 % of a full course of therapy [145]. As a result, the role of interferon-α as an adjuvant treatment in melanoma remains limited and controversial. Interferon-β had been shown to have inhibitory effect on melanoma cells in the Japanese population. However, genetic therapy with interferon-β has not shown significant efficacy in the treatment of metastatic melanoma in the United States [151, 152]. Today, efforts on combining interferon therapy with other immune-based therapies, as will be described later on, are underway [153]. The current standard dosing for interferon-α is an induction phase of 20 million IU/m^2 body surface area, through IV infusion for 5 consecutive days per week for 4 weeks, followed by a maintenance phase of 10 million IU/m^2 body surface area, through subcutaneous injection 3 times per week for 48 weeks.

GM-CSF

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a growth factor that functions as a potent inhibitor of the growth and progression of melanoma [55, 56]. This effect is mediated by the host immunological system and appears to involve activation of dendritic cells [154]. The anti-melanoma effects of GM-CSF have been evaluated in patients with high-risk Stage III and Stage IV disease, demonstrating an increase in disease-free and overall survival in a Phase II study of 48 patients [155]. Other studies have looked at GM-CSF as an adjuvant immunotherapy [156] and in perilesional injection of melanoma sites [157, 158]. Most current work focuses on the use of GM-CSF as an adjuvant therapy to increase immune response in vaccine trials [159].

Imiquimod

Imiquimod is an imidazoquinoline that is prepared in a topical formulation. Imiquimod binds to toll-like receptor 7 (TLR-7) on the surfaces of antigen presenting cells, inducing a signaling cascade that activates nuclear factor-kB (NF-kB). This activation leads to an upregulation of Th1 response cytokines, including TNF-α, IFN-α, IL-12, and IL-18, which

can then activate CD8 cells to become cytotoxic T-lymphocytes [160]. Imiquimod is FDA approved for use in the treatment of actinic keratosis, genital warts, and superficial basal cell carcinoma. Experimentally, imiquimod was found to be capable of inducing apoptosis in melanoma in initial *in vitro* and *in vivo* studies [161]. Subsequently, the off-label use of topical imiquimod for the treatment of melanoma, primarily the lentigo maligna variant of melanoma in situ, has been pursued. To date, the data on imiquimod's success has remained largely anecdotal case series, and studies have been hindered by insufficient patient follow-up time [162–165]. To date, no randomized controlled trials exist that compares imiquimod use versus surgical intervention, traditionally considered the gold standard for treatment of melanoma in situ [166]. Nevertheless, imiquimod remains an off-label option in patients with lentigo maligna who are not candidates for surgical therapy. Though case reports exist on the use of imiquimod for the treatment of metastatic melanoma, imiquimod is not recommended as therapy for invasive primary melanoma or metastatic melanoma [161, 166–169].

Biochemotherapy

Researchers have combined immunotherapy with other treatment modalities in an attempt to increase response rates. Several groups have administered IL-2 and IFN with traditional chemotherapy (i.e. dacarbazine or tamoxifen) for 'biochemotherapy.' Although some initial results were promising, larger trials have failed to show that the combinations selected result in longer survival times [170–173]. Pooled trial data has indicated increased response rates with biochemotherapy, but this comes at the cost of decreased quality of life and increased treatment related toxicity for patients [173].

Cytotoxic T-Lymphocyte Antigen 4 Blockade

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a surface glycoprotein that functions as an inhibitor to T-lymphocyte activation, working in opposition to CD28 [174]. Specifically, T-lymphocyte activation requires a costimulatory signal of the CD28 molecules on their surfaces binding to B7/CD80 on APCs. However, in locations such as the microenvironment surrounding melanoma, T-lymphocytes can express CTLA-4 that binds B7/CD80 instead, inducing T-lymphocyte anergy by preventing the CD28-B7 costimulatory signal. This T-lymphocyte anergy then allows melanoma cells to evade the immune system [21, 175].

Initial studies in murine models demonstrated that antibody inhibition of CTLA-4 could enhance the destruction of certain tumors, particularly those that were more immunogenic [176]. *In vitro* studies in humans also evaluated if inhibition of CTLA-4 could enhance the immune cell response against 'self' antigens, such as gp100 and Mart-1, which are expressed by melanoma cells [177–179]. Overall response rates, defined as tumor shrinkage of at least 30 %, in early trials were found to be around 15 % after treatment with an antibody to CTLA-4 administered alone or with other agents, such as vaccines against tumor antigens, and, as expected, response rates appear to correlate with the development of auto-immunity [180, 181]. In human trials, ipilimumab, a recombinant human monoclonal antibody against CTLA-4, demonstrated a median overall survival benefit of 4 months by itself or in combination with gp100 peptide vaccine, compared to the active control arm that used gp100 peptide vaccine alone [179, 182, 183]. Compared to dacarbazine monotherapy, ipilimumab with dacarbazine increased overall survival [184]. These promising data led to FDA approval of the medication in 2011 for metastatic melanoma treatment. Long-term studies of ipilimumab have also demonstrated a surprisingly durable response with this treatment, with a 3-year survival of 22 % in a multi-study pooled analysis, and a 5-year survival of 18 % in a smaller single trial analysis. Of note, the survival analyses was done irrespective of tumor shrinkage [185, 186]. However, the autoimmune side effects induced by CTLA-4 blockade can be severe, ranging from uveitis, gastroenteritis, colitis, dermatitis, and hypophysitis. Over half of patients treated with ipilimumab developed autoimmune side effects, with 20 % discontinuing treatment due to drug-related adverse events, bringing into question the tolerability of CTLA-4 blockade for a significant number of melanoma patients [182, 187]. Furthermore, ipilimumab may have a very slow onset of action. *Prieto et al*. demonstrated that in clinical trials of ipilimumab, patients who achieved clinical remission did so on average 30 months after the start of their therapy [188, 189]. Ipilimumab is given as an intravenous injection at 3 mg/kg every 3 weeks for a total of 4 doses. In a recent Phase III trial evaluating the use of adjuvant ipilimumab following resection of Stage III melanoma, a dose of 10 mg/kg was used, and while recurrence-free survival was improved, 52 % of patients had to discontinue treatment due to drug-related adverse events and 5 of 475 patients in the treatment arm died from therapy related adverse events [190].

Tremelimumab was another anti-CTLA-4 antibody evaluated as possible immune modifying therapy for melanoma. In clinical trials, while the treatment was tolerable to patients and the durability of treatment response was longer than traditional chemotherapy (i.e. DTIC), tremelimumab offered no survival advantage over DTIC [191, 192].

Programmed Cell Death Receptor (PD) 1 and PD-Ligand (PD-L) 1 Blockade

The PD-1 receptor is a T-lymphocyte surface receptor that binds two ligands, PD-L1 and PD-L2, that are found on the surfaces of tumor cells, including melanoma, renal cell carcinoma, colorectal cancer, and non-small cell lung cancer

[193, 194]. PD-1 and PD-L1 binding in the tumor microenvironment leads to T-lymphocyte exhaustion and inhibition, allowing for tumor evasion of the immune system [195–197]. Nivolumab, a humanized monoclonal antibody targeting the PD-1 receptor to prevent PD-L1 binding, was developed to prevent T-lymphocyte inhibition, thus allowing for continued immune response to tumor. In the initial Phase I trial of nivolumab, 28 % of patients with advanced melanoma enrolled in the study attained tumor regression, with the majority having a durable response to therapy of more than 1 year [198]. In a follow-up study of advanced melanoma patients treated with nivolumab, median overall survival was noted to be 16.8 months, with 1 year and 2 year survival rates of 62 % and 43 %, respectively, in all patients enrolled [198, 199]. Maintenance of response off-therapy was found to be at least 16 weeks in patients who had discontinued treatment [200]. Impressively, nivolumab was noted to be efficacious in patients who had previously failed ipilimumab and vemurafenib [199, 201–203]. Adverse events associated with nivolumab therapy in melanoma patients include fatigue, rash, and diarrhea, and rarely thrombocytopenia [200]. Of all patients treated with nivolumab, 1 % died of drug-induced pneumonitis, although none were patients with melanoma [200]. Nonetheless, nivolumab appears extremely well tolerated in patients with melanoma, with only 7 % of patients requiring discontinuation of treatment due to treatment associated side effects, a significant improvement compared to other melanoma therapies such as dacarbazine or ipilimumab [199, 202, 203]. Recently, a Phase III trial of nivolumab confirmed its high response rate, durable and rapid response, and improved safety profile, leading to FDA approval in December 2014 for melanoma refractory to ipilimumab, vemurafenib, and other available melanoma treatments [202, 204–206]. Dosing of nivolumab is 3 mg/kg intravenously every 2 weeks, until disease progression or side effects become unacceptable.

Pembrolizumb, formerly known as lambrolizumab, is another anti-PD-1 humanized monoclonal antibody. In a Phase I trial, pembrolizumab was shown to have an overall response rate of 38 %, with the response rates reaching 52 % in the study cohort that received the highest dose treatment [205]. This trial did not exclude patients who had previously been exposed to ipilimumab and demonstrated that ipilimumab exposure did not affect response rate to pembrolizumab. In Phase II trials, pembrolizumab increased progression free survival was comparable to traditional chemotherapy in patients with metastatic melanoma who had previously failed ipilimumab and/or BRAF inhibition [207]. Through the FDA Fast Track Program, pembrolizumab was approved in September 2014 [208]. Phase III trials for pembrolizumab are also underway. Pembrolizumab is given to the patient as a 2 mg/kg intravenous infusion every 3 weeks until disease progression or when side effects become unacceptable.

Other attempts to target this pathway include the development of antibodies to PD-L1. A Phase I trial of BMS-936559, a fully humanized monoclonal antibody against PD-L1, demonstrated efficacy in patients with metastatic melanoma, with an average response rate of 17 % in all treated patients with various dosing regimens, and a maximum of 29 % response rate for patients treated at a dose of 3 mg/kg, with more than half of responders having a durable response for more than 1 year [209]. Adverse reactions attributed to treatment included fatigue, infusion reaction, rash, and potential immune mediated reactions such as hypothyroidism, hepatitis, myasthenia gravis, sarcoidosis, endophthalmitis, and diabetes mellitus. 61 % of patients had adverse reactions, although most reactions were low grade, and only 11 % of patients required discontinuation of therapy due to side effects [209].

Combination Therapy

Significant interest exists in combining CTLA-4 blockade with BRAF inhibition in the treatment of BRAF V600E harboring melanoma because of the differences in their mechanisms of action. In addition, pre-clinical studies have shown that BRAF inhibition enhances T-lymphocyte recognition of melanoma, suggesting the combination of the two treatments may be synergistic [210–212]. A Phase I study in the United States of this concurrent therapy was unfortunately terminated due to hepatotoxicity [212]. However, an Italian study has shown that sequential treatment with vemurafenib followed by ipilimumab, or ipilimumab followed by vemurafenib, appears to be more tolerable while retaining improved efficacy over single agent therapies [213]. *Hodi et al*. also demonstrated benefits with combining ipilimumab and GM-CSF. In the study arm with ipilimumab plus GM-CSF, overall survival at 1 year was 68 %, versus 51 % in the study arm with ipilimumab alone. There was no significant difference in adverse events between the different treatment arms in this trial [214].

Combination blockade of both CTLA-4 and PD-1 has been studied in a recent Phase I trial of nivolumab and ipilimumab. *Wolchock et al*. reported that, at maximum acceptable doses of nivolumab and ipilimumab, 53 % patients with metastatic melanoma had an objective responses, all with tumor reduction of greater than 80 % [215].

Similarly, there are significant interests in combining CTLA-4 blockade with PD-1 inhibitors, taking advantage of a complementary blockade of different T-cell checkpoint pathways to enhance antitumor immunity [216]. In a large multicenter, randomized controlled Phase III study of 945 patients with unresectable stage III or IV melanoma, combination nivolumab and ipilimumab therapy led to a median progression-free survival of 11.5 months, compared to 2.9 months with ipilimumab alone, and 6.9 months with ipilimumab [217]. In a subgroup analyses of patients with tumors positive for PD-L1, combination of nivolumab and

ipilimumab therapy led to a progress-free survival of 14.0 months [217]. This combination of therapy, however, was noted to have increased risks of treatment-related adverse events, most commonly diarrhea, fatigue, pruritus, and rash, and these adverse events led to discontinuation of therapy for 36 % of patients, compared to 8 % for nivolumab therapy alone, and 15 % for ipilimumab therapy alone [217].

Adoptive Immunotherapy

Adoptive cell therapy (ACT) against melanoma antigens is achieved by the isolation of immunocompetent T-lymphocytes from patients, *in vitro* expansion of specific subsets of these cells, and then re-infusion of these T-lymphocytes back into patients. The source of infused lymphocytes can be derived from the tumor itself, which yields tumor infiltrating lymphocytes (TILs), from lymph nodes draining the site of the primary lesion, or from the peripheral blood. Several clinical trials have investigated various types of adoptive immunotherapy as treatment for metastatic melanoma, as well as a possible adjuvant therapy for less advanced disease [218–221]. Early studies demonstrated that tumor specific cytotoxic T-cells harvested from lymph nodes draining areas of melanoma showed little or no tumor specific cytotoxic activity, but that culture of these cells with irradiated autologous tumor cells in the presence of IL-2 rendered them cytotoxic to autologous tumor [222]. Treatment regimens for melanoma have included intravenous infusion of tumor infiltrating lymphocytes with IL-2 and intralesional injection of IL-2-cultured lymphoid cells. These methods have shown promise in small clinical trials [223–226]. IFN-α has also been tested in Phase I/II clinical trials as a suitable replacement for IL-2, with a far less toxic side effect profile [227]. More recently, in a pooled analysis, response rates to ACT for metastatic melanoma have been reported to be 50 %, with complete response in 20 % of patients. For complete responders, 95 % remain disease free at 5 year follow-up [228, 229].

One primary limitation of ACT has been the inability to generate a sustained level of tumor infiltrating lymphocytes in patients undergoing therapy. This is a significant setback, as the persistence of tumor infiltrating lymphocytes has been correlated with clinical response for *in vitro* trials [230]. Circulating myeloid cells in the peripheral blood, as well as Treg cells, suppress CD8+ T-lymphocyte function and proliferation, decreasing the clinical response to ACT [231, 232]. As a result, myeloablation and lymphodepletion prior to T-lymphocyte transfer have been shown to improve success with ACT [233, 234].

Chimeric antigen receptors (CARs) are constructed of antibody single-chain variable fragment combined with T-cell receptor (TCR) and T-lymphocyte costimulatory domains. The variable fragment of the antibody single chain can be engineered to target a tumor surface antigen, allowing T-lymphocytes to recognize tumor cells and proliferate independent of MHC function and without the need for additional costimulatory signals [235]. This approach has been successful in hematologic malignancies and studies are ongoing to investigate the coupling of ACT with CAR in metastatic melanoma [228, 235, 236].

Specific Immunotherapy

Specific immunotherapy agents stimulate the host immune system to recognize and destroy neoplastic cells bearing tumor-associated antigens. Also referred to as active specific immunotherapy, this approach often involves vaccination with modified melanoma cells or melanoma-derived antigens. Melanoma vaccines are currently under investigation for the treatment of advanced metastatic melanoma (Stage IV) and as an adjuvant treatment of patients at high risk for metastatic disease after surgical treatment (Stage II and III). Multiple trials with melanoma vaccines have been completed in the past decade, although there has been minimal overall success in Phase III clinical trials.

Autologous Vaccines

One approach to generate a specific host immune response is to vaccinate patients with their own tumor cells or cellular lysates made from their own tumor cells, known as autologous vaccination. For this procedure, melanoma tumor cells are harvested from the patient, processed *in vitro* (often in combination with an immunostimulatory agent), and injected subcutaneously back into the patient as an autologous vaccine. The theoretical advantage of this technique is that it targets the specific antigens found on the patient's tumor cells. Some of the major limitations to this approach are the number of tumor cells required for vaccine preparation and the labor-intensive and technically challenging nature of culturing and expanding the patient's melanoma cells.

Early randomized trials with autologous whole tumor cells plus BCG (Bacille Calmette-Guérin) failed to demonstrate improvement in overall outcomes [237–239]. Modulating a patient's tumor cells to increase the immune response has been attempted by haptenation of tumor cells with dinitroflourobenzene (DNFB). Administering these modified tumor cells, after sensitizing the patient to DNFB, appears to increase immune response and potentially survival in patients who develop cell-mediated immunity to tumor antigens [240, 241]. Additional work has focused on extraction of a patient's heat shock proteins from melanoma cells to create an autologous vaccine [242]. The vaccine, known as OncophageTM, consists of autologous tumor-derived heat shock protein gp96. In clinical trials, HLA restricted T-cells against the vaccine were induced in 50 % of patients, but the tumor response was modest at best [243, 244]. Taking advantage of the anti-tumor properties of GM-CSF by transfecting autologous melanoma cells with the gene for this molecule has also been explored [245, 246].

Using retrovirus mediated gene transfer for transfection, investigators have been able to create an autologous vaccine that increases tumor necrosis and invasion by immune cells in early studies [245, 246].

Allogeneic Vaccines

An alternative approach to autologous melanoma vaccines is the use of tumor vaccines derived from allogeneic melanoma cells. The goal of this approach is to stimulate the host immune system with a variety of common melanoma antigens. In addition to making a vaccine that would be widely available to many patients, the foreign MHC expression on the surface of these tumor cells further augments the patient's antitumor immune response. Multiple investigators have initiated clinical trials utilizing allogeneic vaccines as monotherapy for metastatic disease, as well as adjuvant therapy after surgical excision of the tumor. Improvement in time to disease progression was shown in a double-blind, placebo controlled trial of a polyvalent shed antigen vaccine. This small trial of 38 patients demonstrated a 2.5 times increase in time to disease progression, from 0.6 to 1.5 years, in the vaccine group [247]. Melacine, a vaccine derived from two melanoma cell line lysates administered with a detoxified end toxin adjuvant, has been studied in Phase III clinical trials, with no improvement in outcomes in advanced disease as compared to chemotherapy [248]. In a large trial studying this vaccine as adjuvant therapy in Stage II disease, no improvement was noted in relapse free survival [249], although a statistically significant improvement was seen in patients expressing HLA A2 or HLA C3 [250]. Canavaxin, an allogeneic vaccine derived from three melanoma cells lines mixed with BCG, also failed to show success in Phase III trials [251].

Viral Oncolysates

Administration of viral oncolysates is another form of specific immunotherapy that has shown potential in stimulating a clinical anti-melanoma response when used as adjuvant therapy [252]. Viral oncolysates are produced by infecting cultured melanoma cells with a virus to generate a mixture of lysed cells plus virion that can be administered as vaccines. Vaccinia virus melanoma cell oncolysates have shown promise in Phase I/Phase II studies in patients with Stage I and Stage II melanoma, but not in a Phase III study with Stage III melanoma patients [253]. Previous studies have demonstrated that patients with Stage III metastatic melanoma with palpable lymph node disease have responded to post-surgical vaccination with Newcastle disease virus (NDV) melanoma cell oncolysate [254, 255]. This group of patients treated with allogeneic melanoma cell oncolysates produced by the 73 T NDV strain had a 55 % survival at 15 year follow-up as compared to historical controls with 6–33 % 10 year survival rates [256]. The anti-tumor mechanisms of action triggered by NDV oncolysate are unknown, although some studies indicate that it may act in part through the induction of certain cytokines with anti-tumor properties [257].

Talimogene laherparepvec (T-VEC, OncoVEX) takes a different approach to viral oncolytic vaccination. T-VEC is a bioengineered strain of herpes simplex virus type 1 (HSV-1) that expresses human GM-CSF. After injection directly into melanoma, the virus replicates and causes a direct lytic effect, while the GM-CSF produced by the virus attracts and matures APCs [258, 259]. The APCs, after phagocytosing lysed melanoma cells, prime the T-lymphocyte mediated immune response to melanoma [259]. Of note, the virus has been engineered so as not to infect host cells [260]. In Phase II clinical trials, a 26 % response rate was cited, with 1 year overall survival of 58 % and 2 year overall survival of 52 % [261]. The Oncovex Pivotal Trial in Melanoma (OPTiM) trial is an on-going randomized Phase III trial; preliminary data suggests clinical response with T-VEC is 26 %, compared to 6 % in the treatment group of treatment with GM-CSF. Fatigue, chills, and pyrexia were the most common adverse events [262].

Peptide Vaccines

The identification of genes encoding melanoma specific antigens has led to their use in specific immunotherapy. As previously discussed, these are antigens that are expressed on cells of melanocytic lineage or melanoma cells that have been shown to play a role in tumor immunology. Multiple clinical trials have studied the effect of immunization against melanoma peptides such as gp100 [263], MAGE [264], MART [265] and tyrosinase in patients with advanced melanoma [266]. Positive clinical outcomes with peptide vaccines have been few, as obstacles such as tumor escape, preexisting neutralizing antibodies and antigen loss variants hamper vaccine success [17]. A multicenter randomized controlled trial demonstrated some success with the gp100 vaccine combined with IL-2 in patients with localized advanced Stage III melanoma or Stage IV metastatic melanoma. Clinical responses were 16 % in the treatment group, compared to 6 % in the control group that received IL-2 only, with progression free survival increased from 1.6 to 2.2 months [267].

Recently, tumor specific missense mutations, translated as amino acid substitutions, are being used to stimulate host response to melanoma cells. *Carreno et al*. harvested peptides with amino acid substitution seen on individual melanoma tumors (i.e. tumor neoantigens), isolated peptides that had high HLA-A*02:01 binding, and created vaccines that targeted multiple tumor antigens [119]. The targeting of both dominant tumor specific neoantigens and subdominant neoantigens, in theory, would prevent loss of efficacy from tumor evolution. These vaccines were found to stimulate tumor-specific immunity to specific, individual melanomas,

leading to possibility of personalized immunotherapy [119]. Clinical trials are expected to be underway in the near future.

Ganglioside Vaccines

Other investigators have focused on melanoma gangliosides as vaccine targets. Although expressed in all tissues, gangliosides are often overexpressed in melanoma cells. The minor ganglioside antigen GM2 has been used as a melanoma vaccine, administered in conjunction with BCG [268] or conjugated with keyhole limpet hemocyanin [269], due to its role as the most immunogenic ganglioside [118]. The conjugated form of the vaccine has been studied in a Phase III trial against interferon for adjuvant therapy of high-risk melanomas, but results were disappointing [270]. The major ganglioside antigens GD2 and GD3 have also sparked interest as possible vaccine targets, although both molecules are less immunogenic than GM3. As a result, investigations have focused on methods to increase the antibody response to these molecules [271] or to use anti-idiotypic monoclonal antibody vaccines [272–274].

Dendritic Cell Vaccines

Dendritic cell-based melanoma vaccines are also under investigation as another type of specific immunotherapy for the treatment of advanced melanoma [275]. The rationale behind this strategy is that APCs modified to present specific melanoma antigens will more effectively stimulate naïve T cells to generate an anti-melanoma response. Numerous preclinical studies have demonstrated that various types of APCs can be loaded *ex vivo* with tumor antigens and administered to tumor-bearing hosts to elicit T cell-mediated tumor destruction [276, 277]. An initial study with advanced melanoma patients used professional APCs pulsed *ex vivo* with purified melanoma peptides or tumor lysates, which were then delivered by direct injection into the inguinal lymph nodes of patients [278]. This study paved the way for other clinical trials using APCs exposed to multiple melanoma peptides [279, 280] or autologous tumor lysates [281, 282]. Although it is difficult to compare trials given the use of different antigens and different dendritic cell lineages, it appears that dendritic cell vaccines induce tumor responses in about 9.5 % of patients [275].

Summary

Understanding the immunobiology of melanoma has resulted in meaningful progress in developing effective agents to treat this aggressive malignancy. The recent FDA approval of several novel immunotherapeutic agents for metastatic melanoma has revitalized the field of melanoma immunology. New targets, refinements of current approaches, and improved patient selection criteria are necessary to optimize and develop other clinically efficacious agents for melanoma.

Questions

(Q1) Interleukin-2 therapy is limited by what type of side effects?

(Q2) What is the mechanism of action of ipilimumab?

(Q3) What is the mechanism of action of nivolumab?

(Q4) Can combination immunotherapy be utilized in the treatment of metastatic melanoma?

(Q5) Describe how adoptive cell therapy (ACT) works in immunotherapy of melanoma?

Answers

(A1) Interleukin-2 can lead to severe vasodilation and capillary leak, leading to a septic shock profile in 64 % of patients

(A2) Ipilimumab is an antibody that inhibits cytotoxic T-lymphocyte antigen 4 (CTLA-4). CTLA-4 on T lymphocytes can bind B7 on antigen presenting cells, which leads to T-lymphocyte anergy. With CTLA-4 blocked, T-lymphocyte anergy is thereby inhibited

(A3) Nivolumab is an antibody that targets the Programmed Cell Death Receptor 1 (PD-1). With PD-1 receptor blocked, PD-1 cannot bind PD-L1, which can otherwise lead to T-lymphocyte inhibition

(A4) Yes. In a large multicenter, randomized controlled Phase III study, combination of ipilimumab with nivolumab led to a progression free survival of 11.5 months, which is significantly increased from 2.9 months with ipilimumab alone, and 6.9 months with nivolumab alone

(A5) Immunocompetent tumor infiltrating lymphocytes are isolated from melanoma patients and expanded *in vitro*, then re-infused back into the patient

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