Mechanisms of Irritant and Allergic Contact Dermatitis

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3.1 Introduction

Contact dermatitis describes the skin reaction resulting from exposure to irritants (irritant contact dermatitis) or allergens (allergic contact dermatitis). In most patients, irritant and allergic contact dermatitis (ACD) are clinically indistinguishable. Also histopathologically, no distinguishing markers have been identified. This is in line with the fact that in irritant and ACD, principal inflammatory pathways are essentially similar. The pivotal different factor in ACD is the involvement of "allergen-specific" T cells as initiators of the inflammatory skin reaction. In irritant contact dermatitis (ICD), the inflammatory reaction mainly depends on either or both chemical and physical irritation. The most frequent chemical irritative factors are long-lasting and repetitive contacts to water, detergents, solvents, or a combination of these factors, often aggravated by too high or too low humidity. Inflammatory reactions to irritants are not triggered by one specific substance or cause, and do not show rapid amplification of severity by repeated insults and are thus called "unspecific."

In the following chapter the immunopathological mechanisms of ICD and ACD reactions will be discussed further in detail.

3.2 Irritant Contact Dermatitis

3.2.1 *Skin Barrier Perturbation Can Lead to Irritant Contact Dermatitis*

The skin functions as a barrier protecting an individual from dehydration, mechanical trauma, irradiation, microbial insults, and direct exposure to harmful sensitizing or

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irritant chemicals [1, 2]. Perturbation of the skin barrier can result in ICD. The barrier function is provided by the uppermost layer of the epidermis, the stratum corneum. The epidermis consists of more than 90% keratinocytes. Proliferating basal keratinocytes undergo a commitment to terminally differentiate and, in doing so, form a compact multilayered cellular compartment consisting of, depending on the skin region, approximately eight living cell layers (approximately 50–100 μm thick). As the keratinocytes become more differentiated, they approach the outermost layers and ultimately form the stratum corneum (10–20 μm thick). The stratum corneum consists of dead, terminally differentiated keratinocytes (corneocytes) embedded in extracellular lipid. The corneocytes and the lipid component of the stratum corneum can be considered as bricks and mortar and form the barrier to the environment and potentially harmful substances [3–5]. In order for a potential irritant to cause an irritant reaction, it must first penetrate or damage the stratum corneum to exert its effect on the viable epidermal and dermal layers below.

A chemical can penetrate the skin via three routes: the intercellular lipid route, the transcellular route across cornified cells and lipid bilayers, and via diffusion along hair follicles and sweat glands [4, 6–9]. Chemicals can also penetrate at sites of skin trauma (wounds) and where the barrier function is impaired by other diseases. Hydrophobic substances have the potential to penetrate via the lipid layers, whereas hydrophilic substances preferentially penetrate via the hair follicles and sweat lands. The lipid bilayer is the primary target for common skin damaging factors such as solvents and soaps since these substances degrade the lipid bilayer directly and expose the underlying viable epidermal layers to the irritant. Once an irritant has penetrated the stratum corneum, it may exert cytotoxic effects on the keratinocytes and trigger keratinocytes to release alarm signals in the form of cytokines and chemokines. In this way, the innate immune system is triggered and the ICD reaction is initiated.

3.2.2 Pathogenesis of Acute Irritant Contact Dermatitis

Thus, ICD reflects an innate inflammatory response of the skin to direct injury. Frequency and intensity of skin contacts with harmful agents determine the outcome. For acute ICD, the reaction is often caused by a single exposure to the irritant and the skin manifestations usually disappear within days to weeks. The source of the irritant is most often a chemical or abrasion to the skin. One of the major initial events before skin damage is observed is the release of proinflammatory cytokines. This in turn amplifies the inflammatory reaction by releasing chemokines, resulting in vasodilation and infiltration of cells (e.g., lymphocytes, eosinophils, macrophages, neutrophils, T cells) into the epidermis and dermis. The resulting physiological signs of irritation are damage to the epidermis as observed by spongiosis and microvesicle formation, erythema, induration, and edema leading to localized painful areas of skin [10–13] (Fig. 3.1).

However, the clinical appearance is often very variable and, moreover, difficult to distinguish from ACD [14, 15]. ACD shows all the features of ICD, but in an accelerated and/or augmented fashion due to the involvement of allergen-reactive T cells. Proinflammatory cytokines locally released by the latter, such as IFN-γ, IL-4, and IL-17, as will be discussed below, serve to amplify the overall inflammatory reactivity and protect the body against potentially harmful agents. Indeed, clinical observations show a clear role for irritancy in ACD: virtually all allergens have irritant properties, whereas irritated skin is easier to sensitize than nonirritated skin. During both an ACD and an ICD reaction, alarm signals provided by skin barrier disruption, epidermal cellular changes and cytokine/chemokine release, stimulate the initial trafficking of immune cells to the site under attack.

Core Message

› In acute ICD, similar immunological mechanisms are involved as in acute ACD. However, the crucial difference is the involvement of specific T cells in ACD. Major events in acute contact dermatitis include damage of the epidermal skin barrier by contact irritants and subsequent activation of unspecific innate immune responses.

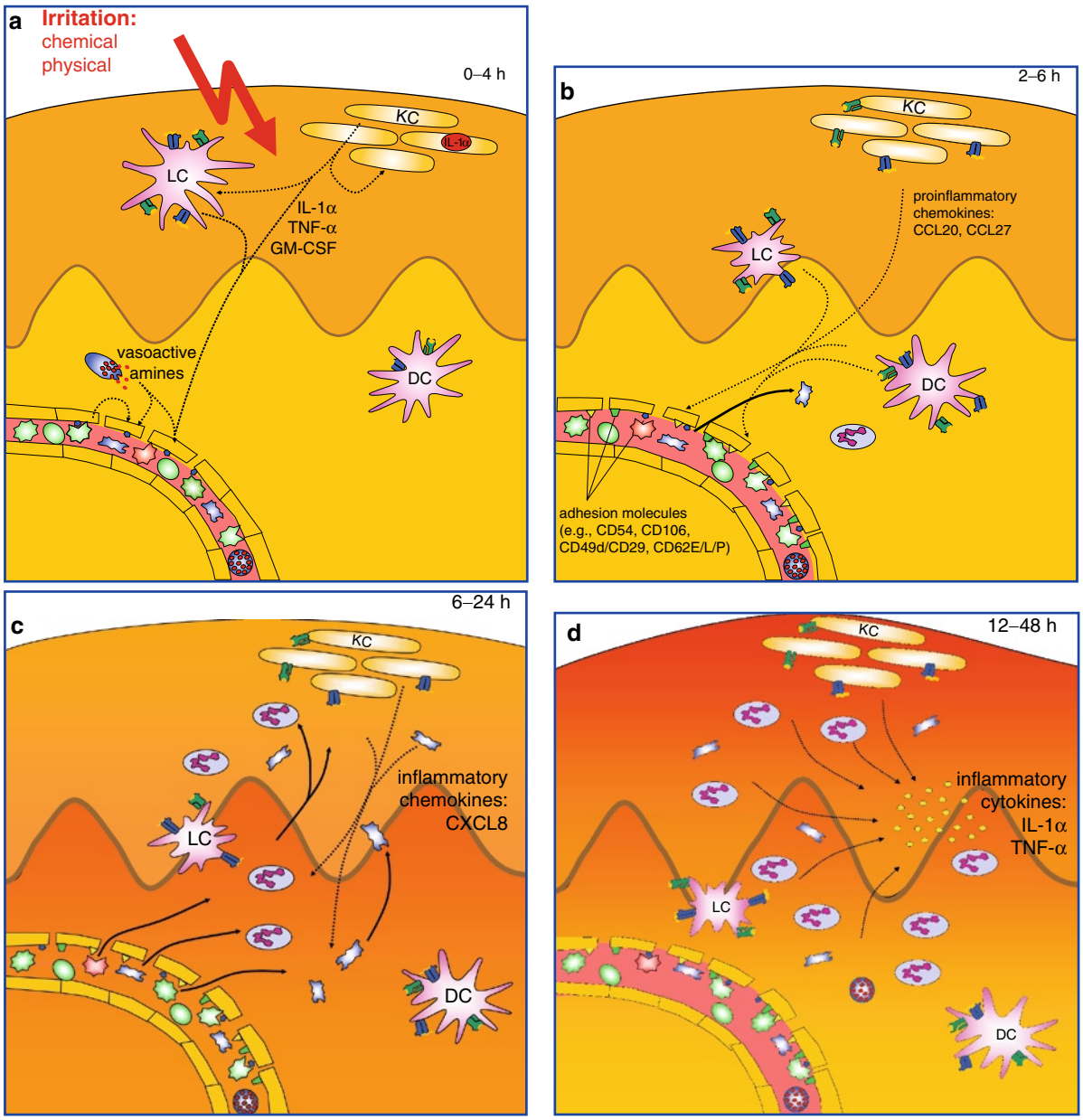

Fig. 3.1 (**a**–**d**) Immunological events in irritant contact dermatitis (ICD). (**a**) Physical and/or chemical irritation triggers the fast release of prestored cytokines and other inflammatory mediators, termed "danger signals." (**b**) In response to the release of these danger signals proinflammatory chemokines from resident epidermal and dermal cells. (**c**) Subsequently, inflammatory chemokines are secreted from resident cells and already infiltrated inflammatory cells. A major cytokine is this process is CXCL8 (formerly known as IL-8). (**d**) As a consequence, from the producion of inflammatory chemokines, more and more inflammatory cells, including neutrophils (), are attracted and, under the influence of inflammatory triggers, secrete inflammatory mediators. This results in the clinically visible acute ICD

3.2.3 Development of Chronic Irritant Contact Dermatitis

Chronic ICD is one of the most frequent forms of ICD and is caused by repeated contact of the skin to weak irritants [16, 17]. Multiple subthreshold skin damaging exposures, each starting before complete recovery from the previous insult, result in this eczematous skin condition (Fig. 3.2). Chronic ICD is characterized by dryness, fissuring, and hyperkeratosis (more pronounced than in acute ICD) and is diagnosed when the ICD persists for longer than 6 weeks. It is often located on the hands, and despite removal of the irritant, the clinical reaction may remain for several years. Factors such as water, detergents, organic solvents, oils, alkalis, acids, oxidizing agents, heat, cold friction all contribute to the elicitation of chronic ICD [16, 17]. Such factors are frequently associated with a wet working environment, and therefore, chronic ICD is a frequent work-related dermatitis [18, 19]. A wet work environment is defined as regular work with the hands in a wet environment for longer than 2 h per day, regular use of occlusive gloves over the same period of time, and / or frequent and intensive hand washing (approximately 20 times per day) [18, 20].

Chronic ICD is a multifactorial disorder in that both exogenous and endogenous factors are involved in its development. The exogenous factors have already been mentioned above and involve direct exposure of the skin to irritants. Endogenous factors are based on the individual's susceptibility to develop chronic ICD. These factors include variations in the skin barrier structure and composition, innate immune reactivity variations, and a skin atopic background. In the past, differences in transepidermal water loss (TEWL), erythema, irritation thresholds, and gender-related differences have been investigated [21–27]. However, no significant differences were found that could explain why one individual develops chronic ICD at the work environment, whereas another individual in the same work environment does not. Recently, much attention has been paid to atopic dermatitis (AD) as a potentially important predisposing factor since a history of AD more than quadruples the risk of hand eczema in cases of skin exposure in a wet work environment [28]. It was shown that penetration of the irritant SLS and subsequent increase in TEWL and erythema was higher in subjects with AD than in healthy individuals indicating that more permeable skin is more susceptible to irritants [29]. Also, genetic risk factors have been linked with the development of AD, which in turn may influence the development of chronic ICD [30–34].

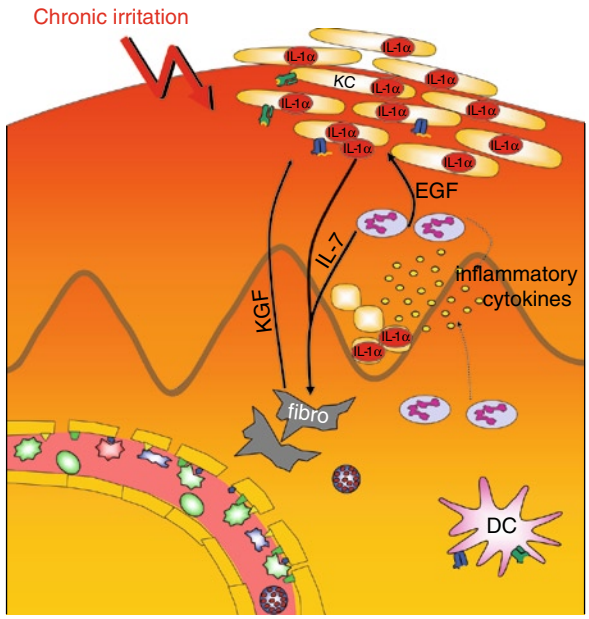

Fig. 3.2 Chronic ICD results from the continuing presence of various inflammatory triggers. Keratinocytes at skin sites of former inflammatory skin reactions contain higher levels of stored IL-1α. Upon exposure to unspecific inflammatory triggers, IL-1α is easily secreted and stimulates the activation of the inflammatory cascade. As a consequence of continuing exposure to inflammatory triggers, epidermal, dermal, and infiltrated inflammatory cells produce different growth factors, including epidermal growth factor (EGF) and keratinocyte growth factor (KGF). These growth factors stimulate proliferation of fibroblasts and keratinocytes, which results in the hyperkeratotic and desquamating clinical phenotype of chronic ICD

Core Message

› In chronic ICD, repetitive skin contacts to different contact irritants cause substantial and prolonged skin barrier damage. A skin atopic background is a strong risk factor for developing chronic ICD.

3.2.4 Genetic Risk Factors in Irritant Contact Dermatitis and Atopic Dermatitis

AD is a hereditary disease and, as already mentioned, is an important predisposing factor for chronic ICD. It is associated with hyperreactivity of the skin to irritants, aero-allergens, microbes, and scratching. Next to overexpression of the epithelial cell and fibroblast-produced cytokine TSLP [35], recently two loss-of-function polymorphisms in the gene encoding for filaggrin have been described as strong predisposing factors for AD [33]. Depending on the disease phenotype, 16–56% of patients with AD carry one or more filaggrin null mutations compared to only 5–10% of the general European population [33, 34, 36–39]. Filaggrin is involved in the formation of corneocytes, and therefore, in the formation of the stratum corneum [37]. Once cornification is complete, filaggrin is degraded into free amino acids. These free amino acids contribute to the natural moisturizing factor component of the stratum corneum by retaining water. Therefore, it is possible that filaggrin null alleles may be responsible in part for the dry skin characteristic of AD. De Jong et al. showed that FLG null alleles are associated with increased susceptibility to chronic ICD; however, whether or not the filaggrin null allele is an independent risk factor needs further study [32].

Until now, no genetic factors have been identified that contribute to hand eczema in the absence of AD. However, it is possible that unidentified polymorphisms in cytokines and chemokines may be involved in chronic ICD. Indeed, such polymorphisms have already been reported in several other inflammatory diseases e.g., rheumatoid arthritis and multiple sclerosis [40]. A case–control study in 197 patients with chronic ICD vs. 217 healthy individuals showed that polymorphisms in several cytokine genes (IL-1α, IL-1β, IL-8, IL-10 and TNF-α) and the two loss-of-function polymorphisms in the filaggrin gene did not provide a substantial risk factor for development of chronic ICD [30]. However, the study did show that (1) both the variant TNFA-308A allele and the filaggrin null alleles predispose to flexural eczema, (2) the variant TNFA-308A allele can increase susceptibility to chronic ICD, and (3) the IL1A-889T allele might protect against hand dermatitis. Here, the ratio of IL-1 receptor antagonist (IL-RA)/IL-1α increased 2–3-fold, corresponding to a reduced level of agonistic IL-1a in the stratum corneum in subjects expressing the variant genotype as compared to the wild type genotype. In conclusion, genetic polymorphisms of TNFA-308 and IL1A-889 may influence the susceptibility of chronic ICD.

3.2.5 Cellular Immunological Changes in Irritant Contact Dermatitis

Next to its barrier function, the skin is recognized as an immunologically active organ. Barrier perturbation results in the generation of the first alarm signal. Skin epidermal cells, notably keratinocytes, melanocytes and Langerhans cells (LC), respond to nonspecific irritant stimuli by producing cytokines, adhesion molecules, and chemotactic factors [41–43]. Keratinocyes are the major source of skin derived cytokines. Epidermal cytokines diffuse into the dermis and trigger dermal cells (e.g., fibroblasts and endothelial cells) to also secrete chemokines [44, 45]. In this way the proinflammatory response is amplified and a chemotactic gradient is introduced directing infiltrating cells into the site of tissue damage. The initial proinflammatory response can result in Langerhans' cell migration out of the epidermis, potentially contributing to allergenicity, and infiltration of monocytes, neutrophils, macrophages, and lymphocytes into the skin. This skin innate immune response is rapid, provides the initial line of defense against damage caused by irritants, is antigen-nonspecific, and lacks immunological memory [13, 46, 47].

As stated, ACD and ICD reactions share alarm signal(s) [14, 48, 49]. This is supported by several in vivo and in vitro studies in which both allergen and irritant exposures result in increased cytokine levels in keratinocytes and fibroblasts [41, 45]. So, which is the initiating cytokine, and is it prestored or does de novo synthesis occur? Of all the cytokines produced by keratinocytes, only IL-1α, IL-1β, and TNF-α activate a sufficient number of effector mechanisms to independently trigger cutaneous inflammation [47]. Furthermore, large stores of preformed and biologically active IL-1α have also been detected as a depot in the stratum corneum and within the epidermis [50, 51]. In contrast, other cytokines such as TNF-α and IL-8 are detectable only at low amounts deep within the stratum corneum

[50, 52]. These results strongly suggest that the release of prestored IL-1α upon barrier perturbation is the initiating cytokine signal, which triggers the induction of other inflammatory mediators. Upon release, IL-1α stimulates further release of IL-1α and the production and release of other cytokines. While resting keratinocytes produce some cytokines constitutively, exposure to irritants induces production of (pro-) inflammatory cytokines (IL-1α, TNF-α), chemotactic cytokines (IL-6, IL-8, CCL20, CCL27), growth-promoting cytokines (GM-CSF, TGF-β), and cytokines regulating specific immune responses (IL-10, IL-12, IL-18) [11, 43]. Thus, via cytokine cascades, an inflammatory response can be rapidly generated. In this way, keratinocytes act as proinflammatory signal transducers, responding to nonspecific external stimuli with the production of inflammatory cytokines, adhesion molecules, and chemotactic factors, stimulating the dermal stroma to amplify the response.

In this context, it should be mentioned that in the skin, TNF-α is stored in dermal mast cells, and following stimulation, it is produced by keratinocytes and LC. Antibodies to TNF-α abolish many inflammatory skin reactions, including allergic and ICD [53]. An in vitro study using a full thickness human skin equivalent model showed that antibodies directed against either TNF-α or IL-1α were able to completely inhibit inflammatory chemokine secretion by dermal fibroblasts [45]. Therefore, taken together, these findings suggest that both TNF-α and IL-1α are pivotal cytokines in mediating irritant induced skin inflammation. In conclusion, several cell types and downstream mechanisms act in concert in inducing different types of skin irritant responses. Determining the cell source, kinetics of production, and the regulation of inflammatory mediators in the skin will be the key to predicting and treating irritant responses arising from different environmental agents (Table 3.1).

Core Message

› Unspecific innate immune reactions cause the development of ICD reactions. Some genetic risk factors including polymorphisms in TNF-α genes have been detected. Further research is needed to unravel the inflammatory innate immune cascades involved in ICD.

Table 3.1 Cytokines, chemokines, and growth factors expressed by epidermal cells and dermal fibroblasts

Cell type	Cytokine/chemokine/growth factors
Epidermal cells Keratinocyte	Cytokines: IL-1α, IL-1β, IL-1RA, IL-3 (mouse), IL-6, IL-7, IL-8 (human), IL-10, IL-12, IL-15, IL-18, IL-20, IL-23, IL-24, IL-33, TNF-α, TGF-α, TGF-β Chemokines: CCL2, CCL5, CCL20, CCL27, CXCL1, CXCL10, CXCL14 (mouse) Growth factors: G-CSF, GM-CSF, M-CSF
Langerhans' cell	Cytokines: IL-1α, IL-1β, IL-6, IL-15, IL-18, IL-23, TNF-α, TGF-β Chemokines: CCL3, CXCL1, CXCL14
Melanocyte	Cytokines: IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12, IL-24, TNF-α, TGF-α, TGF-β Chemokines: CCL2, CCL5, CXCL1, CXCL14 (mouse) Growth factors: G-CSF, GM-CSF, M-CSF
Dermal fibroblast	Cytokines: TNF-α, IL-8, IL-6 Chemokines: CCL2, CCL5, CCL20, CXCL1, CXCL12

Cytokines may be constitutively expressed or induced upon irritant stimuli [11, 41, 43–47]

3.3 Introduction Allergic Contact Dermatitis

During the past few decades, our understanding of why, where, and when ACD might develop has rapidly increased. Critical discoveries include the identification of T cells as mediators of cell-mediated immunity, their thymic origin and recirculation patterns, and the molecular basis of their specificity to just one or few allergens out of the thousands of allergens known. Progress has also resulted from the identification of genes that determine T-cell function and the development of monoclonal antibodies that recognize their products. Moreover, the production of large amounts of recombinant products, e.g., cytokines and chemokines, and the breeding of mice with disruptions in distinct genes (knock-out mice) or provided with additional genes of interest (transgenic mice) have allowed in-depth analysis of skin-inflammatory processes, such as those taking place in ACD.

Although humoral antibody-mediated reactions can be a factor, ACD depends primarily on the activation of allergen-specific T cells [54], and is regarded as a

prototype of delayed hypersensitivity, as classified by Turk [55] and Gell and Coombs (type IV hypersensitivity) [56]. Evolutionarily, cell-mediated immunity has developed in vertebrates to facilitate eradication of microorganisms and toxins. Elicitation of ACD by usually nontoxic doses of small molecular-weight allergens indicates that the T-cell repertoire is often slightly broader than one might wish. Thus, ACD can be considered to reflect an untoward side effect of a well-functioning immune system.

Subtle differences can be noted in macroscopic appearance, time course, and histopathology of allergic contact reactions in various vertebrates, including rodents and man [57]. Nevertheless, essentially all basic features are shared. Since both mouse and guinea pig models, next to clinical studies, have greatly contributed to our present knowledge of ACD, both data sets provide the basis for this chapter.

In ACD, a distinction should be made between induction (also known as sensitization or primary) and effector (also known as elicitation or secondary) phases [58] (Fig. 3.3). The induction phase includes the events following a first contact with the allergen and is complete when the individual is sensitized and capable of giving a positive ACD reaction. The effector phase begins upon elicitation (challenge) and results in clinical manifestation of ACD. The entire process of the induction phase requires at least 4 days to several weeks, whereas the effector phase reaction is fully developed within 1–4 days. Main episodes in the induction phase (steps 1–5) and effector phase (step 6) are:

1. *Binding of allergen to skin components.* The allergen penetrating the skin readily associates with all kinds of skin components, including major histocompatibility complex (MHC) proteins. These molecules, in humans encoded for by histocompatibility antigen (HLA) genes, are abundantly present on epidermal antigen presenting cells, called LC.
2. *Hapten-induced activation of allergen-presenting cells.* Allergen-carrying LC become activated, mature, and travel via the afferent lymphatics to the regional lymph nodes, where they settle as so-called interdigitating cells (IDC) in the paracortical T-cell areas.
3. *Recognition of allergen-modified LC by specific T cells.* In nonsensitized individuals the frequency of T cells with certain specificities is usually far below one per million. Within the paracortical areas, conditions are optimal for allergen-carrying IDC to encounter naïve T cells that specifically recognize the allergen–MHC molecule complexes. The dendritic morphology of these allergen-presenting cells strongly facilitates multiple cell contacts, leading to binding and activation of allergen-specific T cells.
4. *Proliferation of specific T cells in draining lymph nodes.* Supported by interleukin (IL)-1, released by the allergen-presenting cells, activated T cells start producing several growth factors, including IL-2. A partly autocrine cascade follows since at the same time receptors for IL-2 are upregulated in these cells, resulting in vigorous blast formation and proliferation within a few days.
5. *Systemic propagation of the specific T-cell progeny.* The expanded progeny is subsequently released via the efferent lymphatics into the blood flow and begins to recirculate. Thus, the frequency of specific effector memory T cells in the blood may rise to as high as one in a thousand, whereas most of these cells display receptor molecules facilitating their migration into peripheral tissues. In the absence of further allergen contacts, their frequency gradually decreases in subsequent weeks or months, but does not return to the low levels found in naive individuals.
6. *Effector phase.* By renewed allergen contact, the effector phase is initiated, which depends not only on the increased frequency of specific T cells, and their altered migratory capacities, but also on their low activation threshold. Thus, within the skin, allergen-presenting cells and specific T cells can meet, and lead to plentiful local cytokine and chemokine release. The release of these mediators, many of which have a proinflammatory action, causes the arrival of more inflammatory cells, thus further amplifying local mediator release. This leads to a gradually developing eczematous reaction that reaches its maximum after 18–72 h and then declines.

In the following sections, we will discuss these six main episodes of the ACD reaction in more detail. Furthermore, we will discuss local hyperreactivity, such as flare-up and retest reactivity, and hyporeactivity, i.e., upon desensitization or tolerance induction.

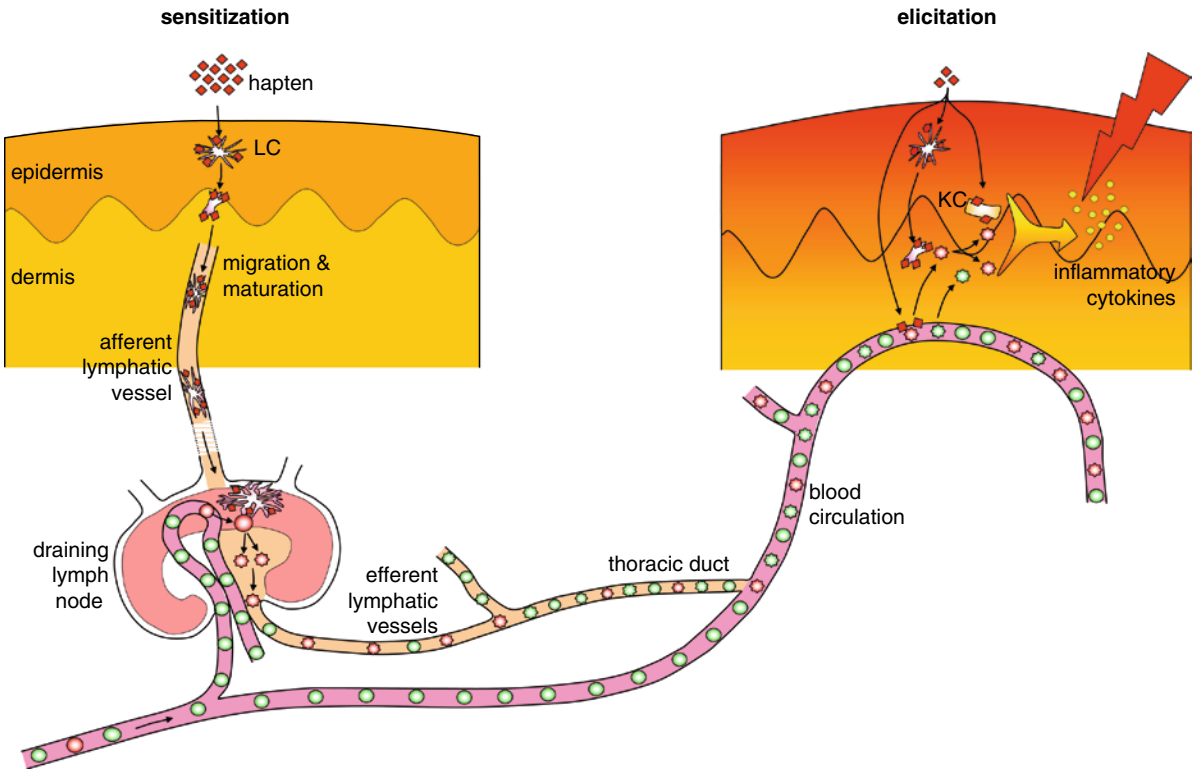

Fig. 3.3 Immunological events in allergic contact dermatitis (ACD). During the induction phase (*left*), skin contact with a hapten triggers migration of epidermal Langerhans cells (*LC*) via the afferent lymphatic vessels to the skin-draining lymph nodes. Haptenized LC home into the T cell-rich paracortical areas. Here, conditions are optimal for encountering naïve T cells that specifically recognize allergen–MHC molecule complexes. Hapten-specific T cells now expand abundantly and generate effector and memory cells, which are released via the efferent lymphatics into the circulation. With their newly acquired homing receptors, these cells can easily extravasate peripheral tissues. Renewed allergen contact sparks off the effector phase (*right*). Due to their lowered activation threshold, hapten-specific effector T cells are triggered by various haptenized cells, including *LC* and keratinocytes (*KC*), to produce proinflammatory cytokines and chemokines. Thereby, more inflammatory cells are recruited further amplifying local inflammatory mediator release. This leads to a gradually developing eczematous reaction, reaching a maximum within 18–48 h, after which reactivity successively declines

3.3.1 Binding of Contact Allergens to Skin Components

3.3.1.1 Chemical Nature of Contact Allergens

Most contact allergens are small, chemically reactive molecules with a molecular weight less than 500 Da [59] (Fig. 3.4). Since these molecules are too small to be antigenic themselves, contact sensitizers are generally referred to as haptens.

Upon penetration through the epidermal horny layer, haptens readily conjugate to endogenous epidermal and dermal molecules. Sensitizing organic compounds may covalently bind to protein nucleophilic groups, such as thiol, amino, and hydroxyl groups, as is the case with poison oak/ivy allergens (reviewed in [60, 61]). Examples of contact allergens containing electrophilic components include aldehydes, ketones, amides, or polarized bonds. Metal ions, e.g., nickel cations, instead form stable metal–protein chelate complexes by coordination bonds [62]. The most reactive nucleophilic side chains are those found in the amino acids lysine, cysteine and histidine [63]. Of note, their degree of ionization and hence nucleophilicity is dependent on the pH of the microenvironment, which is influenced by surrounding amino acids as well as protein location within the epithelium [64]. Predicting the chemicals that can function as haptens in ACD as well as identifying cutaneous proteins

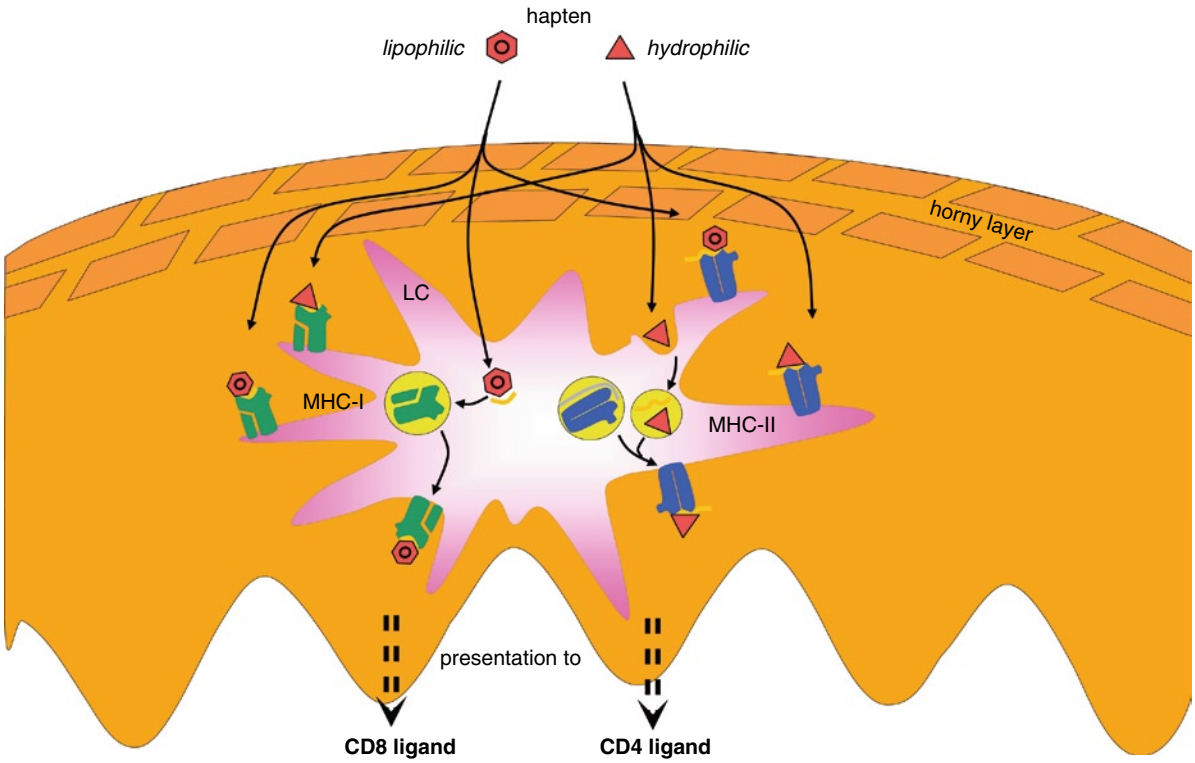

Fig. 3.4 Hapten presentation by epidermal Langerhans cells. Allergen penetrating into the epidermis readily associates with all kinds of skin components, including major histocompatibility complex (*MHC*) proteins, abundantly present on epidermal Langerhans cells (*LC*). Both MHC class I and class II molecules may be altered directly or via intracellular hapten processing and, subsequently, be recognized by allergen-specific $CD8^+$ and $CD4^+$ T cells

involved in hapten–protein complexes is the subject of current intense investigations [65, 66] and discussed in more detail elsewhere in this textbook.

3.3.1.2 Hapten Presentation by Langerhans Cells (LC)

Sensitization is critically dependent on direct association of haptens with epidermal LC-bound MHC molecules, or peptides present in the groove of these molecules. Both MHC class I and class II molecules may be altered this way, and thus give rise to allergen-specific $CD8^+$ and $CD4^+$ T cells, respectively. Distinct differences between allergens can, however, arise from differences in chemical reactivity and lipophilicity (Fig. 3.4), since association with MHC molecules may also result from internalization of the haptens, followed by their intracellular processing as free hapten molecules or hapten–carrier complexes. Lipophilic haptens can directly penetrate into LC, conjugate with cytoplasmic proteins, and be processed along the "endogenous" processing route, thereby favoring association with MHC class I molecules [67]. In contrast, hydrophilic allergens such as nickel ions may, after conjugation with skin proteins, be processed along the "exogenous" route of antigen processing and thus favor the generation of altered MHC class II molecules. Thus, the chemical nature of the haptens can determine to what extent allergen-specific $CD8^+$ and/ or $CD4^+$ T cells will be activated [68–70].

3.3.1.3 Pre and Prohaptens

Whereas most contact allergens can form hapten–carrier complexes spontaneously, some need activation first. Contact allergens requiring activation outside the

body, e.g., by UV-light or oxygen, are called prehaptens [71, 72]. The typical photoallergen tetrachlorosalicylanilide is a prototype of this. Tetrachlorosalicylanilide, which undergoes photochemical dechlorination with UV irradiation, ultimately provides photoadducts with skin proteins [73]. Contact allergens dependent on activation inside the body, e.g., by enzyme-induced metabolic conversion, are referred to as prohaptens. A classical prohapten is *p*-phenylenediamine, which needs to be oxidized by *N*-acetyltransferases to a reactive metabolite that can form a trimer, known as Bandrowski's base [74, 75]. Reduced enzyme activity in certain individuals, related to genetic enzyme polymorphisms, explains the reduced risk of sensitization to prohaptens that need enzymatic activation [76, 77]. Subsequent chapters of this book will present in extensive detail the numerous groups of molecules that have earned disrepute for causing ACD.

Core Message

› Allergenicity depends on several factors determined by the very physicochemical nature of the molecules themselves, i.e., their capacity to penetrate the horny layer, lipophilicity, and chemical reactivity. The sensitizing property of the majority of contact allergens could be predicted from these characteristics [63, 78]. Two other factors, however, further contribute to the allergenicity of chemicals, viz their proinflammatory activity and capacity to induce maturation of LC. These issues will be dealt with in more detail in the following sections.

3.3.2 Hapten-Induced Activation of Allergen-Presenting Cells

3.3.2.1 Physiology of Langerhans Cells

Although originally thought to be neurons based on their staining properties and cellular morphology [79], LC were subsequently surmised to function as "professional" antigen-presenting-cells [80]. They form a contiguous network within the epidermis and represent 2–5% of the total epidermal cell population [81]. Their principal functions are internalization, processing, transport, and presentation of skin-encountered antigens [82, 83]. As such, LC play a pivotal role in the induction of cutaneous immune responses to infectious agents as well as to contact sensitizers [84–86]. Recent studies of LC indicate that this cell type has direct epidermal innervations and can respond to a number of neurotransmitters (among them are calcitonin gene-related peptide, α-melanocyte stimulating hormone, and substance P). Most of the experimental evidence to date indicates a suppressive effect of the neurohormones and neuropeptides on Langerhans cell function and cutaneous inflammation, but it has become evident lately that the timing of exposure to a stimulus is critical to the outcome of the immune response. Thus, administration of a stress hormone or exposure to a stressor before the LC encounters an allergen may diminish the immune response toward that substance, while a stressor may enhance immune function when acting on a maturing LC or before reexposure to the allergen [87]. LC originate from $CD34^+$ bone marrow progenitors, entering the epidermis via the blood stream [88]. Their continuous presence in the epidermis is also assured by local proliferation [89, 90]. They reside as relatively immature DC, characterized by a high capacity to gather antigens by macropinocytosis, whereas their capacity to stimulate naïve T cells is still underdeveloped at this stage [91]. Their prominent dendritic morphology and the presence of distinctive Birbeck granules were observed long ago [79, 92, 93]. In the last decade, their pivotal function in the induction of skin immune responses was explained by high expression of molecules mediating antigen-presentation (e.g., MHC class I and II, CD1), as well as of cellular adhesion and costimulatory molecules (e.g., CD54, CD80, CD86, and cutaneous lymphocyte antigen [CLA]) [94–96].

3.3.2.2 Hapten-Induced LC Activation

Upon topical exposure to contact sensitizers, or other appropriate stimuli (e.g., trauma, irradiation), up to 40% of the local LC become activated [97, 98], leave the epidermis, and migrate, via afferent lymphatic vessels, to the draining lymph nodes [99] (Fig. 3.5). This process of LC migration results from several factors, including contact allergen-induced production of cytokines favoring LC survival [100–102] and loosening from surrounding keratinocytes [103–105]. Thus,

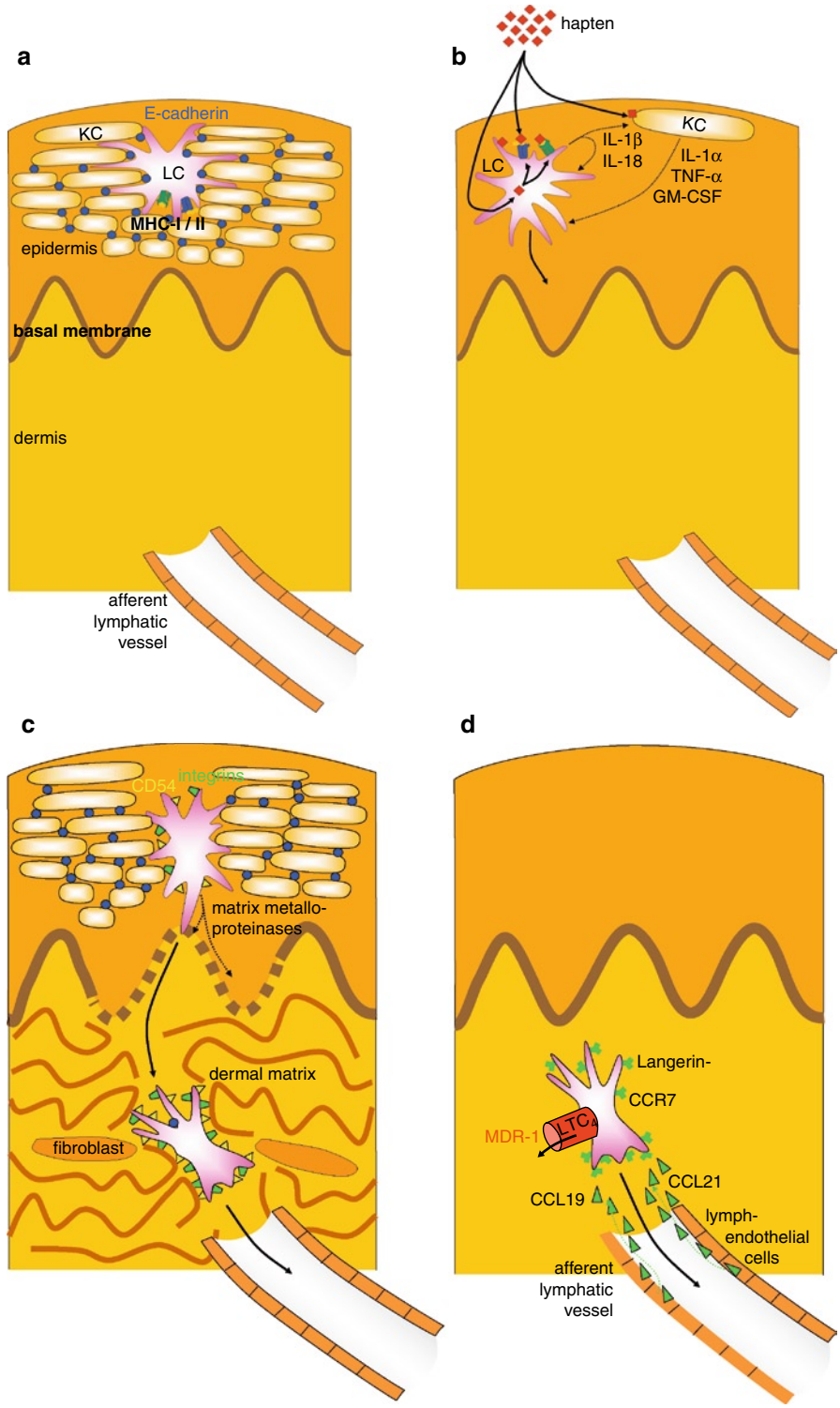

Fig. 3.5 (**a**–**d**) Hapten-induced migration of Langerhans cells. (**a**) In a resting state, epidermal Langerhans cells (*LC*) reside in suprabasal cell layers, tightly bound to surrounding keratinocytes (*KC*), e.g., by E-cadherin. (**b**) Early after epidermal hapten exposure, LC produce IL-1β and IL-18, which induce the release of IL-1α, TNF-α, and GM-CSF from keratinocytes. Together, these three cytokines facilitate migration of LC from the epidermis toward the lymph nodes. (**c**) Emigration of LC starts with cytokine-induced disentanglement from surrounding keratinocytes (e.g., by downregulation of E-cadherin) and production of factors facilitating penetration of the basal membrane (e.g., matrix metalloproteinases) and interactions with extracellular matrix and dermal cells (e.g., integrins and integrin ligands). (**d**) Once in the dermis, LC migration is directed toward the draining afferent lymphatic vessels, guided by local production of chemokines (e.g., CCL19 and CCL21) acting on newly expressed chemokine receptors, such as CCR7, on activated LC. Along their journey, haptenized LC further matures as characterized by their increased dendritic morphology and expression of costimulatory and antigen-presentation molecules

within 15 min after exposure to a contact sensitizer, production of IL-1β mRNA is induced [106, 107]. Along with this, caspase-1, formerly known as interleukin-1-converting enzyme, is activated and cleaves the active IL1β cytokine from the translated precursor-IL1β protein. Caspase-1 activates also IL-18 from its precursor form. These inflammatory processes are now viewed at as making up the "inflammasome" [108]. IL-1β in concert with IL-18 stimulates release of tumor necrosis factor (TNF)-α and granulocyte–macrophage colony-stimulating factor (GM-CSF) from keratinocytes [108]. Together these three cytokines facilitate migration of LC from the epidermis toward the lymph nodes [109]. IL-1β and TNF-α downregulate membrane-bound E-cadherin expression and thus cause disentanglement of LC from surrounding keratinocytes (Fig. 3.5) [104, 105, 110]. Simultaneously, adhesion molecules are upregulated promoting LC migration by mediating interactions with the extracellular matrix and dermal cells, such as CD54, α_6 integrin, and CD44 variants [111–115]. Also, production of the epidermal basement membrane degrading enzyme metalloproteinase-9 is upregulated in activated LC [116].

Next, LC migration is directed by hapten-induced alterations in chemokine receptor levels [117]. Upon maturation, LC downregulate expression of receptors for inflammatory chemokines (e.g., CCR1, 2, 5, and 6), whereas others (including CCR4, 7, and CXCR4) are upregulated (Fig. 2.3) (reviewed by [118] and [119–121]). Notably, CCR7 may guide maturing LC into the draining lymphatics and the lymph node paracortical areas, since two of its ligands (CCL19 and 21) are produced by both lymphatic and high endothelial cells [122, 123]. Importantly, the same receptor-ligand interactions cause naive T cells, which also express CCR7, to accumulate within the paracortical areas [124]. Migratory responsiveness of both cell types to CCR7 ligands is promoted by leukotriene C4, released from these cells via the transmembrane transporter molecule Abcc1 (previously called MRP1) [117, 125–127]. Interestingly, Abcc1 belongs to the same superfamily as the transporter associated with antigen-processing TAP, known to mediate intracellular peptide transport in the "endogenous route" which favors peptide association with MHC Class I molecules. Final positioning of the LC within the paracortical T-cell areas may be due to another CCR7 ligand, EBI1-ligand chemokine (ELC, CCL19), produced by resident mature DC [128].

Core Message

› Along with their migration and settling within the draining lymph nodes, haptenized LC further mature, as characterized by their increased expression of costimulatory and antigen-presentation molecules [129, 130]. In addition, they adopt a strongly veiled, interdigitating appearance, thereby maximizing the chances of productive encounters with naive T lymphocytes and recognition of altered self [131–133].

3.3.3 Recognition of Allergen-Modified Langerhans' Cells by Specific T Cells

3.3.3.1 Homing of Naive T Cells Into Lymph Nodes

More than 90% of naive lymphocytes present within the paracortical T-cell areas have entered the lymph nodes by high endothelial venules (HEV) [134]. These cells are characterized not only by CCR7 but also by the presence of a high molecular-weight isoform of CD45 (CD45RA) [134, 135]. Entering the lymph nodes via HEV is established by the lymphocyte adhesion molecule L-selectin (CD62L), which allows rolling interaction along the vessel walls by binding to peripheral node addressins (PNAd), such as GlyCAM-1 or CD34 [136–138]. Next, firm adhesion is mediated by the interaction of CD11a/CD18 with endothelial CD54, resulting in subsequent endothelial transmigration. Extravasation and migration of naïve T cells to the paracortical T-cell areas are supported by chemokines such as CCL18, 19, and 21 produced locally by HEV and by hapten-loaded and resident DC [125, 139–141]. In nonsensitized individuals, frequencies of contact allergen-specific T cells are very low, and estimates vary from 1 per 109 to maximally 1 per 106 [134, 142]. Nevertheless, the preferential homing of naive T cells into the lymph node paracortical areas and the large surface area of IDC make allergen-specific T-cell activation likely with only few dendritic cells exposing adequate densities of haptenized-MHC molecules [143, 144].

3.3.3.2 Activation of Hapten-Specific T Cells

As outlined in "Binding of Contact Allergens to Skin Components," the chemical nature of the hapten determines its eventual cytoplasmic routing in antigen-presenting cells (APC), and thus whether presentation will be predominantly in context of MHC class I or II molecules (Fig. 3.4). T cells, expressing CD8 or CD4 molecules, can recognize hapten-MHC class I or II complexes showing stabilized MHC membrane expression [145, 146]. Chances of productive interactions with T cells are high since each MHC-allergen complex can trigger a high number of T-cell receptor (TCR) molecules ("serial triggering") [147]. Moreover, after contacting specific $CD4^+$ T cells, hapten-presenting DC may reach a stable superactivated state, allowing for efficient activation of subsequently encountered specific $CD8^+$ T cells [148]. The actual T-cell activation is executed by TCRξ-chain mediated signal transduction, followed by an intracellular cascade of biochemical events, including protein phosphorylation, inositol phospholipid hydrolysis, increase in cytosolic Ca^{2+} [149, 150], and activation of transcription factors, ultimately leading to gene activation (Fig. 3.6) [151].

For activation and proliferation, TCR triggering ("signal 1") is insufficient, but hapten-presenting APC also provide the required costimulation ("signal 2"; Fig. 3.7) [152, 153]. The costimulatory signals may

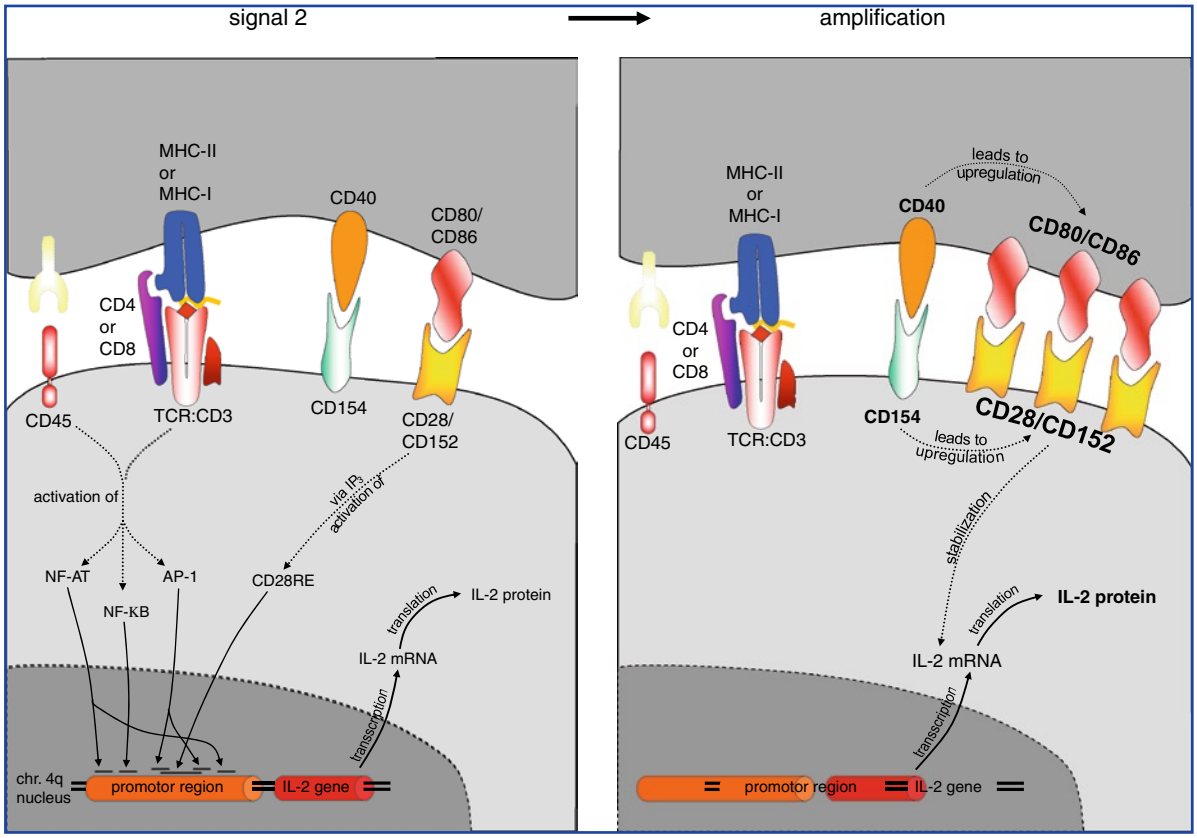

Fig. 3.6 Activation of hapten-specific T cells. T-cell receptor (TCR) triggering by hapten-major histocompatibility complex (*MHC*) complexes ("signal 1") is insufficient for T-cell activation. But "professional" antigen-presenting cells (*APC*), such as Langerhans cells, can provide the required costimulation ("signal 2") involving secreted molecules, such as cytokines, or sets of cellular adhesion molecules present on the outer cellular membranes of APC and T cells. T cells, stimulated in this way, activate nuclear responder elements (e.g., CD28RE). Together with nuclear transcription factors (*NF*) produced upon TCR triggering, these nuclear responder elements enable transcription of T-cell growth factors, e.g., IL-2. APC–T cell interaction gives rise to mutual activation ("amplification"): on APC, ligation of CD40 with CD154 molecules on T cells induces overexpression of several costimulatory molecules, including CD80 and CD86. In turn, these molecules bind to and increase expression of CD28 on T cells. This interaction stabilizes CD154 expression, causing amplified CD154–CD40 signaling, and preserves strong IL-2 production, finally resulting in abundant T-cell expansion

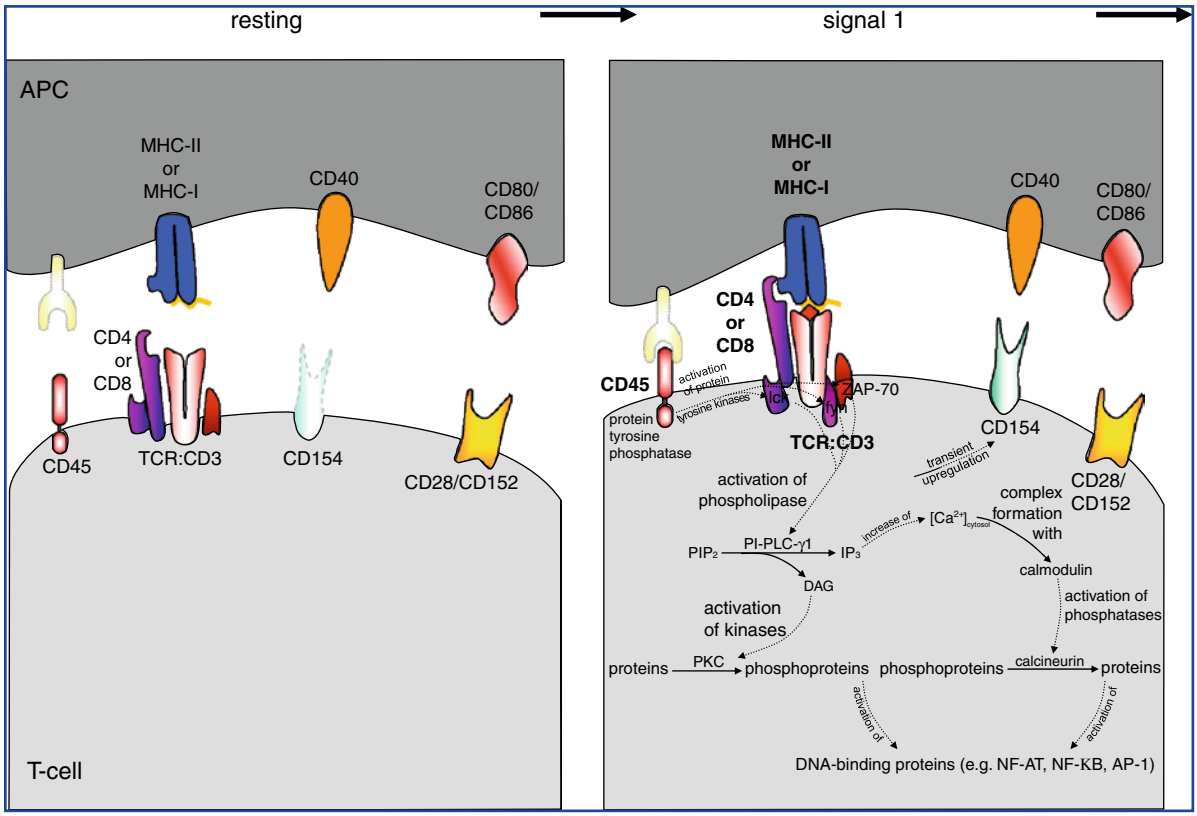

Fig. 3.6 (continued)

involve secreted molecules, such as cytokines (IL-1), or sets of cellular adhesion molecules (CAMs) and their counter-structures present on the outer cellular membranes of APC and T cells. Expression levels of most of these CAMs vary with their activational status, and thus can provide positive stimulatory feedback-loops. For example, as mentioned above, after specific TCR binding and ligation of CD40L (CD154) on T cells with CD40 molecules, APC reach a superactivated state, characterized by overexpression of several CAMs, including CD80 and CD86 (Fig. 3.6) [154, 155]. In turn, these molecules bind to and increase expression of CD28 on T cells. This interaction stabilizes CD154 expression, causing amplified CD154–CD40 signaling [155, 156].

The activational cascade is, as illustrated above, characterized by mutual activation of both hapten-presenting APC and hapten-reactive T cells. While this activation protects the APC from apoptotic death and prolongs their life to increase the chance of activating their cognate T cells, only the latter capitalize on these interactions by giving rise to progeny. As discussed below, to promote T-cell growth, cellular adhesion stimuli need to be complimented by a broth of cytokines, many of which are released by the same APC. Together, elevated expression levels of (co-)stimulatory molecules on APC and local abundance of cytokines overcome the relatively high activation threshold of naive T cells [157].

Core Message

› The intricate structure of lymph node paracortical areas, the differential expression of chemokines and their receptors, the characteristic membrane ruffling of IDC, and the predominant circulation of naïve T lymphocytes through these lymph node areas provide optimal conditions for T-cell receptor binding, i.e., the first signal for induction of T-cell activation [158]. Intimate DC–T cell contacts are further strengthened by secondary signals, provided by sets of CAMs, and growth-promoting cytokines (reviewed in [159, 160]).

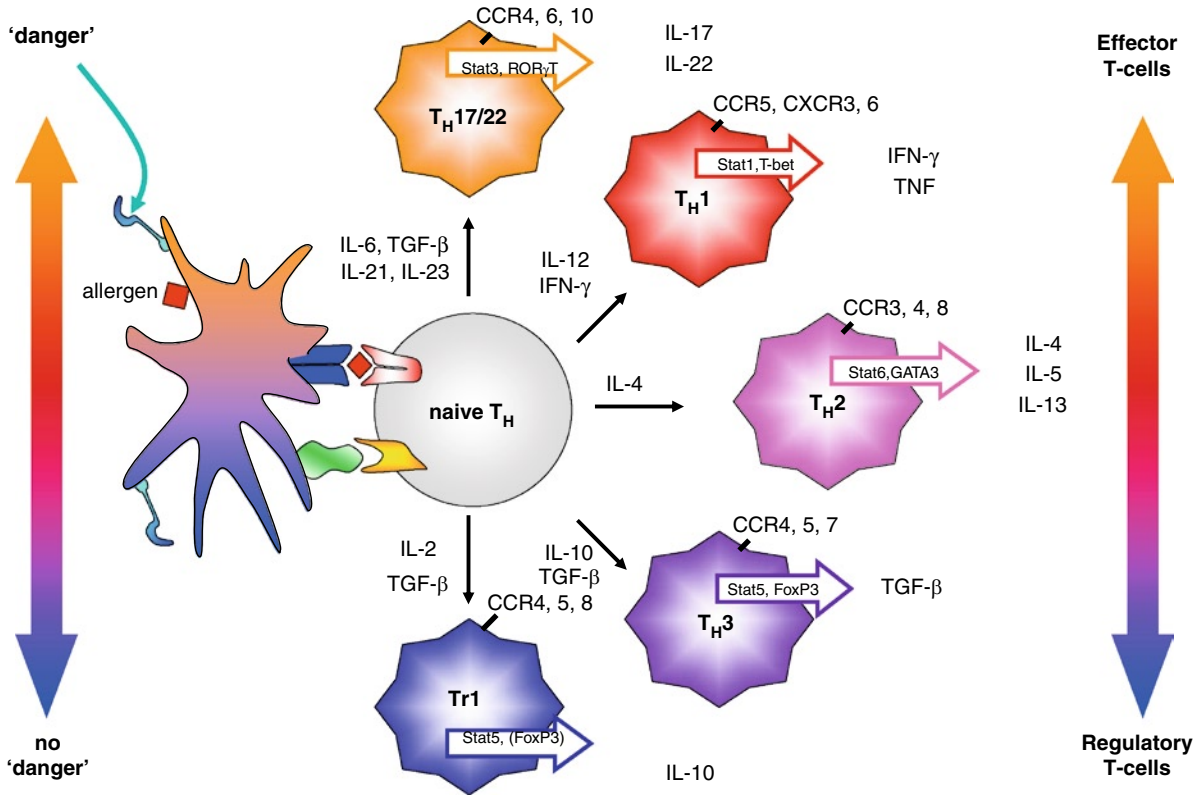

Fig. 3.7 Spectrum of allergen driven CD4⁺ T cell differentiation: current schematic view. Depending on the immunological microenvironment (amount of allergen, danger signals, and other soluble mediators), activated naïve T cells are skewed into distinct phenotypes. The presence of allergen and sufficient danger signals leads to the development of effector T cell phenotypes of ACD. Presence of IL-6, TGF-β, IL-21, and IL-23 stimulates the generation of TH17/Th23 cells. Development of TH1 cells is stimulated by the presence of IL-12 and IFN-γ, and the development of TH2 is favored by IL-4. The absence of sufficient danger signals stimulates the development of tolerogenic phenotypes, including TH3 and Tr1 [165–167, 175, 201, 239, 241]

3.3.4 Proliferation and Differentiation of Specific T Cells

3.3.4.1 T-Cell Proliferation

Upon their activation, naive allergen-specific T cells start producing several cytokines, including IL-2, the classical T-cell growth factor [161, 162]. In particular, ligation of T cell-bound CD28 receptors unleashes full-scale IL-2 production in T cells by increasing IL-2 transcription and mRNA stabilization [163]. T-cell IL-2 production peaks within 24 h, and declines subsequently (Villarino 2007). Concomitant upregulation of the IL-2 receptor α-chain facilitates the assembly of high affinity IL-2 receptor complexes that augment autocrine T-cell responsiveness, thus providing a positive feedback loop leading to T-cell clonal expansions up to 1,000-fold [164]. The process of proliferation can be visible as an impressive, sometimes painful lymph node swelling.

3.3.4.2 T-Cell Differentiation

Whereas allergen-specificity remains strictly conserved along with their proliferation, within few days T cells show distinct expression of transcription factors associated with varying cytokine production profiles and [165–168]. Thus, the recent offspring of allergen-specific CD4⁺ T cells can show at least five distinct cytokine profiles, generally associated with helper/effector or regulatory/suppressive functions (Fig. 3.7). Type 1 Th cells are characterized by a predominant

release of IFN-γ, IL-2, and TNF-β, all known as prototypical proinflammatory and cytotoxic cytokines. Type 2 Th cells secrete IL-4, IL-5, and IL-13, which have distinct proinflammatory activities, but are most prominent in promoting humoral antibody production, e.g., along mucosal surfaces where IgA contributes to exclusion of microbial entry [169, 170]. Next, the Th3 subset is distinguished by its release of transforming growth factor (TGF)-β, which displays anti-inflammatory activities [170]. Recently, Th-17 cells have been recognized as a separate lineage of proinflammatory T cells, characterized by the production of IL-17A and IL-17E, as well as IL-22, all of which play pivotal roles in autoimmune diseases, e.g., by recruiting neutrophils and macrophages [171, 172]. Finally, still another subset of $CD4^+$ T cells is recognized for its strong regulatory role in controlling inflammatory reactivities, i.e., the Tr1 cells or "inducible Tregs," characterized by the secretion of IL-10 [166, 167]. This $CD4^+$ T cell population is phenotypically remarkably heterogeneous, with part of the cells expressing high amounts of the high affinity IL-2 receptor ("$CD25^{high}$"), either or not accompanied by expression of the transcription factor FoxP3 [173–175] (Fig. 3.7). Tr1 cells have essential roles in the maintenance of immune homeostasis, regulating effector T-cell responses and preventing their potentially pathogenic effects by various indirect ways, e.g., by suppressing macrophage functions [176, 177]. Each of these five cytokine profiles is under control of distinct sets of transcription factors that are shown in Fig. 3.7, but are discussed further elsewhere (e.g., [165, 166, 178–180].

To some extent, the same distinct cytokine profiles may develop in CD8+ T cells, where at least type 1 and 2 cytokine releasing $CD8^+$ cells are known to contribute to ACD [68, 181].

Several factors are thought to contribute to the above described polarized cytokine production profiles in allergen-specific T cells, including (1) the site and cytokine environment of first allergenic contact, (2) the molecular nature and concentrations of the allergen, and (3) the neuroendocrine factors.

3.3.4.3 Cytokine Environment

In the skin-draining lymph nodes, allergen-activated LC and dermal dendritic cells rapidly produce large amounts of IL-12, switching off IL-4 cytokine production, thereby promoting the differentiation of Th1 cells [182–184]. Of note, since Th1 cells retain, next to IL12R, high IL-4R expression, they remain sensitive to IL-4 as a growth factor [185]. Thus, they also retain the capacity to shift cytokine production toward the type 2 profile. In contrast, type-2 T cells, e.g., developing in mucosa-draining lymph nodes, rapidly lose the genes encoding the IL-12-R β2 chain and thus type-2 differentiation is irreversible [186, 187].

Early differentiation of type-1 T cells is promoted by microbial danger-signal-induced IL-12 and IL-18, leading to IFN-γ release by nonspecific "bystander" cells, e.g., DC and NK cells, within the lymph nodes [188, 189]. IFN-γ interferes with skewing toward other cytokine profiles. Since Th1 cells rapidly lose functional IFN-γR expression, these cells, in contrast to Th2, Th3, and Th17 cells, become refractory to the growth-inhibitory effects of IFN-γ [190–192]. Interestingly, T-cell skewing may also be facilitated by primary contact-mediated signals, e.g., Th1 skewing by CD154 ligation through APC-bound CD40 [193] or Th2 skewing by ligation of CD134 (OX40) through APC-bound CD252 [194, 195].

In the process of T-cell skewing toward the other major cytokine profiles, TGF-β plays a central role. TGF-β can be produced by various cell types, including Th3 cells themselves, but is most prominently produced by mucosal epithelial cells [166, 192, 196]. Apparently, in conjunction with IL-10 production, e.g., produced by mucosal B cells, allergen-stimulated T cells rapidly initiate endogenous TGF-β production thus revealing the Th3 phenotype [197]. These cells may stimulate IgA production along the mucosae, but elsewhere immunosuppressive activities prevail. Interestingly, in conjunction with abundant local IL-2 production, such as induced by strong antigenic stimulation involving most effective CD28 triggering, TGF-β favors skewing toward IL-10 production, thereby providing an effective immunoregulatory feedback loop [198, 199]. Still, in the presence of strong and persistant microbial molecule-induced danger/growth signals, e.g., IL-6, IL-21, and IL-23, TGF-β induces the development of Th17 and/or Th22 cells, which both have been postulated to contribute to various allergic and autoimmune disorders [168, 172, 192, 200, 201] (Fig. 3.7).

Thus, ACD may be caused by any combination of at least three distinct types of effector T cells, releasing

type-1, -2, and –17/22 cytokines, respectively. Considering that contact allergens will mainly enter via the skin, type-1 proinflammatory T cells are thought to represent the primary effector cells in ACD [202, 203]. Nevertheless, in sensitized individuals, type-2 T cells also play a role, as shown by both IL-4 production and allergen-specific type-2 T cells in the blood and at ACD reaction sites (see Sect. 3.3.6) [204–206]. Their role may increase along with the longevity of sensitization, since several factors contribute to shifting type-1 to type-2 responses, including reversibility of the former and not of the latter T cells, as mentioned above [207, 208]. Still, other sets of cytokines, including IL-17 and/or IL-22, are important in immune defense mechanisms, and thus Th17 and or Th22 cells have also been found to mediate allergic and autoimmune disorders [209]. Given rapid local release of both IL-4 and TGF-β within mucosal tissues, mucosal allergen contacts, if accompanied by strong danger signals, may lead in particular to Th2 and Th17 effector cells. Without these signals, rather immunoregulatory subsets (Th3, Tr1) would develop, as is observed in the induction of "oral tolerance" (see below) [210].

3.3.4.4 Nature of the Allergen

A second factor in determining T-cell cytokine production profiles, although still poorly understood, is the molecular character of the contact allergen itself, and the resulting extent of TCR triggering [211, 212]. For both protein and peptide antigens, high doses of antigen might favor type-2 responses, whereas intermediate/low doses would induce type-1 T-cell responses [211, 213]. Strong antigenic stimulation was also shown to upregulate CD40L expression on T cells and, in combination with microbial-induced IL-6, to promote Th17 differentiation. To what extent this translates to contact allergens is still unclear. Certainly, endogenous capacities of contact allergens to provide danger signals and activate the "inflammasome," in combination with their capacity to induce differentiation-skewing cytokines (in particular IL-4, IL-6, IL-12, and IL-23), will affect the outcome [214, 215]. In this respect, some contact allergens are notorious for inducing type-2 responses, even if their primary contact is by the skin route, e.g., trimellitic acid, which is also known as a respiratory sensitizer [216].

3.3.4.5 Neuroendocrine Factors

Diverse neuroendocrine factors codetermine T-cell differentiation [217–219]. An important link has been established between nutritional deprivation and decreased T cell-mediated allergic contact reactions [220]. Apparently, adipocyte-derived leptin, a hormone released by adequately nourished and functioning fat cells, is required for type-1 T-cell differentiation. Administration of leptin to mice restored ACD reactivity in mice during starvation [220]. Also, androgen hormones and adrenal cortex-derived steroid hormones, e.g., dehydroepiandrosterone (DHEA), promote type-1 T-cell and ACD reactivity. DHEA, like testosterone, may favor differentiation of type-1 T cells by promoting IFN-γ and suppressing IL-4 release [221, 222]. In contrast, the female sex hormone progesterone furthers the development of type-2 $CD4^+$ T cells and even induces, at least transient, IL-4 production and CD30 expression in established type-1 T cells [223, 224]. Type-2 T-cell polarization is also facilitated by adrenocorticotrophic hormone (ACTH) and glucocorticosteroids [225], and by prostaglandin $(PG)E_2$ [226]. PGE_2, released from mononuclear phagocytes, augments intracellular cAMP levels, resulting in inhibition of proinflammatory cytokine, like IFN-γ and TNF-α, production [227–230] and thus can influence the development of effector T cells in ACD.

Core Message

› In healthy individuals, primary skin contacts with contact allergens lead to differentiation and expansion of allergen-specific effector T cells displaying Th1, Th2, and/or Th17 cytokine profiles. The same allergens, if encountered along mucosal surfaces, favor the development of allergen-specific Th2 and Th17 effector cells, and/or Th3 and Tr1 allergen-specific regulatory T cells. While the first two subsets may assist or replace Th1 cells in proinflammatory effector functions, the latter two subsets are mainly known for downregulating immune responsiveness. For most, if not all allergens, along with prolonged allergenic contacts, the role of Th2 cells as effector cells gradually increases given reduced longevity of Th1 responses.

› The respective contributions of similar subsets o f allergen-specific CD8+ T cells are still unknown, but distinct effector roles of allergen-specific Tc1 and Tc2 have been postulated.

3.3.5 Systemic Propagation of the Specific T-Cell Progeny

3.3.5.1 T-Cell Recirculation

Upon sensitization via the skin, the progeny of primed T cells is released via the efferent lymphatic vessels of the skin-draining lymph nodes and the thoracic duct into the blood (Fig. 3.8). If the first encounter with allergen occurs via the intestinal route, (e.g., along with induction of oral tolerance), priming will take place in the Peyer's patches and mesenteric lymph nodes, and primed T cells will be released from there to the circulation. The subsequent recirculation and homing pattern of primed T cells is guided by adhesion molecules and chemokine receptors, which they express on the cell membrane (Table 3.2). As outlined below, expression of these molecules is determined by the site of priming, as well as by the activational state of the T cells. In addition, there is a distinct relationship between the sets of chemokine

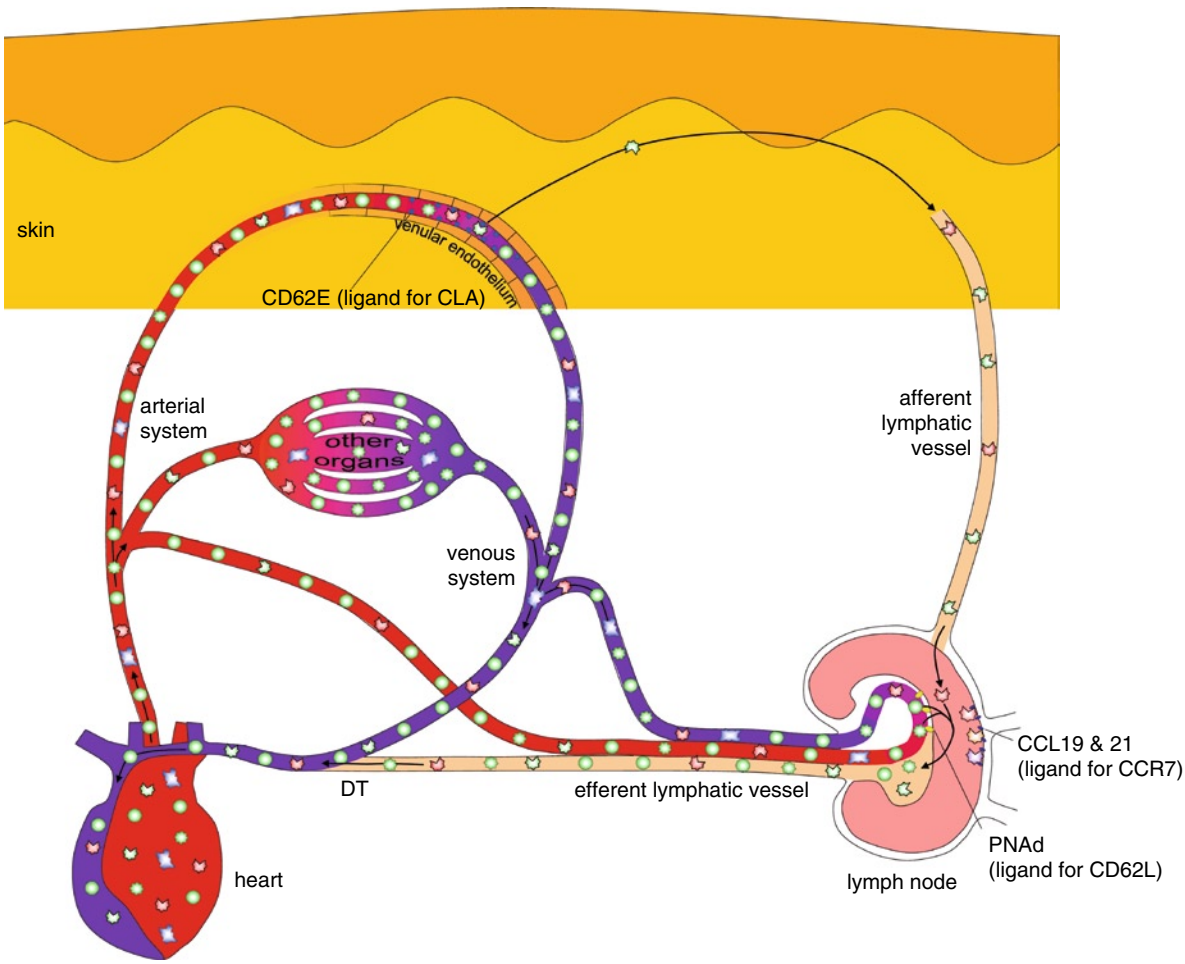

Fig. 3.8 Systemic propagation of hapten-specific T cells. From the skin-draining lymphoid tissue, the progeny of primed T cells is released via the efferent lymphatic vessels and the thoracic duct (*DT*) into the blood and becomes part of the circulation. Like their naïve precursors, these $CCR7^+$ effector/memory T cells can still enter lymphoid tissues and settle in paracorticale areas by binding to its ligands CCL19 and CCL21. But increased expression of skin-homing molecules, e.g., cutaneous lymphocyte antigen (*CLA*), facilitates their spontaneous migration in the skin

Table 3.2 Molecules involved in the migration of hapten-specific T lymphocytes

Receptor/ligand	T-cell	Ligand/receptor	Cell	Tissue	References
CD62L (L-selectin)	T_{naive} T_{CM}	CD34, GlyCAM-1 (PNAd)	HEV	lymph node	Janeway [436], Sallusto [212]
CCR7	T_{naive} T_{CM}	CCL19, CCL21	Stromal cells, DC	lymph node	Sallusto [212]
CD11a/CD18 (αL:β2-integrin, LFA-1)	increased upon activation	CD54, CD102 (ICAM-1, ICAM-2)	Endothelial cells	Skin	Janeway [436]
CD49d (α4:β1-integrin, VLA-4)	increased upon activation	CD106, fibronectin (VCAM-1)	Endothelial cells	Skin	Janeway [436]
CD162 (P- selectin ligand, PSGL-1)	increased upon activation	CD62P (P-selectin)	Endothelial cells	Skin	Woodland [461]
CLA	skin homing	CD62E (E-selectin)	Cutaneous endothelial cells	Skin	Woodland [461]
CCR4	Th2	CCL17 (TARC)	Keratinocytes	Skin	Woodland [461]
CCR5	Th1	CCL2 (MCP-1)? CCL3 (MIP-1α)	Keratinocytes (a.o.)	Skin	Meller [479], Gaga [490]
CCR6	Th17	CCL20 (MIP-3α / LARC)	Langerhans cells Endothelial cells	Skin	Larsen [455] Meller [479]
CCR10	Th22, CLA+	CCL27 (CTACK)	Keratinocytes Langerhans cells	Skin	Duhe [462], Homey [491], Kagami [492], Woodland [461]
CXCR3	Th1	CXCL9 and CXCL10 (Mig and IP-10)	Keratinocytes	Skin	Moed [285] Meller [479]
α4:β7-integrin	gut homing	MAdCAM-1	Endothelial cells	gut	Janeway [436]
CCR9	T-cells	CCL25 (TECK)	Epithelial cells	gut	Grimm [493]; Miles [460]

and homing receptors expressed by T cells and their type of differentiation.

First, primed T cells have different homing receptors depending on the site of priming, a process called "imprinting" [231, 232]. During priming of allergen-specific T cells in the skin-draining lymph nodes, both $CD4^+$ and $CD8^+$ T cells are stimulated to express CLA [233] and the chemokine receptors CCR4 and CCR10, a phenotype that predisposes for eventual migration to the skin. In the mesenteric lymph nodes, on the other hand, T cells are stimulated to express the integrin α4:β7 and the chemokine receptor CCR9, a phenotype which predisposes for gut homing. An instructive role of the peripheral tissues in this imprinting process was demonstrated in a mouse model on T cell priming by dendritic cells, where either dermal or intestinal cells were added to the cultures, resulting in T cells expressing mouse "CLA" or α4:β7 integrin, respectively [231]. For the imprint of gut homing, retinoic acid was identified as a crucial factor, while for the imprint of skin homing, the active metabolite of vitamin D3 was shown to be essential, because it induces CCR10 expression in T cells [234]. Still, for induction of CLA and thus for establishing the full skin-homing profile, cell–cell contact and/or other mediators, like IL-12, seem to be required [231].

After priming and imprinting, circulating gut homing memory T lymphocytes, bearing the α4:β7 integrin, can attach to intestinal endothelial cells by binding to the mucosal vascular addressin MAdCAM-1. Further infiltration in the mucosa is guided by chemokines, such as CCL25, produced by small intestinal epithelial cells [235]. Thus, along the gut, T lymphocyte progeny is attracted that has been generated in other mucosal tissues. Likewise, in the skin, CLA-positive cells that have been generated in skin-draining lymph nodes are attracted. CLA binds to E-selectin (CD62E) on dermal endothelial cells, while CCR4 and CCR10 expression allow the lymphocytes to migrate in the skin toward CCL17 and CCL27 produced by keratinocytes in the epidermis.

At least as important for the recirculation and homing characteristics of T cells is the activational state of the cells. In this respect, primed T cells can be divided into two main subsets: the central memory T cells (T_{CM}) and the effector memory T cells (T_{EM}). Like their naive precursors, T_{CM} can still enter the peripheral lymphoid tissues due to the fact that they continue to express CD62L and CCR7, allowing for binding to HEV in the lymph nodes and migration into the paracortical areas. T_{EM}, on the other hand, have lost these molecules and migrate, due to simultaneous upregulation of several other adhesion molecules, preferentially to peripheral inflamed tissues. T_{EM} are characterized by rapid effector function upon antigenic stimulation, but, in the absence of antigenic stimuli, T_{EM} eventually convert to T_{CM} by reacquiring CCR7 and CD62L. In turn, T_{CM} may convert to T_{EM} upon antigenic restimulation [167, 232, 236, 237].

Peripheral endothelial binding and extravasation of T cells to inflamed tissues require the expression of both selectins and integrins on the T cell membrane, such as LFA-1, VLA-4, and PSGL-1. The vascular expression of their respective ligands (Table 3.2) is strongly increased by cytokines released at inflammatory sites. The density of adhesion molecules on the T cell membrane is generally upregulated upon activation, in particular in T_{EM}. Since their expression is highest only for short periods after activation, only recently activated T cells show a unique propensity to enter skin sites and exert effector functions.

Third, the differentiation of T cells (Th1, Th2 etc.) is clearly associated with distinct homing characteristics. T cells biased toward a proinflammatory phenotype show a higher propensity to enter skin sites, as compared to mucosal tissues [233, 238]. [201, 239] In mice, the early influx of type-1 T cells into delayed-type hypersensitivity (DTH) reactions was found to be more efficient than that of type-2 T cells, although both cell types expressed CLA. Here, CD162, highly expressed by type-1 T cells, was found to be important for this preferential homing [240]. Also, the pattern of chemokine receptors differs between the Th subsets (Table 3.2). Some receptors, such as CXCR3, are preferentially expressed on Th1 cells, whereas others, such as CCR4 and CCR8, are in particular expressed by Th2 cells [167, 175, 241, 242]. The latter chemokine receptors are not only overexpressed on type-2 cytokine-producing T cells, but also on basophils and eosinophils. Together these cells strongly contribute to local immediate allergic hyperresponsiveness. The more recently described Th17 and Th22 lymphocyte subsets expressing CCR4, CCR6, and CCR10 [239, 241] are attracted to the skin by epidermal CCL17, CCL20, and CCL27, respectively (Table 3.2). Overall, results obtained thus far favor the view that the proinflammatory subsets (Th1 and Th17/22) will be the first to enter skin sites upon local inflammatory stimuli, their primary function being an early control of antigenic pressure, e.g., through amplification of macrophage effector functions. The ACD reaction is, however, a dynamic process, in which the first influx of cells influences the local chemokine environment and determines the type of subsequent infiltrating cells. Thus, upon repeated exposure to contact allergens, gradually Th2 cells and regulatory cells may dominate [243]. Interestingly, also at the T cell level modulation of the cytokine and chemokine receptor profiles may occur, thereby maintaining plasticity of the immune response [167, 180]. The actual composition of the T cell infiltrate in ACD skin lesions does not only depend on the influx of lymphocytes, but should rather be regarded as the resultant of infiltration, apoptosis and retention of lymphocytes, next to their emigration to the lymphatics.

Finally, the antigen specificity of T cells contributes to their migration pattern. Allergens penetrated via the epidermis and displayed at the dermal endothelial surface may be recognized by allergen-specific T cells, thereby resulting in activation, immobilization, and transendothelial migration of these cells at sites of allergen exposure [241].

Core Message

› Priming via the skin results in CLA-positive T cells, which upon inflammatory stimuli preferentially enter the skin; on the other hand, gut homing T cells have been primed and generated along mucosal surfaces. Upon priming, T cells loose much of their capacity to recirculate via the lymph nodes, but gain the capacity to enter the tissues. In particular, recently activated T cells will enter skin-inflammatory sites. ACD reactions are primarily infiltrated by CD4 and/or CD8 proinflammatory cells, later reactions may be dominated by Th2 cells and regulatory T cells. Skin infiltration by T cells is fine tuned by sets of adhesion molecules and chemokine receptors, whose expression is not rigid, but can be modulated by microenvironmental factors.

3.3.5.2 Allergen-Specific T-Cell Recirculation: Options for In Vitro Testing

The dissemination and recirculation of primed, allergen-specific T cells in the body suggests that peropheral blood offers a most useful and accessible source for T-cell based in vitro assays for ACD. A major advantage of in vitro testing would be the noninterference with the patient's immune system, thereby eliminating any potential risk of primary sensitization and boosting by in vivo skin testing. Although such tests have found several applications in fundamental research, e.g., on recognition of restriction elements, cross-reactivities, and cytokine profile analyses, their use for routine diagnostic purposes is still limited. Even in highly sensitized individuals, frequencies of contact allergen-specific memory/effector cells may still be below 1 per 10^4 [244–246]. Given the relatively small samples of blood obtainable by venepuncture (at only one or a few time points), numbers of specific T cells in any culture well used for subsequent in vitro testing would typically be below 100 cells/well. For comparison, in vivo skin test reactions recruit at least 1,000 times more specific T cells from circulating lymphocytes passing by for the period of testing, i.e., at least 24 h [247].

Therefore, the sensitivity of in vitro assays, e.g., allergen-induced proliferation or cytokine production, may not always be sufficient to pick up weak sensitization. Intermediate or strong sensitization is, however, readily detected in vitro by both proliferation and cytokine production assays [245, 248–250]. With respect to the latter, both the "Elispot" assay, where allergen-induced cytokine production is evaluated at the single cell level, and the cytokine evaluation in allergen-stimulated culture supernatants provide adequate information [249, 251, 252]. Notably with respect to cytokine production, type-2 cytokines appear to provide most specific parameters for contact sensitization in these assays, [251, 253] although generally both Th1 and Th2 cytokines are being produced in vitro by allergic individuals, upon allergen exposure [250, 254].

Importantly, most of the above mentioned successful in vitro studies evaluated hydrophilic allergens, such as nickel, chromium, and palladium salts. Reports on successful in vitro assays with other hydrophobic and more toxic allergens are scarce [250, 255, 256]. Appropriate allergen presentation is a major hurdle in in vitro studies because of the broad range of requirements for different allergens with unique solubilities, toxicities, and reactivity profiles. Moreover, in the absence of LC, monocytes are the major source of APC, and their numbers in peripheral blood vary substantially within and between donors. Of note, optimal APC function is particularly critical for in vitro activation of resting memory T cells, since in the absence of repeated allergenic contacts, activated effector memory T cells (T_{EM}) may finally revert to a more naïve phenotype, with a higher threshold for triggering [236, 257]. Supplementing in vitro test cultures with appropriate mixtures of cytokines may, however, compensate for suboptimal APC function [250, 251, 258].

Core Message

› After antigenic activation the progeny of primed T cells is released via the efferent lymphatics into the bloodstream. Circulating allergen-specific cells can be challenged in vitro to provide diagnostic parameters for contact hypersensitivity. At least for water-soluble

allergens, such as metal salts, the degree of allergen-specific proliferation and cytokine production, in particular type-2 cytokines, correlates with clinical allergy. For routine application of a broad spectrum of allergens, culture conditions still need to be improved. For mechanistic in vitro studies in ACD, however, with selected sets of relatively nontoxic allergens, peripheral blood provides an excellent source of lymphocytes and APC.

3.3.6 The Effector Phase of Allergic Contact Dermatitis

3.3.6.1 Elicitation of ACD

Once sensitized, individuals can develop ACD upon reexposure to the contact allergen. Positive patch test reactions mimic this process of allergen-specific skin hyperreactivity. Thus, skin contacts induce an inflammatory reaction that, in general, is maximal within 2–3 days and, without further allergen supply, declines thereafter (Fig. 2.8). Looked at superficially, the mechanism of this type of skin hyperreactivity is straightforward: allergen elicitation or challenge leads to the (epi)dermal accumulation of contact allergen-specific memory/effector T lymphocytes, which, upon encountering allergen-presenting cells, are reactivated to release proinflammatory cytokines. These, in turn, spark the inflammatory process, resulting in macroscopically detectable erythema and induration. As compared to immediate allergic reactions, developing within a few minutes after mast-cell degranulation, ACD reactions show a delayed time course, since both the migration of allergen-specific T cells from the dermal vessels and local cytokine production need several hours to become fully effective. Still, the picture of the rise and fall of ACD reactions is far from clear. Some persistent issues are discussed below, notably: (1) irritant properties of allergens, (2) role of early phase reactivity, (3) T-cell patrol and specificity, (4) effector T-cell phenotypes, and (5) downregulatory processes.

3.3.6.2 Irritant Properties of Allergens

Within a few hours after allergenic skin contact, immunohistopathological changes can be observed, including vasodilatation, upregulation of endothelial adhesion molecules [259, 260], mast-cell degranulation [261, 262], keratinocyte cytokine and chemokine production, [45, 263] influx of leucocytes [264, 265], and LC migration toward the dermis [112, 266–268]. These proinflammatory phenomena, which are also observed in nonsensitized individuals [269] and in T cell-deficient nude mice [270], strongly contribute to allergenicity [58]. Clearly most, if not all, of these effects can also be caused by irritants and, therefore, do not unambiguously discriminate between irritants and contact allergens [45, 271–273]. Apparently, true differences between these types of compounds depend on whether or not allergen-specific T cells become involved. Thus, only after specific T-cell triggering, distinctive features might be observed, e.g., local release of certain chemokines such as the Th1 associated chemokines CXCL9, CXCL10 (IP-10), and CXCL11 (I-TAC/IP-9) [263, 274] or the Th2 related chemokines CCL11, CCL17, and CCL22 [263, 274]. Certainly, proinflammatory effects of contact allergens increase, in many ways, the chance of allergen-specific T cells meeting their targets. The first cells affected by skin contact, i.e., keratinocytes and LC, are thought to represent major sources of pivotal mediators such as IL-1β and TNF-α [106, 275]. First, as described in "Hapten-Induced Activation of Allergen-Presenting Cells," these cytokines cause hapten-bearing LC to mature and migrate toward the dermis [94, 131, 268]. But these cytokines also cause (over)expression of adhesion molecules on dermal postcapillary endothelial cells, and loosen intercellular junctions. In that way, extravasation of leucocytes, including allergen-specific T cells, is strongly promoted [241, 275–278]. Moreover, haptens can stimulate nitric oxide (NO) production of the inducible NO-synthase (iNOS) of LC and keratinocytes, which contributes to local edema, vasodilatation, and cell extravasation [279, 280].

Histopathological analyses support the view that the major causative events take place in the papillary dermis, close to the site of entry of allergen-specific T cells, for instance at hair follicles, where haptens easily penetrate and blood capillaries are nearby [281]. Here, perivascular mononuclear cell infiltrates develop, giving the highest chance of encounters between

allergen-presenting cells and specific T cells. Once triggered, extravasated T cells will readily enter the lower epidermal layers, in which haptenized keratinocytes produce lymphocyte-attracting chemokines, such as CXCL9/10, CCL17, CCL20, and CCL27 ([201, 232, 263, 274]; Table 3.2). Subsequently, since effector memory T cells can also be triggered by "nonprofessional" APC, including KC, fibroblasts, and infiltrating mononuclear cells, ACD reactivity is amplified in the epidermis [157, 159, 269]. Together, these events result in the characteristic epidermal damage seen in ACD, such as spongiosis and hyperplasia. Notably, in ongoing ACD reactions, the production of chemokines attracting lymphocytes and monocytes/macrophages, in addition to the production of cytokines, adds to the nonspecific recruitment and activation of leucocytes [119, 282, 283]. Thus, like the very early events in the effector phase reaction, the final response to a contact allergen is antigen-nonspecific. It is, therefore, not surprising that allergic and irritant reactions are histologically alike.

3.3.6.3 Early Phase Reactivity

In the elicitation phase allergen-specific T cells are triggered by MHC-bound allergen, just like in the afferent phase. The role of LC in allergen presentation upon elicitation is, however, less prominent, and also other cells such as mast cells, macrophages, and keratinocytes may now contribute, since effector T cells are easily triggered and do not require professional antigen presentation. The role of keratinocytes in the onset of the ACD reaction is important because of the cytokines and chemokines they produce upon hapten application [237, 263], thereby facilitating the influx of effector T cells. In addition, a variety of other cells and mediators may contribute to the initiation of the ACD reaction, as summarized below.

The role of neutrophils in the onset of ACD reactions has not been well-established, though recent studies in mice demonstrate that skin reactivity to haptens largely depends on CXCL1, released from endothelial cells when the first hapten-specific CD8 T cells encounter the allergen and produce IL-17. CXCL1 may then attract neutrophils to the elicitation site, thus facilitating further influx of allergen-specific T cells [284]. In the human system, neutrophil infiltration was also observed in skin biopsies from nickel patch tests, presumably as a result of IL-17/IL-22 mediated inflammation [201]. Moreover, it has been shown that IL-8/CXCL8, a potent neutrophil chemoattractant, is readily produced by human antigen-presenting cells upon hapten exposure [285]; this could also contribute to an early influx of neutrophils in ACD reactions.

The role of an antibody-mediated early-phase reaction in the development of ACD is still unclear in man, although Askenase and his colleagues have generated robust data to support this view in murine models [286]: Hapten-specific IgM, produced upon sensitization by distant hapten-activated B-1 cells, can bind antigen early after challenge and activate complement. The resulting C5a causes the release of serotonin and TNF-α from local mast cells and platelets, leading to vascular dilatation and permeabilization, detectable as an early ear swelling peaking at 2 h [287]. Furthermore, C5a and TNF-α induce the upregulation of adhesion molecules on local endothelial cells [288, 289], thereby contributing to the recruitment of T cells in hapten challenge sites [289, 290]. In addition, human T cells were found to express the C5a receptor and are chemoattracted to endothelium-bound C5a [291]. However, against most contact allergens, including nickel, no antibodies have been detected in man, arguing against humoral mechanisms playing more than a minor role in clinical ACD [292, 293]. Interestingly in mice, immunoglobulin light chains, which have long been considered as the meaningless remnants of a spillover in the regular immunoglobulin production of B cells, were discovered to mediate very early hypersensitivity reactions by mast cell activation [294].

In addition to an auxiliary role of B cells and antibodies, natural killer (NK) cells have been reported to play a role in the onset of ACD reactions. Mice lacking both T and B cells (RAG2−/−) could still be sensitized to contact allergens, and Thy1+ NK cells were identified here as effector cells with a prominent role for the activating NK receptor NKG2D [295]. Interestingly, another NK-like cell, the invariant NKT cell, that recognizes CD1d bound glycolipids resulting in rapid IL-4 and IFN-γ release, was also found to play a role in the elicitation of contact sensitivity in mice: blocking of CD1d prevented both sensitization and elicitation by contact allergens [296]. Notably, in human ACD reactions relatively high frequencies of invariant NKT cells have been observed, ranging from 1.7 to 33% of total infiltrating T cells, which is 10–100-fold higher than the frequency found in the circulation [297]. Also, other

T cells with relatively restricted TCR repertoire, such as Tγδ cells, have been reported to contribute in a nonantigen-specific, probably non-MHC-restricted manner, to (early) elicitation responses [298].

To conclude, using various mouse models, different types of early allergen-specific reactivity have been claimed to play initiating roles in ACD, but clinical evidence for such mechanisms is still lacking.

3.3.6.4 T-Cell Patrol and Specificity of T-Cell Infiltrates

Whereas early nonspecific skin reactivity to contact allergens is pivotal for both sensitization and elicitation, full-scale development of ACD, of course, depends on allergen-specific T cells within the (epi) dermal infiltrates. In healthy skin there is a constant flow of memory T cells ending up in the draining lymph nodes: about 200 T cells/h/cm^2 skin [115]. Since one single antigen-specific T cell can already trigger visible skin inflammation [299, 300], randomly skin-patrolling memory/effector T cells might account for the initiation of the allergen-specific effector phase. However, since frequencies of hapten-specific T cells in sensitized individuals may still remain below 1 in 10,000, this does not seem to be a realistic scenario. Thus, augmented random and/or specific T-cell infiltration accompanies the development of ACD. Apparently, local chemokine release upon allergen contact is pivotal in this respect (see *T-Cell recirculation*; 482). Chemokine gene expression evaluated 48 h after NiSO4 application was increased for both Th1 related cytokines (CXCL9, CXCL10, and CXCL11) and Th2 related cytokines (CCL11, CCL17, and CCL22). On the other hand, CCL27 that attracts preferentially CCR10 bearing Th17/22 cells is constitutively produced in resting skin, but is rapidly released upon allergen contact to accumulate in the draining lymph nodes.

The question concerning the specificity of ACD T-cell infiltrates has so far received little attention. In a guinea pig model, preferential entry of dinitrochlorobenzene (DNCB)-specific T cells was observed within 18 h after elicitation of skin tests with DNCB, as compared to nonrelated compounds [301]. Probably, extravasation of hapten-specific T cells benefits from T-cell receptor-mediated interactions with endothelial MHC molecules, presenting hapten penetrated from the skin [241]. Within minutes after epicutaneous application, hapten can indeed be found in dermal tissues and on endothelial cells [259, 302, 303]. Indeed, the frequency of allergen-specific cells in positive patch tests to urushiol was found to be 10–100-fold higher than in the blood [246]. Interestingly, whereas preferential entry may already contribute to relatively high frequencies of allergen-specific T cells (within 48 h up to 10%) [205, 299], at later stages, when the ACD reaction fades away, the local frequency of allergen-specific T cells may increase even further, due to allergen-induced proliferation and rescue from apoptosis. Thus, at former skin reaction sites, these cells can generate "local skin memory" (see Sect. 3.3.7).

3.3.6.5 Effector T-Cell Phenotypes

The debate on phenotypes of effector T cells in ACD is still ongoing and the number of T cell subsets potentially involved is growing every year (Fig. 3.7). Consensus exists, however, on the phenotype of the skin-homing T cell, i.e., CLA positive. This molecule enables binding to cutaneous endothelial cells via E-selectin (CD62E) and thus migration into the dermis.

Since cutaneous infiltrates show a clear preponderance of CD4$^+$ T cells, it is not surprising that these cells have most often been held responsible for mediating ACD. In nickel allergic individuals, indeed, allergen responding cells were found to be CD4$^+$CLA$^+$ memory T cells [304]. Other studies, however, revealed CD8$^+$CLA$^+$ nickel reactive T cells as most discriminating for allergic individuals, since CD4$^+$ nickel reactive T cells were also found in healthy controls [244]. While the effector mechanism of CD4$^+$T cells is mainly based on cytokine production, CD8+ T cells may mediate skin inflammation also through killing of hapten-bearing target cells. In mice, generally CD8$^+$ T cells are found to cause contact sensitivity reactions, certainly to strong allergens, like DNFB [284, 297]. In mice CD4$^+$ T cells are rather found to be regulatory, as shown by the fact that contact sensitization to weak allergens succeeded only after depletion of the CD4$^+$ T cells [305]. Of note, most model allergens studied in mice are hydrophobic molecules such as DNFB and oxazolone, whereas in human studies, very often, water-soluble metal salts, such as NiSO4, are used as model allergen. This could, at least partly, explain the

different T cell subsets involved (Fig MHCI/II presentation). So, taken together, it has become clear that both $CD4^+$ and $CD8^+$ T cells can act as effector cells in DTH and ACD reactions. Likewise, neither of these subsets can be regarded simply as regulatory or suppressor cells, although both of these subsets may, depending on the allergen models and read-out assays, play such roles [68, 306].

An essentially similar conclusion holds true for T-cell subsets (whether $CD4^+$ or $CD8^+$), releasing type-1, type-2, or type-17 cytokines or combinations thereof. While type-1 cytokines, in particular IFN-γ, display well-established proinflammatory effects by fi increasing MHC and ICAM-1 expression [284, 307], thereby contributing to improved allergen presentation and infiltration, IL-4, a hallmark type-2 cytokine, can cause erythema and induration, when released in the skin [308, 309]. Indeed, blockage of IL-4 can interfere with ACD [309]. IL-17 plays a role in recruitment and activation of neutrophils. It was shown to be produced both by $CD8^+$ T cells (in mouse models with DNFB; 483) and by $CD4^+$ T cells (in human nickel patch tests; 456). The latter study shows, interestingly, that within a few hours after challenge, CCL20 expression is upregulated in the skin, attracting CCR6 positive cells. Since all Th17 cells do express this receptor, an early preferential influx of Th17 and, as a consequence, IL-17 and IL-22 production could be an essential early event in the development of the ACD reaction.

Thus, a picture emerges in which ACD reactions can be caused both by allergen-specific type-1, type-2, and type-17 T cells [168, 201, 244, 251, 297, 304]. In retrospect, the downregulatory effects of IL-4 on ACD reactions observed earlier in some mouse models [310] might be ascribed to accelerated allergen-clearance, rather than to blunt suppression. Still, both with time and repeated allergen-pressure, type-2 responsiveness may rapidly take over [243, 311]. Allergen-specific T cells isolated from skin test sites of sensitized individuals, as compared to blood, showed a strong bias toward type-2 cytokine profiles [204]. Additional local IFN-γ release seems, however, indispensable, since for a broad panel of contact allergens, clinical ACD reactions were characterized by increased expression of mRNA encoding IFN-γ-inducible chemokines [274]. In addition, transgenic mice expressing IFN-γ in the epidermis showed strongly increased ACD reactivity [312].

3.3.6.6 Downregulatory Processes

Resolution of ACD reactions and risk factors for the development of chronicity are not yet fully understood. Of course, if the allergen source is limited, as with skin testing, local concentrations of allergen usually rapidly decrease, thus taking away the critical trigger of the ACD reaction cascade. Since even ACD reactions due to chronic exposure to allergen seldomly result in permanent tissue destruction and scarification, immunoregulatory factors most likely contribute to prevention of excessive cytotoxicity and fatal destruction of the basal membrane. Both IL-1 and heparinase, secreted from activated keratinocytes and T cells, protect keratinocytes from TNF-α-induced apoptosis [313, 314]. Moreover, activated effector T cells can undergo activation-induced cell death (AICD) during the resolution phase [315]. Notably, proinflammatory type-1 T cells, expressing high levels of Fas-ligand (CD95L) and low amounts of apoptosis-protecting FAP-1 protein, are more susceptible to AICD than type-2 cells [316]. This may partly explain the shift toward type-2 reactivity that is observed upon prolonged allergen exposure [311]. Moreover, during the late phase of ACD, keratinocytes, infiltrated macrophages, and T cells start producing IL-10 [317–319], which has many anti-inflammatory activities, including suppression of antigen-presenting cell and macrophage functions [320, 321]. In addition, the release of factors, such as PGE_2 and TGF-β, derived from activated keratinocytes and infiltrated leucocytes, e.g., type-3 T cells, contribute to dampening of the immune response [322, 323]. Release of PGE_2, on the one hand, inhibits production of proinflammatory cytokines [230, 324] and, on the other hand, activates basophils [325]. These may constitute up to 5–15% of infiltrating cells in late phase ACD reactions [326] and are also believed to contribute to downregulation of the inflammatory response [327, 328]. TGF-β silences activated T cells and inhibits further infiltration by downregulating the expression of adhesion molecules on both endothelial and skin cells [236]. Regulatory cells producing these suppressive mediators might even predominate in skin sites, frequently exposed to the same allergen, and known to show local (allergen-specific) hyporesponsiveness [329]. It is of interest in this context that $CD4^+$ memory T cells expanded from late DTH reactions could be educated to become $CD4^+CD25^{++}$ regulatory T cells expressing Foxp3.

Core Message

› ACD reactions can be mediated by classical effector cells, i.e., allergen-specific $CD4^+$ type-1 T cells, which, upon triggering by allergen-presenting cells, produce IFN-γ to activate non-specific inflammatory cells such as macrophages. However, $CD8^+$ T cells and other cytokines, including IL-4, IL-17, and IL-22, can also play major roles in ACD. The conspicuous difference with DTH reactions induced by intradermal administration of protein antigens, i.e., the epidermal infiltrate, can largely be attributed to hapten-induced chemokine release by keratinocytes.

3.3.7 Flare-Up and Retest Reactivity

3.3.7.1 Local Allergen Retention

Flare-up reactivity of former ACD and patch test reaction sites is sometimes observed [330–332]. From the basic mechanisms of ACD, it can be inferred that allergen-specific flare-up reactions depend either on local allergen or T-cell retention at these skin sites. Upon short-lasting, low-dose contacts, e.g., by skin testing, local allergen retention usually does not exceed a 2-week period, which is actually long enough to exceed the time required for active sensitization. In experimental guinea pig studies, we observed that skin tests with DNCB, chromium, or penicillin could become positive even if primary sensitization was postponed to 1 week after skin testing. Apparently effector T cells released into the circulation at that late time still detected sufficient residual allergen at the former skin test sites to cause flare-up reactivity (Scheper et al., unpublished results). Maximum allergen-persistence for around 14 days was also reported by Saint-Mezard et al., [58] using the hapten fluorescein-isothiocyanate in a mouse model for flare-up reactivity. Also in humans flare-up reactions due to locally persisting allergen can be observed, when from about 4–6 days after primary sensitization, peripheral effector T cell frequency increases [333]. Clinically, this phenomenon can explain anomalous results from patch testing with multiple contact allergens. When a patient suspected for penicillin allergy was patch tested with cross-reactive penicillin derivatives, a regular 24–72 h reaction was only observed to one of the penicillins, but all others also became positive from about 8–9 days after skin testing. The first penicillin derivative turned out to release formaldehyde to which the patient was found to be allergic. Positive reactivity to formaldehyde apparently had potentiated primary sensitization to penicillin, causing the other previously negative reaction sites to flare-up (Neering, personal communication). Thus, skin test sites may occasionally flare-up if the testing dose itself led to the release or activation of sufficiently high numbers of effector T cells in the circulation.

3.3.7.2 Local T-Cell Retention

In contrast, allergen-specific T cells may persist for at least several months in the skin causing "local skin memory" (Figs. 3.9 and 3.10) [334, 335]. Thus, locally increased allergen-specific hyperreactivity, detectable through either accelerated "retest" reactivity (after repeated allergenic contact at the same skin site) or flare-up reactivity (after allergen entry from the circulation, e.g., derived from food ingestion), may be observed for long periods of time at former skin reaction sites [336–338]. Typically, the erythematous reactions peak between 2 and 6 h after contact with the allergen. Histological examination of such previously positive skin reaction sites shows that the majority of remaining T cells is $CD4^+$ $CCR10^+$ [335]. The remarkable flare-up reactivity at such sites can be understood by considering that just one specific effector T cell can be sufficient to generate macroscopic reactivity [300]. Moreover, a very high frequency of the residual T cells may be specific for the allergen, as discussed above in Sect. 3.3.6. Apparently, local specific T cell retention is highly advantageous in combating microbial infections, since memory T cells localized in peripheral tissues contribute to robust protection, e.g., to viral infections [232]. Only in highly sensitized individuals unrelated skin test sites may also show flare-up reactions [334] and even generalized erythematous macular eruptions can be observed with higher allergen doses [339]. The latter reactivities probably relate to the fact that recently activated T cells show strong expression of adhesion and homing molecules,

e.g., CLA and chemokine receptors such as CCR5, facilitating random migration into peripheral tissues and thus allergen-specific T cell patrol in the skin [232, 340]. Upon subsequent allergen entry from the circulation, these allergen-specific T cells could mediate generalized erythematous reactions [331].

Interestingly, local allergen-specific T cell retention/ "local skin memory" can be clinically exploited to discriminate between simultaneous sensitization to different sensitizers ("concomitant sensitization") and cross-reactivity between different sensitizers [341–343]. Using several different combinations of contact allergens in a guinea pig model, we retested guinea pigs previously sensitized to DNCB and methyl methacrylate (MMA), with the same allergens and some other methacrylate congeners. Accelerated retest reactivities were observed with the latter congeners on the former MMA, but not DNCB, patch test sites [341]. Thus, with preferential local retention of MMA-specific T cells at the MMA skin test site, no accelerated retest reactivity could be elicited with DNCB, but to varying degrees with all four MMA-related compounds. In clinical practice using this approach, Matura [342] confirmed positive cross-retest reactions for cloprednol and tixocortol pivalate, both belonging to group A, and budesonide, amcinonide, and triamcinolone, all belonging to group B corticosteroids (see also [344]).

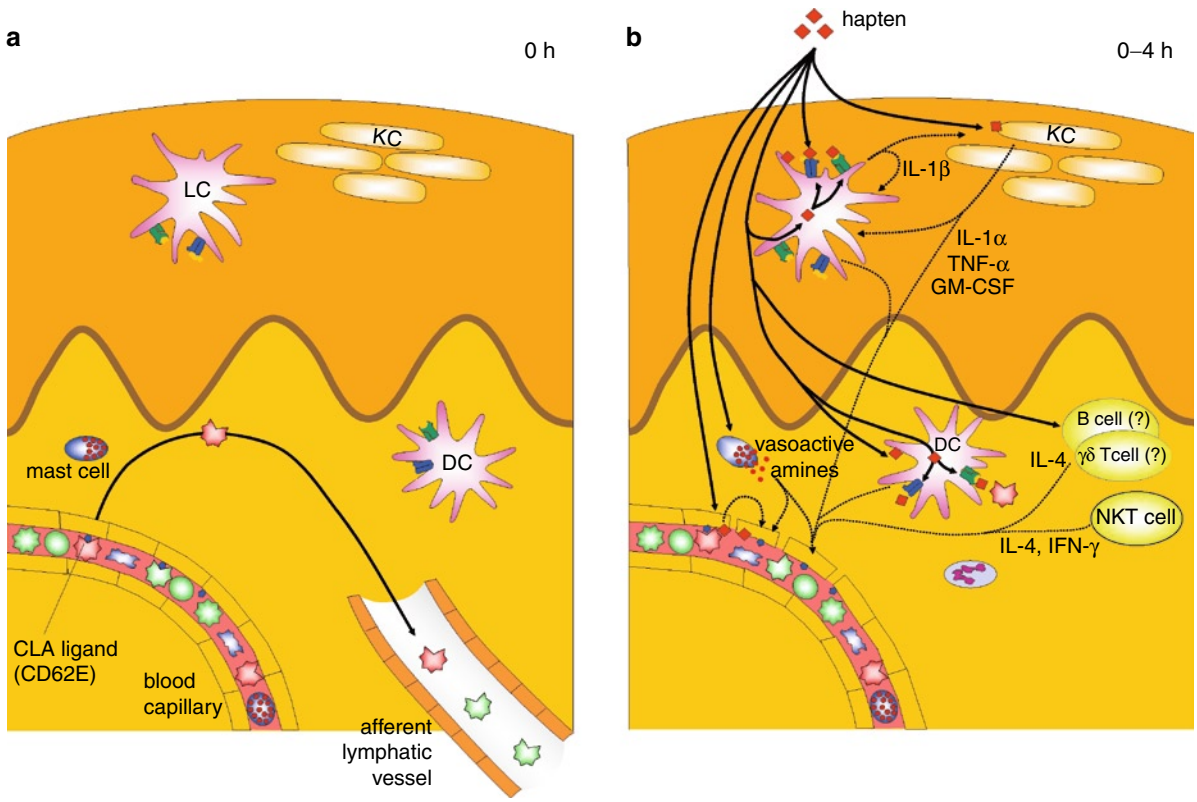

Fig. 3.9 (**a**–**c**) The effector phase of allergic contact dermatitis. (**a**) *0 h*: In resting skin relatively few randomly patrolling, skin-homing CLA$^+$ T cells are present. (**b**) *0–4 h*: Reexposure of the contact allergen, binding to (epi)dermal molecules and cells, induces release of proinflammatory cytokines. (**c**) *2–6 h*: Influenced by inflammatory mediators, activated epidermal Langerhans cells (*LC*) start migrating toward the basal membrane and endothelial cells express increased numbers of adhesion molecules. Endothelial cell-bound hapten causes preferential extravasation of hapten-specific T cells, which are further guided by inflammatory chemokines. (**d**) *4–8 h*: Hapten-activated T cells release increasing amounts of inflammatory mediators, amplifying further cellular infiltration. (**e**) *12–48 h*: The inflammatory reaction reaching its maximum, characterized by (epi) dermal infiltrates, edema, and spongiosis. (**f**) *48–120 h*: Gradually, downregulatory mechanisms take over, leading to decreased inflammation and disappearance of the cellular infiltrate. Finally, primordial conditions are reconstituted except for a few residual hapten-specific T cells causing the local skin memory. *KC* keratinocyte; *DC* dendritic cell

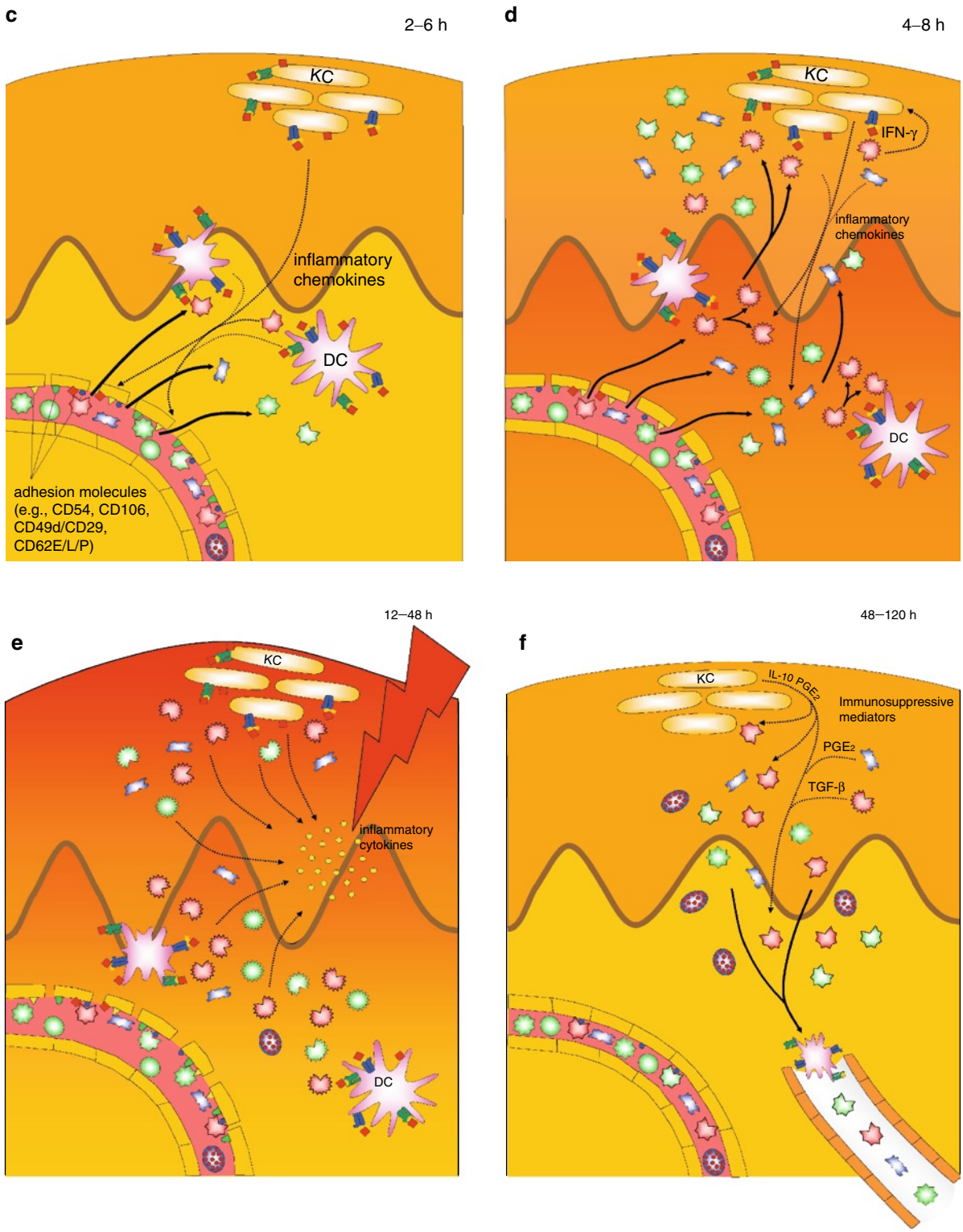

Fig. 3.9 (continued)

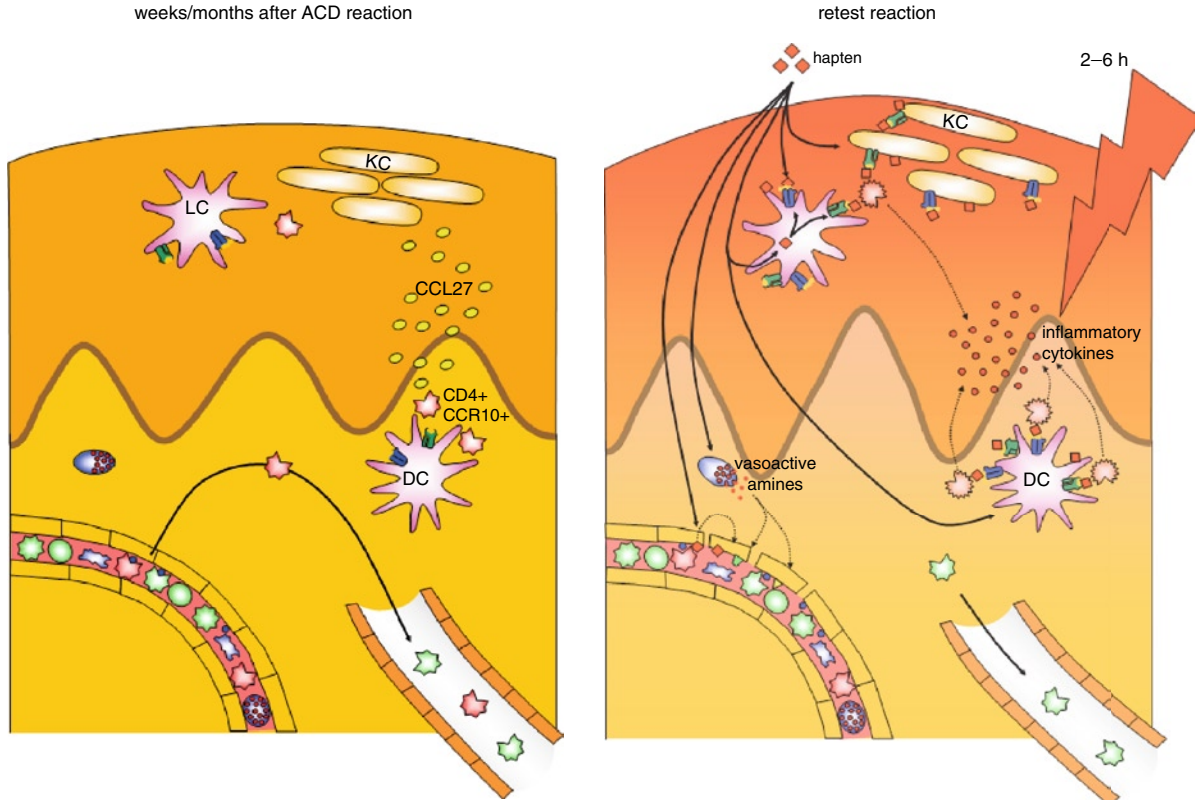

Fig. 3.10 Local skin memory. In former allergic contact dermatitis sites, a few hapten-specific T cells can remain, mainly close to dermal dendritic cells (*DC*). Retest reaction: renewed hapten contact can induce a rapid onset of an erythematous reaction, sparked off by the residual hapten-specific T cells. *KC* keratinocyte; *LC* Langerhans cell

3.3.8 Hyporeactivity: Tolerance and Desensitization

Of course, uncontrolled development and expression of T cell-mediated immune function would be detrimental to the host. During evolution, several mechanisms developed to curtail lymph node hyperplasia or prevent excessive skin damage upon persisting antigen exposure.

3.3.8.1 Regulation of Immune Responses

First, allergen contacts, e.g., by oral or intravenous administration, may lead to large-scale presentation of allergen by cells other than skin DC (Fig. 3.11). In the absence of appropriate costimulatory signals (as described above in Sect. 3.3.3), allergen presented by, e.g., immature Langerhans' cells may anergize naive T cells, i.e., cause receptor-downregulation associated with an unresponsive state, eventually leading to their death by apoptosis (Fig. 3.12) [345–347]. With increasing densities of MHC-antigen complexes on the surface of professional APC, at least three different levels of T-cell tolerance may be induced, characterized by active suppression, anergy, or deletion [348, 349]. Unresponsiveness of T cells, induced by allergenic contacts at skin sites where LC/DC functions have been damaged, e.g., by UV irradiation, or are naturally absent, e.g., in the tail skin of mice, may be ascribed to T-cell anergy, frequently associated with TCR/CD4 or CD8 downregulation, and apoptosis/deletion [350, 351]. Whereas anergy and deletion reflect "passive" unresponsiveness, tolerance by active suppression may also be induced under similar circumstances [352].

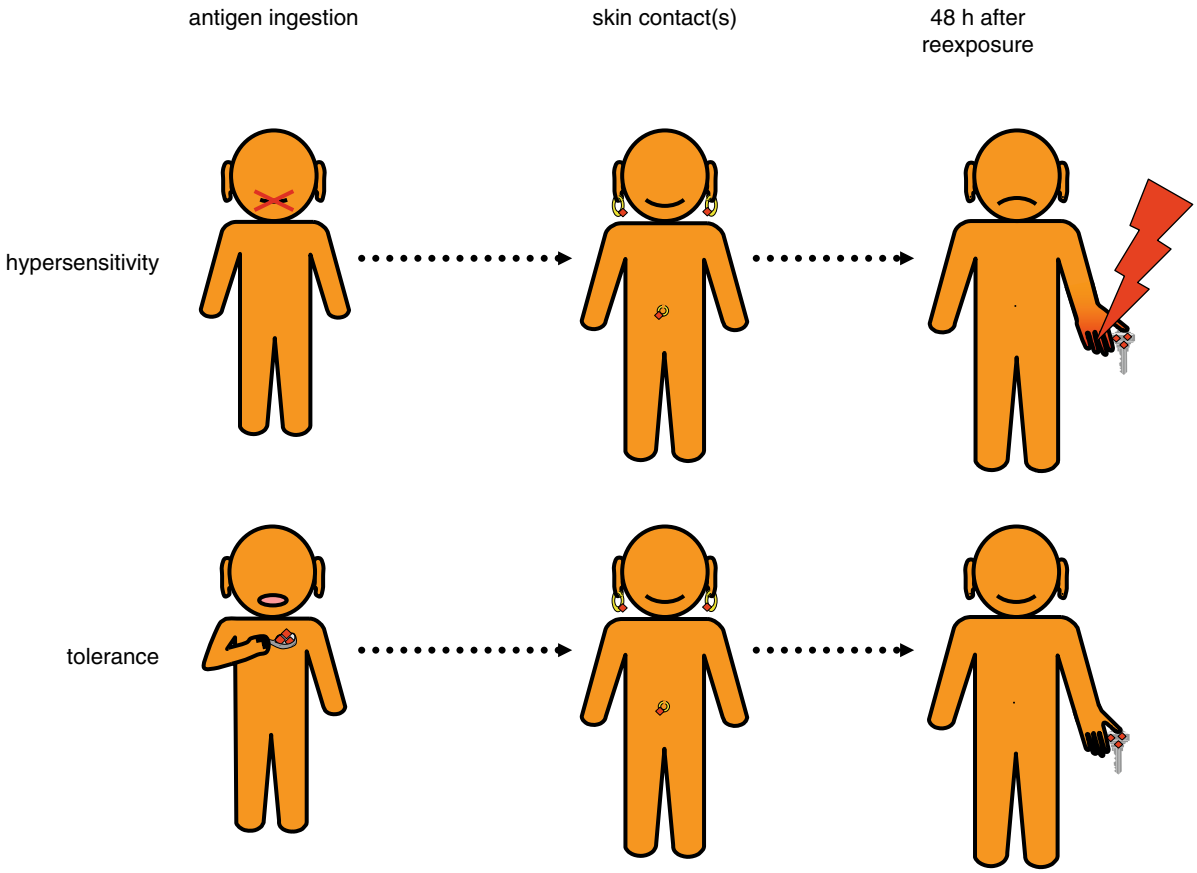

Fig. 3.11 Induction of oral tolerance. Hapten ingestion, prior to potential sensitizing skin contact(s), can induce hapten-specific tolerance

Actually, with increasing dose and exposure times, even regular epicutaneous allergenic contacts induce not only T effector cells but also lymphocytes controlling T-cell proliferation (afferently acting regulatory cells) and/or causing decreased skin reactivity (regulatory cells of effector phase). Thus, allergic contact hypersensitivity is the resultant of a delicate balance between effector and regulatory mechanisms [329, 353].

3.3.8.2 Cellular Basis of Active Tolerance

Upon preferential stimulation of regulatory cells, e.g., by feeding nonprimed, naïve individuals with contact allergens, strong, and stable allergen-specific, active tolerance may develop [354–356]. The concept of active regulatory ("suppressor") cells controlling ACD is based on the fact that in experimental animal models, such allergen-specific tolerance can be transferred by lymphoid cells from tolerant to naive animals [298, 357]. Active suppression, as revealed by these adoptive cell transfers, is a critical event in regulating T-cell responses to contact sensitizers and to all possible peptide/ protein antigens, including bacterial, autoimmune, and graft rejection antigens [358–360].

Like effector T cells in ACD, regulatory cells are not a single subpopulation of cells. As outlined above, depending on, e.g., the nature of the allergen and route of exposure, ACD can be mediated by both $CD4^+$ and $CD8^+$ T cells, either or both releasing Th1, Th2, Th3, Th17/22 cytokines. With distinct effector phenotypes for particular allergens, each of the other phenotypes can act as regulatory cells ([361, 362]: CD8+ Treg). Notwithstanding, type-2 cytokine-producing cells are prominent in regulating ACD, with allergic contact hypersensitivity enhanced and tolerance reversed by interfering with type-2 T cell functions [363–366]. Also, interferons and IL-12, both impairing Th2 and Th17/22 cells, were shown to inhibit regulatory cells

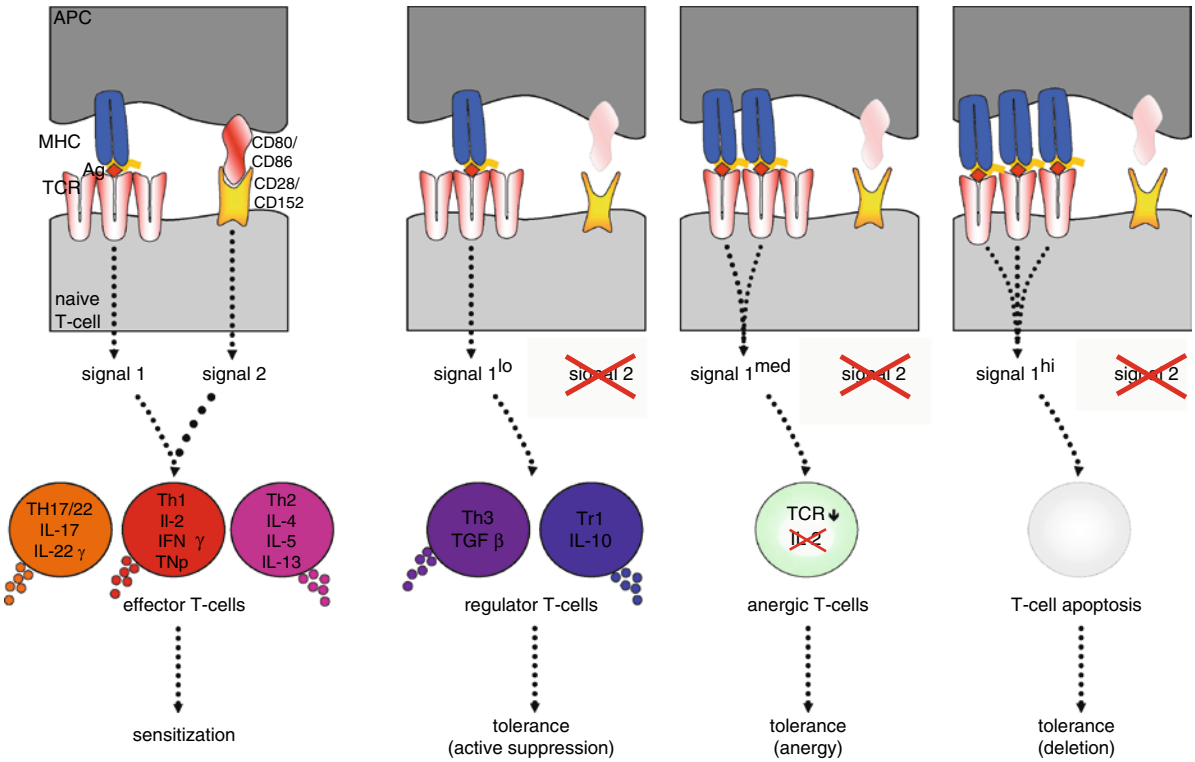

Fig. 3.12 The character of the APC–T cell interaction determines the immunological outcome. Sensitization: Naïve T cells, activated by antigen-presenting cells (*APC*) providing both hapten-specific ("signal 1") and appropriate costimulatory ("signal 2") signals, develop into effector T cells, characterized by Th-17/22, -1, and -2 cytokine secretion profiles. Tolerance: In the absence of appropriate costimulatory signals, immunological tolerance may develop. With increasing density of MHC–hapten complexes on the surface of APC, activating "signal 1" T-cell pathways, multiple levels of T-cell tolerance might be induced

and stimulate effector cell functions in mouse models [367–369]. In particular, after mucosal allergen contact stimulation, T cells producing IL-10 and/or TGF-β (type-3 cytokine profile), many of which coexpressing CD4, CD25, and the transcription factor Foxp3 (Treg), may act as regulatory cells [174, 370, 371]. These T cells promote anti-inflammatory immunity, e.g., by switching antibody production to IgA, which mediates secretory immunity and thus contributes to antigen exclusion in the lumen, e.g., of the gastro-intestinal tract [372]. Of note, TGF-β strongly suppresses development of both type-1 and -2 effector T cells, and can silence T cells in a seminaïve state [236].

3.3.8.3 Regulatory Mechanisms of the Effector Phase

A critical feature of the regulatory principles involving mutual regulation of T-cell subpopulations by Th1, Th2, Th3, and Th17/22 cytokines is that regulatory functions are most effective during initiation of immune responses (Fig. 3.7). Thus, once established, effector T cell and cytokine profiles show remarkable stability and refractoriness to regulatory forces. Downregulation of allergic skin reactions may, therefore, take considerable time. Of course, the preliminary factor facilitating decreased allergic skin reactivity is the removal of hapten by exudate and innate immune cells of the inflammatory infiltrate. But, at chronically exposed sites, specific regulatory mechanisms can also be involved, such as $CD8^+$ T cells, acting either as regulator/ suppressor ($CD28^-CD11b^+$) or cytotoxic ($CD28^+CD11b^-$) T cells [373, 374], which may downregulate skin reactivity by targeting allergen-presenting DC [374]. Multiplicity and redundancy of regulatory mechanisms have thus far hampered development of robust clinical treatments exploiting regulatory T cell functions to provide for allergen-specific downregulation of the effector phase of ACD. The development of potential therapeutic

applications of regulatory cells in various disorders, such as ACD and autoimmune diseases, therefore, needs much more time than envisioned earlier [375].

3.3.8.4 Redundancy of Tolerance Mechanisms

Besides regulatory T cells, producing different cytokines or exerting distinct cytotoxicities, other mechanisms may also contribute to immune regulation and tolerance. Clearly, the risk of excessive immune reactivity should be very low. These mechanisms involve allergen-specific T cells shedding truncated TCRs, acting as antagonists and blocking allergen presentation [376], and high-dose allergen-induced anergic T cells [349]. Possibly, the latter cells, by actively suppressing DC functions, can function as "active" suppressor cells [377, 378]. Interestingly, DC, becoming suppressive by this mechanism [378] or by suppressive cytokines like IL-10 and PGE_2 [230, 379, 380], can, in turn, act themselves as suppressor cells by conferring antigen-specific anergy to subsequently encountered T cells [377, 378, 381]. Although, at present, consensus has been reached about a critical role of regulatory/ suppressor cells in the development and expression of ACD, the relative contributions of each of the various mechanisms are still far from clear.

3.3.8.5 Induction of Lasting Tolerance Only in Naive Individuals

Both clinical and experimental findings indicate that full and persistent tolerance can only be induced prior to any sensitizing allergen contacts [356, 382, 383]. Upon primary allergenic contacts, naive T cells differentiate to produce polarized cytokine profiles (Figs. 3.7 and 3.11). Once polarized, however, T-cell profiles are irreversible, due to loss of cytokine (receptor) genes, or at least very stable, due to the mutually suppressive activities of T-cell cytokines. An important corollary of the latter concept of active suppression is the bystander effect, in which the response to any antigen can be downregulated by immunosuppressive cytokines acting in a local tolerogenic microenvironment [384]. The latter was observed for both protein antigens [385] and methacrylate contact allergens [357]. Stable polarization/ skewing may also explain why even low, nonsensitizing doses of nickel applied to the skin prevented subsequent tolerance induction by feeding the metal allergen [386]. Apparently, the progeny of naïve allergen-specific cells, once "on the stage," has been triggered to a "subclinical" degree toward effector cell differentiation and becomes refractory to regulatory cell action. This may also have contributed to incomplete tolerance induction in earlier clinical studies when feeding with poison ivy-/oak-derived allergens [387]. Indeed, to our knowledge, permanent reversal of existing ACD in healthy individuals has, as yet, never been achieved. Nevertheless, as described above, effector cells still seem susceptible, though transiently, to the downregulation of allergen reactivity, as was observed in desensitization procedures [386, 388].

3.3.8.6 Transient Desensitization in Primed Individuals

For dermatologists, methods by which patients might be desensitized for existing ACD would be a welcome addition to the currently prevailing symptomatic therapies, and investigators have made a wide variety of attempts to achieve this goal. Unfortunately, as mentioned above, therapeutic protocols involving ingestion of poison ivy allergen, penicillin, or nickel sulfate were of only transient benefit to the patients [387–391]. Similarly, in animal models, only a limited and transient degree of hyposensitization was obtained by Chase [392] when feeding DNCB-contact-sensitized guinea pigs with the allergen, whereas for achieving persistent chromium-unresponsiveness in presensitized animals, Polak and Turk [393] needed a rigorous protocol involving up to lethal doses of the allergen. As outlined above, mechanisms underlying specific desensitization in ACD probably depend on direct interference of allergen with effector T-cell function by blocking or downregulating TCRs, leading to anergy and apoptosis [394]. As the onset of desensitization is immediate, no suppressor mechanisms may initially be involved. Apparently in the absence of LC, MHC class II-positive keratinocytes can serve as APC and are very effective in rendering allergen-specific effector cells anergic [395]. Moreover, at later stages active suppression may come into play resulting from secondary inactivation of DC function by anergized T cells [350]. Nevertheless, major problems with in vivo desensitization procedures relate to the

refractoriness of effector T cells to regulatory cell functions, and the rapid replacement of anergized effector cells by naïve T cells from relatively protected peripheral lymphoid tissues provides a source of new effector cells upon sensitizing allergen contacts. The same conclusions can be drawn from attempts to achieve local desensitization. It was found that local desensitization by repeatedly applying allergen at the same skin site did not result from local skin hardening or LC inactivation, as local reactivity to an unrelated allergen at the site was unimpaired [329]. Persistence of cellular infiltrates, in the absence of erythematous reactivity, at a desensitized skin site could reflect local anergy, but also locally active regulatory cells. Upon discontinuation of allergen exposure, however, local unresponsiveness was rapidly (within 1 week) lost. Collectively, this data illustrate the problems encountered in attempting to eradicate established effector T-cell function, not only in ACD but also in autoimmune diseases [356, 360].

3.4 Summary and Conclusions

Extensive research has led to a better understanding of the mechanisms of ICD and ACD. The primary role of innate immune cells in coping with exogenous potential harmful threats is rapidly being uncovered. Also, the basic immunology of ACD is now well-defined, including T-cell migratory patterns, recognition of distinct allergens, interactions with other inflammatory cells to generate inflammation, and cytokine profiles. But new complexities have emerged. For instance, in contrast to earlier belief, many of the currently known T-cell subpopulations can act either or both as effector and regulatory cells, depending on the nature of the allergen, the route of entry, frequency of exposure, and many other still ill-defined factors. In particular, the poor understanding of regulatory mechanisms in ACD still hampers further therapeutic progress. So far, no methods of permanent desensitization have been devised.

Nevertheless, next to the established anti-inflammatory drugs, recently defined cellular interaction molecules and mediators provide promising targets for new generations of anti-inflammatory drugs, some of which have already entered clinical trials. Clearly, drugs found to be effective in preventing severe T-cell-mediated conditions, e.g., rejection of a vital organ graft, should be very safe before their use in ACD would seem appropriate. To date, prudence favors alternative measures to prevent ICD and ACD, be it through legal action to outlaw the use of certain materials or through avoiding personal contact with these materials. In the meantime, for difficult-to-avoid allergens, further studies on the potential value of tolerogenic treatments prior to possible sensitization seem warranted.

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