Immunity/Immunopathology

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Contents

Abstract

For appreciable time, the pathophysiology leading to ultimate melanocyte destruction remained uncertain. This is because skin-infiltrating T cells involved in melanocyte loss are few in number and are only observed in actively depigmenting skin; thus, such infiltrates are easily overlooked. Moreover, T cells were more difficult to distinguish before antibodies became readily available for immunohistology. Ample support exists for autoimmunity as a chief etiopathological factor in vitiligo: susceptible individuals exhibit polymorphisms in immune regulatory genes which promote autoimmunity in the cutaneous microenvironment; additionally,

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M. Picardo, A. Taïeb (eds.), *Vitiligo*, https://doi.org/10.1007/978-3-319-62960-5_28

vitiligo has a well-established association with other autoimmune diseases. Among the possibly involved cell populations, Langerhans cells might contribute to depigmentation on-site (perhaps through continued melanocyte antigen presentation to cytotoxic T cells), thereby preventing viability of any melanocytes attempting to repopulate the skin. The HSP-native protein complexes can trigger a local immune response directed at the cells from which the native proteins originate. Upon melanocyte stress and subsequent HSP70i release, antigen-presenting cells will recruit an initial cohort of melanocyte-reactive T cells that produce IFN-γ upon antigen recognition. This would lead to CXCL10 production and further recruitment to the epidermis. The absence of Tregs in vitiligo skin is likewise best explained by differential chemokine expression in lesional skin, mainly involving CCL22.

Key Points

- Progressive depigmentation in vitiligo relies on skin-infiltrating cytotoxic T cells specific for melanocyte self-antigens.
- Melanocytes in perilesional skin are also sensitive to self-reactive antibodies.
- Regulatory T cells (Tregs) are present in decreased numbers in vitiligo-affected skin.
- Stress proteins including inducible heat shock protein 70 (HSP70i) link initial skin trauma to the adaptive immune responses that follow.
- Interferon-gamma (IFN-γ) signaling through the JAK-STAT pathway drives vitiligo pathogenesis by recruiting cytotoxic T cells to the skin.
- Stress protein upregulation, activation of antigen-presenting cells, recruitment of melanocyte-reactive T cells, and a paucity of regulatory T cells are intertwined phenomena that lie at the heart of vitiligo pathogenesis.
- Promising immunotherapeutic strategies are being considered for vitiligo treatment including modified HSP70 delivery, chemokine receptor blockade, JAK-STAT inhibitor application, adoptive Treg transfer, and anti-cytokine therapies.
- Vitiligo development is considered a positive prognostic factor in melanoma, as it serves as a marker of generation of robust anti-melanocyte tumor responses.

28.1 Establishment of Vitiligo as an Autoimmune Disease Entity

The disease course of vitiligo is variable, and it affects between 0.5% and 1% of the population [1]. The clinical finding of depigmented skin patches correlates with the finding of nearly complete absence of melanocytes histologically [2, 3]. This observation prompted studies that would explain disease etiology in terms of melanocyte destruction rather than suppression of pigmentation. For an appreciable time, the pathophysiology leading to ultimate melanocyte destruction remained uncertain. This is because skin-infiltrating T cells involved in melanocyte loss are few in number and are only observed in actively depigmenting skin; thus, such infiltrates are easily overlooked. Moreover, T cells were more difficult to distinguish before antibodies became readily available for immunohistology [4, 5], and, initially, most attention was directed toward possible humoral involvement in vitiligo [6, 7]. Observations including the transmission of disease through adoptive transfer of antibodies [8, 9], as well as reduced surface expression of factors such as decay-accelerating factor (DAF) that otherwise protects from complement-mediated destruction, also led to antibodies being considered as the culprits in melanocyte destruction [10]. For the morphology of cultured melanocytes required for such studies, see Fig. 28.1. However, as the target antigens identified by these studies are not necessarily expressed in the cell membrane, anti-melanocyte

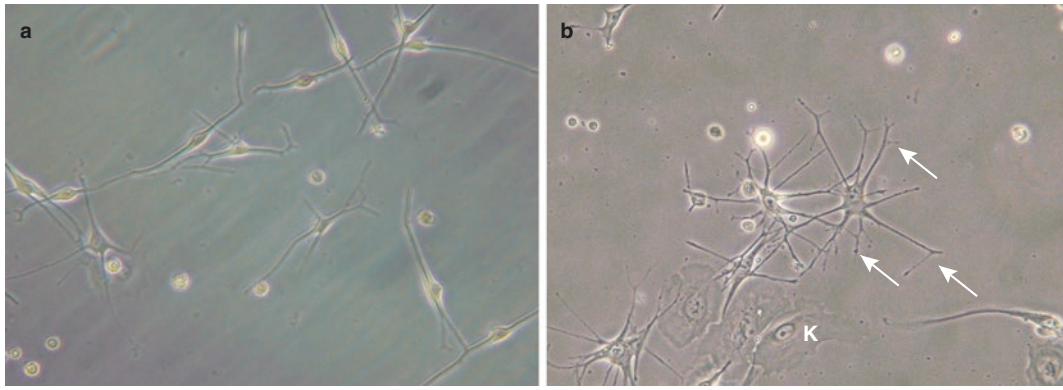

Fig. 28.1 Dendritic morphology of recently plated melanocytes in culture. Cells were isolated from (C) neonatal foreskin or (V) a scalp biopsy from a vitiligo patient by overnight enzymatic treatment and plated in replete media. Note that vitiligo melanocytes exhibit multiple, branched dendrites (arrows) in contrast to the more bipolar morphology of healthy neonatal melanocytes. *K* keratinocytes

antibodies were ultimately considered an epiphenomenon of the disease. Since B cell activation and T cell activation are intimately related and mutually supportive [11–13], renewed attention to B cell and antibody involvement is to be expected.

While many environmental factors can contribute to disease precipitation, the consensus reached by the Vitiligo European Task Force (VETF) is that autoimmunity contributes to all cases of vitiligo [14]. Interestingly, this includes segmental vitiligo [15], which was initially considered a developmental defect [16]. Vitiligo likely manifests itself when certain environmental factors affect individuals with a genetic predisposition for the condition. Interestingly, many of the proposed vitiligo susceptibility genes have immune modulatory functions, including interleukin-2 receptor alpha chain (IL2RA), ubiquitin-associated and SH3 domain-containing A protein (UBASH3A), and C1q and tumor necrosis factor-related protein (C1QTNF6) [17]. Human leukocyte antigens (HLA)-A2 [17, 18] and HLA-DR4 [17, 19] have also been linked with vitiligo development. Indeed, MHC involvement is a hallmark of autoimmunity [20]. In the following sections, we describe the interplay between the cutaneous microenvironment and immunologic factors that lead to ultimate melanocyte destruction in vitiligo.

28.2 Cellular Immunity in Vitiligo

Ample support exists for autoimmunity as a chief etiopathological factor in vitiligo. As discussed above, susceptible individuals exhibit polymorphisms in immune regulatory genes which promote autoimmunity in the cutaneous microenvironment. Additionally, vitiligo has a well-established association with other autoimmune diseases [6], the most common of which is Hashimoto's thyroiditis [21]. In fact, this association is common enough to warrant checking antithyroid antibodies, including antithyroid peroxidase antibody and anti-thyroglobulin levels, in addition to baseline thyroid-stimulating hormone (TSH) levels in newly diagnosed vitiligo patients [22, 23]. Other associated autoimmune diseases include alopecia areata, diabetes mellitus type I, pernicious anemia, and Addison's disease [21] (see Chap. 13). Some of the more pertinent findings supporting contributory autoimmune mechanisms in vitiligo involve the inflammatory infiltrates consistently identified in newly vitiligo-afflicted skin. Specifically, lymphocytic infiltrates exhibiting a decreased ratio of $CD4^+/CD8^+$ T cells have been identified in perilesional areas of patients with active depigmentation [24, 25]. The cytotoxic ($CD8^+$) T cells present in perilesional areas are activated [26] and are located in close proximity to melanocytes and melanocyte fragments in the basal layer of the epidermis

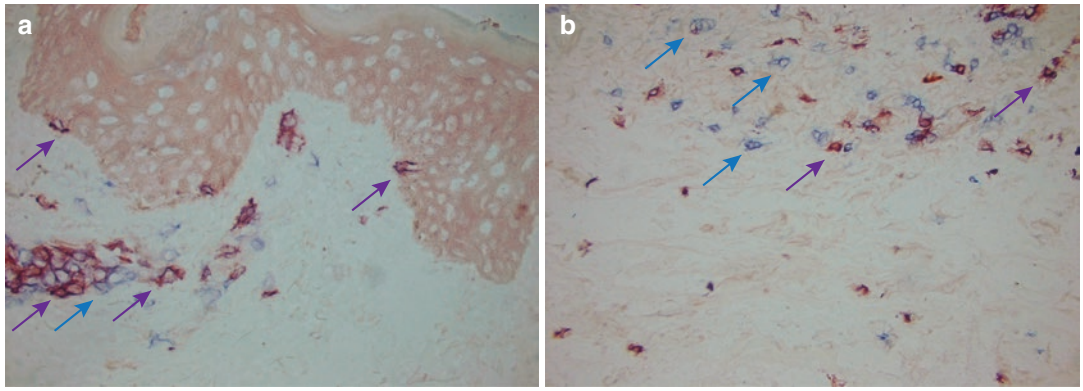

Fig. 28.2 T cells in vitiligo and melanoma. Sections of (**a**) depigmenting vitiligo skin and (**b**) a melanoma tumor metastasis were stained with antibodies to CD3 (blue) and CD8 (red). Note infiltrates consisting of primarily CD8+ T cells in vitiligo skin with some cytotoxic T cells approaching the basal layer of the epidermis. A larger proportion of the tumor-infiltrating T cells belongs to the $CD4^+$ subset of helper and regulatory T cells (blue)

(Fig. 28.2). Notably, their presence correlates with melanocyte disappearance [25]. These cytotoxic T cell infiltrates are found in perilesional skin of vitiligo patients [24], suggesting a role in melanocyte destruction. Indeed, these skin-infiltrating T cells are reactive with melanocyte self-antigens [27, 28]. Circulating melanocyte antigen-specific T cells have been found in increased levels in vitiligo patients as well [29], but, given their location, the skin-homing T cells are of greater pathogenic significance. Further supporting the role of antigen-specific T cells in vitiligo pathogenesis is their epidermal tropism [30, 31].

28.3 Antigen-Presenting Cells in Vitiligo

The development of cutaneous immune responses begins with antigen-presenting cells (APCs) residing in the epidermis and dermis. Indeed, these APCs ultimately promote T cell activation, which, in turn, begets both further T cell responses (cellular immunity), as well as B cell activation and antibody formation (humoral immunity). Numerous types of professional antigen-presenting cells reside in the skin, including macrophages, Langerhans cells (LC), and dermal dendritic cells [32]. Dendritic cells (DCs) play a particularly important role in cutaneous immune responses by patrolling for pathogens. Pathogenic signals such as microbial peptides interact with receptors on resident dendritic cells, resulting in DC maturation and antigen presentation. This maturation involves a marked increase in a number of MHC class II and costimulatory molecules on the DC cell surface [33] and allows DCs to process antigens and activate T cells efficiently [34]. Two important receptor families which commonly stimulate DC maturation are the toll-like receptors (TLRs) and tumor necrosis factor (TNF) receptors [35–39]. Dendritic cells then endocytose, process, and present antigenic peptides on the cell surface in the context of major histocompatibility complex (MHC) molecules [40]. After migrating to skin-draining lymph nodes, they interact with, activate, and recruit cytotoxic and helper T cells specific to the antigens presented [41, 42]. Thus, antigen-presenting cells govern the type of helper T cell response elicited. Through cross-presentation, melanocyte-specific antigens displayed via MHC class I molecules give rise to the cytotoxic T cell cohort known to mediate melanocyte loss in vitiligo. Interestingly, a viral trigger may exist in some cases of vitiligo [43].

Langerhans cells are epidermal antigen-presenting cells that form the first line of defense against foreign antigens entering via the skin. They can stimulate $CD4^+$ T cells [44] and are largely responsible for inducing contact hypersensitivity reactions [45]. Sensitization to contact allergen was not observed in Langerhans cell-deficient skin [46]. The role of Langerhans cells in vitiligo is not as clearly established. Interestingly,

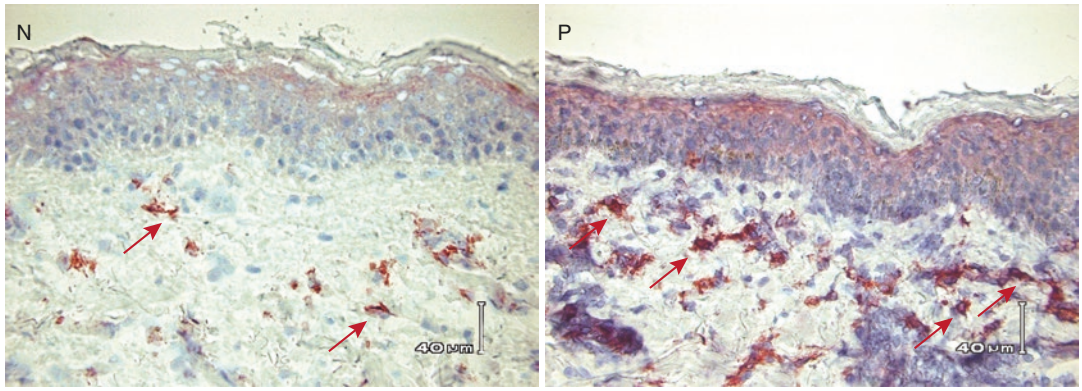

Fig. 28.3 Abundance of CD36⁺ cells in perilesional vitiligo skin. Overexpression of the thrombospondin receptor CD36 has been associated with monocyte-to-macrophage differentiation and activation of the phagocytic process. CD36 can be expressed by other cells including thrombocytes, endothelial cells, and melanocytes, yet the morphology and location of the majority of cells observed in the dermis of this patient's non-lesional and perilesional skin (detectable as a red AEC precipitate, red arrows) support the reported abundance of macrophages in depigmenting vitiligo skin (P) as compared to non-lesional skin (N)

Langerhans cells reposition themselves, settling into the basal layer of the epidermis in melanocyte-depleted vitiliginous skin [47]. Furthermore, repigmentation in response to treatment can be associated with Langerhans cell depletion [48]. Thus, Langerhans cells might contribute to depigmentation on-site (perhaps through continued melanocyte antigen presentation to cytotoxic T cells), thereby preventing viability of any melanocytes attempting to repopulate the skin. This model could be likened to a delayed type hypersensitivity response, whereby the melanocyte self-antigens represent the "contact allergen." Indeed, peptide-pulsed Langerhans cells can stimulate and expand CD8⁺ MART-I specific T cells [44]. However, as Langerhans cells are relatively inefficient at processing antigen [49, 50], their actual role in recruiting melanocyte-reactive T cells and initiating disease remains to be firmly established. Macrophages have been implicated in autoimmune disease development [51], and diabetes will not develop in macrophage-deficient mice [52]. Macrophages may likewise be pathogenic in vitiligo as they consistently infiltrate depigmenting perilesional vitiligo skin (Fig. 28.3), and their appearance correlates with melanocyte loss [24, 25]. However, the role of macrophages in vitiligo has not been clearly established [53]. Macrophages may phagocytize already apoptotic melanocytes or may actively contribute to melanocyte destruction. Once activated by interferon-gamma (IFN-γ) [54], macrophages secrete proinflammatory cytokines. Among these, TNF-α can contribute to melanocyte apoptosis [55]. Moreover, phagocytic macrophages [56] generate proinflammatory cytokines, such as IL-8, when engulfing apoptotic cells [57]. They may also serve as APCs to promote continued activation of autoreactive T cells. Taken together, there is ample cause for further studies into the pathogenic role of macrophages in vitiligo.

28.4 Heat Shock Proteins in Immune Activation

Heat shock proteins (HSPs) play an integral role in the development of several autoimmune diseases, including vitiligo [58, 59]. Expression of HSPs is induced by stress [60, 61]. HSPs bind native proteins to prevent misfolding and cellular apoptosis [62]. In addition to facilitating cellular self-preservation, these HSP-native protein complexes can also trigger a local immune response directed at the cells from which the native proteins originate [63]. Once the HSP-protein complexes are released into the microenvironment, they bind receptors located on antigen-presenting cells [64, 65]. Surrounding APCs subsequently process the bound peptides and cross-present

them to CD8+ T cells [66], eliciting immune responses toward the chaperoned antigens. Inducible heat shock protein 70 (HSP70i) can also be secreted from live cells under stress as a "chaperokine" [67]. HSP70 and other stress proteins can activate dendritic cells and subsequent T cell responses, leading to autoimmunity. HSP70 mediates development of autoimmune diabetes in mice [68] and likely plays a role in other autoimmune diseases as well. Overexpression of inducible HSP70 is sufficient to precipitate vitiligo disease [69], and vitiligo does not develop in mice that lack expression of HSP70i [70]. Importantly, the stress protein is overexpressed in vitiligo skin [71, 72]. These findings support a critical role for HSP70i in T cell-mediated melanocyte destruction.

Stress exposures that precipitate and worsen vitiligo include trauma to the affected area (koebnerization) [73], psychological stress [74], UV irradiation (oxidative stress) [75], and exposure to bleaching phenols [76]. HSP70 is upregulated in melanocytes after exposure to bleaching phenols [77], which have a well-established role in precipitating and worsening depigmentation [76, 78]. Other heat shock proteins are less likely to induce vitiligo as they are released only after cell death [79]. HSP70i is unique in that it is secreted by viable cells under stress, including melanocytes [80–82], which are ultimately later killed as the result of HSP70i-mediated cellular immune activation.

28.5 T Cell Subset Imbalance in Vitiligo

Infiltrates with decreased CD4+/CD8+ T cell ratios are found in actively depigmenting vitiligo patient skin [24, 25, 83]. T cells infiltrating vitiligo skin were shown to express the cutaneous lymphocyte antigen (CLA), a skin-homing receptor [25]. Circulating T cells in vitiligo patients are reactive with several melanocyte-specific antigens, including gp-100, tyrosinase, and Melan-A/MART-1 [83, 85]. Moreover, a T cell receptor reactive with the gp100 antigen has been characterized and cloned from patient skin [86]. T cells derived from vitiligo patient skin secrete predominantly type I cytokines in response to melanocytes, again indicative of cytotoxic T cell involvement [28].

In addition to increased numbers of skin-homing effector T cells [83], decreased skin homing of their inhibitory counterparts, the regulatory T cells (Tregs), is observed [87]. Others have reported a decreased abundance of Tregs in the circulation [88] or mentioned that Treg function may be reduced among circulating Tregs [89], but such deficiencies are expected to accompany more systemic autoimmune consequences. Decreased numbers or impaired function of Tregs have been established in systemic autoimmune disease [90]. This deficiency can well explain why tolerance to self-antigens is inadequately maintained [91]. The T cell imbalance in vitiligo skin thus creates the perfect environment for depigmentation. Destruction of melanocytes in the skin not only results in clinical depigmentation but also leads to a large number of resident memory T cells capable of recognizing melanocytic self-antigens [92, 93]. These self-reactive T cells have been found to persist in the skin long after melanocyte destruction [93]. This might well explain disease relapse in patients following periods of repigmentation.

28.6 T Cell Trafficking

In order for vitiligo to develop, melanocyte-specific cytotoxic T cells must migrate toward the epidermis in response to chemoattractant cytokines. The presence of IFN-γ is crucial for active depigmentation to ensue [94]; it induces the expression of the Th1 chemokine, CXCL10 [95], which is produced by numerous cell types, including keratinocytes, macrophages, fibroblasts, neutrophils [95, 96], and, importantly, activated T cells [97, 98]. Upon melanocyte stress and subsequent HSP70i release, antigen-presenting cells will recruit an initial cohort of melanocyte-reactive T cells that produce IFN-γ upon antigen recognition. This would lead to CXCL10 production and further T cell recruitment to the epidermis. Indeed, neutralization of CXCL10 prevented depigmentation and promoted repigmentation in mice [99]. Aside from T cells, CXCL10 can recruit monocytes/

macrophages and natural killer (NK) cells [96]. Yet, the primary contribution of CXCL10 to vitiligo development lies in skin recruitment of self-reactive T cells expressing its receptor CXCR3, as found in vitiligo patient skin [99].

The absence of Tregs in vitiligo skin is likewise best explained by differential chemokine expression in lesional skin. Cells expressing the Treg chemoattractant CCL22 are present in markedly decreased numbers in vitiligo as compared to control skin [82]. CCL22 is secreted by macrophages and dendritic cells [100]. As the CCL22 receptor, CCR4, is expressed in similar amounts by circulating Tregs from patients and controls, the decreased amounts of CCL22 may be primarily responsible for the significant underrepresentation of Tregs in vitiligo skin.

28.7 Cytokines in Vitiligo Skin

Activated perilesional vitiligo T cells secrete cytokines and chemokines, which either propagate immune mechanisms or contribute directly or indirectly to melanocyte destruction. Vitiligo development is critically dependent on IFN-γ in mouse models of vitiligo [94, 101]. Similarly, T cells derived from vitiligo patient skin secrete IFN-γ in response to melanocytes, again indicative of a type 1 cytokine response and cytotoxic T cell involvement [28]. IFN-γ-responsive effector chemokines, including CXCL9, CCL5, and especially CXCL10, are reportedly elevated in the vitiligo skin [102] and can serve to facilitate T cell trafficking toward resident melanocytes. IFN-γ also increases T cell expression of CXCR3, activates macrophages, and increases ICAM expression on endothelial cells [54, 103–105]. Each of these factors can facilitate melanocyte destruction. Importantly, IFN-γ also inhibits Treg generation through STAT1-signaling [106]. However, in mice knockout for IFN-γ, spontaneous depigmentation was restored when Tregs were depleted from the circulation [107], supporting the concept that IFN-γ involvement is not the sole pathogenic cytokine in vitiligo progression.

TNF-α contributes to vitiligo pathogenesis by supporting cytotoxic T cell development [108] and enhancing IFN-γ secretion [109]. TNF-α also inhibits melanocyte proliferation in vivo [110] and promotes melanocyte apoptosis in vitro [111]. However, its presence is not required for disease development, as vitiligo development ensued in TNF-α knockout mice [107]. Cytotoxic T cell-mediated apoptosis occurs through either the perforin-granzyme pathway [112] or the Fas/Fas ligand pathways [112–114]. Because melanocytes proved resistant to Fas ligand-mediated cell death [115], and most perilesional T cells in vitiligo skin are perforin and granzyme-B immunoreactive [25], this has implicated perforin-granzyme-mediated cell death in vitiligo. However, mice knockout for perforin can still develop vitiligo [100, 107], as can granzyme knockout mice [115]. This implies that neither mechanism is exclusively responsible for depigmentation.

IL-17 is a cytokine with controversial involvement in vitiligo pathogenesis. IL-17 is produced by T cells and natural killer cells [116] and is increased both in vitiligo patient serum and in the lesional skin [117]. Furthermore, IL-17A mRNA and IL-17A$^+$ T cells were present in increased quantities in perilesional compared to non-lesional vitiligo skin [118]. In mice, adoptive transfer of Th17 cells induced vitiligo, supporting a possible role for their cytokines, including IL-17, in vitiligo pathogenesis. However, a role for IFN-γ cannot be ruled out [119]. Thus, focusing on the blockade of production or action of the latter cytokine may be of greater utility.

28.8 Additional Players in Cellular Immunity

Importantly, melanocytes from vitiligo patients are increasingly sensitive to cellular stress, as evidenced by dilation of the endoplasmic reticulum [120] and increased levels of oxidative byproducts [121–123]. When melanocytes release stress signals into the microenvironment (reactive oxygen species and HSP70i), this activates pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) [124]. In addition to induction of adaptive immune responses via dendritic cell activation, there is evidence to support that HSP70 also stimulates local innate immune responses. Indeed, HSP70$^+$ exosomes were found

to stimulate activation and migration of natural killer (NK) cells in vitro [125]. This likely translates to human disease, as patients with vitiligo were found to have increased amounts of natural killer cells in both lesional and non-lesional skin compared to their non-vitiligo counterparts [126]. This likely renders vitiligo patients capable of generating robust innate immune responses. Interestingly, NKs taken from lesional skin of vitiligo patients expressed high levels of granzyme B [126], the serine protease that can mediate melanocyte destruction. Inflammatory dendritic cells are also increased in vitiliginous skin [59, 72] and are induced by HSP70i in vitro [127]. Gene transcription evaluation in Smyth chickens that develop spontaneous vitiligo revealed increased transcription of innate immune response genes in response to oxidative stress [128], further supporting the role of innate immune responses in vitiligo.

The role of humoral immunity in vitiligo pathogenesis may be less contributory than cellular immune responses, but it likely fuels disease progression. Vitiligo patients have elevated anti-melanocyte antibody titers [29, 129]. Such antibodies could mediate cytotoxicity toward melanocytes [130]. Moreover, B cells may be involved in propagating T cell activation [11–13] to drive depigmentation. A noteworthy observation to discriminate between causative and bystander roles for autoantibodies is that of rarely reported congenital vitiligo [131, 132], where depigmentation was postulated to result from transplacental transfer of melanocyte-specific IgG antibodies in utero.

28.9 Opportunities for Immunotherapy

- *Phototherapy*
- Ultraviolet radiation (UVR) has immunosuppressive properties and is utilized to treat many skin conditions including psoriasis, atopic dermatitis, cutaneous T cell lymphoma, and vitiligo [133]. Immunosuppression is mediated by keratinocyte secretion of Th2 cytokines, primarily IL-10 and IL4 [134]. Specifically, IL-10 appears to decrease the ability of APCs to activate Th1 cells [134], inhibits development of delayed type hypersensitivity responses [135], and decreases T cell production of type I cytokines, including IL-12, IL-8, and interferon-γ [136]. Importantly, UVB was shown to inhibit phosphorylation of STAT-1, inhibiting IFN-γ signaling [137]. Narrowband UVB (nb-UVB) also markedly increases lesional and perilesional abundance of the Treg transcription factor FoxP3 [138], implying that nb-UVB increases the number of Tregs in the cutaneous environment. This likely contributes to the observed treatment success of nb-UVB in the clinic [139]. The immunosuppressive properties of UVR are evidenced by blunted T cell immune-mediated responses as well as decreased immune surveillance of UV-induced skin cancers following exposure [140–144].
- In addition to its immunosuppressive effects, UVR has direct effects on melanocyte function. Specifically, UVR increases melanosome accumulation and transfer to keratinocytes [145]; treatment with UVR also achieves clinical repigmentation. Narrowband UVB resulted in increased keratinocyte release of bFGF and ET-1, which induce melanocyte proliferation; nb-UVB also resulted in increased melanocyte expression of $p125^{FAK}$ and MMP-2, which enhance melanocyte migration [146]. Therefore, UVR may induce melanocyte stem cell proliferation, differentiation, and migration from their reservoir site upon exposure. The source of repigmenting melanocytes is presumably the hair follicle, which would clinically explain the appearance of perifollicular repigmentation as the first sign of treatment response in UVR-treated vitiligo patients. Inactive (Dopa-negative, non-melanin-producing) melanocyte stem cells are preserved in the outer root sheath of the hair follicles of vitiligo skin [147] and do not yet express the target molecules recognized by pathogenic T cells. When clinical repigmentation was noted, the first signs microscopically were an increased number of melanocytes in the outer root sheath of the hair follicle, migrating toward the epidermis and undergoing maturation [147]. UVA and nb-UVB

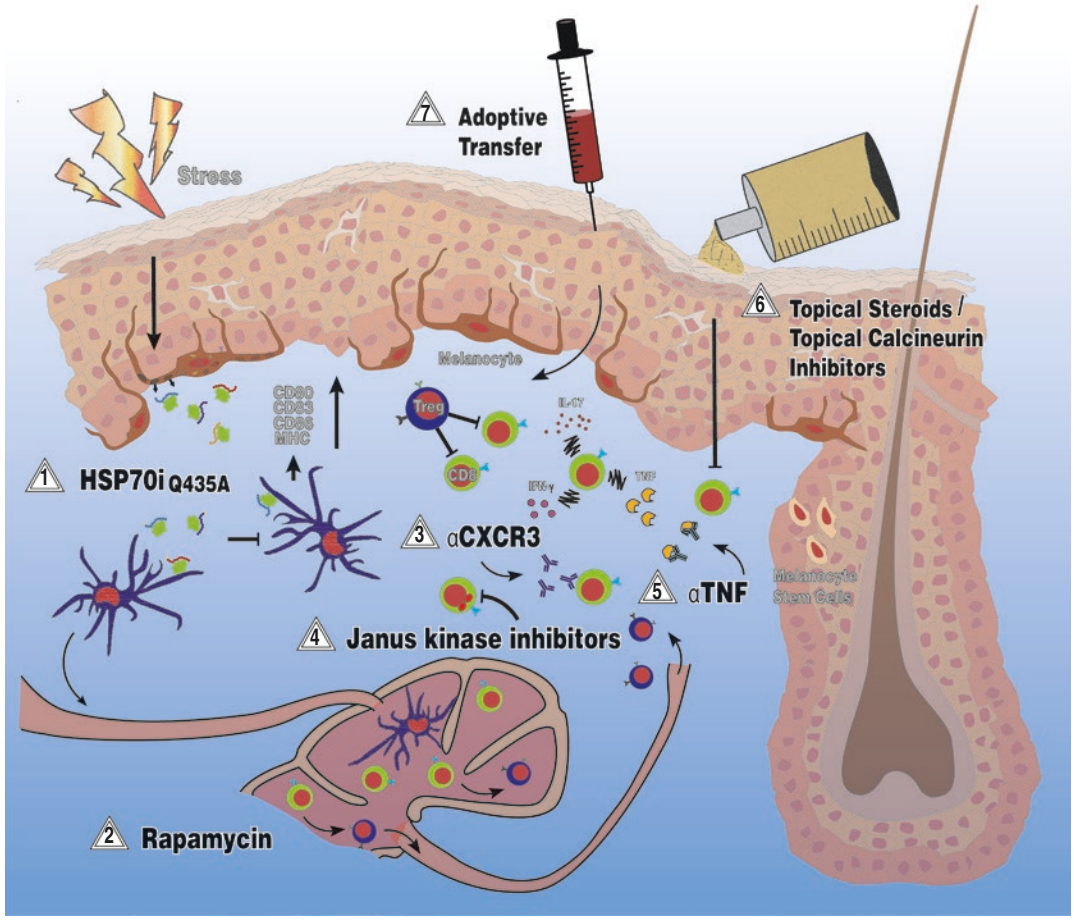

Fig. 28.4 Immunotherapeutic approaches for vitiligo. Stressed melanocytes can secrete inducible HSP70. (1) Activation of dendritic cells can be inhibited by overexpressing a modified version of inducible heat shock protein, namely, $HSP70i_{Q435A}$. Migratory dendritic cells transporting melanocyte-specific antigens can activate and recruit T cells from draining lymph nodes. (2) However, rapamycin can favor the development of Tregs over inflammatory IL-17-producing T cells and prevent effector T cell development and recruitment. (3) By introducing inhibitors of chemokine receptors such as anti-CXCR3, migration of effector T cells to the skin can be avoided. When encountering their target antigens presented by dendritic cells or upon arrival in the skin, T cells become activated. (4) Such T cell activation can be inhibited by JAK inhibitors. Within the skin, activated T cells will secrete cytokines that can promote ongoing autoimmunity. (5) Inhibitors of type 1 cytokines can neutralize such immune enhancement. The local environment is conducive to effector responses in the absence of sufficient regulatory T cells. (6) By topical application of steroids or calcineurin inhibitors, regulatory responses are encouraged, and autoimmune responses can be brought to a halt in early stages of disease. (7) Adoptive transfer of Tregs can similarly counterbalance ongoing effector responses. In preclinical models, most therapeutics not only halt depigmentation but also allow for subsequent repigmentation of the skin if a reservoir of stem cells is available

(311 nm) and excimer laser (308 nm) have all been employed and have each met with varying degrees of success in treating vitiligo patients [148, 149]. While phototherapy is an efficacious and well-tolerated treatment option for vitiligo [139], it carries some limitations including limited accessibility to patients and its blunted efficacy in more resilient body sites. Therefore, development of additional immunotherapies is prudent. The principles of UV-based and other immunotherapeutic strategies are depicted in Fig. 28.4.

- *Modified HSP70*
- To be successful, treatment should both halt ongoing melanocyte destruction and stimulate repigmentation. HSP70 lends way

to melanocyte destruction by activating dendritic cells, which subsequently leads to T cell recruitment and an anti-melanocyte immune response. $HSP70i_{Q435A}$ is a variant of inducible HSP70i of potential therapeutic benefit in vitiligo treatment [71] (=Sci. Transl. Med. 2013). Vitiligo-prone mice treated with DNA encoding $HSP70i_{Q435A}$ exhibited markedly reduced depigmentation [69]. This treatment is currently being applied to Sinclair swine with human-like skin and vitiligo lesions that develop in response to regressing melanomas [148]. In this application, the gene gun has been replaced by needle-less injectors, which are more translatable to use in the clinic and which are less traumatic than the gene gun delivery method [149]. The latter is important in vitiligo given its tendency to koebnerize. Such studies as well as further safety testing will be have to be completed before a clinical trial can be considered. By preventing dendritic cell activation, autoimmunity is prevented, and repigmentation can occur in the absence of infiltrating T cells.

- *Inhibiting T cell recruitment*
- Effector T cells engage the chemokine receptor, CXCR3, and its ligand, CXCL10, to migrate to the skin [99, 101, 152]. Recently, serum CXCL10 levels have been found to correlate with progressive disease, whereas levels decreased following successful treatment [153]. It follows that one proposed treatment strategy includes blocking the chemokine receptor, CXCR3, from binding its ligand [154]. This intervention has proven efficacious in vitiligo mice in preventing and reversing disease [99]. CXCL10 is secreted by cytotoxic T cells in vitiligo skin [97, 98]. Though anti-CXCL10 monoclonal antibodies have been tested in treating rheumatoid arthritis and ulcerative colitis [155, 156], their efficacy has not yet been tested in vitiligo patients. It is possible that chemokine depletion will be relatively less successful due to its redundancy with, in particular, CXCL11. Overall, however, interfering with effector T cell homing offers an exciting therapeutic approach in vitiligo.
- *JAK inhibitors*
- Janus kinases (JAKs) are tyrosine kinases which transmit extracellular cytokine signals to the intracellular space via their association with cytokine receptors [157]. Through their activation of the STAT transcription pathway (JAK-STAT pathway) [158], the JAK kinases mediate signaling of type I cytokines and interferons [159–161]. This ultimately affects lymphocyte activation and proliferation [160, 162, 163]. There are four mammalian JAK kinases [160, 161]. JAK 1 and 2 are clinically relevant to vitiligo, as they mediate IFN-γ signal transduction [164, 165]. Transcription of IFN-γ-inducible genes leads to CXCL10 production [166]. A JAK 1/3 inhibitor is currently FDA-approved for the treatment of moderate to severe rheumatoid arthritis. Oral treatment has also been successfully used for alopecia areata [167] and psoriasis [168], and systemically administered treatment can prevent alopecia areata in mice [169]. Randomized controlled trials are required to further define their utility and safety, but given the mechanistic relevance of JAK inhibition to vitiligo pathogenesis, their use to treat vitiligo holds promise.
- An exciting new treatment proposal entails the use of lipid-lowering statins to treat vitiligo. HMG-CoA reductase inhibitors can target a different aspect of the JAK-STAT signaling pathway than the JAK inhibitors, by inhibiting STAT1 activation [170]. Indeed, statins can prevent progression of vitiligo and promote repigmentation in mice [171] and likewise promoted repigmentation in a vitiligo patient [172].
- *Treg-based therapy*
- The paucity of skin-homing regulatory T cells [87] promotes a local environment whereby cytotoxic T cells may freely attack melanocytes. Cutaneous Treg recruitment fails in vitiligo patients due to decreased levels of the Treg chemokine CCL22 [87]. In fact, replenishing CCL22 was sufficient to overcome depigmentation [173]. Vitiligo-prone mice [174] exhibited reduced depigmentation following adoptive Treg transfer [107]. Animals experienced prolonged inhibition of depig-

mentation, while transferred Tregs migrated to the skin [107]. Likewise, vitiligo was successfully treated with rapamycin, which promoted Treg development [107, 175]. When administered with all-trans retinoic acid, rapamycin was able to confer both expansion of Tregs and biological stability of such Tregs in an inflammatory environment [176]. Rapamycin as monotherapy also conferred protection against depigmentation for up to 6 weeks after cessation of therapy in mice, highlighting its stabilizing effects on expanded Tregs [107]. Thus, creating a quantitative increase in Tregs in the skin via adoptive Treg transfer or rapamycin administration may serve as a potentially efficacious treatment modality in halting progressive vitiligo in humans.

- *Cytokine inhibition*
- The presence of minute immune infiltrates in depigmenting vitiligo skin warrants the application of antibodies to neutralize the effects of cytokines generated by activated lymphocytes. IFN-γ-neutralizing antibodies were shown to markedly reduce self-reactive cytotoxic T cell accumulation in the skin and to prevent depigmentation in a mouse model of vitiligo [94]. In the absence of IFN-γ, T cell activation is prevented, as is IFN-γ-induced melanocyte apoptosis [177]. Furthermore, melanocytes will downregulate the expression of MHC, including MHC class II [178]. TNF-α likely contributes to active depigmentation via its activation of $CD8^+$ T cells. This is supported by the finding that TNF-α inhibition has been successful in halting progressive vitiligo [179–181], in which $CD8^+$ T cells play an active role [25]. Paradoxically, some individuals developed de novo vitiligo when treated with anti-TNF-α agents. This may be explained by the fact that TNF-α can also increase Treg activity; the scale may be tipped in favor of depigmentation as a result of Treg depletion in these exceptional cases [182].

28.10 Vitiligo and Melanoma T Cell Responses

After decades without marked progress, several immune-based treatments are currently emerging for the treatment of malignant melanoma. Their efficacy is often associated with depigmentation of the skin. The development of vitiligo is a positive prognostic factor in melanoma patients [183]. This is likely the case because the presence of vitiligo denotes efficient anti-melanocyte T cell responses against shared tumor and normal melanocyte antigens [184], including MART-1 and gp100 [185]. A comparison of T cell infiltrates in vitiligo and melanoma tissue is shown in Fig. 28.2. Meanwhile, a large retrospective cohort study revealed that patients with vitiligo have a threefold decreased lifetime risk of developing melanoma [186]. Adoptive transfer of cytotoxic T cells specific for melanoma differentiation antigens has been associated with vitiligo development [31, 174, 187], again supporting a pathogenic role of autoimmune T cells in vitiligo. Interestingly, the presence of normal or increased levels of regulatory T cells in the skin of melanoma patients is associated with less effective clearing of melanoma tumors [188]; depleting these Tregs is required in order for sufficient cytotoxic T cell-mediated melanocyte destruction to ensue [93]. As vitiligo represents autoimmune melanocyte destruction in the skin, dermatologists must consider the origin of generation of these responses. While vitiligo often does not have a readily identifiable trigger by history, the appearance of this entity beyond adolescence might be a harbinger of the body generating immune responses against an underlying melanoma. This concept is similar to the clinical implications of halo nevi as a potential marker of dysplastic nevi or malignant melanocytic neoplasms [189, 190]. Therefore, new-onset vitiligo in this age group warrants a total body skin exam to screen for melanoma..

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