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Review

Patho-immunological mechanisms of vitiligo: the role of the innate and adaptive immunities and environmental stress factors

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Summary

Epidermal melanocyte loss in vitiligo, triggered by stresses ranging from trauma to emotional stress, chemical exposure or metabolite imbalance, to the unknown, can stimulate oxidative stress in pigment cells, which secrete damage-associated molecular patterns that then initiate innate immune responses. Antigen presentation to melanocytes leads to stimulation of autoreactive T-cell responses, with further targeting of pigment cells. Studies show a pathogenic basis for cellular stress, innate immune responses and adaptive immunity in vitiligo. Improved understanding of the aetiological mechanisms in vitiligo has already resulted in successful use of the Jak inhibitors in vitiligo. In this review, we outline the current understanding of the pathological mechanisms in vitiligo and locate loci to which therapeutic attack might be directed.

Keywords: antibodies, autoimmunity, cytokines, cytotoxic T cells, Th1/Th2 cells

Abbreviations: UV, Ultraviolet; TYRP1, tyrosinase-related protein-1; ROS, reactive oxygen species; DAMP, damage-associated molecular patterns; PRRs, pattern recognition receptors; NOD, nucleotide oligomerization domain; NLRs, nucleotide oligomerization domain-like receptors; PAMPs, pathogen-associated molecular patterns; GZMB, granzyme-B; NK, natural killer; HSP, heat shock protein; IL, interleukin; TNF-α, tumour necrosis factor-α; HMGB1, High-mobility group protein B1; pDCs, plasmacytoid dendritic cells; ILCs, innate lymphoid cells; NLRP3, NLR family pyrin domain containing 3; NF-kB, nuclear factor kB; ASC, apoptosis-associated speck-like protein containing a CARD; TRPM2, transient receptor potential cation channel subfamily M member 2; CXCR3, C-X-C chemokine receptor type 3; JAK, janus kinase; Treg, Regulatory T; Foxp3, forkhead box P3; CAR, chimeric antigen receptor; NB-UVB, narrow band-ultraviolet B; Trm, memory T; ICAM-1, intercellular adhesion molecule-1; CTLA4, cytotoxic T lymphocyte antigen-4; NLRP1, NACHT leucine-rich-repeat protein 1; IFIH1, interferon-induced helicase C domain 1; CASP7, caspase-7; MC1R, melanocortin 1 receptor; Tc, cytotoxic T cell; TYRP1, tyrosinase-related protein-1; PRRs, pattern recognition receptors; HMGB1, high-mobility group protein B1; IFN-γ, interferon-γ

Introduction

Melanocyte damage and destruction is the underlying pathological event in vitiligo, a skin disease characterized by depigmented patches. Vitiligo has a worldwide prevalence of about one percent, and can be classified into non-segmental, segmental, mixed, and unclassifiable/undetermined types [1, 2]. Differentiating subtypes may be important as they might have different aetiologies. Segmental vitiligo often maps to a blaschkoid or dermatomal distribution [3]. Vitiligo can affect any gender, race, or geographic region [4].

Although non-life threatening, vitiligo can have a serious psychological impact on sufferers [5]. Vitiligo patients commonly experience feelings of stress, fear of disease exacerbation, embarrassment, negative self-image, and self-consciousness [6]. Moreover, patients with vitiligo often experience depression, anxiety, and discrimination and stigmatization from others, resulting in low self-esteem and social isolation [7].

In some countries, vitiligo is still confused with leprosy and patients are stigmatized [8]. Vitiligo can also have a negative impact on the marital prospects of those affected [9–11]. Sufferers with decreased quality of life at treatment initiation face a lower response rate to a given therapy [12]. Development of specific psychological intervention and quality of life measures may benefit treatment outcome and enhance self-esteem [13].

A variety of factors may trigger vitiligo. These include emotional stress, physical trauma, and chemical exposure to imbalances in endogenous neural factors, metabolites, cytokines, or hormones. All of these can stimulate autoimmune responses, in individuals with the appropriate genetic susceptibilities, that ultimately target melanocytes [2]. The melanocytes in vitiligo are highly vulnerable to damage and apoptosis under the action of triggering factors [14]. The treatment of vitiligo, including topical steroids, calcitonin-inhibitors, phototherapies, and surgical procedure, in the past

has frequently failed to achieve satisfactory repigmentation, but recently, the Jak inhibitors have shown promise [15].

Histopathological features of vitiligo and melanocyte degeneration

The affected skin in vitiligo demonstrates melanin loss and absence of or reduced numbers of melanocytes in the epidermis [16]. This decrease varies according to the disease stage [13]. Melanocytes as revealed by an appropriate monoclonal antibody technique or the Fontana Masson stain, remain present, though they disappear from affected skin of vitiligo as the disease progress [17]. Immunohistochemical studies of vitiligo lesions show the absence of melanocytes from the basal layer, but they may exist in decreased numbers and can demonstrate degenerative changes [18].

Even though it is clear that depigmentation in vitiligo results from melanocyte loss from the skin, it remains unclear whether this occurs through a degenerative or autoimmune process. Defective *in vitro* growth of melanocytes derived from individuals with vitiligo [19, 20] and increased susceptibility of vitiligo melanocytes to exogenous stimuli suggests that degeneration may precede melanocyte loss [21].

Melanocytes from vitiligo subjects were shown to poorly proliferate compared to healthy normal melanocytes [19] and also show inadequate antioxidant activity with high levels of superoxide dismutase and low levels of catalase [22]. Under normal circumstances, superoxide dismutase cata-lyses the first step of dismutation by converting the superoxide anion into oxygen and hydrogen peroxide and then the catalase enzyme transforms hydrogen peroxide into water and oxygen, protecting cells from reactive oxygen species (ROS). In fact, melanocytes synthesize high ROS levels as by-product of melanogenesis. Therefore, compensatory media supplements such as growth factors or catalase are required to culture melanocytes derived from vitiligo patients [20, 23]. Also, increased expression of hydrogen peroxide and elevated oxidative by-products within vitiligo patient skin has been reported [22, 24, 25].

In addition, melanocytes from vitiligo patients have been shown to be more sensitive to *in vitro* oxidative therapies such as cumene hydroperoxide and ultraviolet B irradiation [26, 27]. However, exogenous treatment with catalase in the form of pseudocatalase, which was proposed to cure vitiligo by regulating reactive oxygen species (ROS), was ineffective in treating vitiligo lesions [28]. Thus, dysregulated redox balance in vitiligo might be a consequence, but not a cause, of vitiligo.

Melanocytes from vitiligo patients show morphological and physiological abnor-malities. Those in peri-lesional borders are seen to be enlarged with longer dendritic ends than normal melanocytes [29]. However, rapid regimentation of the skin following engrafting of human vitiligo lesional skin on nude mouse was achieved, indicating that the intrinsic melanocyte defect was not the only cause of melanocyte destruction in vitiligo [30]. Histochemical and immunohistochemical examination shows infiltration of a large number of T lymphocytes at the edge of vitiligo lesions with complete microscopic loss of melanin in lesional skin [31]. Therefore, it is clear that vitiligo melanocytes are abnormal compared to healthy melanocytes.

Responses to stress in vitiligo

Melanocytes in the epidermis are regularly exposed to various environmental stressors e.g. ultraviolet (UV) radiation,

pollution, microorganisms, and oxidizing chemicals, all of which can stimulate ROS production [32]. ROS consist of a number of oxygen-based free radicals such as superoxide and hydrogen peroxide, formed during multiple physiological and pathological processes [33]. Such free radicals are constantly scavenged by antioxidants such as superoxide dismutase, catalase, vitamin C, and vitamin E. As mentioned, in vitiligo patients, high levels of superoxide dismutase and low levels of catalase have been observed in the skin [34].

Hydrogen peroxide created from superoxide anion can easily cross melanocyte membranes causing cellular damage [33]. Even though melanin present in the skin protects melanocytes as well as adjacent keratinocytes through its ability to absorb UV radiation, its synthesis likewise results in higher amount of intracellular ROS, causing to be melanocytes more vulnerable to oxidative stress [35, 36].

In addition, a decrease in the stability of tyrosinase-related protein-1 (TYRP1), which is required for melanin synthesis, has been observed in vitiligo melanocytes, allowing accumulation of melanin intermediates [37] (Fig. 1). The build-up of intermediate products increases the risk of protein misfolding, hence activating the unfolded protein response (Fig. 1). This in turn induces the restoration of endoplasmic reticulum homeostasis through the halting of protein translation, inducing misfolded protein degradation and promoting the synthesis of chaperons to facilitate protein folding, sustained activation of which leads to apoptosis [2]. Disturbance of UPR can contribute to the development of auto-immune diseases through formation of antigens during misfolded protein degradation, secretion of neo-antigens by apoptotic cells or disruption of immune tolerance [38]. Engagement of UPR in vitiligo pathogenesis is proposed by genetic studies, which revealed that polymorphisms in the gene-encoding X-box-binding protein 1 (*XBP1*) are correlate with an elevated risk of developing vitiligo [39]. *XBP1* is a transcription factor that modulates various downstream UPR targets [39]. Studies showed that exposure of melanocytes to phenolic compounds, known as triggers of vitiligo, activate XBP1, which in turn activates the UPR and increases the expression of cytokines IL6 and IL8 [40] (Fig. 1). Increased levels of IL6 and IL8 were indeed found in the skin and serum of vitiligo patients, indicating sustained UPR activation [40, 41]

Intrinsic defects may also render vitiligo melanocytes vulnerable to oxidative stress. Observed anomalies include a dilated endoplasmic reticulum, mitochondrial dysfunction, and an abnormal melanosome structure, all of which suggests that these pigment cells are less capable of dealing with such stressors than those from healthy individuals [32].

ROS-mediated stress has been directly linked with generation of neoantigenic epitopes within beta islet cells [42], and likewise melanocyte stress may generate neoantigens. Elevated ROS in melanocytes of vitiligo subjects has been correlated with peroxidation, and thus it is likely that ROS generates melanocyte neoantigens via protein carbonylation and oxidation [43].

Cellular stress has been found to develop in healthy melanocytes exposed to phenolic derivatives such as 4-tertiary butylphenol and monobenzyl ether of hydroquinone (Fig. 1) [40]. Once melanocytes become stressed, they promote the secretion of inducible heat shock protein 70 (iHSP70), which has been detected in vitiligo melanocytes and seen to correlate with active disease [44] (Fig. 1).
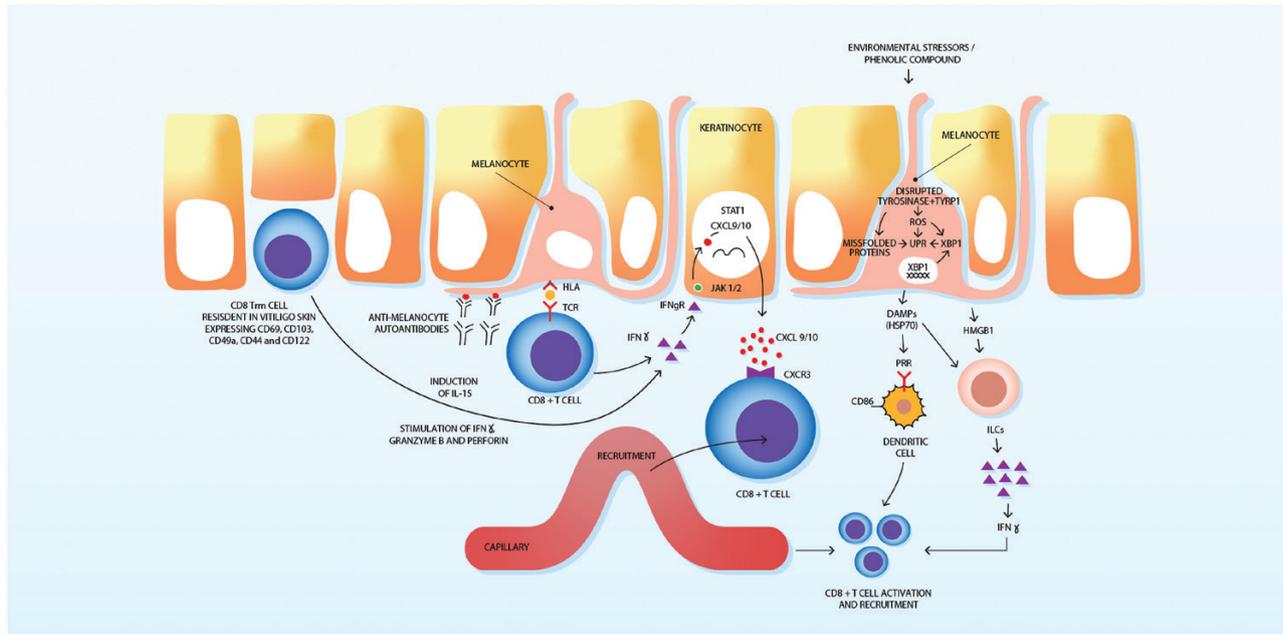
Fig. 1 Melanocytes are regularly exposed to environmental insults such as phenolic compounds can stimulate a state of melanocyte stress through interacting with tyrosinase and TYRP1, leading to production of DAMPs. DAMPs can then stimulate nearby dendritic cells through PRRs. Activated dendritic cells locally synthesize cytokines, inducing CD8+ T cell activation and recruitment to the skin. HMGB1 can activate ILCs with subsequent release of IFN-γ. In the progressive phase of vitiligo, melanocyte-reactive CD8+T cells produce interferon-gamma on encountering melanocyte antigens. These induce keratinocytes to secrete CXCL 9 and CXCL 10, resulting in additional recruitment of lymphocytes to the site through the CXCR3 receptor. CD8+ T cells, resident in vitiligo skin, are involved through the induction of IL-15, which results in the stimulation of IFN-γ, granzyme B and perforin. DAMPs; pathogens or damage-associated molecular patterns, TYRP1; tyrosinase-related protein-1, PRRs; pattern recognition receptors, HMGB1; high-mobility group protein B1, ILCs; innate lymphoid cells, IFN-γ; interferon (IFN)-γ, CXCR3; C-X-C chemokine receptor type 3, CXCL 9/10; C-X-C motif chemokine ligand 9/10, IL-15; interleukin-15.

Pathogens or damage-associated molecular patterns (DAMP), which can evoke inflammation via pattern recognition receptors (PRRs) including Toll-like receptors and nucleotide oligomerization domain (NOD)-like receptors (NLRs). Indeed, NLRP1 has been linked with vitiligo in a linkage study [45]. Innate immunity is activated by the release of DAMPs from stressed cells. DAMPs are likely to be constantly released from stressed melanocytes resulting in skin inflammation in patients with vitiligo [46]. In agreement with this, uninvolved skin of vitiligo patients shows increased numbers of lymphocytes in comparison with healthy controls [47].

Innate immunity

Innate immunity is based on the ability of PRRs to detect pathogen-associated molecular patterns found in pathogens or DAMPs released by damaged cells [48]. ROS and iHSP70 produced by stressed melanocytes serve as DAMPs in vitiligo, and PRRs initiate the innate response [46] (Fig. 1). Innate immune cells such as natural killer (NK) cells, macrophage, and dendritic cells show aberrant activation in vitiligo skin and granzyme-B (GZMB)-expressing activated NK cells have been found [47, 49].

Vitiligo skin shows an increase in NK cells activating receptors (CD16+CD56+ and CD3+CD16+CD56+), an upregulation in CLEC2B, an activating ligand of NK cells, and a decrease in the inhibitory receptors (CD16+CD158a+). Vitiligo skin also demonstrates increased numbers of dendritic cells, which can destroy melanocytes when activated by iHSP70 [49]. While chemicals can trigger vitiligo by inducing

melanocyte stress, adding HSP70i alone aggravates vitiligo in a mouse model, probably via activation of dendritic cells in the skin [50]. In addition, increased iHSP70 expression in the skin of vitiligo patients causes melanocyte loss [50]. Mice lacking expression of iHSP70 will not develop experimental depigmentation, suggesting a role for iHSP70 in vitiligo [49]. In addition, mutant HSP70i delivery, which interferes with the signalling pathway of endogenous HSP70i, could inhibit depigmentation in mouse and swine models of vitiligo by interfering with dendritic cell activation [49, 51]. Thus, DAMPs, in particular HSP70i, can directly initiate vitiligo in animal models by activating dendritic cells. iHSP70 has been found to have potent adjuvant and chaperone properties [52].

Under conditions of oxidative stress, genetically compromised melanocytes secrete melanosomal peptides-chaperoned iHSP70 that can activate dendritic cells and release the inflammatory signal that initiates the immune response in vitiligo [49]. Secreted iHSP70 from stressed vitiligo melanocytes has been reported to induce dendritic cells to elevate the expression of coactivation markers CD80 and CD86, stimulating immune responses to melanocytes [53, 54] (Fig. 1). Therefore, it is likely that melanocyte stress can contribute to the instigation of autoimmunity through both neoantigen generation and the activation of innate immunity [43]. iHSP70 is thus a link between oxidative stress, as a trigger, and the onset of the autoimmune reaction in vitiligo. Other DAMPs such as calreticulin and high-mobility group protein B1 (HMGB1) have been proven to take a part in the pathogenesis of vitiligo. Some studies found that calreticulin levels were higher in patients with vitiligo than in healthy controls, and its levels were positively correlated with vitiligo lesional area [55].

Calreticulin was shown to induce the secretion of IL-6 and tumour necrosis factor-α (TNF-α), suggesting that calreticulin may stimulate the immune response, thus affecting melanocytes via cellular-mediated immunity [55].

In addition, studies have revealed that HMGB1 expression was also increased in both blood and lesional samples from vitiligo patients [56]. Moreover, serum level of HMGB1 was elevated in patients with active progressive vitiligo, whereas no change in HMGB1 serum level was shown in patients with slowly progressive vitiligo [57]. HMGB1 is generally located in the cell nucleus and involved in the regulation of gene transcription through interaction with chromatin structures of DNA [58]. Cui *et al.* (2019) reported abnormal localization of HMGB1 in the cytoplasm of remaining melanocytes in perilesional areas of vitiligo skin. Additionally, *in vitro* studies have shown that HMGB1 could be released from melanocytes into the extracellular space due to oxidative stress, and then stimulate inflammation via integrating with PRRs [57] (Fig. 1). HMGB1 could induce the expression of inflammatory factors, such as CXCL16, from keratinocytes, which could stimulate the maturation of dendritic cells and the infiltration of cutaneous CD8+ cells [57].

Activated, dendritic cells locally synthesize cytokines, inducing T-cell activation and recruitment to the skin and, in local lymph nodes, the recruitment of cytotoxic T cells, thus bridging the innate with the adaptive immunity [59]. Therefore, delivery of mutant HSP70i may offer a potential treatment for vitiligo by altering innate immunity. The connection in vitiligo between cellular stress and cell-based immunity was illustrated when melanocytes, stressed by exposure to 4-tertiary butyl phenol, were noted to facilitate activation of dendritic cells thus rendering the latter melanocytotoxic *in vitro* [53].

Other studies have demonstrated that stressed melanocytes can activate melanocyte-specific CD8+ T cells, resulting in an autoimmune response and consequent pigment cell destruction [60]. In addition, perilesional skin of patients with active vitiligo showed infiltrates of plasmacytoid dendritic cells (pDCs), which are associated with a local IFN-α response [61]. Recently, increased presence of innate lymphoid cells (ILCs) have been demonstrated in the skin and blood of vitiligo patients [62] (Fig. 1). Interestingly, ILCs from vitiligo patients synthesized higher amount of IFN-γ when exposed to oxidative stress or stimulated by HSP70 and HMGB1, compared to healthy individuals [62]. ILCs are initial source of IFN-γ, a signature vitiligo cytokine, which is involved in T-cell-mediated destruction of pigment cells [62].

Recently, perilesional keratinocytes from vitiligo skin, under oxidative stress *in vitro*, have been shown to exhibit increased expressions of NLR family pyrin domain containing 3 (NLRP3) and downstream cytokine IL-1β, an inflammasome regulator that may modulate an innate immune attack on melanocytes [63]. NLRP3 is a cytoplasmic NLR and is an essential constituent of the inflammasome in the innate immunity. The NLRP3 inflammasome is activated by a variety of extracellular inflammatory stimuli, such as bacteria, viruses, and yeasts, in a nuclear factor kB (NF-kB)-mediated signalling manner [64]. In addition, the NLRP3 inflammasome can be activated in response of a wide range of endogenous molecules such as lysosomal destabilization, oxidized mitochondrial DNA, extracellular ATP, potassium efflux and intracellular calcium levels [64]. The priming step induces NLRP3

expression and activates licensing receptors. Importantly, the activation of NLRP3 inflammasome also can be controlled by tyrosine kinase and JNK or Syk kinases, via the recruitment of caspase-1 and regulation of apoptosis-associated speck-like protein containing a CARD (ASC) oligomerization, respectively [64]. In addition, the NLRP3 inflammasome can be activated through transient receptor potential cation channel subfamily M member 2 (TRPM2)-induced intracellular and mitochondrial calcium influx in H_2O_2-treated keratinocytes [63]. In vitiligo, the NLRP3 inflammasome can be activated by monobenzone, and H_2O_2. van den Boorn *et al.* showed that monobenzone induces a melanocyte-specific immune response characterized by the activation of NK cells and the infiltration of macrophages. Indeed, NK cell recruitment to the ear, during treatment with monobenzone, was significantly suppressed in *Nlrp3*-deficient mice, indicating a key role for the NLRP3 inflammasome in monobenzone-induced inflammation in melanocytes [65]. This suggests that the NLRP3 inflammasome and its downstream inflammatory cytokines could be promising targets for vitiligo therapy. Recently, it has been shown that the activation of the NLRP3 inflammasome is required to stimulate innate immunity in keratinocytes [63]. In addition, NLRP3 inflammasome deactivation diminishes recruitment of CD8+ T cells and suppresses cytokine production in T cells derived from patients with vitiligo [63].

Once activated, the NLRP3 inflammasome mediates caspase-1 cleavage which promotes synthesis of IL-β [66, 67]. Subsequently, the function of CD8+ and CD4+ T cell is strengthened through the IL1-β/IL-1R signalling pathway [63]. IL1-β has been observed to elevate expression of CXCR6 and CXCR3 in CD8+ T cells from vitiligo patients. Similarly, IL1-β has been found to increase synthesis of IL17A/F in CD4+ T cells and interferon (IFN)-γ in both CD8+ and CD4+ T cells [63]. IL1-β in stressed keratinocytes has been also noted to stimulate expression of CXCL10 and CXCL16, ligands of CXCR3 and CXCR6 through NF-kB pathway, which promote migration of cytotoxic T cells into vitiligo areas [63].

Adaptive immunity

Infiltrating T cells and their role in vitiligo

Following proinflammatory signalling in the skin, melanocyte antigens can be processed and presented by dendritic cells in the draining lymph node, resulting in the production of melanocyte-specific cytotoxic T lymphocytes and the generation of melanocyte-specific antibodies by B lymphocytes [68]. Histological studies of vitiligo lesions have shown infiltration of lymphocytes at the border of depigmented lesions. These infiltrates consist mainly of CD8 + T cells, preferentially located in the dermo-epidermal borders neighbouring the melanocytes [69, 70]. CD8+ T cells have been implicated in the destruction of melanocytes in vitiligo.

Melanocytotoxic CD8+ T cells that express the skin homing marker, cutaneous lymphocyte-association antigen, infiltrate the lesional borders of vitiligo skin, and their number correlates with disease extension and severity [71–73].

Furthermore, self-reactive CD8+ T cells in infiltrating perilesional areas of vitiligo recognize the antigens Melan-A/MART, tyrosinase (TYR), and melanocyte-specific protein, all of which are involved in the melanogenic pathway [74]. Vitiligo patients have elevated numbers of CD8+ T cells in their peripheral blood compared to healthy individuals [72].

Moreover, the peri-lesional skin in vitiligo is highly infiltrated by melanocyte-specific CD8+ T cells [70] – cells which are able to kill melanocytes *in vitro* [70, 75, 76]. CD8+ T cells isolated from the peri-lesional skin of vitiligo patients, have been demonstrated to infiltrate explants of autologous healthy pigmented vitiligo skin and eliminate melanocytes in a manner similar to that observed in the clinical pathology of the disease [76]. Importantly, isolated CD4+ T cells were incapable of inducing melanocyte apoptosis in autologous skin explants [76].

In vitro culture of CD8+ and CD4+ T cells derived from peri-lesional vitiligo skin secreted high levels IFN-γ, a proinflammatory cytokine required for melanocyte-specific autoreactive CD8+ T-cell recruitment [77]. These findings provide robust evidence that CD8+ T cells are essential and sufficient for the destruction of melanocytes in vitiligo.

IFN-γ signalling pathway acts as a key driver of CD8+ T-cell recruitment and function in vitiligo

The mechanism by which CD8+ T cells trigger melanocytes apoptosis requires their secretion of proinflammatory cytokines IFN-γ from CD8+ T cells [76]. Analysis of gene expression in human vitiligo lesional skin demonstrated increased expression of the interferon-response gene [78, 79], as well as genes induced by IFN-γ and these include C-X-C chemokine receptor type 3 (*CXCR3*), a T-cell chemokine receptor, and its ligands: *CXCL9*, *CXCL10*, and *CXCL11* [79]. In agreement with this finding, skin biopsies from vitiligo patient lesions and from a mouse model of vitiligo also show prominent lymphocyte infiltrates that are primarily CXCR3+ [61, 77, 79, 80]. In addition, melanocyte-specific CD8+ T cells isolated from the skin and blood of human vitiligo subjects predominantly express CXCR3 receptor, and CXCL9 is a specific skin biomarker of active vitiligo [73]. Studies in mouse models of vitiligo have established a critical role for this pathway in the pathogenesis of the disease, since IFN-γ, CXCR3, and CXCL10 are all essential for its development [79, 81]. The neutralization of IFN-γ with antibody treatment or the lack of *CXCR3* expression on T cells prevents the migration of autoreactive T cells into the skin, which therefore do not cause depigmentation [79, 81]. Studies employing chemokine reporter mice have shown that CXCL9 and CXCL10 are mostly produced by keratinocytes, and functional studies have demonstrated that keratinocytes are primarily responsible for the recruitment of autoreactive T cells [82]. CXCL9 seems predominantly responsible for the bulk recruitment of T cells to the skin in a mouse model of vitiligo, since, when it is absent, the number of melanocyte-autoreactive T cells within lesional vitiligo skin is decreased tenfold [79]. However, in spite of reduced a number of T cells, vitiligo severity remains unchanged, indicating that the overrecruitment of T cells occurs during the disease process. In contrast, when CXCL10 is absent in mice with established widespread depig-mentation, the incidence and severity of the depigmentation are decreased, even though bulk recruitment of T cells is unchanged [79]. Interestingly, in the Cxcl10–/– mice, the quantity of T cells shown in the epidermis compared to the dermis in the skin is reduced, signifying that CXCL10 is responsible for T-cell localization within the skin and their effector function [79]. Thus, T cells produces IFN-γ, which stimulates the production of CXCL9 and CXCL10 from keratinocytes to recruit more T cells and induce the progression of vitiligo [43]. As well as a role in initiation and progression, the IFN-γ-chemokine pathway is also needed for the maintenance of established vitiligo lesions, as knocking out CXCR3 or blocking the action of CXCL10 in mouse models of vitiligo prevents and reverses depigmentation in the disease [79] (Fig. 1). Indeed, ruxolitinib, a janus kinase (JAK)-1,2 inhibitor, which interferes with IFN-γ signalling pathway through preferential inhibition of JAK1 and JAK2, shows promise in vitiligo. A phase 2 trial of ruxolitinib cream was associated with significant repigmentation of vitiligo up to 52 weeks of treatment, and was well tolerated [83].

Regulatory T cells are suppressors of autoreactive effectors and inhibit depigmentation

Regulatory T (Treg) cells are key factors in the maintenance of appropriate peripheral tolerance, via suppression of self-reactive effector T cells, and so maintain immune homeostasis. Treg cells are a subgroup of CD4+ T cells that primarily represent a phenotype of CD4+, CD25+ and forkhead box P3 (Foxp3), and play a key role in preventing autoimmunity. Patients who suffer from immuno-dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, lack Treg cells because of a mutation in the *FOXP3* gene and, as a consequence, suffer from other autoimmune diseases, including vitiligo [84, 85]. Likewise, scurfy mutant mouse strain with defective FOXP3 lack Treg cells and shows widespread autoimmunity, underlining a critical role for Treg cells in the maintenance of tolerance to self-antigens [86]. In a mouse model of vitiligo repigmentation was accompanied by an elevated infiltration of Tregs into the skin, possibly resulting in the prevention of immune responses against melanocytes [87]. In addition, some authors have reported an increase in vitiligo severity when Treg cells were depleted using either CD4 or CD25 antibodies [88, 89]. One study showed an increase in cutaneous Treg cell infiltration and a decrease in depigmentation when the expression of CCL22 was induced in the skin [90]. Another report revealed that adoptive transfer of exogenous Treg cells to vitiligo-prone mice at 3 weeks of age led to elevated number of cutaneous Treg cells and prevented vitiligo [89]. When vitiligo-prone mice were injected with PD-L1P-Fc, Treg cell accumulation in the skin was enhanced and depigmentation was reversed [91]. These findings support the theory that the number of Treg cells in the skin is critical for reducing depigmentation driven by T effectors and therefore helping to control vitiligo progression. Interestingly, recent studies used GD3-reactive chimeric antigen receptor (CAR) Tregs to treat vitiligo in a TCR transgenic mouse model of spontaneous vitiligo [92]. These CAR Tregs demonstrated elevated IL-10 production in response to antigen, suppressed T-cell cytotoxicity towards melanocytes, and they significantly delayed depigmentation compared to untransduced Tregs, thus providing local immune tolerance in perilesional skin of vitiligo [92]. These results were also associated with a greater number of Tregs and melanocytes in CAR Treg treated mice as compared to mice treated with untransduced Tregs, indicating an important role for antigen-specific Tregs in controlling progressive depigmentation [92]. Several research groups have reported disruption of Treg cells function, but there is no consensus as to where the disruption exactly lies: is it in the density of Treg cells, suppressive effects, or in the immigration ability to the skin. A study has reported that peripheral Treg cells isolated from vitiligo patients have shown a decreased ability to inhibit CD8+ T-cell proliferation and activation *in vitro* [93]. However, another study showed normal

Treg cell activity in vitiligo patients but a decreased number in the skin, suggesting that reduced cutaneous localization of Treg cells to the skin, rather than diminished function of Treg cells, contributes to the pathogenesis of vitiligo [94].

Immunohistochemical studies have revealed no significant reduction in Treg cell number in areas of vitiligo [95, 96], whereas another report showed a significant decrease in these cells [97]. It is therefore not clear how the function of Treg is disrupted in vitiligo patients, but effector T-cell phenotype in human vitiligo also indicates the presence of defective Treg T cells. Analysis of the phenotype of peripheral blood mononuclear cells indicates that in vitiligo patients [98]. Further studies are required to reliably determine how a deficiency of Treg cells contributes to the development of vitiligo, and to examine the potential for improving Treg cell function as a treatment.

Immune-dysregulation of T helper cells in vitiligo

The numbers of circulating Th1 cells are increased in vitiligo compared to controls [99]. A mouse model of vitiligo shows a predominantly Th1-mediated pattern with a dominant role of CXCL10 [80]. Recently, decreased frequencies of circulating Th1/Tc1, Th17/Tc17, and Th1/Th17-Tc1/Tc17 cells were shown in patients with vitiligo, suggesting possible migration of these cells into the skin [100]. Indeed, Th1/Th17 and Tc1/Tc17 cells have been touted as potential targets for therapy [101]. Importantly, one study has demonstrated that the production of IL-17 by T cells from vitiligo perilesional skin remains similar to the amount secreted by T cells from healthy skin, indicating that the Th17 pathway does not play an important role in melanocyte destruction [77]. In addition, more recent studies, evaluating the use of the anti-IL-17A biological agent secukinumab for the treatment of vitiligo, have confirmed that IL-17 or Th17 cells had no direct pathogenic role in vitiligo [102, 103].

Resident memory T cells and their function in vitiligo

The loss of pigmentation in therapeutically repigmented skin is common in patients with vitiligo, and is often seen in the first year following cessation of treatment [104]. One study showed that vitiligo patients treated with narrow band-ultraviolet B (NB-UVB) experienced such loss within 2 years of stopping treatment [105]. Notably, relapse occurs in the previously repigmented areas, suggesting that autoimmune memory plays a role in the skin that is not cleared by current treatments [43]. Resident memory T (Trm) cells are a subset of long-lived T cells that persist within most nonlymphoid tissues after inflammation or infection driven by T cells [106, 107].

Trm cells of the skin are known for their long-term residence in the skin. They patrol their surroundings, the epidermis and papillary dermis, and rapidly respond when they encounter their cognate antigen [108]. They are defined by the cell surface expression of CD69, CD103, CD49a, and CD44 [109]. CD103 is implicated in binding E-cadherin expressed on epidermal cells, thereby promoting Trm cell retention [110]. CD69 is required for the development of Trm cells in non-lymphoid tissues, including the skin [111]. Moreover, CD69 has been demonstrated to inhibit the function of sphingosine 1-phosphate receptor 1 (S1PR1) [111]. S1PR1 is required for lymphocyte exit from peripheral tissues, hence its suppression maintains Trm cell residence in the skin [112].

Upregulated expression of CD103 and CD96 on Tm cells is crucial for their development and survival as their deletion on virus-specific T cells caused a significant decrease in the quantity of Trm cell in the skin following a virus infection [113]. Different subsets of Trm cells were identified in vitiligo skin. One study found that stable and active vitiligo perilesional skin was highly infiltrated with a population of CD8 Trm cells that express both CD69 and CD103, compared with control normal human skin [77].

CD8 Trm cells that remain in stable vitiligo could be a vital mediator for disease flares [77]. In addition, CXCR3 was also reported to define a subset of Trm cells in vitiligo [77]. Vitiligo skin was shown to be enriched with CD8 Trm cells expressing CXCR3. Interestingly, high frequencies of melanocyte-specific CD8 cells were demonstrated within CXCR3+ subsets [77].

A study showed that lesional skin from vitiligo patients contained an accumulation of a subset of CD8+ Trm cells characterized by CD49a+ [114]. Activated CD8+ CD49a+ Trm cells were found to be involved in the stimulation of IFN-γ, granzyme B and perforin due to the induction IL-15, and thus promoting a high cytotoxic response [114] (Fig. 1). One study found that human and mouse Trm cells in vitiligo expressed high level of CD122, a subunit of IL-15 receptor, in the blood and lesional skin, and that lesional keratinocytes upregulated CD215 expression [115]. Interestingly, IL-15 is secreted in different peripheral tissues via myeloid and stroma cells and it is most frequently trans-presented to T cells on the surface of myeloid and stromal cells that express CD215, which is required to display the cytokine on the cell surface to induce activation of T cells [116].

Importantly, melanocyte-specific T cells from mouse and human vitiligo express significantly higher levels of CD122 than endogenous memory T cells, indicating that autoreactive T cells are more reliant on this cytokine than are non-autoreactive T cells [115]. Treatment with anti-CD122 antibody reversed disease in mouse vitiligo, inhibited Trm secretion of IFN-γ and depleted Trm cell from skin lesions, while leaving endogenous memory T cells unaffected [115]. Both systemic and local short-term treatment, through injection with anti-CD122 antibody, provided durable repigmentation [115]. These findings are in agreement with there being an essential role for IL-15 in Trm cell maintenance in vitiligo and we propose that targeting CD122 could be highly effective treatment strategy for patients with vitiligo [43].

Very recently, NKG2D was found to define a subset of effector memory T cells with proinflammatory functions in vitiligo [117]. NKG2D+ CD8+ effector memory T cells were characterized by an activated phenotype and have a great ability to secrete high amount of both IFN-γ and TNF-α [117]. The NKG2D ligands MICA-MICB were shown to be expressed by dendritic cells that infiltrated vitiligo skin upon inflammation and could signify a cell subset capable of promoting activation of NKG2D+ CD8+ effector memory T cells in the skin [117]. Interaction of NKG2D with its corresponding ligands MICA-MICB potentiated the synthesis of IFN-γ and TNF-α by CD8+ effector memory T cells [117]. These results infer that NKG2D may play an important role in vitiligo progression, and it could be an interesting target to treat vitiligo [117].

As Trm cells are able to live in tissues for long periods and rapidly evoke immunity reactions against viruses, they were considered strong candidates for stimulating relapse in vitiligo [43]. Trm cell function has been the subject of debate. In

some models, these cells sufficiently control viral titres during re-infection with no need for recirculating cells [118, 119]. In other contexts, they have been seen to mainly synthesize cytokines for effector T cell recruitment from the circulation. However, CD8+ Trm cells are shown to have low cytotoxic potential.

In normal human skin, CD8+ Trm cells demonstrated low levels of granzyme B and perforin [120]. Moreover, the effect of CD8+ Trm cells isolated from normal human skin on lysing allogenic target cells were significantly poorer than circulating effector memory T cells [121]. Trm cells mediate the immune response to melanoma via inhibiting tumour outgrowth instead of tumour elimination, indicating that Trm cells lack cytotoxic ability [122]. Based on their low cytotoxic activity and efficient ability of producing cytokines, Trm cells may be involved in recruitment of effector cells from circulation [43]. In vitiligo mouse model, it was found that blockade of T-cell migration to the skin or depletion of recirculating memory T cells reversed vitiligo, although the number of Trm cells was not affected [123]. It was therefore concluded that Trm cells cannot independently maintain vitiligo in the absence of recruitment of further T cells [123]. In this vitiligo mouse model, autoreactive melanocyte-specific CD8+ Trm cells were shown to synthesize IFN-γ and CXCL10, probably employing the IFN-γ-chemokine pathway to maintain vitiligo lesions [123]. Thus, Trm cells are likely to mediate the long-term maintenance and relapse of the disease via cytokine-mediated recruitment of circulating T cells [43].

Notably, patients with stable vitiligo are considered to have a continuing immune response, as evidenced by the existence of Trm cells in perilesional skin [77, 115]. These may be involved during a flare-up, as, by analogy, in psoriasis [124].

Humoral immunity in vitiligo

Besides cellular immunity, humoral (antibody-mediated) immunity adds supportive evidence for a pathological role of autoimmunity in vitiligo. Different antibodies to melanocytes have been identified at significantly elevated levels in the sera of vitiligo patients as compared to healthy controls [125–128], and their levels are directly associated with the extent and activity of vitiligo [126, 129, 130], being present in 93% of patients with wider depigmentation (5–10% skin area involved) and in only 50% of patients with minimal pigment loss (<2% skin area involved) [131]. These anti-melanocyte antibodies belong to the immunoglobulin G (IgG) class [128], including subclasses IgG1, IgG2, and IgG3 [132], though IgA anti-melanocyte antibodies have also been detected [133].

Immunoprecipitation studies using mela-nocyte protein extracts have shown that antibodies in vitiligo patients are most frequently directed to antigens with molecular weights of 35, 40–45, 75, 90, and 150 kDa, these being found on the cell surface [134]. Some of these proteins, including those of 40–45, 75, and 150 kDa, appear to be common tissue antigens, whereas the 35 and 90 kDa proteins are preferentially expressed on melanocytes [135]. Other researchers have identified vitiligo antibody targets of 45, 65, 70, 88, and 110 kDa, specifically expressed in melanocytes [136]. Various melanocyte-associated autoantigens have been reported. Antibodies against tyrosinase, a melanocyte-specific protein, have been extensively detected [137–139], as have antibodies against other proteins of the melanogenic pathway such as L-dopachrome tautomerase, TYRP1, and PMEL, albeit at a low prevalence [140–142]. Considering this autoantibody

response, rituximab, a monoclonal directed against CD20 protein expressed on the B cell surface, has shown promise in a clinical trial [143]. A variety of circulating organ-specific antibodies, for example, against gastric parietal cells, pancreatic islet cells, thyroid and adrenal glands, are common in vitiligo patients' sera, though these are not recognized as major melanocyte antigens [144].

Phage display technology has identified other targets such as melanin-concentrating hormone receptor 1, tyrosine hydroxylase, heat-shock protein 90, osteopontin, ubiquitin-conjugating enzyme, translation-initiation factor 2, GTP-binding protein Rab38, γ-enolase, α-enolase, and lamin A [145–147], as well as four novel autoantigens glycoprotein non-metastatic melanoma protein b, melanocortin 1 receptor, OCA2-encoded P protein and GTP-binding protein Rab27A (unpublished data). Vitiligo-associated antibodies are capable of melanocytotoxicity *in vitro* and *in vivo* by complement-mediated cytotoxicity and by antibody-dependent cellular cytotoxicity [148, 149]. Melanin-concentrating hormone receptor 1 blocks the function of the receptor, though it is not known if this affects melanocyte function [150]. Melanocyte expressed of HLA-DR and intercellular adhesion molecule-1 (ICAM-1) induced by anti-melanocyte antibodies may make melanocytes a target for cytotoxic T cells [151]. Presently, it is unclear whether pigment cells are a primary or secondary target of the humoral immunity in vitiligo. Autoantibodies might arise from a genetic susceptibility to immune dysregulation at the T or B lymphocyte level, with lack of tolerance to pigment cell antigens, or from an immune response against melanocytes damaged by other mechanisms, such as T-cell destruction [152].

Repigmentation observed in vitiligo patients receiving immunosuppressive treatments supports the notion of an immune-mediated process in vitiligo [13]. Tacrolimus, a reagent that suppresses T cells by blocking cytokine gene-activating cofactor calcineurin, works in vitiligo [153, 154], as do topical corticosteroids, which suppress T lymphocyte activity and B-cell antibody responses [155]. Phototherapy reduces the number of Langerhans cells in the skin and downregulates expression of vitiligo-associated melanocyte antigens [156].

Pathogenic mechanisms of autoimmunity in vitiligo

The ability of vitiligo-associated antibodies to destroy melanocytes has been demonstrated *in vitro* by both complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity [148, 150, 157].

In vivo, the administration of IgG from vitiligo patients into human skin grafted onto nude mice has been shown to induce melanocyte destruction [149]. In a reconstructed epidermis model, sera from 9 out of 13 (69%) vitiligo patients induced the detachment of melanocytes, although this was not related to disease extent nor activity [158]. Vitiligo patient antibodies against MCHR1 were demonstrated to block the function of the receptor [150]. However, it is not known if or how this activity could affect the melanocyte function [150]. Moreover, IgG anti-melanocyte antibodies are able to induce the expression of HLA-DR and ICAM-1 on melanocytes and the release of IL-8 from melanocytes [151]. By enhancing the antigen-presenting activity of melanocytes in this way, they become targets for cytotoxic T cells [159]. Interestingly, the normally intracellular melanocyte antigens TYRP1 and PMEL can be expressed on the cell surface and

so can be accessible by antibodies [160, 161]. In addition, pigment cell antibodies in vitiligo might be secondary to destruction of the melanocyte from another immune cause such as T cells, but that once triggered the antibodies are themselves destructive to melanocytes.

While the potential for cytotoxic CD8+ T cells to eliminate melanocytes in both vitiligo and the immunotherapy of malignant melanoma is clear, the exact mechanism that these cells use is not fully understood. Several cytotoxic effector proteins such as perforin, granzyme, Fas ligand, and cytokines can be used to destroy target cells [162]. It is believed that cytotoxic T cells mainly use perforin and granzyne as fast-acting method of destroying cancer cells or virus-infected cells, whereas Fas ligand-driven killing mechanism may act as slower-acting alternative process [163].

In fact, several intracellular signalling pathways promote cytotoxic T-cell killing via perforin, granzyme, and Fas ligand, and therefore it is currently unclear how these pathways are selectively used by T cells and how they communicate [162]. T-cell-mediated killing mechanism in autoimmune vitiligo may differ from that of cancer or viral-infected cells. Therefore, how melanocytes in vitiligo are eliminated is not yet clear. This requires further studies in order to reveal the exact mechanism involved in details. Genes implicated in antigen presentation confer a significant risk for the development of vitiligo [164]. One hypothesis suggests that modified proteins called neo-antigen can be extremely immunogenic, stimulating an immune response against them, since thymic epithelial cells responsible for T-cell education do not synthesize such proteins [165]. This leads to the formation of highly self-reactive T cells with high-affinity receptors that target neo-antigens [165]. Malignant melanoma is immunogenic owing to somatic DNA mutation that result in neo-antigen generation [166]. However, self-reactive T cells are unlikely to target mutated proteins in vitiligo, since untransformed melanocytes do not have the ability to mutate their DNA [43]. Several biochemical processes have been involved in neo-antigen formation as well such processes as deamidation, carbanylation, citrullination, oxidation, and alternative mRNA splicing [42]. These activities are implicated in generating neo-antigens in untransformed beta islet cells [42].

Cytokines imbalance and their role in vitiligo pathogenesis

Cytokine imbalance has been shown in vitiligo skin [167]. Elevated serum levels of soluble IL-2 receptor, which correlates with disease activity in vitiligo patients, have been reported, as have increased synthesis of IL-6, a cytokine that induces ICAM-1 expression on melanocytes which could facilitate leukocyte–melanocyte interaction, in addition to elevated levels of IL-8, a recruiter of neutrophils, T lymphocytes, and basophils [168, 169]. Recently, it was described that T helper (TH) 1 (IL-2, IFN-γ, TNF-β), Th2 (IL-4, IL-5, IL-10, IL-13), and Th17 (IL-17, IL-23)-innate cytokines were elevated in the sera of all 44 vitiligo patients examined, with a higher ratio of Th1/Th2 cytokines [170]. Expression of the pro-inflammatory cytokine TNF-α is significantly high in lesional and peri-lesional vitiligo patient skin, whereas a variety of melanogenic mediators such as endothelin-1, stem cell factor, basic fibroblastic growth factor, and granulocyte monocyte-colony stimulating factor are expressed at lower levels in vitiligo patches [171]. Melanocyte adhesion is disrupted in vitiligo skin through increased levels of MMP-9, produced by keratinocytes in response to IFN-γ and TNF-α, and which disturbs E-cadherin on the pigment cells [172]. In both human vitiligo and in a mouse model, high levels of IFN-γ, the cytokine required for the cutaneous recruitment of melanocyte-specific autoreactive CD8+ T lymphocytes, and of IFN-γ-induced cytokine CXCL10, and its receptor CXCR3, found on autoreactive CD8+ T cells, have been demonstrated [79]. Knocking out CXCR3 or blocking CXCL10 action prevents and reverses depigmentation in vitiligo [173].

Malignant melanoma-associated vitiligo

Malignant melanoma is a type of skin cancer resulted from uncontrolled growth of melanocytes. Although the exact mechanism implicated in the pathogenesis of vitiligo associated with malignant melanoma is still unclear, the immune reactivity against malignant melanoma cells, especially CD8+ T cells, is thought to play a critical role [174].

Malignant melanoma-associated vitiligo may arise from immune response directed against antigens expressed by both melanocytes and malignant cells. Indeed, antibodies reactive to tyrosinase [175], TYRP1, dopachrome tautomerase, and Pmel17 [176] have been found in the sera of some patients with malignant melanoma. These melanocyte-specific antigens were recognized by self-reactive CD8+ T cells [177]. Following immunotherapy for metastatic malignant melanoma, enhanced efficacy is associated with CD8+ T cells. Immunotherapy for malignant melanoma involves blocking T-cell checkpoint inhibitors, which interfere with T-cell tolerance in tissues, and adoptive cell therapy, in which T cells that infiltrate autologous tumour are expanded *ex vivo* for therapeutic reinjection [43]. Importantly, tumour infiltration with CD8+ T cells is vital in the effectiveness of both strategies [178]. These cells are thought to regulate malignant melanoma via perforin-dependent cytolysis [162].

New-onset vitiligo occurs in about 4% of patients treated with immunotherapy [179]. In patients suffering from melanoma, treatment with the immune checkpoint inhibitors; anti-PD-1 therapy, is correlated with development of vitiligo [180]. One study showed that vitiligo occurs following anti-DP-1 treatment in one of four melanoma patients but not in other cancers, indicating that vitiligo is melanocyte lineage-specific immune adverse event [180]. The same study revealed that development of vitiligo in patients suffering from melanoma is correlated with greater response rates to anti-PD-1 therapy [180].

Notably, vitiligo patches initiated by malignant melanoma immunotherapy are packed with CD8+ T cells that are specific to melanocytes, in a similar manner to the situation in idiopathic vitiligo [181]. More recent study demonstrated that in patients with melanoma, anti-PD-1 treatment resulted in an expansion of melanocyte-specific CD8+ T cells and only 5 of 13 responders developed vitiligo [182]. Thus, CD8+ T cells are crucial to both the eradication of malignant melanoma and the pathogenesis of vitiligo. Therefore, the immune response in malignant melanoma patients which causes melanocyte damage is suggested to be cell-mediated, driven by CD8+ T cells and not humoral. Melanocyte-specific antibodies in malignant melanoma are likely to arise as a secondary immune response after melanocyte destruction via cell-mediated effects. The serum titres of malignant melanoma-related antibodies are low and their levels do not differ in patients with and without malignant melanoma-associated vitiligo. Vitiligo-like lesions in malignant

melanoma patients receiving immunotherapy are considered as a good prognostic factor, correlated with a longer survival [183]. Therefore, the relationship between vitiligo and melanoma is suggested to be the result of an immune response to antigens shared by normal melanocytes and melanoma cells. However, it is not clear whether the autoimmune response against normal melanocyte antigens contributes to clearance of melanoma or whether development of vitiligo is merely a correlating adverse event [182]. In addition, there appears to be mutually exclusive relationship between a predisposition to vitiligo and a predisposition to melanoma, which implies a genetic dysregulation of the immune surveillance affecting melanocytic system [184]. Of particular interest is the association found in European-derived whites between vitiligo and SNPs in *TYR*, the gene encoding tyrosinase [185]. The causal genetic variation of *TYR* is inversely correlated between vitiligo and malignant melanoma [186], thus suggesting vitiligo may develop from the dysregulation of normal immune surveillance against melanoma [186].

Interestingly, a correlation between the minor *TYR* allele, 402Q and predisposition to malignant melanoma has been described [187], suggesting that such a variant may be less accessible to the immune defences. Therefore, in melanoma cases with the 402Q variant, these neoplastic melanocytes perhaps evade immune surveillance [185, 186]. On the other hand, the *TYR* 402R variant possibly presented to the immune defences more effectively than 402Q variant leading to a more efficient immunological responses to neoplastic melanocytes, but equally contributes to susceptibility to vitiligo [185, 186]. Better understanding of immunity against melanocytes in patients with melanoma, who develop vitiligo, may help in the identification of more targets for immunotherapy against both melanoma and vitiligo.

Genetic factors

Genetic involvement in vitiligo is obvious from a simple examination of family histories: 15–20% of vitiligo patients have at least one affected first-grade relative [188]. However, vitiligo does not show a Mendelian mechanism of inheritance, but is polygenic with multiple susceptibility loci [164].

The concordance rate in monozygotic twins is 23%, suggesting involvement of non-genetic factors [189]. Genome-wide association studies have revealed that approximately half of vitiligo susceptibility genes encode immune-regulatory proteins, while the remainder encode melanocyte-specific proteins [164].

Several studies have shown that multiple loci contribute to vitiligo susceptibility (Table 1). An observed association between HLA types and vitiligo, and other autoimmune disorders, can be elucidated by several pathways that ultimately result in disruption in self-antigen recognition. This can lead to autoreactive T-cell development and/or failure to produce effective Tregs population [68]. Genome-wide association studies have also reported a subset of other immune regulatory genes that are key mediators of adaptive immunity, such as *CD80* (activation of T cells), cytotoxic T lymphocyte antigen-4; *CTLA4* (inhibition of T cells), *GZMB* (cytotoxicity of T cells), forkhead box protein P3; *FOXP3* (development and function of regulatory T cells), lymphoid protein tyrosine phosphatase non-receptor type 22; *PTPN22* (downregulation of T-cell activation), and arginine–glutamic acid dipeptide repeats protein – *RERE* (regulation of cell apoptosis) [190]. An association of vitiligo with genes that play a role in innate immunity, such as NACHT leucine-rich-repeat protein 1 (*NLRP1*), interferon-induced helicase C domain 1 (*IFIH1*) and caspase-7 (*CASP7*), has also been found in genome-wide association studies [191]. In addition to immune regulatory genes, several genes that are only expressed in melanocytes and involved in melanocyte function have been identified as vitiligo susceptibility loci. These include *TYR*, *PMEL*, melanocortin 1 receptor (*MC1R*), *OCA2* [192]. Such genes encode for enzymes or proteins recognized as autoantigens in vitiligo, facilitating an anti-melanocyte autoimmune response [139, 141].

Neuronal theory, linking neuropeptides and the immune response, and the convergence theory

Neuronal elements were originally thought to have a role in vitiligo through catecholamine released from epidermal nerve endings, which might be cytotoxic to melanocytes, or by autonomic dysfunction producing pigment cell destruction [223, 224]. Clinical pointers towards neural involvement included the distribution along blaschkoid lines in segmental vitligo, and the occasional observation of a true dermatomal appearance, e.g. in the trigeminal areas [190, 224].

Ultrastructurally, vitiligo skin shows degeneration of fine cutaneous nerves in 42% of cases, with Schwann cells showing thickened basement membrane in 75% of instances and axonal damage in a half [223, 225]. Changes in neuropeptides, notably neuropeptide Y, are evident at the margins of vitiligo patches [225]. This led to the postulation that neuropeptides, potentially neuropeptide Y, which has effects on the immune system through receptors located on several immunologically active cells including T cells, NK cells, dendritic cells, granulocytes, and macrophages, released from nerve ending release next to melanocytes, could provoke a local immune reaction and melanocyte destruction [226, 227].

Treg cells, seen as central to the mechanism of melanocyte destruction in vitiligo, can be induced by vasoactive intestinal polypeptide, and can alter the Th1/cytotoxic T-cell (Tc)1 balance that is skewed in vitiligo [228]. Hence, the neural and neuro-endocrine systems, neuro-peptides and neurotransmitters, inter-acting with the immune systems, need to be taken into account in the causation of vitiligo [229].

The convergence theory attempts to link together the potential causal mechanisms of vitiligo [230]. It suggests multiple sequential stages in pathogenesis beginning with an elicitation stage perhaps due to mechanical friction and skin trauma, emotional stresses, chemical exposure or imbalances in endogenous neural factors, metabolites, cytokines or hormones [5, 226, 231–234]. Such factors, in an individual whose genetic make-up predisposes to immune activation and melanocyte destruction, it is proposed, result in oxidative stress within melanocytes which subsequently express HSP70 and chaperoned melanocyte antigens, presented by dendritic cells to T cells in regional lymph nodes, resulting in proliferation of melanocyte-specific cytotoxic T cells and melanocyte destruction – the stage of immune activation [60, 152, 189]. Absent or reduced fully-functional skin-infiltrating Tregs contribute to the on-going immune response and disease spread [95]. Antibodies against melanocyte-specific proteins such as tyrosinase, generated in response to melanocyte destruction, damage pigment cells by activating complement or by antibody-dependent cellular cytotoxicity [139, 150].

Table 1. A summary of genes associated with susceptibility to vitiligo

Type	Gene	Protein	Function	References
HLA	*HLA-A*	HLA class I histocompatibility antigen, A	Peptide antigen presentation to the immune system	[185, 193, 194]
	HLA-DRB1 and DQA1	HLA class II histocompatibility antigen, DRB1 and DQA1	Peptide antigen presentation to the immune system	[185, 195]
	HLA class III	HLA class III histocompatibility antigen	Involved in inflammation and cytokine production	[196]
Immune-regulatory	*AIRE*	Autoimmune regulator	Maintenance of immune tolerance	[197, 198]
	BACH2	Transcription regulator protein BACH2	B cell transcriptional repressor encodes a transcriptional repressor of B cells, which is a key regulator of CD4+ T-cell differentiation that prevents inflammatory disease by controlling the balance between tolerance and immunity	[191]
	C1QTNF6	Complement C1q tumour necrosis factor-related protein 6	Induces monocyte IL-10 expression	[185]
	CASP7	Caspase-7	Executor protein of apoptosis and inflammation	[191]
	CCR6	Chemokine-cytokine receptor 6	Regulates differentiation and function of B and T lymphocytes and dendritic cells	[185, 196]
	CD44	CD44 antigen	Involves in T-cell development	[191]
	CD80	T lymphocyte activation antigen CD80	Co-stimulates activation of T cells	[191]
	CLNK	Cytokine-dependent hematopoietic cell linker	Positively regulates immune-receptor signalling	[191]
	CTLA4	Cytotoxic T lymphocyte protein 4	Inhibition of T cells	[199–201]
	CXCR5	C-X-C chemokine receptor type 5	Involves in B lymphocyte migration	[192]
	FASLG	FAS ligand	Regulate immune apoptosis	[202]
	FOXP1	Forkhead box protein P1	Regulates B cell development	[203]
	FOXP3	Forkhead box protein P3	Regulates development and function of regulatory T cells	[85]
	GZMB	Granzyme B	Mediates the process of immune-induced cell death with contribution of cytotoxic T lymphocytes and natural killer cells	[185, 204]
	IFIH1	Interferon-induced helicase C domain 1	Regulates innate immune response	[191]
	IKZF4	Zinc finger protein Eos	Regulates T-cell activation	[191, 192]
	IL2RA	Interleukin2 receptor subunit alpha	Regulates regulatory T cells	[185, 192]
	NLRP1	NACHT leucine-rich-repeat protein 1	Regulates innate immune system	[45, 205–207]
	PTPN22	Lymphoid protein tyrosine phosphatase non-receptor type 22	Negative regulation of T-cell activation	[185, 208–213]
	SH2B3	SH2B adapter protein 3	Development of B and T lymphocytes	[191]
	SLA	Src-like-adapter	Regulates antigen receptor signalling	[191]
	TOB2	Protein TOB2	Involves in T-cell tolerance	[191]
	TSLP	Thymic stromal lymphopoietin	Cytokine regulator of maturation of skin dendritic cells and T cells	[85]
	UBASH3A	Ubiquitin-associated and SH3 domain–containing A protein	Suppresses T-cell receptor signalling	[185]
	XBP1	Xbox-binding protein 1	Regulator of major histocompatibility complex class II	[39, 85]

Table 1. Continued

Type	Gene	Protein	Function	References
Melanocyte function	ASP	Agouti signalling protein	Melanogenesis regulator via MC1R	[214]
	FOXD3	Forkhead box D3	Regulator of melanoblast development	[215]
	MC1R	Melanocortin 1 receptor	Regulator of melanogenesis	[191, 214]
	OCA2	OCA2 gene	Melanosomal transporter	[191]
	PMEL	Melanocyte-specific protein PMEL	Structural organization of premelanosomes	[192]
	TYR	Tyrosinase	Regulator of melanogenesis	[185, 216]
	ZMIZ1	Zinc finger protein MIZ type 1	Regulates development, function and survival of melanocytes	[196, 217]
Metabolism-related genes	ACE	Angiotensin-converting enzyme	Regulator of inflammation and blood pressure	[218]
	CAT	Catalase	Protects cells from oxidative stress by breakdown of hydrogen peroxide	[219–221]
	EDN-1	Endothelin-1	Regulator of melanocyte growth and function	[222]
	LPP	Lipoma-preferred partner	Potential coactivator	[185]
	RERE	Arginine–glutamic acid dipeptide repeats protein	Regulates cell apoptosis	[185]
	RNASET2	Ribonuclease T2	Oxidative stress regulator	[196]

Conclusion and prospects

Sufficient is now understood about the pathogenesis of vitiligo to permit targeted pharmacological intervention at the appropriate immunological steps. However, the exact modus by which the genetic interacts with the environment, and with the immune system still requires considerable elucidation. What can be said is that both environmental factors and cell-intrinsic defects instigate stress responses in melanocytes, resulting in the synthesis of DAMPs that elicit innate immune cells, which in turn activate adaptive immunity that ultimately targets melanocytes.

A genetic predisposition to autoimmunity may underlie inappropriate immune responses in vitiligo, but immune responses may occur secondarily as a result of melanocyte damage by other factors, as when autoantibodies have been observed directed against intracellular pigment cell proteins – exposure of cryptic epitopes and protein modification occurring during apoptosis [235]. Following processing by dendritic cells, antigenic proteins can be presented to either autoreactive T cells, which have evaded clonal deletion, or to naive T cells, which have not been tolerized against cryptic epitopes [236]. In consequence, antibodies can be secreted following autoreactive B-cell stimulation by activated autoreactive CD4+ T lymphocytes [236], which may then act to further aggravate vitiligo.

However, it is possible that antibodies play no part in the pathogenesis of vitiligo, but might indicate the existence of autoreactive anti-melanocyte T cells capable of destroying melanocytes, a scenario that merits further investigation.

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