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Regulatory T cells in vitiligo: Implications for pathogenesis and therapeutics

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Abbreviations: FoxP3, Foxhead box 3P; CTLA-4, cytotoxic T lymphocyte antigen-4; GM-CSF, granulocyte-macrophage colony-stimulating factor; I κ B, NF- κ B inhibitor; iTregs, inducible regulatory T cells; IL, interleukin; IL-2R, interleukin-2 receptor; IFN, interferon; NFAT, nuclear factor of activated T cells; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; nTregs, natural regulatory T cells; TGF- β , transforming growth factor- β ; Tregs, regulatory T cells

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ABSTRACT

Vitiligo is a hypomelanotic autoimmune skin disease arising from a breakdown in immunological self-tolerance, which leads to aberrant immune responses against melanocytes. Regulatory T cells (Tregs) are crucial to the development of self-tolerance and so are major foci in the study of autoimmune pathogenesis of vitiligo. This review will summarise recent findings concerning the role of Tregs in the pathogenesis of vitiligo. In addition, as antigen-specific Tregs are a potential route for the reinstatement of immune tolerance, new strategies that expand or induce *de novo* generation of Tregs and which are currently being investigated as therapies for other autoimmune diseases, will be discussed. These approaches will highlight the opportunities for Treg cell-based therapeutics in vitiligo.

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1. Introduction

Vitiligo is a hypomelanotic autoimmune skin disease resulting from loss of functional melanocytes from the skin with a prevalence of 1-2% [1]. The disease can affect individuals of any race or sex and manifests before the age of 20 years in approximately half of all cases [1]. Although vitiligo might be considered a minor disorder, the psychological effects of the disease are frequently considerable. Individuals with the disease may experience feelings of stress, embarrassment, self-consciousness and low self-esteem particularly when in professional or social situations [2]. This is particularly the case in people with darkly pigmented skin where the appearance of depigmented patches is very prominent and also where vitiligo develops on visible areas of the body such as the face, hands and arms [3]. To date, no universally effective and safe therapy for the disease exists. Many treatment options have been developed, but challenges persist as not all patients respond to the available therapies, relapse is common and complete repigmentation is rarely accomplished [4].

The exact aetiology and detailed pathogenesis of vitiligo is not fully understood, but autoimmunity has been strongly implicated in the development of the disease [5]. The current 'state-of-the-art' thinking is summarised in the convergence theory which suggests that several factors can act synergistically or independently to induce the disappearance of melanocytes from the skin [5]. In the elicitation phase of vitiligo, triggers including physical trauma to the skin, emotional stresses, and imbalances of endogenous neural factors, metabolites or hormones lead to oxidative stress within melanocytes which then respond by actively secreting heat-shock protein 70 and chaperoned melanocyte antigens [6]. During the immune activation

stage, these 'danger' signals promote the activation of antigen-presenting dendritic cells with the subsequent activation and recruitment of anti-melanocyte autoreactive cytotoxic T lymphocytes to the skin [7] (Fig. 1).

The importance of autoimmunity in the aetiology of vitiligo is backed by recently identified susceptibility genes which include those involved in regulating the immune response such as *PTPN22*, *CTLA-4*, *FOXP1*, and those encoding certain major histocompatibility complex antigen specificities [8]. In addition, several studies have identified a pivotal role for cytotoxic T cells in inducing melanocyte-specific destruction in vitiligo [7], including the findings that CD8⁺ T cells isolated from vitiligo skin are cytotoxic to melanocytes, recognise melanocyte-specific autoantigens [7], and induce autologous melanocyte apoptosis through IL-6 and IL-13 [9] (Fig. 1). In addition, there is a global expansion and widespread activation of the CD8⁺ T cell population in vitiligo patients [10-12]. So far, however, the exact mechanisms underlying the induction and activation of autoreactive CD8⁺ T cells and the loss of tolerance to melanocyte autoantigens in vitiligo are not clear, but as with other autoimmune disorders, it seems likely that this loss of tolerance to self must involve some defect in regulatory T cell (Tregs) function [10,11,13].

Regulatory T cells are a subset of CD4⁺ lymphocytes that play a key role in maintaining peripheral tolerance *in vivo* through the active suppression of self-reactive T cell activation and expansion (Fig. 1) [14]. They maintain order in the immune system by enforcing a dominant negative regulation on other immune cells including actively suppressing the activation and expansion of autoreactive T cells that have escaped clonal deletion in the thymus [14]. According to their site of maturation, two classes of Treg cells have been identified: natural

Tregs (nTregs) and inducible Tregs (iTregs) [15]. Natural Tregs originate from the thymus as CD4⁺ cells expressing high levels of CD25 together with the transcription factor and lineage marker FoxP3. They represent approximately 5–10% of the total CD4⁺ T cell population and are positively selected thymocytes with a relatively high avidity for self-antigens [15].

Inducible Tregs originate from the thymus as single CD4⁺ cells and differentiate into CD25⁺ and FoxP3⁺ iTregs following adequate antigenic stimulation in the presence of cognate antigen and cytokines such as transforming growth factor- β (TGF- β), and interleukin (IL)-10 [15]. Inhibition of effector T cells proceeds via cell-cell contact or via the secretion of inhibitory cytokines, mainly TGF- β [15].

This review will address recent findings concerning the role of Tregs in the pathogenesis of vitiligo. In addition, as antigen-specific Tregs are a potential route for the reinstatement of immune tolerance, new strategies which expand or induce *de novo* generation of Tregs and which are currently being investigated as therapies for other autoimmune diseases, will be discussed and will highlight the opportunities for Treg-based therapeutics in vitiligo.

2. Regulatory T cells in vitiligo

An initial indication that a defect in Treg cell function could contribute to the progressive depigmentation of the skin came from studies of murine melanoma where the depletion of Tregs caused the activation of anti-melanoma cytotoxic T cells that destroyed melanoma tumours but also generated vitiligo as a side-effect [16]. More recently, repigmentation in a mouse model of spontaneous epidermal depigmentation was found to be accompanied by an

increased Treg cell infiltration, suggesting their importance in preventing an ongoing immune response against melanocytes [17]. To date, several studies have indicated perturbations in Treg cell numbers and/or function in vitiligo patients [10-27]. Such alterations might lead to the higher levels and activation of cytotoxic T cells (Fig. 1) which have been reported in individuals with the disease [10,11]. Currently, however, studies examining any reciprocal relationship between CD4⁺CD25⁺FoxP3⁺ Tregs and CD8⁺ in vitiligo progression are lacking.

2.1. Regulatory T cell numbers

Several studies have been carried out to look for alterations in Treg cell numbers in vitiligo (Table 1) [10-23,26,27]. Assessment of circulating Tregs by flow cytometric analysis has revealed a decrease in their numbers in vitiligo patients compared to controls [10,11,13]. Reduced peripheral Treg cell numbers have also been reported in early age-of-onset patients (1-20 years) compared to those with late onset vitiligo, and decreased circulating Treg cell counts have been demonstrated in patients with active vitiligo as compared to those with stable disease [11]. Moreover, a striking reduction in the number of Tregs in the marginal and lesional skin of vitiligo patients has been observed [18,19].

Interestingly, some studies have demonstrated that peripheral or lesional skin CD4⁺CD25⁺FoxP3⁺ Treg cell numbers remain unaltered in vitiligo [20,21,23], and even that either may be increased [10,22].

2.2. Regulatory T cell function

Significant reductions in the function of peripheral Tregs have been demonstrated in progressive vitiligo using assays which measure how well these cells can inhibit the proliferation of and cytokine production from stimulated autologous CD8⁺ T cells (Table 1) [10, 13]. The possible causes of Treg cell functional defects in vitiligo have been investigated and these are summarised below.

2.2.1. Forkhead box P3

The transcription factor Forkhead box P3 (FoxP3) is a specific marker for Tregs and serves as the dedicated mediator governing Treg cell development and function [28-30]. FoxP3 down-regulates T cell activation and cytokine genes (e.g., encoding IL-2, IL-4), and upregulates immunosuppressive cell-surface molecules (e.g., CD25, CTLA-4) and, in doing so, contributes to both the hyporesponsive and suppressive Treg cell phenotype [31,32]. FoxP3 suppresses the effector functions of T helper cells by directly inhibiting the activity of two key transcription factors, nuclear factor of activated T cells (NFAT) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which are essential for cytokine gene expression and T cell functions [30]. In mice, the depletion of FoxP3⁺ Tregs leads to the induction of autoimmunity by specifically ablating Tregs [33].

Several observations indicate that there is a defect in FoxP3 expression in vitiligo patients that could impair the function of Treg cells. Firstly, FoxP3 expression is significantly decreased in CD4⁺CD25⁺ Tregs from vitiligo patients compared to controls [11]. Secondly, the

mean percentage area of FoxP3-positive immunostaining in lesional skin and the levels of FoxP3-positive cells in the peripheral blood are significantly lower in vitiligo patients compared to controls [24]. Finally, *FOXP3* mRNA levels in lesional and perilesional skin are significantly reduced in vitiligo patients when compared with skin from healthy individuals [25]. In contrast, significantly higher levels of *FOXP3* mRNA expression have been found to occur in lesional vitiligo skin compared to non-lesional vitiligo skin [13], suggesting the recruitment of Tregs into the affected site to balance the autoimmune response.

Several studies have indicated that genetic defects in *FOXP3* may lead to its altered levels and functionality and, indeed, autoimmune disorders have been related to various deleterious mutations in *FOXP3* [34-38]. With respect to vitiligo, polymorphisms of *FOXP3* have been associated with predisposition to the development of the disease (Table 2) [39-41].

2.2.2. Cytotoxic T lymphocyte antigen-4

Cytotoxic T lymphocyte antigen-4 (CTLA-4 or CD152) is a T cell surface molecule involved in regulation of T cell activation and plays a critical role in the maintenance of tolerance to self-antigens [42]. Moreover, knock-out mouse models have shown that CTLA-4 plays a key role in Treg cell function and maintaining peripheral tolerance [43-45]. It is therefore not surprising that the abnormal function of the molecule has been implicated in the aetiology of several autoimmune diseases including vitiligo [46-48]. Indeed, both soluble (sCTLA-4) and full-length (fICTLA-4) *CTLA-4* mRNA have been found at decreased levels in vitiligo patients, which could perturb the normal suppressive capacity of Tregs [46]. Vitiligo has also been associated with the

presence of the 3' untranslated region CT60GG (rs3087243) allelic variation of *CTLA-4*, this in turn being correlated with the reduction in *CTLA-4* mRNA levels [46].

2.2.3. Transforming growth factor- β

Transforming growth factor- β is important for imposing a regulatory phenotype on the Treg cell subset [49-51]. Studies have reported that TGF- β has the ability to induce CD4⁺CD25⁻ cells to become CD4⁺CD25⁺ Tregs *in vitro* [52,53], and TGF- β can induce FoxP3 expression in iTregs [54,55]. Additionally, other studies have clearly demonstrated that the suppressive capacity of FoxP3⁺ Tregs *in vivo* is via a TGF- β -dependent mechanism [56,57].

A number of studies indicate that decreased TGF- β serum and tissue levels are found in vitiligo patients, and this could have a deleterious effect upon Treg cell function in these individuals [58-60]. In addition, serum concentrations of TGF- β have been shown to be decreased in cases of active but not stable vitiligo, indicating that lower levels of the cytokine are positively correlated with disease progression [61]. Furthermore, the reduced levels of TGF- β secreted by vitiligo patient Tregs are negatively correlated with the percentage of depigmented body area [62]. Interestingly, three single nucleotide polymorphisms of the TGF- β receptor 2 gene (*TGFBR2*) (rs2005061, rs3773645, rs3773649) are associated with vitiligo susceptibility [63], although it is not yet clear how these allelic variants influence the expression of TGF- β .

2.2.3. Interleukin-10

Tregs induced by IL-10 contribute to Treg cell-mediated immunosuppression principally by producing IL-10 [64-65]. Several pieces of evidence suggest a role for IL-10 in the pathogenesis of vitiligo. For example, decreased IL-10 levels have been demonstrated in cases of active but not stable vitiligo, suggesting that IL-10 might play an important role in disease progression [61]. In addition, our recent study has also suggested a decrease in *IL-10* mRNA expression in vitiligo patients as compared to healthy controls (Dwivedi et al., unpublished results). Interestingly, increased IL-10 expression in vitiligo lesions has been shown to occur after topical tacrolimus treatment suggesting that the inhibition of melanocyte destruction is triggered by unchecked Th1 pathways in vitiligo [66]. Furthermore, genetic susceptibility to vitiligo has been reported to be associated with polymorphisms in the *IL-10* gene in Saudi patients: the GG genotype at -1082 and the CC genotype at positions -592 and +819 were significantly more prevalent in vitiligo patients compared to controls [67]. However, it has not been established whether these promoter polymorphisms play a role in the altered IL-10 expression.

Animal models also indicate a role for IL-10 in vitiligo development. Studies of Smyth line chickens, an animal model for human autoimmune vitiligo, have demonstrated that elevated leukocyte infiltration in early and active vitiligo is accompanied by increased levels of cytokine expression, especially in interferon (IFN)- γ , IL-10, and IL-21 [68]. Recently, in a mouse model of spontaneous melanoma, Treg cell-depletion and IL-10 neutralisation led to an increased occurrence of vitiligo which correlated with a decreased incidence of melanoma metastases [69].

2.2.4. Cell-homing capacity

Klarquist et al. [23] reported a discrepancy between the relative abundance of Treg cells present in the circulation of vitiligo patients as compared to their skin. The authors suggested that this may be explained by a failure of Tregs to migrate into the skin. However, their experiments demonstrated that expression of receptors previously shown to dictate homing of Treg cells to the skin compartment was similar among the patient and control populations. Further investigations indicated that reduced expression of chemoattractant CCL22 within vitiligo patient skin is primarily responsible for impaired migration of Tregs into the tissue.

3. Regulatory T cells as therapeutic targets for vitiligo

The studies discussed above certainly render Treg cells as crucial immune regulators involved in vitiligo pathogenesis. Approaches to correct defects in numbers or functions of Tregs may, therefore, be valuable in the treatment of autoimmune vitiligo.

3.1. Adoptive transfer therapy using regulatory T cells

Systemic treatments for autoimmune disorders directed at augmenting Treg cell activities *in vivo* may be associated with significant off-target effects, including a generalised immunosuppression that may compromise beneficial immune responses. Therefore, the adoptive transfer of *in vitro*-generated, antigen-specific iTregs is a potential therapy option for autoimmune disorders. Treg cells can indeed be expanded *ex vivo* with allogeneic B cells [70]

and these express very high levels of FoxP3, maintain an anergic phenotype, and are potent suppressors capable of inhibiting activated T cell responses [70]. To generate the sufficient antigen-specific Tregs for adoptive transfer, Brusco et al. [71] have developed a novel technique involving lentiviral T cell receptor gene transfer into *in vitro* expanded polyclonal nTreg cell populations, indicating the feasibility of major histocompatibility complex class I-restricted T cell receptor transfer as a promising strategy to redirect the functional properties of Tregs and provide for a more efficacious adoptive Treg cell therapy.

Several previous studies have indeed demonstrated that the adoptive transfer of iTregs can suppress disease development in a lupus syndrome-like model [72, 73], experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis [74], an autoimmune gastritis model [75-76], spontaneous development of type 1 diabetes in mice [77], Th1-mediated colitis [78], Th17-mediated diseases [79], and house dust mite-induced allergic pathogenesis in an asthmatic mouse model [80].

Recently, Chatterjee et al. [81] have reported the first animal study proposing Tregs as a therapeutic for vitiligo. Using transgenic mice that carry T cells with a human leukocyte antigen (HLA)-A2-restricted human tyrosinase peptide-reactive T cell receptor and that develop spontaneous vitiligo from an early age, adoptively transferred Tregs were found to induce a lasting remission of vitiligo in mice treated at the onset of disease, or in mice with established disease. The authors concluded that reduced regulatory responses are pivotal to the development of vitiligo in disease-prone mice, and that a quantitative increase in the Treg cell population may be therapeutic for vitiligo patients with active disease.

3.2. Adoptive transfer therapy using heat-shock protein-specific regulatory T cells

Heat-shock proteins play an important role in immune activation and may participate in dysregulation of immune homeostasis. The presence of heat-shock protein activates dendritic cells which respond by recruiting immune cells to the site of inflammation. Recently, a role for induced heat-shock protein has been reported in the pathogenesis of vitiligo revealing its potential as a significant therapeutic target for the disease [6,82,83]. Indeed, the suppressive capacity of heat-shock protein-specific Treg cells has been demonstrated in studies performed in animal models and in human clinical trials for autoimmune and autoinflammatory diseases [84-86], but not yet for vitiligo.

3.3. Use of probiotics for the induction of regulatory T cells

The administration of microbes or microbial metabolites for the prevention and treatment of aberrant immune response is gaining importance [87]. The term probiotic refers to 'living microorganisms which, when consumed in adequate amounts, confer a health effect on the host' [88]. The protective effects associated with these microbes are mediated by multiple mechanisms involving T cells, dendritic cells and epithelial cells. An increasingly recognised feature of immunoregulatory microbes is their ability to induce Treg cells [87]. It has been demonstrated that oral consumption of *Bifidobacterium infantis* 35624 is associated with enhanced IL-10 secretion and FoxP3 expression in human peripheral blood [89]. Murine studies have demonstrated that *B. infantis* administration results in an increase in CD4⁺CD25⁺FoxP3⁺

lymphocytes [90]. Moreover, it has been seen that, *in vitro* human dendritic cells stimulated with *B. infantis* selectively promoted the upregulation of FoxP3 expression in naïve lymphocytes [89]. Furthermore, *B. infantis* has been demonstrated to reduce symptom severity in patients with inflammatory bowel syndrome in two placebo-controlled human clinical studies [91,92]. Thus, the induction of FoxP3⁺ Treg cells by *B. infantis* seems to be a robust and reproducible phenomenon, which could be useful in the treatment of autoimmune diseases including vitiligo.

Recently, it has been suggested that probiotic (*Lactobacillus reuteri* DSM 17938)-facilitated development of Tregs might play an important role in the prevention of necrotising enterocolitis, and Feleszko et al. [93] showed that probiotic bacteria (*L. rhamnosus* GG or *Bifidobacterium lactis* Bb-12) inhibit subsequent allergic sensitisation and airway disease in a murine model of asthma by inducing Tregs associated with increased TGF- β production. Furthermore, the probiotics mixtures (*L. acidophilus*, *L. casei*, *L. reuteri*, *Bifidobacterium*, and *Streptococcus thermophilus*) induce the generation of CD4⁺FoxP3⁺ Tregs from the CD4⁺CD25⁻ population and increased the suppressor activity of naturally occurring CD4⁺CD25⁺ Tregs in mice [94]. Interestingly, the report suggests that conversion of T cells into FoxP3⁺ Tregs is directly mediated by regulatory dendritic cells that express high levels of IL-10 and TGF- β . In addition, administration of the probiotics had therapeutical effects in experimental inflammatory bowel disease, atopic dermatitis and rheumatoid arthritis [94]. Recently, the effects of *L. rhamnosus* on the progression of the clinical signs of atopic dermatitis to allergic asthma has been shown by suppressing Th2 and Th17 responses via CD4⁺CD25⁺FoxP3⁺ Tregs [95]. Finally, a mixture of three *Lactobacilli* strains (*L. paracasei* DSM 13434, *L. plantarum* DSM 15312 and DSM 15313) suppressed the progression and reversed the clinical and histological signs of experimental

autoimmune encephalomyelitis by inducing CD4⁺CD25⁺FoxP3⁺ Tregs and enhancing production of serum IL-10 and TGF- β [96].

Overall, these findings indicate the therapeutic potential of oral administration of a combination of probiotics in the management of autoimmune diseases and they therefore have similar potential for the treatment of vitiligo. However, further animal as well as human studies are required to provide evidence for the possible use of probiotics in the therapy of skin depigmentation.

3.4. Other approaches used for the induction of regulatory T cells

A number of other approaches have been used for the induction of Treg cells, and these are summarised in this section. However, their full potential for treatment of autoimmune diseases including vitiligo remains to be explored.

*3.4.1. Acidic polysaccharide of *Panax ginseng**

An acidic polysaccharide of *Panax ginseng* (APG) has multiple immunomodulatory effects through instigating the production of a variety of anti-inflammatory cytokines. Studies have shown that APG significantly ameliorates the progression of experimental autoimmune encephalomyelitis by inhibiting the proliferation of autoreactive T cells and promoting the generation of immunosuppressive Tregs through the activation of FoxP3 [97]. Recently, Ahmad et al. [98] showed that 5-aminoisoquinolinone treatment significantly up-regulated the number

of Tregs, I κ B (NF- κ B inhibitor)- α levels and mRNA expression of anti-inflammatory mediators, thereby reducing the arthritis scores in adjuvant-induced arthritis in mice.

3.4.2. Interleukin-5

Tran et al. [99] have shown that IL-5 is able to promote the induction of antigen-specific CD4⁺CD25⁺ Tregs that suppress autoimmunity. In particular, treatment of experimental autoimmune neuritis with IL-5 markedly reduced the infiltration of CD4⁺ (Th1 and Th17) and CD8⁺ T cells, and macrophages in nerves with expansion of antigen-specific CD4⁺CD25⁺FoxP3⁺ Tregs. Moreover, the depletion of CD25⁺ Tregs or blocking of IL-4 abolished the benefits of IL-5 treatment [99]. Overall, the study suggested IL-5 therapy may be able to induce antigen-specific tolerance in autoimmune disease.

3.4.3. Interleukin-2 and interleukin-2 receptor

Several observations have suggested that novel targets for Treg cell targeted treatment for autoimmune disease may lie within the IL-2/IL-2 receptor (R) pathway. Recently, Rouse et al. [100] demonstrated a role for IL-2 in the activation and expansion of Tregs in the amelioration of experimental autoimmune encephalomyelitis. Furthermore, defects in IL-2R-signaling have been shown to contribute to the diminished maintenance of FoxP3 expression in Tregs in patients with type 1 diabetes [101]. In addition, allogeneic stem cell transplantation was found to lead to full recovery from primary biliary cirrhosis where peripheral blood lymphocytes were completely deficient in the α -subunit of the IL-2R (CD25) [102].

3.4.4. Self antigen-derived peptides

Recent experimental work and preclinical studies have provided proof-of-concept for the induction of self-tolerance through the modulation of Tregs using self antigen-derived peptides which then promote suppression of hyperactive CD4⁺ T cells and the production of pathogenic autoantibodies [103]. Interestingly, cytokine-like vasoactive intestinal peptide treatment to human CD4⁺CD25⁻ T cells resulted in the induction of an anergic CD4⁺CD25⁺FoxP3⁺ T cell subset displaying potent regulatory activities against allospecific effector T cells [104]. This study suggested that alloantigen-specific vasoactive intestinal peptide-generated Tregs could be a valuable tool in therapeutic interventions to promote immunotolerance toward allogeneic grafts [104]. Analogous approaches could be developed for specific autoimmune diseases.

3.4.5. Anti-CD3 monoclonal antibody

The stimulation of T cell receptors with modified anti-CD3 monoclonal antibody has been applied to expand the CD8⁺ T cell population and induce CD8⁺CD25⁺ Tregs in patients with type 1 diabetes [105]. The study highlighted the clinical efficacy of this method of treatment of an autoimmune disease which prevented the loss of insulin production over the first two years of the disease and reduced insulin usage.

3.4.6. Granulocyte-macrophage colony-stimulating factor

Sheng et al. [106] showed that the treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF) resulted in amelioration of experimental autoimmune myasthenia

gravis, accompanied by a down-regulation of autoreactive T cell and pathogenic autoantibody responses, a mobilisation of dendritic cells with a tolerogenic phenotype, and an expansion of Tregs that potently suppressed acetylcholine receptor-stimulated T cell proliferation *in vitro*. The study provided evidence that the mobilization of antigen-specific Tregs *in vivo* using pharmacologic agents, like GM-CSF, can modulate ongoing immune responses against specific antigens capable of suppressing antibody-mediated autoimmunity.

3.5. B cell-mediated pathways

It is known that B cells participate in the immune system through antibody production. Interestingly, Walters et al. [107] have recently reported that intrathymic B cells are involved in production of CD4⁺FoxP3⁺ nTregs. Such information offers the possibility of using the B cell-mediated pathway of nTreg cell generation in the Treg cell-mediated therapy of vitiligo.

3.6. Regulatory T cell/Th17 cell balance

The transcription factors STAT5 and STAT3 are known to control the differentiation of Tregs and Th17 cells, respectively. Inhibiting STAT3 using siRNA has been shown to significantly increase the proportion of Treg cells and decrease the proportion of Th17 cells in the CD4⁺ T cell population from the peripheral blood and synovial fluid of patients with rheumatoid arthritis [108,109]. The inhibition of STAT5 has the counter effects [108,109]. In the same study, no change was found to be induced by STAT3 or STAT5 siRNA in the production of Th1 *versus* Th2

signature cytokines [108], suggesting that these signalling pathways could provide novel target molecules for the control of Treg cells in the treatment of autoimmune disease, including vitiligo.

3.7. Enhanced killing activity of regulatory T cells

An alternative approach for using Treg cells therapeutically has been to enforce their pro-apoptotic activity by decorating them with Fas-L protein [110]. The Fas-L-labelled Tregs showed sustained inhibitory activity in relation to T cell proliferation *in vitro* and were also found to be efficient in preventing the progression in mouse models of autoimmune insulinitis and chronic colitis [110]. Though promising, the efficacy of such an approach has yet to be studied in relation to the treatment of human autoimmune disorders including vitiligo.

4. Conclusions

The exact role of Tregs in the development of vitiligo has yet to be established unequivocally. Particularly, there are still conflicts in the data regarding Treg cell numbers and function in vitiligo patients. Future studies will reveal detailed mechanisms by which Treg cells are involved in maintaining tolerance against pigment cell-specific antigens, such that alternative therapeutic approaches which could manipulate Treg cells and specifically dampen B and T cell autoreactivity towards melanocytes without affecting general immunity may be a way forward.

Conflict of interest

The authors declare no conflict of interest.

Take-home messages

- Vitiligo patients present with defects in the function and numbers of regulatory T cells which may lead to increased levels of CD8⁺ T cells with anti-melanocyte activity.
- Antigen-specific adoptive transfer of Tregs and other approaches for inducing Treg cells seem to be efficient in ameliorating autoimmune disease, and thus may also serve as therapeutics for vitiligo.
- Further studies for determining the exact role of Tregs in the pathogenesis of vitiligo are required to pave the way for improved treatments.

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References

- [1] Taïeb A, Picardo M. Epidemiology, definitions and classification. In: Taïeb A, Picardo M, editors. Vitiligo. Berlin: Springer-Verlag; 2010, p. 13-24.
- [2] Taïeb A, Picardo M. Management overview. In: Taïeb A, Picardo M, editors. Vitiligo. Berlin: Springer-Verlag; 2010, p. 319-23.
- [3] Ongenaë K, Beelaert L, van Geel N, Naeyaert JM. Psychosocial effects of vitiligo. *J Eur Acad Dermatol Venereol* 2006;20:1-8.
- [4] Ongenaë K, Dierckxsens L, Brochez L, van Geel N, Naeyaert JM. Quality of life and stigmatization profile in a cohort of vitiligo patients and effect of the use of camouflage. *Dermatology* 2005;210:279-85.
- [5] Le Poole I, Luiten R. Autoimmune etiology of generalized vitiligo. *Curr Dir Autoimmun* 2008;10:227-43.
- [6] Mosenson JA, Zloza A, Klarquist J, Barfuss AJ, Guevara-Patino JA, Poole IC. HSP70i is a critical component of the immune response leading to vitiligo. *Pigment Cell Melanoma Res* 2012;25:88-98.
- [7] van den Boorn JG, Konijnenberg D, DelleMijn TA, van der Veen JP, Bos JD, Melief CJ, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. *J Invest Dermatol* 2009;129:2220-32.
- [8] Spritz RA. Six decades of vitiligo genetics: genome-wide studies provide insights into autoimmune pathogenesis. *J Invest Dermatol* 2012;132:268-73.
- [9] Wu J, Zhou M, Wan Y, Xu A. CD8⁺ T cells from vitiligo perilesional margins induce autologous melanocyte apoptosis. *Mol Med Rep* 2013;7:237-41.

- [10] Lili Y, Yi W, Ji Y, Yue S, Weimin S, Ming L. Global activation of CD8⁺ cytotoxic T lymphocytes correlates with an impairment in regulatory T cells in patients with generalized vitiligo. *PLoS One* 2012;7:e37513.
- [11] Dwivedi M, Laddha NC, Arora P, Marfatia YS, Begum R. Decreased regulatory T-Cells and CD4⁺/CD8⁺ ratio correlate with disease onset and progression in patients with generalized vitiligo. *Pigment Cell Melanoma Res* 2013;26:586-91.
- [12] Wankowicz-Kalinska A, van den Wijngaard RM, Tigges BJ, Westerhof W, Ogg GS, Cerundolo V et al. Immunopolarization of CD4⁺ and CD8⁺ T cells to type-1-like is associated with melanocyte loss in human vitiligo. *Lab Invest* 2003;83:683-95.
- [13] Ben Ahmed M, Zaraa I, Rekik R, Elbeldi-Ferchiou A, Kourda N, Belhadj Hmida N et al. Functional defects of peripheral regulatory T lymphocytes in patients with progressive vitiligo. *Pigment Cell Melanoma Res* 2012;25:99-109.
- [14] Levings M, Sangregorio R, Roncarolo M. Human CD25(+)CD4(+) T regulatory cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. *J Exp Med* 2001;193:1295-302.
- [15] Maloy K, Powrie F. Regulatory T-cells in the control of immune pathology. *Nat Immunol* 2001;2:816-22.
- [16] Zhang P, Côté AL, de Vries VC, Usherwood EJ, Turk MJ. Induction of postsurgical tumor immunity and T-cell memory by a poorly immunogenic tumor. *Cancer Res* 2007;1:6468-76.

- [17] Eby JM, Kang HK, Klarquist J, Chatterjee S, Mosenson JA, Nishimura MI, et al. Immune responses in a mouse model of vitiligo with spontaneous epidermal de- and repigmentation. *Pigment Cell Melanoma Res* 2014;doi:10.1111/pcmr.12284.
- [18] Abdallah M, Lotfi R, Othman W, Galal R. Assessment of tissue FoxP3⁺, CD4⁺ and CD8⁺ T-cells in active and stable nonsegmental vitiligo. *Int J Dermatol* 2014;53:940-6.
- [19] Ono S, Tanizaki H, Otsuka A, Endo Y, Koyanagi I, Kataoka TR, et al. Coexistent skin lesions of vitiligo and psoriasis vulgaris. Immunohistochemical analyses for IL-17A-producing cells and regulatory T cells. *Acta Derm Venereol* 2014;94:329-30.
- [20] Terras S, Gambichler T, Moritz RK, Altmeyer P, Lambert J. Immunohistochemical analysis of FOXP3⁺ regulatory T cells in healthy human skin and autoimmune dermatoses. *Int J Dermatol* 2014;53:294-9.
- [21] Zhou L, Li K, Shi YL, Hamzavi I, Gao TW, Henderson M, et al. Systemic analyses of immunophenotypes of peripheral T cells in non-segmental vitiligo: implication of defective natural killer T cells. *Pigment Cell Melanoma Res* 2012;25:602-11.
- [22] Abdallah M, Saad A. Evaluation of circulating CD4⁺CD25^{high}FoxP3⁺ T lymphocytes in active non-segmental vitiligo. *J Pan-Arab League Dermatol* 2009;20:1.
- [23] Klarquist J, Denman CJ, Hernandez C, Wainwright DA, Strickland FM, Overbeck A, et al. Reduced skin homing by functional Treg in vitiligo. *Pigment Cell Melanoma Res* 2010;23:276-86.
- [24] Elela MA, Hegazy RA, Fawzy MM, Rashed LA, Rasheed H. Interleukin 17, interleukin 22 and FoxP3 expression in tissue and serum of non-segmental vitiligo: A case- controlled study on eighty-four patients. *Eur J Dermatol* 2013;23:350-5.

- [25] Hegazy RA, Fawzy MM, Gawdat HI, Samir N, Rashed LA. T helper 17 and Tregs: a novel proposed mechanism for NB-UVB in vitiligo. *Exp Dermatol* 2014;23:283-6.
- [26] Moftah NH, El-Barbary RA, Ismail MA, Ali NA. Effect of narrow band-ultraviolet B on CD4⁺ CD25^{high} FoxP3⁺ T-lymphocytes in the peripheral blood of vitiligo patients. *Photodermatol Photoimmunol Photomed* 2013;doi:10.1111/phpp.12104.
- [27] Richetta A, D'Epiro S, Salvi M, Campoli M, Giancristoforo S, Mattozzi C, et al. Serum levels of functional T-reg in vitiligo: our experience and mini-review of the literature. *Eur J Dermatol* 2013;23:154-9.
- [28] Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor FOXP3. *Science* 2003;299:1057-61.
- [29] Yagi H, Nomura T, Nakamura K, Yamazaki S, Kitawaki T, Hori S, et al. Crucial role of FOXP3 in the development and function of human CD25⁺CD4⁺ regulatory T-cells. *Int Immunol* 2004;16:1643-56.
- [30] Bettelli E, Dastrange M, Oukka M. Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. *Proc Natl Acad Sci USA* 2005;102:5138-43.
- [31] Zheng Y, Rudensky AY. FoxP3 in control of the regulatory T cell lineage. *Nat Immunol* 2007;8:457-62.
- [32] Fontenot JD, Gavin MA, Rudensky AY. FoxP3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat Immunol* 2003;4:330-6.

- [33] Kim J, Lahl K, Hori S, Loddenkemper C, Chaudhry A, de Roos P, et al. Cutting edge: depletion of FoxP3⁺ cells leads to induction of autoimmunity by specific ablation of regulatory T cells in genetically targeted mice. *J Immunol* 2009;183:7631-4.
- [34] Bennett C, Christie J, Ramsdell F, Brunkow M, Ferguson P, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001;27:20-1.
- [35] Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet* 2002;39:537-45.
- [36] van der Vliet HJ, Nieuwenhuis EE. IPEX as a result of mutations in FOXP3. *Clin Dev Immunol* 2007;2007:89017.
- [37] Bassuny WM, Ihara K, Sasaki Y, Kuromaru R, Kohno H, Matsuura N, et al. A functional polymorphism in the promoter/enhancer region of the FOXP3/Scurfin gene associated with type 1 diabetes. *Immunogenetics* 2003;55:149-56.
- [38] Inoue N, Watanabe M, Morita M, Tomizawa R, Akamizu T, Tatsumi K, et al. Association of functional polymorphisms related to the transcriptional level of FOXP3 with prognosis of autoimmune thyroid diseases. *Clin Exp Immunol* 2010;162:402-6.
- [39] Song P, Wang XW, Li HX, Li K, Liu L, Wei C et al. Association between FOXP3 polymorphisms and vitiligo in a Han Chinese population. *Br J Dermatol* 2013;169:571-8.
- [40] Jahan P, Cheruvu R, Tippisetty S, Komaravalli PL, Valluri V, Ishaq M. Association of FOXP3 (rs3761548) promoter polymorphism with nondermatomal vitiligo: A study from India. *J Am Acad Dermatol* 2013;69:262-6.

- [41] Birlea SA, Jin Y, Bennett DC, Herbstman DM, Wallace MR, McCormack WT, et al. Comprehensive association analysis of candidate genes for generalized vitiligo supports XBP1, FOXP3, and TSLP. *J Invest Dermatol* 2011;131:371-81.
- [42] Gribben JG, Freeman GJ, Boussiotis VA, Rennert P, Jellis CL, Greenfield E, et al. CTLA4 mediates antigen-specific apoptosis of human T cells. *Proc Natl Acad Sci USA* 1995;92:811-5.
- [43] Romo-Tena J, Gómez-Martín D, Alcocer-Varela J. CTLA-4 and autoimmunity: new insights into the dual regulator of tolerance. *Autoimmun Rev* 2013;12:1171-6.
- [44] Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP et al. Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. *Science* 1995;270:985-8.
- [45] Chen Z, Stockton J, Mathis D, Benoist C. Modeling CTLA4-linked autoimmunity with RNA interference in mice. *Proc Natl Acad Sci USA* 2006;103:16400-5.
- [46] Dwivedi M, Laddha NC, Imran M, Shah BJ, Begum R. Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) in isolated vitiligo: a genotype-phenotype correlation. *Pigment Cell Melanoma Res* 2011;24:737-40.
- [47] Gerold KD, Zheng P, Rainbow DB, Zerneck A, Wicker LS, Kissler S. The soluble CTLA-4 splice variant protects from type 1 diabetes and potentiates regulatory T-cell function. *Diabetes* 2011;60:1955-63.
- [48] Saverino D, Brizzolara R, Simone R, Chiappori A, Milintenda-Floriani F, Pesce G, et al. Soluble CTLA-4 in autoimmune thyroid diseases: Relationship with clinical status and possible role in the immune response dysregulation. *Clin Immunol* 2007;123:190-8.

- [49] Bala KK, Moudgil KD. Induction and maintenance of self tolerance: the role of CD4⁺CD25⁺ regulatory T cells. Arch Immunol Ther Exp (Warsz) 2006;54:307-21.
- [50] Joetham A, Takeda K, Taube C, Miyahara N, Matsubara S, Koya T, et al. Naturally occurring lung CD4(+)CD25(+) T cell regulation of airway allergic responses depends on IL-10 induction of TGF- β . J Immunol 2007;178:1433-42.
- [51] Zhu J, Paul WW. CD4 T cells: fates, functions, and faults. Blood 2008;112:1557-69.
- [52] Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S, Horwitz DA. Generation *ex vivo* of TGF- β -producing regulatory T cells from CD4⁺CD25⁻ precursors. J Immunol 2002;169:4183-9.
- [53] Fu S, Zhang N, Yopp AC, Chen D, Mao M, Chen D, et al. TGF- β induces FoxP3⁺ T-regulatory cells from CD4⁺CD25⁻ precursors. Am J Transplant 2004;4:1614-27.
- [54] Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. Cutting edge: TGF- β induces a regulatory phenotype in CD4⁺CD25⁻ T cells through FoxP3 induction and down-regulation of Smad7. J Immunol 2004;172:5149-53.
- [55] Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4⁺CD25⁺ cells educate CD4⁺CD25⁻ cells to develop suppressive activity: the role of IL-2, TGF- β , and IL-10. J Immunol 2004;172:5213-21.
- [56] Liang S, Alard P, Zhao Y, Parnell S, Clark SL, Kosiewicz MM. Conversion of CD4⁺ CD25⁻ cells into CD4⁺CD25⁺ regulatory T cells *in vivo* requires B7 costimulation, but not the thymus. J Exp Med 2005;201:127-37.
- [57] Chen W, Konkel JE. TGF- β and 'adaptive' FoxP3⁺ regulatory T cells. J Mol Cell Biol 2010;2:30-6.

- [58] Basak PY, Adiloglu AK, Ceyhan AM, Tas T, Akkaya VB. The role of helper and regulatory T cells in the pathogenesis of vitiligo. *J Am Acad Dermatol* 2009;60:256-60.
- [59] Khan R, Gupta S, Sharma A. Circulatory levels of T-cell cytokines interleukin (IL)-2, IL-4, IL-17, and transforming growth factor- β in patients with vitiligo. *J Am Acad Dermatol* 2012;66:510-1.
- [60] Moretti S, Spallanzani A, Amato L, Hautmann G, Gallerani I, Fabiani M, et al. New insights into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. *Pigment Cell Res* 2002;15:87-92.
- [61] Tembhre MK, Sharma VK, Sharma A, Chattopadhyay P, Gupta S. T helper and regulatory T cell cytokine profile in active, stable and narrow band ultraviolet B treated generalized vitiligo. *Clin Chim Acta* 2013;424:27-32.
- [62] Tu CX, Jin WW, Lin M, Wang ZH, Man MQ. Levels of TGF- β (1) in serum and culture supernatants of CD4(+)CD25(+) T cells from patients with non-segmental vitiligo. *Arch Dermatol Res* 2011;303:685-9.
- [63] Yun JY, Uhm YK, Kim HJ, Lim SH, Chung JH, Shin MK et al. Transforming growth factor beta receptor II (TGFB2) polymorphisms and the association with nonsegmental vitiligo in the Korean population. *Int J Immunogenet* 2010;37:289-91.
- [64] O'Garra A, Vieira PL, Vieira P, Goldfeld AE. IL-10-producing and naturally occurring CD4⁺ Tregs: limiting collateral damage. *J Clin Invest* 2004;114:1372-8.
- [65] DeJaco C, Duftner C, Grubeck-Loebenstien B, Schermer M. Imbalance of regulatory T cells in human autoimmune diseases. *Immunol* 2006;117:289-300.

- [66] Taher ZA, Lauzon G, Maguiness S, Dytoc MT. Analysis of interleukin-10 levels in lesions of vitiligo following treatment with topical tacrolimus. *Br J Dermatol* 2009;161:654-9.
- [67] Abanmi A, Al Harthi F, Zouman A, Kudwah A, Jamal MA, Arfin M, et al. Association of Interleukin-10 gene promoter polymorphisms in Saudi patients with vitiligo. *Dis Markers* 2008;24:51-7.
- [68] Shi F, Erf GF. IFN- γ , IL-21, and IL-10 co-expression in evolving autoimmune vitiligo lesions of Smyth line chickens. *J Invest Dermatol* 2012;132:642-9.
- [69] Pommier A, Audemard A, Durand A, Lengagne R, Delpoux A, Martin B, et al. Inflammatory monocytes are potent antitumor effectors controlled by regulatory CD4⁺ T cells. *Proc Natl Acad Sci USA* 2013;110:13085-90.
- [70] Chen LC, Delgado JC, Jensen PE, Chen X. Direct expansion of human allospecific FoxP3⁺CD4⁺ regulatory T cells with allogeneic B cells for therapeutic application. *J Immunol* 2009;183:4094-102.
- [71] Brusko TM, Koya RC, Zhu S, Lee MR, Putnam AL, McClymont SA, et al. Human antigen-specific regulatory T cells generated by T cell receptor gene transfer. *PLoS One* 2010;5:e11726.
- [72] Lan Q, Fan H, Quesniaux V, Ryffel B, Liu Z, Zheng SG. Induced FoxP3(+) regulatory T cells: a potential new weapon to treat autoimmune and inflammatory diseases? *J Mol Cell Biol* 2012;4:22-8.
- [73] Zheng SG, Wang JH, Koss MN, Quismorio F Jr, Gray JD, Horwitz DA. CD4⁺ and CD8⁺ regulatory T cells generated ex vivo with IL-2 and TGF-beta suppress a stimulatory graft-versus-host disease with a lupus-like syndrome. *J Immunol* 2004;172:1531-9.

- [74] Selvaraj RK, Geiger TL. Mitigation of experimental allergic encephalomyelitis by TGF-beta induced FoxP3⁺ regulatory T lymphocytes through the induction of anergy and infectious tolerance. *J Immunol* 2008;180:2830-8.
- [75] Nguyen TL, Sullivan NL, Ebel M, Teague RM, DiPaolo RJ. Antigen-specific TGF-beta-induced regulatory T cells secrete chemokines, regulate T cell trafficking, and suppress ongoing autoimmunity. *J Immunol* 2011;187:1745-53.
- [76] DiPaolo RJ, Brinster C, Davidson TS, Andersson J, Glass D, Shevach EM. Autoantigen-specific TGFbeta-induced FoxP3⁺ regulatory T cells prevent autoimmunity by inhibiting dendritic cells from activating autoreactive T cells. *J Immunol* 2007;179: 4685-93.
- [77] Weber SE, Harbertson J, Godebu E, Mros GA, Padrick RC, Carson BD, et al. Adaptive islet-specific regulatory CD4 T cells control autoimmune diabetes and mediate the disappearance of pathogenic Th1 cells in vivo. *J Immunol* 2006;176:4730-9.
- [78] Fantini MC, Becker C, Tubbe I, Nikolaev A, Lehr HA, Galle P, et al. Transforming growth factor beta induced FoxP3⁺ regulatory T cells suppress Th1-mediated experimental colitis. *Gut* 2006;55:671-80.
- [79] Huter EN, Stummvoll GH, DiPaolo RJ, Glass DD, Shevach EM. Cutting edge: antigen specific TGF beta-induced regulatory T cells suppress Th17-mediated autoimmune disease. *J Immunol* 2008;181:8209-13.
- [80] Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. Conversion of peripheral CD4⁺CD25⁻ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF-beta induction of transcription factor FoxP3. *J Exp Med* 2003;198:1875-86.

- [81] Chatterjee S, Eby JM, Al-Khami AA, Soloshchenko M, Kang HK, Kaur N, et al. A quantitative increase in regulatory T cells controls development of vitiligo. *J Invest Dermatol* 2014;134:1285-94.
- [82] Mosenson JA, Eby JM, Hernandez C, Le Poole IC. A central role for inducible heat-shock protein 70 in autoimmune vitiligo. *Exp Dermatol* 2013;22:566-9.
- [83] Mosenson JA, Zloza A, Nieland JD, Garrett-Mayer E, Eby JM, Huelsmann EJ, et al. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. *Sci Transl Med* 2013;5:174ra28.
- [84] Van Herwijnen MJ, Van Der Zee R, Van Eden W, Broere F. Heat shock proteins can be targets of regulatory T cells for therapeutic intervention in rheumatoid arthritis. *Int J Hyperthermia* 2013;29:448-54.
- [85] Bluestone JA, Tang Q. Therapeutic vaccination using CD4⁺CD25⁺ antigen-specific regulatory T cells. *Proc Natl Acad Sci USA* 2004;101:14622-6.
- [86] Wright GP, Notley CA, Xue SA, Bendle GM, Holler A, Schumacher TN, et al. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. *Proc Natl Acad Sci USA* 2009;106:19078-83.
- [87] Konieczna P, Akdis CA, Quigley EM, Shanahan F, O'Mahony L. Portrait of an immunoregulatory *Bifidobacterium*. *Gut Microbes* 2012;3:261-6.
- [88] Fuller R. Probiotics in man and animals. *J Appl Bacteriol* 1989;66:365-78.
- [89] Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, Shanahan F, et al. *Bifidobacterium infantis* 35,624 administration induces FoxP3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut* 2012;61:354-66.

- [90] O'Mahony C, Scully P, O'Mahony D, Murphy S, O'Brien F, Lyons A, et al. Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NFkappaB activation. *PLoS Pathog* 2008;4:e1000112.
- [91] O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005;128:541-51.
- [92] Whorwell PJ, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, et al. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35,624 in women with irritable bowel syndrome. *Am J Gastroenterol* 2006;101:1581-90.
- [93] Feleszko W, Jaworska J, Rha RD, Steinhausen S, Avagyan A, Jaudszus A, et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy* 2007;37:498-505.
- [94] Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, et al. Generation of regulatory dendritic cells and CD4⁺FoxP3⁺ T cells by probiotics administration suppresses immune disorders. *Proc Natl Acad Sci USA* 2010;107:2159-64.
- [95] Kim HJ, Kim YJ, Lee SH, Yu J, Jeong SK, Hong SJ. Effects of Lactobacillus rhamnosus on allergic march model by suppressing Th2, Th17, and TSLP responses via CD4(+)CD25(+)FoxP3(+) Tregs. *Clin Immunol* 2014;153:178-86.
- [96] Lavasani S, Dzhabazov B, Nouri M, Fåk F, Buske S, Molin G, et al. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. *PLoS One* 2010;5:e9009.

- [97] Hwang I, Ahn G, Park E, Ha D, Song JY, Jee Y. An acidic polysaccharide of Panax ginseng ameliorates experimental autoimmune encephalomyelitis and induces regulatory T cells. *Immunol Lett* 2011;138:169-78.
- [98] Ahmad SF, Zoheir KM, Bakheet SA, Ashour AE, Attia SM. Poly(ADP-ribose) polymerase-1 inhibitor modulates T regulatory and IL-17 cells in the prevention of adjuvant induced arthritis in mice model. *Cytokine* 2014;68:76-85.
- [99] Tran GT, Hodgkinson SJ, Carter NM, Verma ND, Plain KM, Boyd R, et al. IL-5 promotes induction of antigen-specific CD4⁺CD25⁺ T regulatory cells that suppress autoimmunity. *Blood* 2012;119:4441-50.
- [100] Rouse M, Nagarkatti M, Nagarkatti PS. The role of IL-2 in the activation and expansion of regulatory T-cells and the development of experimental autoimmune encephalomyelitis. *Immunobiology* 2013;218:674-82.
- [101] Long SA, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, Zhang S, et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)/CD25(+) regulatory T-cells of type 1 diabetic subjects. *Diabetes* 2010;59:407-15.
- [102] Aoki CA, Roifman CM, Lian ZX, Bowlus CL, Norman GL, Shoenfeld Y, et al. IL-2 receptor alpha deficiency and features of primary biliary cirrhosis. *J Autoimmun* 2006;27:50-3.
- [103] Singh RP, Hahn BH, La Cava A. Tuning immune suppression in systemic autoimmunity with self-derived peptides. *Inflamm Allergy Drug Targets* 2008;7:253-9.
- [104] Pozo D, Anderson P, Gonzalez-Rey E. Induction of alloantigen-specific human T regulatory cells by vasoactive intestinal peptide. *J Immunol* 2009;183:4346-59.

- [105] Bisikirska B, Colgan J, Luban J, Bluestone JA, Herold KC. TCR stimulation with modified anti-CD3 mAb expands CD8⁺ T cell population and induces CD8⁺CD25⁺ Tregs. *J Clin Invest* 2005;115(:2904-13.
- [106] Sheng JR, Li LC, Ganesh BB, Prabhakar BS, Meriggioli MN. Regulatory T cells induced by GM-CSF suppress ongoing experimental myasthenia gravis. *Clin Immunol* 2008;128:172-80.
- [107] Walters SN, Webster KE, Daley S, Grey ST. A role for intrathymic B cells in the generation of natural regulatory T cells. *J Immunol* 2014;193:170-6.
- [108] Ju JH, Heo YJ, ChoML, Jhun JY, Park JS, Lee SY, et al. Modulation of STAT-3 in rheumatoid synovial T cells suppresses Th17 differentiation and increases the proportion of Treg cells. *Arthritis Rheum* 2012;64:3543–52.
- [109] Noack M, Miosse P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmunity Rev* 2014;13:668-677.
- [110] Askenasy N. Enhanced killing activity of regulatory T cells ameliorates inflammation and autoimmunity. *Autoimmunity Rev* 2013;12:972-975.

Table 1.

Quantitative and functional status of Treg cells in vitiligo patients.

Treg cell numbers	Treg cell function	Reference
Decreased in blood and increased in skin	Lower suppressive capacity of Tregs toward CD8 ⁺ T cells and increased IFN- γ and tumour necrosis factor- α levels	[10]
Decreased in blood	Decreased FoxP3, CTLA-4 and IL-10 levels	[11]
Unaltered in skin and decreased in blood of active cases only	Lower suppressive capacity of peripheral Tregs toward CD8 ⁺ T cells and decreased FoxP3 expression in skin	[13]
Decreased in skin	Not analysed	[18]
Decreased in skin	Not analysed	[19]
Unaltered in skin	Not analysed	[20]
Unaltered in blood	Not analysed	[21]
Increased in blood	Not analysed	[22]
Decreased in skin and unaltered in blood	Normal suppressive capacity of peripheral Tregs	[23]
Not analysed	Decreased FoxP3 expression in blood and skin	[24]
Not analysed	Decreased FoxP3 expression in skin	[25]
Increased in blood	Reduction in peripheral Tregs after NB-UVB phototherapy	[26]
Decreased in blood	Not analysed	[27]

Table 2.Genetic polymorphisms of *FOXP3* studied in vitiligo.

Polymorphism	Association	Population	Reference
rs2232365 (A/G promoter)	$P = 0.004$	Han Chinese	[39]
rs3761548 (A/C promoter)	$P = 0.033$	Han Chinese	[39]
rs3761547 (A/G promoter)	$P = 0.002$	Indian	[40]
rs11798415 (A/T promoter)	$P = 1.8 \times 10^{-3}$	Non-Hispanic white	[41]
rs11798415 (A/T promoter)	$P = 5.8 \times 10^{-4}$	Non-Hispanic white	[41]

Figure legend

Fig. 1. Proposed role of regulatory T cells in melanocyte destruction in vitiligo. The schematic representation indicates the potential Treg cell defects which may lead to melanocyte loss in vitiligo. In particular, the decreased number of Tregs, which may result from defects in positive or negative selection in the thymus leading to a peripheral imbalance of Tregs or genetic susceptibility leading to abnormal Treg cell development and function, fails to suppress the increased number of autoreactive CD8⁺ and CD4⁺ T cells. The increase in CD8⁺ T cells may then destroy melanocytes through the granzyme B or perforin pathway or through cognate help via cytokines such as IL-6 and IL-13 resulting in melanocyte loss through apoptosis. Tregs not capable of suppressing autoreactive CD8⁺ T cells could also lead to melanocyte loss: the suppressive capacity of Tregs can be diminished by decreased expression of Treg cell-related proteins such as FoxP3, IL-10, TGF- β and CTLA-4. Genetic defects in these genes may result in altered expression of these proteins. Moreover, the perturbed homeostasis of calcium in vitiligo signifies the alteration of calmodulin and calcineurin activation which may result in altered NFAT expression and activation leading to a reduction in the suppressive capacity of Tregs. Moreover, deficient IL-2 production by T effector cells leads to decreased Treg cell numbers or function as low surface CD25 expression delivers deficient IL-2 signals resulting in impaired survival and function of resident Tregs. Apart from CD8⁺ T cells, increased CD4⁺ T cells can also lead to activation of CD8⁺ T cells which will not be suppressed by defective Tregs, thus leading to a hyper-autoreactive T cells response towards melanocytes. The unknown triggering factors such as altered melanocyte antigens, neo-antigens and defective apoptosis may serve as the activators of autoreactive B cells and T cells resulting into melanocyte loss. However, if the

ongoing immune response towards melanocytes is not checked by Tregs, then the potential loss of melanocytes can result in vitiligo.

Figure 1

