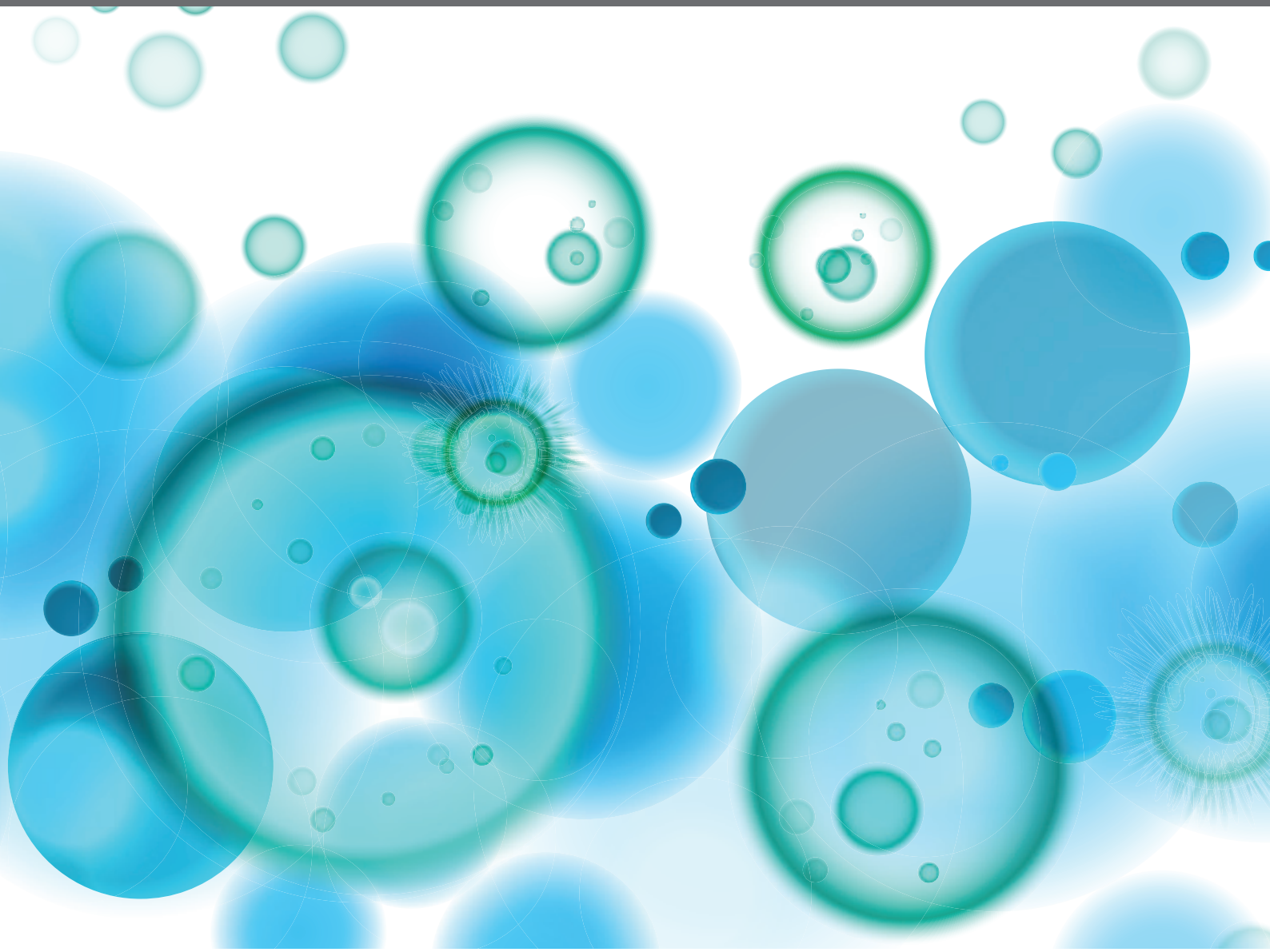
IMMUNOLOGY OF VITILIGO

EDITED BY: Katia Boniface, Isabelle Caroline Le Poole, John E. Harris, Julien Seneschal, Thierry Passeron and Reinhart Speeckaert

PUBLISHED IN: Frontiers in Immunology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-230-4
DOI 10.3389/978-2-88971-230-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

IMMUNOLOGY OF VITILIGO

Topic Editors:
Katia Boniface, Université de Bordeaux, France
Isabelle Caroline Le Poole, Northwestern University, United States
John E. Harris, University of Massachusetts Medical School, United States
Julien Seneschal, Université de Bordeaux, France
Thierry Passeron, Université Côte d'Azur, France
Reinhart Speeckaert, Ghent University Hospital, Belgium

Topic Editor Prof. John E. Harris is Scientific Founder of Villaris Therapeutics, Inc. Topic Editor Prof. Julien Seneschal received financial support from Sanofi Genzyme and Calypso Biotech. Topic Editor Prof. Caroline Le Poole is the CSO for Temprian Therapeutics, a company hoping to bring an HSP70iQ435A-based treatment to clinical trials for vitiligo. The approach for this treatment is different and separate from the proposed Research Topic. Please note that studies investigating HSP70iQ435A-based treatment thus fall out of the remit of this project. All other Topic Editors declare no competing interests with regards to the Research Topic subject.

Citation: Boniface, K., Le Poole, I. C., Harris, J. E., Seneschal, J., Passeron, T., Speeckaert, R., eds. (2021). Immunology of Vitiligo. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-230-4

Table of Contents

frontiers in Immunology

EDITORIAL
published: 24 June 2021
doi: 10.3389/fimmu.2021.711080

Editorial: Immunology of Vitiligo

Julien Seneschal[1,2], *John E. Harris*[3], *I. Caroline Le Poole*[4], *Thierry Passeron*[5,6], *Reinhart Speeckaert*[7] *and Katia Boniface*[1]*

[1] Univ. Bordeaux, INSERM, BMGIC, U1035, Bordeaux, France, [2] Department of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Disorders, Hôpital Saint-André, Bordeaux, France, [3] Department of Dermatology, University of Massachusetts Medical School, Worcester, MA, United States, [4] Departments of Dermatology, Microbiology and Immunology/Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, United States, [5] INSERM U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Côte d'Azur University, Nice, France, [6] Department of Dermatology, CHU Nice, Côte d'Azur University, Nice, France, [7] Department of Dermatology, Ghent University Hospital, Ghent, Belgium

Keywords: vitiligo, melanocytes, innate immunity, adaptive immunity, oxidative stress, translational research, tolerance

Editorial on the Research Topic

Immunology of Vitiligo

Disappearance of melanocytes is the pathogenic hallmark of vitiligo. Progressive depigmentation of the skin has a high negative impact on patients' quality of life. To date, vitiligo remains a therapeutic challenge (1).

Several theories have been proposed to explain disease pathogenesis, considering the roles of increased inflammatory and cytotoxic immune responses, neuropeptides, microvascular anomalies, intrinsic abnormalities in melanocyte and keratinocyte adhesion, as well as oxidative stress (2). Over the past decades, clinical, basic, and translational research on patient samples as well as *in vitro* and *in vivo* models have tremendously improved our understanding of the pathophysiology of the disease and highlighted its complexity. Such progress is of utmost importance to identify appropriate therapeutic targets and treatments to halt progression of the disease and to induce repigmentation. "Immunology of vitiligo" is a collection of six review articles and four original articles focusing on complementary aspects of the immune pathways involved in vitiligo, from a pathophysiologic to a therapeutic perspective.

Mechanisms leading to the loss of melanocytes include genetic predispositions and environmental triggers, as well as metabolic and immune alterations (3, 4). Epigenetic modifications may also be involved in vitiligo pathogenesis, as suggested in the Pu et al. study that identifies altered methylation levels of key genes involved in oxidation-reduction, inflammatory, or pigmentation processes in vitiligo melanocyte cell lines. Most of the published studies focused on the role of the immune response in non-segmental vitiligo (or vitiligo), which accounts for approximately 90% of clinical forms; segmental vitiligo being less well-studied. In their review, Speeckaert et al. emphasize the role of autoimmunity in segmental vitiligo, likely involving a targeted immune response against melanocytes carrying a somatic mutation (5). Indeed, as shown by Yang et al., transcriptomic analysis of lesional skin of segmental vitiligo versus non-segmental vitiligo showed similar changes in dysregulated immune pathways.

The innate immune response is important for the initiation of the disease. Jadeja et al. discuss the role of the endoplasmic reticulum stress-induced unfolded protein response as a bridge between oxidative stress and the development of autoimmunity in vitiligo. Danger signals (DAMPs and PAMPs) together with the role of innate immune cells in vitiligo pathogenesis is further reviewed by

OPEN ACCESS

Edited and reviewed by:
Betty Diamond, Feinstein Institute for Medical Research, United States

***Correspondence:**
Katia Boniface
katia.boniface@u-bordeaux.fr

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *17 May 2021*
Accepted: *09 June 2021*
Published: *24 June 2021*

Citation:
Seneschal J, Harris JE, Le Poole IC, Passeron T, Speeckaert R and Boniface K (2021) Editorial: Immunology of Vitiligo. Front. Immunol. 12:711080. doi: 10.3389/fimmu.2021.711080

Boniface et al., important for subsequent activation of adaptive T cell immune responses, leading to the loss of melanocytes. Indeed, CD8+ T cells infiltrating the skin of vitiligo patients play a direct role in melanocyte disappearance, through their cytotoxic activity and the release of type 1 cytokines, in particular IFNγ and TNFα (6, 7). More recent studies also described the role of resident memory T cells expressing the IL-15 receptor, CXCR3, and NKG2D, and targeting this subset appears to prevent disease progression, flares, and to maintain repigmentation after treatment (2, 8). In this context, Plaza-Rojas and Guevara-Patiño discuss the role of the NKG2D/NKG2D ligand axis, and Willemsen et al. the relevance of targeting the PD-1/PD-L1 axis in vitiligo. Disruption of tolerance is a hallmark of autoimmunity and previous studies describe dysregulation of regulatory T cell function and/or number in patients (9, 10). In their original article, Mukhatayev et al. elegantly investigated the potential of using CAR Tregs as a therapeutic strategy in a pre-clinical humanized mouse model prone to develop vitiligo. The importance of using appropriate *in vitro* and *in vivo* pre-clinical models to perform mechanistic and translational studies is outlined in the Katz and Harris review. Animal models with spontaneous development of vitiligo are not optimal to fully understand the complexity of the human disease. In their case series, Egbeto et al. described a similar immune profile between canine and human autoimmune pigmentary disorders, suggesting that findings in one model could be relevant to the other.

In conclusion, the prominent role of autoimmunity in vitiligo pathogenesis is now evident, as exemplified with recent clinical studies evaluating the efficacy of drugs targeting the immune response, such as JAK inhibitors (11, 12). Yet prediction of drug efficacy is important for personalized therapeutic management of patients. Yang et al. identified biomarkers of innate and adaptive immune responses associated with favorable response to therapy. It will be also important to assess whether new therapies induce durable repigmentation of vitiligo, or whether maintenance therapies will be required to prevent disease relapse.

AUTHOR CONTRIBUTIONS

JS, JH, IP, TP, RS, and KB wrote the editorial and invited authors to participate in the collection. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. *Lancet* (2015) 386:74–84. doi: 10.1016/S0140-6736(14)60763-7
2. Frisoli ML, Essien K, Harris JE. Vitiligo: Mechanisms of Pathogenesis and Treatment. *Annu Rev Immunol* (2020) 38:621–48. doi: 10.1146/annurev-immunol-100919-023531
3. Picardo M, Dell'Anna ML, Ezzedine K, Hamzavi I, Harris JE, Parsad D, et al. Vitiligo. *Nat Rev Dis Primers* (2015) 1:15011. doi: 10.1038/nrdp.2015.11
4. Boniface K, Seneschal J, Picardo M, Taïeb A. Vitiligo: Focus on Clinical Aspects, Immunopathogenesis, and Therapy. *Clin Rev Allergy Immunol* (2018) 54:52–67. doi: 10.1007/s12016-017-8622-7
5. van Geel N, Mollet I, Brochez L, Dutré M, De Schepper S, Verhaeghe E, et al. New Insights in Segmental Vitiligo: Case Report and Review of Theories. *Br J Dermatol* (2012) 166:240–6. doi: 10.1111/j.1365-2133.2011.10650.x
6. Boniface K, Seneschal J. Vitiligo as a Skin Memory Disease: The Need for Early Intervention With Immunomodulating Agents and a Maintenance Therapy to Target Resident Memory T Cells. *Exp Dermatol* (2019) 28:656–61. doi: 10.1111/exd.13879
7. Riding RL, Harris JE. The Role of Memory Cd8+ T Cells in Vitiligo. *J Immunol* (2019) 203:11–9. doi: 10.4049/jimmunol.1900027
8. Seneschal J, Boniface K, D'Arino A, Picardo M. An Update on Vitiligo Pathogenesis. *Pigment Cell Melanoma Res* (2021) 34:236–43. doi: 10.1111/pcmr.12949
9. Dwivedi M, Kemp EH, Laddha NC, Mansuri MS, Weetman AP, Begum R. Regulatory T Cells in Vitiligo: Implications for Pathogenesis and Therapeutics. *Autoimmun Rev* (2015) 14:49–56. doi: 10.1016/j.autrev.2014.10.002
10. Mukhatayev Z, Ostapchuk YO, Fang D, Le Poole IC. Engineered Antigen-Specific Regulatory T Cells for Autoimmune Skin Conditions. *Autoimmun Rev* (2021) 20:102761. doi: 10.1016/j.autrev.2021.102761
11. Rosmarin D, Pandya AG, Lebwohl M, Grimes P, Hamzavi I, Gottlieb AB, et al. Ruxolitinib Cream for Treatment of Vitiligo: A Randomised, Controlled, Phase 2 Trial. *Lancet* (2020) 396:110–20. doi: 10.1016/S0140-6736(20)30609-7
12. Passeron T. First Step in a New Era for Treatment of Patients With Vitiligo. *Lancet* (2020) 396:74–5. doi: 10.1016/S0140-6736(20)30747-9

Conflict of Interest: JH is a Scientific Founder of Villaris Therapeutics, Inc. JS and KB received financial support from Sanofi Genzyme and Calypso Biotech that is separate from this proposed editorial. IP is the CSO for Temprian Therapeutics, a company hoping to bring an HSP70iQ435A-based treatment to clinical trials for vitiligo. The approach for this treatment is different and separate from the proposed Research Topic.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 27 October 2020
doi: 10.3389/fimmu.2020.568447

Autoimmunity in Segmental Vitiligo

Reinhart Speeckaert[1], Jo Lambert[1], Vedrana Bulat[2], Arno Belpaire[1], Marijn Speeckaert[3] and Nanja van Geel[1]*

[1] Department of Dermatology, Gent University Hospital, Gent, Belgium, [2] Department of Dermatology, University Hospital Centre Zagreb, Zagreb, Croatia, [3] Department of Nephrology, Gent University Hospital, Gent, Belgium

The autoimmune basis of segmental vitiligo (SV) has only recently been recognized. Systemic autoimmune diseases are less frequently associated compared to non-segmental vitiligo (NSV), but localized skin disorders – in particular linear morphea – have been repeatedly observed in patients with SV. The inflammatory response is documented on a clinical level with cases displaying erythematous borders or a hypochromic stage, on a histopathological level with predominantly CD8 lymphocytes migrating toward the basal layer and by flow cytometry demonstrating the antimelanocyte specificity of these cytotoxic T cells. The increased risk for halo naevi and NSV in these patients further underline the immune-mediated mechanisms of SV. Nonetheless, the localized and unique distribution pattern points to somatic mosaicism. This places SV in a category of similar diseases such as lichen striatus, blaschkitis, linear lupus erythematosus, and linear scleroderma where an immune reaction against genetically mutated skin cells is believed to be the underlying cause. All these disorders are characterized by a young age of onset, a temporary disease activity with spontaneous resolution, limited response to treatment, and often long-term sequelae. Although challenging, genetic research proving this genetic mosaicism could offer crucial insights into the pathogenesis of both segmental and non-segmental vitiligo.

Keywords: segmental vitiligo, immunology, mosaicism, linear morphea, lichen striatus

OPEN ACCESS

Edited by:
Kutty Selva Nandakumar, Southern Medical University, China

Reviewed by:
Mehdi Rashighi, University of Massachusetts Medical School, United States
Rasheedunnisa Begum, Maharaja Sayajirao University of Baroda, India

****Correspondence:***
Reinhart Speeckaert
Reinhart.Speeckaert@UGent.be

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *01 June 2020*
Accepted: *08 October 2020*
Published: *27 October 2020*

Citation:
Speeckaert R, Lambert J, Bulat V, Belpaire A, Speeckaert M and van Geel N (2020) Autoimmunity in Segmental Vitiligo. Front. Immunol. 11:568447. doi: 10.3389/fimmu.2020.568447

INTRODUCTION

The prevalence of vitiligo is reported to be around 0.5-1% of the population, although remarkable regional differences exist (1). Segmental vitiligo (SV) accounts for 5-16% of all vitiligo cases and has a relatively equal gender distribution. However, some studies report a slight predominance of females (2, 3). SV has several unique characteristics compared to non-segmental vitiligo (NSV). SV usually involves only one body area and displays a sharp demarcation around the midline of the body. It can develop at all ages although a predominance around 4-10 years exists (4). Progression mostly occurs in the first year with a spontaneous stop of new depigmentations after 1.5-2 years in the majority of patients. In some patients, SV stabilizes even after a few days (8/141; 16.7%) (5). However, this is not always the case as in one study, 36.8% of patients still reported progression after 2 years (6). The development of SV areas on opposite body sites has been termed bilateral SV. Mixed vitiligo is the concomitant presence of both SV and NSV in the same patient (7). In contrast to NSV, poliosis is often visible at an early stage and far more frequent compared to NSV. When examined in detail, almost all SV patients exhibit leukotrichia (8). Itch might be an underrecognized symptom being present in 20% of SV patients (9).

Melanocytes carrying a mosaic mutation which elicits a targeted immune response are suspected as the underlying cause of SV (10). Additionally, several other hypotheses have been put forward in an

attempt to explain SV including neurogenic mechanisms, genetic mosaicism, and oxidative stress. In the last years, research suggests that different theories likely play a combined role.

During the progressive phase, topical anti-inflammatory treatments including corticoids or immunomodulators are advised (11). UVB or excimer treatment can be initiated but the repigmentation rates are lower compared to NSV (12). Nonetheless, UVB/excimer therapy can be considered in patients with recent disease onset or combined with pigment cell transplantation. Shah et al. reported a 75% repigmentation in 78% of patients with 35.6% showing complete repigmentation after excimer light combined with topical tacrolimus (13). Both marginal and perifollicular repigmentation patterns can be observed (14).

AUTO-IMMUNITY IN SEGMENTAL VITILIGO

NSV is characterized by multiple auto-immune disorders with thyroid disease and alopecia areata being the most prevalent. The rates of auto-immune disorders are higher in NSV compared to SV, especially concerning thyroid disease (15). Lim et al. found positive thyroid peroxidase antibodies in 24.7% of NSV patients while this was only 15.1% in SV (16). A systematic review documented a prevalence of thyroid disorders of 3/69 (4.3%) in SV which was not increased compared to healthy controls (17). This underlines the difference in pathogenesis between both disorders. While in NSV, a genetic background of increased risk to autoimmunity has been uncovered by genome-wide analyses [e.g. a major histocompatibility complex (MHC) enhancer polymorphism], this is less expected in its segmental counterpart (18). A relatively small whole-genome expression study (20 SV; 20 NSV; 20 healthy controls) found more differentially expressed genes involved in the adaptive immune response in SV while in NSV regulation of the innate immune response, B cell differentiation and activation was more prominent (19). This suggests that the melanocyte immune response in SV is more targeted and involves a narrower inflammatory activation profile (e.g. less evidence for autophagy) compared to NSV. The inflammatory response in SV is short-lived and localized, but evidence for an immune-based cytotoxic destruction of SV melanocytes is increasing. From a clinical perspective, 2 cases of inflammatory SV have been reported (20–22). In a relatively small study, immunohistochemistry revealed a lesional lymphocytic infiltrate in 8/12 of progressing SV patients comparable to NSV with a predominance of $CD8^+$ T cells migrating toward the basal layer. A non-significant increase in IFN-γ was observed (23). In a larger study (SV n = 50), histopathological inflammatory features were noticed in 78% (38/50) of patients. In 42% (21/50), small clusters of lymphocytes were found around melanocytes. In 36% (18/50) of SV patients, a focal lichenoid inflammation was present and in 22% (11/50) post-inflammatory changes were observed (24). Our group analyzed the lymphocytic infiltrate in a progressive case of SV more in-depth using HLA-peptide tetramers (MART-1, tyrosinase, gp100). We discovered both in lesional and non-lesional skin CD8 lymphocytes with a high production of IFN-γ and TNF-α. An increased percentage of CD8 lymphocytes targeting the melanocyte differentiation peptide gp100 was observed both in lesional and non-lesional skin (25).

Nonetheless, this antimelanocyte-specific response can explain why halo naevi can develop and why progression to NSV can occur. Deregulation of chemotactic signals of CCL11, CCL17, CCL22, CCL24, CCL27, CXCL10, and CXCL12 was found in SV which was associated with a prominent inflammatory infiltrate. CD11c+ dendritic cells were abundantly present together with CD8 T cells (26). In another study, no difference in lesional versus non-lesional SV levels of sICAM-1 and GM-CSF was detected (n=16) (27). However, this study did not specify whether it concerned stable or progressive SV vitiligo lesions which might explain the lack of difference in inflammatory markers. SV skin contains increased epidermal oxidative stress (H_2O_2/ONOO(-)) (28). Similar to NSV, increased levels of superoxide dismutase (SOD), decreased antioxidative enzymes such as catalase, and increased lipid peroxidation levels were found (29). Whether this concerns a primary cause or a secondary phenomenon due to inflammation, remains a topic of debate. Up till now, reproducible results of effective SV treatment with antioxidants are lacking.

Poliosis is an important and early sign of SV. This indicates that the immune-privileged environment around the hairs is less protective in SV compared to NSV. As the reservoir of melanocyte precursors around the hair bulb is often depleted in SV, repigmentation is less impressive when phototherapy is initiated (12). Several immune checkpoints [e.g. programmed death ligand-1 (PD-L1), indoleamine 2,3-dioxygenase (IDO)] have been found to regulate the immune privilege of the hair follicle. Inhibition of these factors by immunotherapy for melanoma results often in the development of vitiligo-like lesions very similar to NSV (30, 31). It is therefore plausible to speculate that SV is less dependent on changes in factors regulating immune privilege compared to NSV (32).

Mixed vitiligo has been reported in a subset of SV patients (7). In our experience, depigmented areas are in the majority of patients limited, but progression to extensive vitiligo vulgaris is possible. Usually, SV precedes the development of NSV (7, 33). Halo naevi are relatively frequent in SV although variable percentages have been reported in the literature (5.3%-24.2%) (34). The frequency strongly depends on the area of SV involvement (trunk > face) (5). Halo naevi and leukotrichia are strong predictors of progression to mixed vitiligo (OR= 24.8 and 25.7, respectively) (33). The presence of halo naevi in SV is associated with a positive family history of vitiligo and thus linked to the genetic background (33). Several theories for the subsequent development of NSV can be proposed. Healthy individuals carry circulating melanocyte-specific T cells with an anergic phenotype (35). This cell population is believed to be a protective mechanism against the development of melanoma when the balance is shifted from anergic to active. Regulatory T cells (Tregs) are implied in the maintenance of this anergic state. During the pathogenesis of SV, these cells may become activated by losing coinhibitory molecules [e.g. cytotoxic T lymphocyte antigen-4 (CTLA-4)] and gaining a higher T cell receptor affinity. This can lead to the active destruction of naevoid and epidermal

melanocytes illustrated by the development of halo naevi and NSV in genetically predisposed patients. Another mechanism can be epitope spreading where immune cells involved in targeting SV melanocytes acquire specificity for naevoid or epidermal melanocytes.

CASES OF SEGMENTAL VITILIGO DISPLAYING ADDITIONAL SKIN DISORDERS

Although the rate of autoimmune disorders is not markedly increased in SV, some interesting cases have been described with other inflammatory and non-inflammatory disorders co-occurring at the same site (**Table 1**). A literature search was performed using Embase and Pubmed databases from conception up to May 10, 2020. All original articles, reviews, and different types of articles including letters to the editors, conference papers, and posters were included. Embase was searched using the terms "segmental vitiligo" OR "segmental vitiligo"/exp. Pubmed was searched using "segmental vitiligo" [All fields] OR "segmental vitiligo" [Mesh]. All types of articles, in any language, were included. Articles without full-text availability were excluded. Articles with both SV and NSV were only included in case results on SV were described in detail. The goal was to include all case reports mentioning comorbid disorders in patients with SV or drug-induced SV. The literature search was done by 2 independent reviewers (RS and MS) and was uploaded with Zotero software. The data were extracted by RS. Overlapping studies were excluded based on the author list and content.

In total, 564 and 449 results were found for Embase and Pubmed, respectively. For the Embase search, 3 duplicates were removed based on the same content. Titles and abstracts of all articles were screened. One case was excluded due to doubt about the clinical diagnosis of segmental vitiligo based on the clinical picture. Combining both searches, 38 reports could be included describing patients with SV and a comorbid disorder comprising 21 patients with auto-immune disorders, 5 cases of drug-induced vitiligo, 5 cases with benign or malignant pigment disorders, 3 cases with neurological disorders, and 6 other cases

A remarkable number of case-reports with morphea and lichen sclerosus have been published (39, 40, 44, 70). 11 different reports were found which makes it by far the most frequently observed comorbidity [57.1% (12/21 patients) of reported patients with autoimmune comorbidities]. In most cases, the morphea lesions affected the same body area as the SV, although multifocal morphea was also documented (36–46). Cases with lichen striatus are interesting because of the striking similar pathogenesis (48) (**Figure 1**). A cytotoxic lymphocyte attack against postzygotic mutant keratinocytes has been proposed as the underlying mechanism of lichen striatus. This inflammatory response is especially pronounced in the basal and suprabasal layers which explains the development of postlesional hypopigmentation (71). Interestingly, also other localized skin disorders were reported in SV including segmental lichen planus, zosteriform lichen planus pigmentosus, lichen nitidus, and linear psoriasis (48–52). While relatively common in NSV, alopecia areata was only reported in 2 cases (10, 47).

Drug-induced SV is rare, especially infliximab is interesting given the dual effect TNF-α inhibition exerts on NSV (53, 54). TNF-alpha blockers are linked to the new development of NSV whereas also a stabilizing effect on existing NSV has been found (72, 73). A 34-year old patient with ulcerative colitis developed SV 4 months after initiation of infliximab (54). Another 46-year old patient receiving infliximab for rheumatoid arthritis developed SV after 2 months (53). Spreading of pre-existing SV occurred after immunotherapy

TABLE 1 | Segmental vitiligo cases with autoimmune and other comorbidities.

Comorbidity	Age (years)	Authors
Morphea/Parry-Romberg/lichen sclerosus		
Morphea en coup de sabre	22	Ubaldo et al. (36)
Circumscribed morphea	10	Lee et al. (37)
Morphea at same body area	10, 10	Dev et al. (38)
Morphea	10	Kim et al. (39)
Segmental morphea	18	Yadav P. et al. (40)
Morphea en coup de sabre	46	Janowska et al. (41)
Parry-Romberg syndrome	46	Wolek et al. (42)
Morphea en coup de sabre	9	Bowen et al. (43)
Lichen sclerosus	41	Weisberg et al. (44)
Linear morphea	21	Bonifati et al. (45)
Parry-Romberg syndrome	11	Creus et al. (46)
Other autoimmune diseases		
Alopecia areata, segmental lichen planus	12	Kumar et al. (47)
Lichen striatus	4, 5, 6	Correia et al. (48)
Zosteriform lichen planus pigmentosus	22	Sawatkar et al. (49)
Linear psoriasis	47	Valbuena et al. (50)
Alopecia areata, psoriasis	25	van Geel et al. (10)
Lichen nitidus (and Down's syndrome)	4	Agarwal et al. (51)
Segmental lichen planus	14	Sardana et al. (52)
Drug-induced		
Infliximab for rheumatoid arthritis	46	Carvalho et al. (53)
Infliximab for ulcerative colitis	34	Ryu et al. (54)
Immunotherapy for house dust mite	10	Shin et al. (55)
Isotretinoin	17	Avelar-Caggiano et al. (21)
Interferon alpha and ribavirin	60	Tinio et al. (56)
Co-occurring benign or malignant pigment conditions		
Cerci et al. (57)	37	Metastatic melanoma
Tiwary et al. (58)	9	Segmental naevus spilus
Kuruvilla et al. (59)	10	Segmental lentiginosis
Luo et al. (60)	8	Naevus of ota
Hofmann et al. (61)	6	Congenital naevus
Linked neurologic disorders		
Singh et al. (62)	13	Encephalitis
Yacubian et al. (63)	3	Rasmussen encephalitis
Jang et al. (64)	56	Schwannoma
Other		
Utaş et al. (65)	14	Baboon syndrome
Rajashekar et al. (66)	20	Twenty nail dystrophy
Kandpur et al. (67)	14, 23	Twenty nail dystrophy
Muramatsu et al. (68)	22	Naevoid basal cell carcinoma syndrome
Tay et al. (69)	/	Porencephaly, nasofrontal mucoceles, hypertelorism

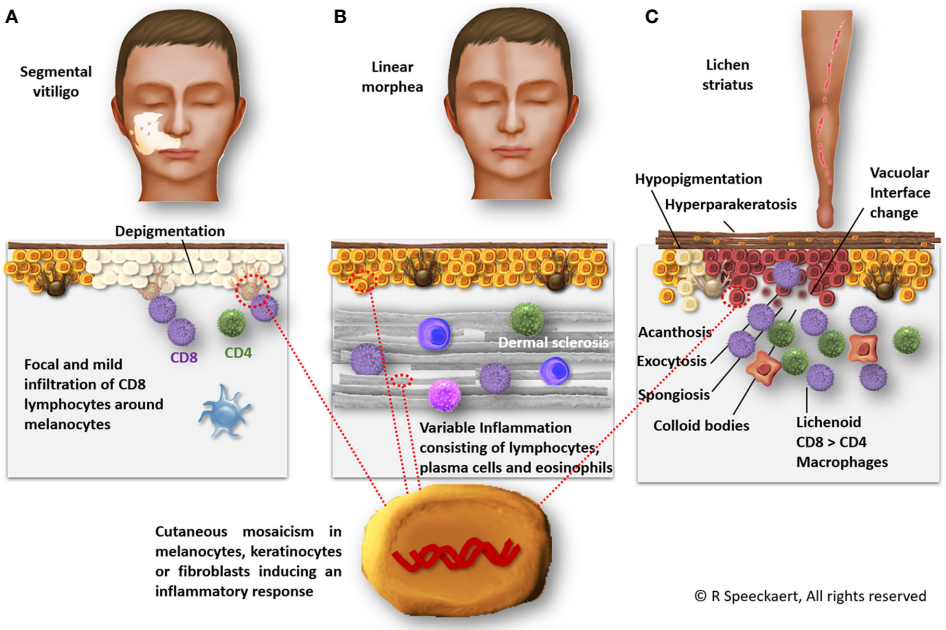


FIGURE 1 | Segmental vitiligo, linear morphea, and lichen striatus are presumed to share a similar pathogenesis based on a genetic mosaicism causing an inflammatory response. In segmental vitiligo **(A)**, a CD8-dominant infiltrate around melanocytes is observed ultimately leading to melanocyte destruction. Linear morphea **(B)** is characterized by dermal sclerosis and an inflammation of variable extent consisting of lymphocytes, plasma cells, and occasional eosinophils. Lichen striatus **(C)** exhibits a focal lichenoid lymphocytic infiltrate with macrophages. Hyperparakeratosis, acanthosis, exocytosis, spongiosis, vacuolar interface dermatitis, and colloid bodies can be observed.

with house dust mite (55). Finally, interferon-α – a well-known trigger of NSV - induced SV in a patient with hepatitis C (56).

Besides halo naevi, other pigment disorders are rare in SV. Only isolated reports of SV patients with co-existing segmental lentiginosis, segmental naevus spilus, and a naevus of Ota were found (58–61).

Overall, most co-occurring mosaic disorders in SV patients are characterized by a cutaneous inflammation which may be triggered by the immunologic events in active SV (or vice versa, the SV may be induced by the other inflammatory mosaic disorder). Another important group (e.g. segmental lentiginosis, neurologic disorders) consists of genetic or non-genetic diseases affecting melanocytes or their precursor cells.

EVIDENCE OF SOMATIC MOSAICISM

The characteristic distribution patterns of SV suggest a genetically mutated population of melanocytes which is targeted by a cytotoxic cell response. Different patterns of segmental vitiligo in the face and on the trunk have been detected and can easily be recognized in clinical practice (74, 75). By comparing different unilateral and localized skin disorders, we found that the SV pattern does not follow a dermatomal distribution but displays a unique distribution pattern that shares the largest similarity with segmental lentiginosis, followed by epidermal naevus verrucosus (76). Due to the remarkable overlap with segmental lentiginosis, the contribution of other skin cells such as keratinocytes, or alterations in the dermis are less plausible in SV. Additionally, segmental vitiligo follows the embryonal migration lines of melanocyte precursors. During embryogenesis, precursors of melanoblasts migrate from the neural crest in a dorsolateral way. During this migration, an impressive number of cell proliferations takes place enhancing the chance of genetic mosaicism. Another subpopulation of adult melanocytes originates from precursor cells with Schwan cell/melanoblast potential that migrate on a ventrolateral pathway. In mice, 65% of melanocytes of hair follicle melanocytes were linked to this separate group of melanocyte progenitors (77, 78). This might explain why long-lasting perifollicular repigmentation is sometimes possible in SV (14). This reservoir of perifollicular melanocytes and melanocyte precursors is however often not spared after prolonged periods of disease activity and phototherapy early in the disease (<6 months) gives better results (12). Interestingly, the frequency of SV on the back is lower compared to the lateral area, followed by the abdomen which aligns nicely with the migration route of melanoblasts (76). The temporary inflammation followed by a stable course in most SV patients is a clear differentiating factor between SV and NSV. This supports an inherent genetic defect carried by melanocytes in the affected segment. After removal of the susceptible SV melanocytes, the inflammation subsides. This is illustrated by

the high success rate of pigment cell transplantations in stable SV compared to NSV (79).

NEUROGENIC THEORY

For many years, SV was described to follow a dermatomal distribution and consequently, the involvement of neurogenic factors was suspected. Several cases with encephalitis preceding SV have been reported (62, 63). It should, however, be noted that this is also the case for NSV (80). Especially in NSV, elevated levels of neuropeptides [e.g. neuropeptide Y (NPY)] have been discovered. This does not confirm the involvement of neurogenic mechanisms as neuropeptides are also released following skin inflammation in other skin diseases such as psoriasis (81). Nonetheless, neuropeptides control regional skin immunity and some have important effects on melanocytes [e.g. α melanocyte-stimulating hormone (α-MSH)]. 2 cases of SV patient with schwannomas were found. This offers however more a clue toward somatic mosaicism given their common origin from the neural crest than an argument for a neurogenic cause of SV (64). Schwann cells can even *in vitro* be dedifferentiated to glia/melanocyte precursors illustrating their common origin (82). A study including 76 patients with SV found a slower nerve conduction velocity on the lesional side of the body compared to the contralateral side which was more pronounced in stable SV (83). Although the difference was small, it could be shed new light on the neurogenic involvement in SV if these results can be confirmed.

FUTURE PERSPECTIVES

Overall, research on SV remains limited as only 4.8% of vitiligo research discusses findings on SV on Pubmed. This is remarkable as SV is likely to be a less multi genetic disorder compared to NSV which might offer interesting information to the key factors making melanocytes vulnerable for immune-based destruction. The number of publications on SV is even declining in recent years in contrast to the rising yearly numbers on NSV.

Based on previous research, an inflammatory response has been proven in the majority of SV patients. The lack of markedly increased rates of associated systemic autoimmune comorbidities highlights the chance of a susceptible skin environment. As discussed above, the distribution patterns of SV render genetic mosaicism is very likely. This suggests that SV follows a similar pathogenesis compared to lichen striatus, blaschkitis, linear morphea, and linear lupus erythematosus. All these conditions share an inflammatory response in a particular distribution pattern usually at a young age. In most cases, a spontaneous stop of disease activity occurs with limited recurrences during adult life. Lichen striatus has the additional similarity of leaving hypopigmented skin areas which can resolve spontaneously (84). Linear morphea usually presents as a broad linear band of induration with often additional dyspigmentation (both hyper- and hypopigmentation) (85).

However, the underlying genetic mutations have currently not been discovered despite several research projects that have been initiated. This is mainly due to the fact that melanocytes of the inflammatory border of vitiligo are difficult to isolate and proliferate in the lab probably because of their compromised skin environment. Altered E-cadherin expression in SV melanocytes may be a primary deficiency which could be one of the initiating factors of SV hampering the *in vitro* culture of SV melanocytes (86). As the distribution patterns in the face and the trunk have been mapped in detail, future projects could focus on analyzing skin areas that are not (yet) affected but are likely to contain genetically identical melanocytes. Early SV rarely involves the whole skin area which is supposed to be populated by melanocytes originating from the same melanocyte precursor and often leaves skipped areas of normally pigmented skin.

Based on the published case reports, the co-occurrence of (localized) morphea and SV is striking. Although morphea has also been associated with NSV, linear morphea seems particularly linked to SV. Linear morphea follows Blaschko's lines and genetic mosaicism is therefore believed to be the underlying cause (87). The distribution patterns of SV and linear morphea are clearly different which can be explained by the different skin cell types which are likely responsible for these conditions (melanocytes vs keratinocytes or fibroblasts) (**Figure 1**). Both morphea and SV have been linked to traumata or local enhanced immune responses and therefore one autoimmune condition can elicit the other. It is remarkable that 2 mosaic disorders affecting cell types of different embryogenic origins have a tendency to co-occur in the same body area. Despite some case reports, this phenomenon remains however extremely rare and the differential diagnosis with hypopigmented lesions caused be morphea can be challenging (85).

In conclusion, SV research merits new attention as it may provide interesting data for the pathogenesis and treatment of vitiligo in general. To date, evidence points to a temporary cytotoxic-response targeting mosaic melanocytes. Associated autoimmune diseases are less prevalent compared to NSV, although a remarkable overrepresentation of case reports with (linear) morphea is present. A genetic predisposition (e.g. family history of NSV) may enhance the chance of SV and the subsequent development of halo naevi and NSV. Screening for thyroid disease is less valuable in SV compared to NSV.

AUTHOR CONTRIBUTIONS

RS: Conception or design of the work, Data collection, Drafting the article. JL: Critical revision of the article. VB: Critical revision of the article. AB: Critical revision of the article. MS: Critical revision of the article, Data collection. NG: Critical revision of the article. All authors contributed to the article and approved the submitted version.

FUNDING

The research activities of RS and NG are supported by the Scientific Research Foundation-Flanders (Krediet aan Navorsers: 1504718N and FWO Senior Clinical Investigator: 18B2721N and 1831512N, respectively).

REFERENCES

1. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. *Lancet* (2015) 386:74–84. doi: 10.1016/S0140-6736(14)60763-7
2. Silverberg NB. Update on childhood vitiligo. *Curr Opin Pediatr* (2010) 22:445–52. doi: 10.1097/MOP.0b013e32833b6ac3
3. Hann SK, Lee HJ. Segmental vitiligo: clinical findings in 208 patients. *J Am Acad Dermatol* (1996) 35:671–4. doi: 10.1016/s0190-9622(96)90718-5
4. Li Y, Xu A. Segmental vitiligo in children: a clinical epidemiologic study in China. *J Eur Acad Dermatol Venereol* (2013) 27:1056–7. doi: 10.1111/jdv.12003
5. van Geel N, De Lille S, Vandenhaute S, Gauthier Y, Mollet I, Brochez L, et al. Different phenotypes of segmental vitiligo based on a clinical observational study. *J Eur Acad Dermatol Venereol* (2011) 25:673–8. doi: 10.1111/j.1468-3083.2010.03847.x
6. Park J-H, Jung M-Y, Lee J-H, Yang J-M, Lee D-Y, Park KK. Clinical course of segmental vitiligo: a retrospective study of eighty-seven patients. *Ann Dermatol* (2014) 26:61–5. doi: 10.5021/ad.2014.26.1.61
7. Ezzedine K, Gauthier Y, Léauté-Labrèze C, Marquez S, Bouchtnei S, Jouary T, et al. Segmental vitiligo associated with generalized vitiligo (mixed vitiligo): a retrospective case series of 19 patients. *J Am Acad Dermatol* (2011) 65:965–71. doi: 10.1016/j.jaad.2010.08.031
8. Lee D-Y, Kim C-R, Park J-H, Lee J-H. The incidence of leukotrichia in segmental vitiligo: implication of poor response to medical treatment. *Int J Dermatol* (2011) 50:925–7. doi: 10.1111/j.1365-4632.2011.04914.x
9. Vachiramon V, Onprasert W, Harnchoowong S, Chanprapaph K. Prevalence and Clinical Characteristics of Itch in Vitiligo and Its Clinical Significance. *BioMed Res Int* (2017) 2017:5617838. doi: 10.1155/2017/5617838
10. van Geel N, Mollet I, Brochez L, Dutré M, De Schepper S, Verhaeghe E, et al. New insights in segmental vitiligo: case report and review of theories. *Br J Dermatol* (2012) 166:240–6. doi: 10.1111/j.1365-2133.2011.10650.x
11. van Geel N, Speeckaert R. Segmental Vitiligo. *Dermatol Clin* (2017) 35:145–50. doi: 10.1016/j.det.2016.11.005
12. Majid I, Imran S. Excimer light therapy in childhood segmental vitiligo: Early treatment gives better results. *Dermatol Ther* (2020) 33:e13408. doi: 10.1111/dth.13408
13. Shah S, Sakhiya J, Deshpande P, Sakhiya D, Inamadar AC. Safety and Efficacy of the Combination of 308-nm Monochromatic Excimer Light and Topical 0.1% Tacrolimus Ointment in Segmental Vitiligo: An Open-label Study. *J Clin Aesthet Dermatol* (2020) 13:E69–75.
14. Gan EY, Gahat T, Cario-André M, Seneschal J, Ezzedine K, Taïeb A. Clinical repigmentation patterns in paediatric vitiligo. *Br J Dermatol* (2016) 175:555–60. doi: 10.1111/bjd.14635
15. Yang Y, Lin X, Fu W, Luo X, Kang K. An approach to the correlation between vitiligo and autoimmune thyroiditis in Chinese children. *Clin Exp Dermatol* (2010) 35:706–10. doi: 10.1111/j.1365-2230.2009.03671.x
16. Lim HK, Bae MI, Jeong KH, Shin MK, Lee M-H. Positivity rates of antithyroid antibody, antinuclear antibody and thyroid peroxidase antibody in different types of vitiligo. *Clin Exp Dermatol* (2016) 41:242–7. doi: 10.1111/ced.12771
17. Fan K-C, Yang T-H, Huang Y-C. Vitiligo and thyroid disease: a systematic review and meta-analysis. *Eur J Dermatol* (2018) 28:750–63. doi: 10.1684/ejd.2018.3449
18. Roberts GHL, Santorico SA, Spritz RA. The genetic architecture of vitiligo. *Pigment Cell Melanoma Res* (2020) 33:8–15. doi: 10.1111/pcmr.12848
19. Wang P, Li Y, Nie H, Zhang X, Shao Q, Hou X, et al. The changes of gene expression profiling between segmental vitiligo, generalized vitiligo and healthy individual. *J Dermatol Sci* (2016) 84:40–9. doi: 10.1016/j.jdermsci.2016.07.006
20. Bonifazi E. Segmental vitiligo with inflammatory border. *Eur J Pediatr Dermatol* (2010) 20:276–6. doi: 10.26326/2281-9649.26.1.1203
21. Avelar-Caggiano MF de S, de Castro CCS, Dellatorre G. Inflammatory segmental vitiligo during oral isotretinoin use: a casual association? *Bras Dermatol* (2020) 95:399–400. doi: 10.1016/j.abd.2019.07.014
22. van Geel N, Grine L, De Wispelaere P, Mertens D, Prinsen CAC, Speeckaert R. Clinical visible signs of disease activity in vitiligo: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol* (2019) 33:1667–75. doi: 10.1111/jdv.15604
23. Shin J, Kang HY, Kim KH, Park CJ, Oh SH, Lee SC, et al. Involvement of T cells in early evolving segmental vitiligo. *Clin Exp Dermatol* (2016) 41:671–4. doi: 10.1111/ced.12852
24. Attili VR, Attili SK. Segmental and generalized vitiligo: both forms demonstrate inflammatory histopathological features and clinical mosaicism. *Indian J Dermatol* (2013) 58:433–8. doi: 10.4103/0019-5154.119949
25. van Geel NAC, Mollet IG, De Schepper S, Tjin EPM, Vermaelen K, Clark RA, et al. First histopathological and immunophenotypic analysis of early dynamic events in a patient with segmental vitiligo associated with halo nevi. *Pigment Cell Melanoma Res* (2010) 23:375–84. doi: 10.1111/j.1755-148X.2010.00703.x
26. El-Domyati M, Hosam W, Rezk A, Uitto J, Alexeev V. Deregulation of chemotactic signals in segmental and non-segmental vitiligo [Society of Investigative Dermatology Congress abstract 614]. *J Invest Dermatol* (2015) 136:S109. doi: 10.1016/j.jid.2016.02.654
27. Yang X, Yan L, Ha D, Qu L, Liu L, Tao Y. Changes in sICAM-1 and GM-CSF levels in skin tissue fluid and expression of IL-6, IL-17 and TNF-α in blood of patients with vitiligo. *Exp Ther Med* (2019) 17:408–12. doi: 10.3892/etm.2018.6937
28. Schallreuter KU, Salem MAEL, Holtz S, Panske A. Basic evidence for epidermal H2O2/ONOO—mediated oxidation/nitration in segmental vitiligo is supported by repigmentation of skin and eyelashes after reduction of epidermal H2O2 with topical NB-UVB-activated pseudocatalase PC-KUS. *FASEB J* (2013) 27:3113–22. doi: 10.1096/fj.12-226779
29. Shajil EM, Begum R. Antioxidant status of segmental and non-segmental vitiligo. *Pigment Cell Res* (2006) 19:179–80. doi: 10.1111/j.1600-0749.2006.00299.x
30. Bertolini M, McElwee K, Gilhar A, Bulfone-Paus S, Paus R. Hair follicle immune privilege and its collapse in alopecia areata. *Exp Dermatol* (2020) 29:703–25. doi: 10.1111/exd.14155
31. Suo A, Chan Y, Beaulieu C, Kong S, Cheung WY, Monzon JG, et al. Anti-PD1-Induced Immune-Related Adverse Events and Survival Outcomes in Advanced Melanoma. *Oncol* (2020) 25:438–46. doi: 10.1634/theoncologist.2019-0674
32. Speeckaert R, van Geel N. Targeting CTLA-4, PD-L1 and IDO to modulate immune responses in vitiligo. *Exp Dermatol* (2017) 26:630–4. doi: 10.1111/exd.13069
33. Ezzedine K, Diallo A, Léauté-Labrèze C, Séneschal J, Prey S, Ballanger F, et al. Halo naevi and leukotrichia are strong predictors of the passage to mixed vitiligo in a subgroup of segmental vitiligo. *Br J Dermatol* (2012) 166:539–44. doi: 10.1111/j.1365-2133.2011.10709.x
34. Martins CP dos S, Hertz A, Luzio P, Paludo P, Azulay-Abulafia L. Clinical and epidemiological characteristics of childhood vitiligo: a study of 701 patients from Brazil. *Int J Dermatol* (2020) 59:236–44. doi: 10.1111/ijd.14645
35. Maeda Y, Nishikawa H, Sugiyama D, Ha D, Hamaguchi M, Saito T, et al. Detection of self-reactive $CD8^+$ T cells with an anergic phenotype in healthy individuals. *Science* (2014) 346:1536–40. doi: 10.1126/science.aaa1292
36. Ubaldo HDC, de Castro CCS. Coexistence of segmental vitiligo, scleroderma en coup de sabre and cleft lip on the same hemiface: association with mosaicism? *Bras Dermatol* (2019) 94:248–50. doi: 10.1590/abd1806-4841.20198110
37. Lee JS, Park HS, Cho S, Yoon HS. Concurrence of circumscribed morphea and segmental vitiligo: A case report. *Ann Dermatol* (2018) 30:708–11. doi: 10.5021/ad.2018.30.6.708
38. Dev T, Bhari N, Sethuraman G. Co-Localization Of Segmental Vitiligo And Morphea: A Feature Of 'Twin-Spotting' Type Of Cutaneous Mosaicism? *Pediatr Dermatol* (2017) 34(Supplement 1):S190–1.
39. Kim HJ, Shin D, Oh SH. A Case of Segmental Vitiligo with Generalized Morphea Stabilized by Antimalarial Medication. *Ann Dermatol* (2016) 28:249–50. doi: 10.5021/ad.2016.28.2.249
40. Yadav P, Garg T, Chander R, Nangia A. Segmental vitiligo with segmental morphea: An autoimmune link? *Indian Dermatol Online J* (2014) 5:S23–25. doi: 10.4103/2229-5178.144517
41. Janowska M, Podolec K, Lipko-Godlewska S, Wojas-Pelc A. Coexistence of Parry-Romberg syndrome with homolateral segmental vitiligo. *Postepy Dermatol Alergol* (2013) 30:409–11. doi: 10.5114/pdia.2013.39441

42. Wolek M, Podolec K, Lipko-Godlewska S, Wozniak W, Wojas-Pelc A. Parry-Romberg syndrome and homolateral segmental vitiligo. *Przegląd Dermatol* (2012) 99:446.
43. Bowen C, Kobayashi TT. What is your diagnosis? Segmental vitiligo and en coup de sabre. *Cutis* (2010) 85(230):237–8.
44. Weisberg EL, Le LQ, Cohen JB. A case of simultaneously occurring lichen sclerosus and segmental vitiligo: connecting the underlying autoimmune pathogenesis. *Int J Dermatol* (2008) 47:1053–5. doi: 10.1111/j.1365-4632.2008.03623.x
45. Bonifati C, Impara G, Morrone A, Pietrangeli A, Carducci M. Simultaneous occurrence of linear scleroderma and homolateral segmental vitiligo. *J Eur Acad Dermatol Venereol* (2006) 20:63–5. doi: 10.1111/j.1468-3083.2005.01336.x
46. Creus L, Sanchez-Regaña M, Salleras M, Chaussade V, Umbert P. [Parry-Romberg syndrome associated with homolateral segmental vitiligo]. *Ann Dermatol Venereol* (1994) 121:710–1.
47. Kumar P, Gosai A, Mondal AK, Debbarman P, Lal NR, Gharami RC, et al. Association of alopecia areata with segmental vitiligo and segmental lichen planus: a case report. *J Pakistan Assoc Dermatol* (2012) 22:55–8.
48. Correia LD, Silva de Castro CC. Association between bilateral segmental vitiligo and lichen striatus: an expression of mosaicism? *Int J Dermatol* (2018) 57:992–3. doi: 10.1111/ijd.13964
49. Sawatkar GU, Kumaran MS, Narang T, Parsad D. Zosteriform Lichen Planus Pigmentosus Altering Segmental Vitiligo. *Skinmed* (2018) 16:133–5.
50. Valbuena MC, Patiño LA. Psoriasis lineal y vitiligo segmentario, manifestación sobrepuesta de dos enfermedades frecuentes. *Rev Argent Dermatol* (2013) 49:0–0.
51. Agarwal S, Guglani V, Kumar B. Down's syndrome with lichen nitidus and segmental vitiligo. *Indian J Dermatol Venereol Leprol* (2009) 75:627–9. doi: 10.4103/0378-6323.57738
52. Sardana K, Sharma RC, Koranne RV, Mahajan S. An interesting case of colocalization of segmental lichen planus and vitiligo in a 14-year-old boy. *Int J Dermatol* (2002) 41:508–9. doi: 10.1046/j.1365-4362.2002.01552_2.x
53. Carvalho CLDB, Ortigosa LCM. Segmental vitiligo after infliximab use for rheumatoid arthritis–a case report. *Bras Dermatol* (2014) 89:154–6. doi: 10.1590/abd1806-4841.20142887
54. Ryu TH, Lee DW, Choi JE, Ahn HH, Kye YC, Seo SH. A Type II Segmental Vitiligo Developed under Infliximab Treatment for Ulcerative Colitis. *Ann Dermatol* (2017) 29:826–7. doi: 10.5021/ad.2017.29.6.826
55. Shin S, Shin JY, Lee H, Oh SH. Spreading of pre-existing segmental vitiligo after immunotherapy with house dust mite in a patient with atopic dermatitis. *Clin Exp Dermatol* (2015) 40:920–1. doi: 10.1111/ced.12443
56. Tinio P, Hadi S, Al-Ghaithi K, Al-Qari H, Rudikoff D. Segmental vitiligo and hair curling after interferon alpha and ribavirin treatment for hepatitis C. *Skinmed* (2006) 5:50–1. doi: 10.1111/j.1540-9740.2006.04772.x
57. Cerci FB, Mesquita LA, Silva de Castro CC. Segmental vitiligo-like hypopigmentation associated with metastatic melanoma. *Cutis* (2017) 99:E1–2.
58. Tiwary AK, Kumar P. Vitiligo developing in congenital segmental speckled lentiginous nevus: Another example of immunocompromised cutaneous district due to immunological assault on aberrant melanocytes? *Indian J Dermatol Venereol Leprol* (2017) 83:612. doi: 10.4103/ijdvl.IJDVL_1055_16
59. Kuruvila S, Dalal M. Segmental lentiginosis with ipsilateral segmental vitiligo – a case report. *J Pakistan Assoc Dermatol* (2016) 21:125–7.
60. Luo X-Y, Xu A-E, Li J-C, Guan C-P. Naevus of Ota associated with segmental vitiligo: a case report. *J Eur Acad Dermatol Venereol* (2010) 24:611–2. doi: 10.1111/j.1468-3083.2009.03443.x
61. Hofmann UB, Bröcker E-B, Hamm H. Simultaneous onset of segmental vitiligo and a halo surrounding a congenital melanocytic naevus. *Acta Derm Venereol* (2009) 89:402–6. doi: 10.2340/00015555-0654
62. Singh A, Kornmehl H, Milgraum S. Segmental vitiligo following encephalitis. *Pediatr Dermatol* (2010) 27:624–5. doi: 10.1111/j.1525-1470.2010.01319.x
63. Yacubian EM, Rosemberg S, Garrido Neto TL, Marie SK, Valério RM, Jorge CL. Rasmussen encephalitis associated with segmental vitiligo of the scalp: clinicopathologic report. *J Child Neurol* (2001) 16:374–7. doi: 10.1177/088307380101600513
64. Jang HS, Kim MR, Hann S-K, Oh SH. Segmental vitiligo and facial palsy associated with a concurrent ipsilateral acoustic schwannoma. *J Dermatol* (2013) 40:291–2. doi: 10.1111/1346-8138.12073
65. Utaş S, Ferahbaş A. Baboon syndrome and segmental vitiligo coexistence. *Turk J Pediatr* (2009) 51:392–4.
66. Rajashekar TS, Singh G, Rajkumar V. Segmental vitiligo and twenty-nail dystrophy: an unusual association. *Indian J Dermatol Venereol Leprol* (2008) 74:661–2. doi: 10.4103/0378-6323.45122
67. Khandpur S, Reddy BS. An association of twenty-nail dystrophy with vitiligo. *J Dermatol* (2001) 28:38–42. doi: 10.1111/j.1346-8138.2001.tb00084.x
68. Muramatsu S, Suga Y, Mizuno Y, Haseegawa T, Komuro Y, Kubo Y, et al. A Japanese case of naevoid basal cell carcinoma syndrome associated with segmental vitiligo. *Br J Dermatol* (2005) 152:812–4. doi: 10.1111/j.1365-2133.2005.06504.x
69. Tay CH. Porencephaly, nasofrontal mucoceles, hypertelorism and segmental vitiligo–report of a new neurocutaneous disorder. *Singapore Med J* (1970) 11:253–7.
70. Lee H, Kim YJ, Oh SH. Segmental vitiligo and extragenital lichen sclerosus et atrophicus simultaneously occurring on the opposite sides of the abdomen. *Ann Dermatol* (2014) 26:764–5. doi: 10.5021/ad.2014.26.6.764
71. Taieb A, El Youbi A, Grosshans E, Maleville J. Lichen striatus: A Blaschko linear acquired inflammatory skin eruption. *J Am Acad Dermatol* (1991) 25:637–42. doi: 10.1016/0190-9622(91)70245-W
72. Webb KC, Tung R, Winterfield LS, Gottlieb AB, Eby JM, Henning SW, et al. Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. *Br J Dermatol* (2015) 173:641–50. doi: 10.1111/bjd.14016
73. Bae JM, Kim M, Lee HH, Kim K-J, Shin H, Ju HJ, et al. Increased Risk of Vitiligo Following Anti-Tumor Necrosis Factor Therapy: A 10-Year Population-Based Cohort Study. *J Invest Dermatol* (2018) 138:768–74. doi: 10.1016/j.jid.2017.11.012
74. Kim D-Y, Oh SH, Hann S-K. Classification of segmental vitiligo on the face: clues for prognosis. *Br J Dermatol* (2011) 164:1004–9. doi: 10.1111/j.1365-2133.2010.10202.x
75. van Geel N, Bosma S, Boone B, Speeckaert R. Classification of segmental vitiligo on the trunk. *Br J Dermatol* (2014) 170:322–7. doi: 10.1111/bjd.12652
76. van Geel N, Speeckaert R, Melsens E, Toelle SP, Speeckaert M, De Schepper S, et al. The distribution pattern of segmental vitiligo: clues for somatic mosaicism. *Br J Dermatol* (2013) 168:56–64. doi: 10.1111/bjd.12013
77. Mort RL, Jackson IJ, Patton EE. The melanocyte lineage in development and disease. *Development* (2015) 142:620–32. doi: 10.1242/dev.106567
78. Adameyko I, Lallemend F, Aquino JB, Pereira JA, Topilko P, Müller T, et al. Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. *Cell* (2009) 139:366–79. doi: 10.1016/j.cell.2009.07.049
79. Olsson MJ, Juhlin L. Long-term follow-up of leucoderma patients treated with transplants of autologous cultured melanocytes, ultrathin epidermal sheets and basal cell layer suspension. *Br J Dermatol* (2002) 147:893–904. doi: 10.1046/j.1365-2133.2002.04837.x
80. Haitao R, Huiqin L, Tao Q, Xunzhe Y, Xiaoqiu S, Wei L, et al. Autoimmune encephalitis associated with vitiligo? *J Neuroimmunol* (2017) 310:14–6. doi: 10.1016/j.jneuroim.2017.05.019
81. Saraceno R, Kleyn CE, Terenghi G, Griffiths CEM. The role of neuropeptides in psoriasis. *Br J Dermatol* (2006) 155:876–82. doi: 10.1111/j.1365-2133.2006.07518.x
82. Dupin E, Real C, Glavieux-Pardanaud C, Vaigot P, Le Douarin NM. Reversal of developmental restrictions in neural crest lineages: transition from Schwann cells to glial-melanocytic precursors in vitro. *Proc Natl Acad Sci USA* (2003) 100:5229–33. doi: 10.1073/pnas.0831229100
83. Zhou J, Zhong Z, Li J, Fu W. Motor nerve conduction velocity is affected in segmental vitiligo lesional limbs. *Int J Dermatol* (2016) 55:700–5. doi: 10.1111/ijd.13171
84. Hauber K, Rose C, Bröcker EB, Hamm H. Lichen striatus: clinical features and follow-up in 12 patients. *Eur J Dermatol* (2000) 10:536–9.
85. Sung JJ, Chen TS, Gilliam AC, McCalmont TH, Gilliam AE. Clinicohistopathological correlations in juvenile localized scleroderma: studies on a subset of children with hypopigmented juvenile localized scleroderma due to loss of epidermal melanocytes. *J Am Acad Dermatol* (2011) 65:364–73. doi: 10.1016/j.jaad.2010.02.065
86. Grill C, Benzekri L, Rubod A, Aktary Z, Ezzedine K, Taïeb A, et al. Epidermal melanocytes in segmental vitiligo show altered expression of E-cadherin, but not P-cadherin. *Br J Dermatol* (2018) 178:1204–6. doi: 10.1111/bjd.16352

87. Weibel L, Harper JI. Linear morphoea follows Blaschko's lines. *Br J Dermatol* (2008) 159:175–81. doi: 10.1111/j.1365-2133.2008.08647.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 06 November 2020
doi: 10.3389/fimmu.2020.579022

Targeting the PD-1/PD-L1 Axis in Human Vitiligo

Marcella Willemsen[1]*, *Cornelis J. M. Melief*[2], *Marcel W. Bekkenk*[1] *and Rosalie M. Luiten*[1]

[1] Department of Dermatology and Netherlands Institute for Pigment Disorders, Amsterdam University Medical Centers, location AMC, University of Amsterdam, Cancer Center Amsterdam, Amsterdam Infection & Immunity Institute, Amsterdam, Netherlands, [2] ISA Pharmaceuticals, Leiden, Netherlands

Autoreactive $CD8^+$ T cells play a pivotal role in melanocyte destruction in autoimmune vitiligo. Immunotherapy for melanoma often leads to autoimmune side-effects, among which vitiligo-like depigmentation, indicating that targeting immune checkpoints can break peripheral tolerance against self-antigens in the skin. Therapeutically enhancing immune checkpoint signaling by immune cells or skin cells, making self-reactive T cells anergic, seems a promising therapeutic option for vitiligo. Here, we review the current knowledge on the PD-1/PD-L1 pathway in vitiligo as new therapeutic target for vitiligo therapy.

Keywords: vitiligo, programmed cell death 1 receptor, B7-H1 antigen, immune tolerance, autoimmunity

OPEN ACCESS

Edited by:
Katia Boniface,
Université de Bordeaux, France

Reviewed by:
Mehdi Rashighi,
University of Massachusetts Medical School, United States
Marijn M. Speeckaert,
Ghent University Hospital, Belgium

***Correspondence:**
Marcella Willemsen
m.willemsen@amsterdamumc.nl

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *01 July 2020*
Accepted: *19 October 2020*
Published: *06 November 2020*

Citation:
Willemsen M, Melief CJM, Bekkenk MW and Luiten RM (2020) Targeting the PD-1/PD-L1 Axis in Human Vitiligo. Front. Immunol. 11:579022. doi: 10.3389/fimmu.2020.579022

BACKGROUND

Vitiligo is a common, acquired skin disease characterized by loss of epidermal melanocytes, resulting in sharply, depigmented macules (1). While there is still an extensive debate on the initiating steps, experts agree on immune-mediated melanocyte destruction, with melanocyte-specific $CD8^+$ T cells being able to kill melanocytes (2–4) and anti-melanocyte antibodies found in the sera of patients with vitiligo (5). Especially IFN-γ holds a crucial role in the pathogenesis of vitiligo (6). Moreover, regulatory T cells are impaired, both in numbers and function, in patients with vitiligo (7–9). Recently, tissue-resident memory T (T_{RM}) cells were shown to have a prominent role in disease development and flare-up in human vitiligo. Vitiligo affected skin was shown to be enriched for $CD8^+$ T_{RM} cells compared to healthy, unaffected donor skin, together indicating immune disturbance in patients with vitiligo (10, 11).

Current treatment modalities aim to block melanocyte destruction and/or induce repigmentation of the skin. Topical corticosteroids or immunosuppressants have shown good outcome, while this might not be the most effective approach (12). Moreover, clinical efficacy of current treatment modalities is not satisfactory and, therefore, new therapeutic strategies should be evaluated and tested for vitiligo patients.

Immune checkpoints play a pivotal role in immune evasion by tumors and immunotherapy for melanoma shows that targeting of immune checkpoints is sufficient to break peripheral tolerance and re-activate melanoma-specific cytotoxic T cells in a proportion of patients. Clinically opposite to melanoma, vitiligo patients might benefit from induced peripheral tolerance, turning the autoimmune response into an anergic response. In this review, we therefore propose that exploiting the PD-1/PD-L1 axis may be effective as a treatment strategy for vitiligo.

AUTOIMMUNITY AND TUMOR IMMUNITY

Similarities exist between skin autoimmunity and tumor immunity, observed in vitiligo and melanoma. Immunization of mice bearing B16 melanoma with gp100 antigen and tumor-specific $CD8^+$ T cells resulted in tumor destruction, but also caused vitiligo development (13). Similarly, in melanoma patients, vitiligo can occur spontaneously or upon immunotherapy. Immunotherapy for melanoma often leads to autoimmune side-effects, including vitiligo-like depigmentation. This depigmentation is caused by activated anti-melanoma immunity, that targets not only malignant cells, but also healthy melanocytes and correlates with prolonged survival (14–18). Conversely, vitiligo patients have 3-fold less risk of developing melanoma during life (19, 20). Nevertheless, this risk is suggested to be influenced by individual skin type (21). Melanocytes are far more protective in dark skin types and, therefore, loss of melanocytes leads to a greater skin cancer risk in these patients. Contrary, in patients with light skin types, loss of protective melanocytes seems to be less important and increased immunosurveillance contributes to an overall decreased risk of developing skin cancer. Melanoma susceptibility in vitiligo patients may, therefore, be adjusted for race. Also, immunotherapy for melanoma using a skin-depigmenting compound induced local and systemic anti-melanoma immunity (22). Conversely, a proportion of metastatic cutaneous melanoma patients develop vitiligo-like depigmentation (16, 17). Considering this melanoma/vitiligo relationship, vitiligo patients could benefit from anti-melanocyte tolerance, observed in melanoma, and exploiting immune checkpoints might influence peripheral tolerance against self-antigens in the skin.

PD-1/PD-L1 SIGNALING

PD-1/PD-L1 Pathway

Programmed cell death 1 (PD-1 or CD279) is an inhibitory cell-surface molecule that suppresses T cells. PD-1 has emerged as a key player in immune regulation and is constitutively expressed by regulatory T cells and can be, upon activation, expressed by effector T cells, natural killer cells, and B cells (23). Immune suppression requires PD-1 binding on T cells to PD-1 ligands (PD-L1 and PD-L2) on other cell types. PD-L1 is constitutively expressed by various immune cells, e.g., lymphocytes, DCs and macrophages, and its expression can be induced on non-immune cells, including cancer cells. Ligation of PD-1/PD-L1 represses the activation and function of autoreactive T cells, inhibits their proliferation and induces apoptosis, thereby regulating both central and peripheral tolerance to hamper and regulate inflammatory responses and autoimmune diseases (24).

Melanoma Immunotherapy

Signaling *via* the PD-1/PD-L1 axis is commonly involved in tumor immune evasion. In melanoma, tumor-associated PD-L1 can functionally suppress T cell responses to melanoma and promote T cell apoptosis (25). Consequently, $PD\text{-}L1^+$ melanomas have been identified as a subset that tends to be more aggressive and is associated with poor prognosis (26).

Targeting these immune checkpoint molecules has become one of the therapeutic options (27). PD-1-targeting monoclonal antibodies have been approved for clinical use and are currently among the first-line treatment options for advanced melanoma patients and many other cancers (28, 29). In a fraction of melanoma patients (ranging from 3.4 to 28%), vitiligo-like depigmentation (or melanoma-associated leukoderma) occurs as an adverse effect of immune checkpoint inhibition, indicating the breaking of tolerance to melanocytic self-antigens (30, 31). Nevertheless, skin depigmentation has now been reported in anti-PD-1/PD-L1 treated patients with other metastatic cancers as well (32–37). Skin depigmentation is significantly associated with a favorable prognosis in melanoma patients (17, 18). Likewise, PD-L1-targeting antibodies, e.g., atezolizumab, are currently being investigated in metastatic melanoma patients (38). Considering vitiligo-like depigmentation in (melanoma) patients receiving anti-PD-1 monotherapy, vitiligo patients may benefit from induced peripheral tolerance to self-proteins by exploiting the T cell immunosuppression mediated by these molecules.

PD-1/PD-L1 in Autoimmune Diseases

Emerging evidence demonstrates that impaired PD-1/PD-L1 function is involved in a variety of autoimmune diseases, among which type 1 diabetes, inflammatory bowel diseases, and rheumatoid arthritis (39). Manipulating PD-1/PD-L1 signaling appears to elicit significant outcomes in disorders with aberrantly-regulated immune system function. More specifically, PD-L1-expressing DCs can induce anergy of otherwise active T cells and, thereby, hinder induction of autoimmune encephalomyelitis, an animal model of multiple sclerosis (40). Also, intravenous injection of recombinant adenovirus expressing full-length mouse PD-L1 (Ad.PD-L1) gene can suppress lupus-like syndrome in BXSB mice, when combined with anti-ICOSL(B7h) antibody to block ICOS-mediated co-stimulation (41). In this study, it was suggested that the protective effect of Ad.PD-L1 was through suppression of autoreactive T cells at the target organ. Furthermore, severe psoriatic inflammation was induced in PD-1-null mice and PD-L1-Ig fusion protein was shown effective in inhibiting inflammatory skin γδ-low T cell activity *in vitro* (42), indicating the rationale to test the therapeutic potential of increasing PD-1/PD-L1 signaling in skin autoimmunity. Raising our knowledge on PD-1/PD-L1 functions and signaling may thus enable us to develop new therapeutic strategies to manipulate the inhibitory function of PD-1.

PD-1/PD-L1 IN VITILIGO

PD-1/PD-L1 Expression in Vitiligo

While abundantly studied in melanoma, the PD-1/PD-L1 axis in vitiligo has received far less attention thus far, but deserves

exploration as a therapeutic target. Similarly to melanoma cells, PD-L1 can be expressed by melanocytes, especially in the case of inflammatory responses (43). PD-L1-expressing melanocytes co-localize with infiltrating immune cells and PD-L1 strongly correlates with immune cell infiltration in both nevi and malignant melanoma. Also, CD45 and IFN-γ mRNA were detected in PD-L1$^+$ melanomas, while IFN-γ could not be detected in PD-L1$^-$ tumors. Expression of PD-L1 can be upregulated on various cell types upon stimulation with pro-inflammatory cytokines, including IFN-γ (44). Although IFN-γ-producing CD8$^+$ T cell are abundantly present in lesional vitiligo skin, cytokine-induced PD-L1 expression might not be occurring or insufficient in driving a negative feedback loop.

Vitiligo patients also have increased PD-1 expression on peripheral CD3$^+$ CD4$^+$ and regulatory T cells (45) compared to healthy individuals, suggesting involvement of PD-1 in disease regulation and pathogenesis. Blocking PD-L1 signaling *in vitro* led to expansion of regulatory T cells (46), implicating that PD-1/PD-L1 engagement may negatively regulate regulatory T cell function. The authors, therefore, hypothesized that PD-1$^+$ regulatory T cells may become exhausted by PD-L1-expressing autoreactive T cells, hence leading to a deficiency of regulatory T cells in vitiligo (45). PD-L1 expression by autoreactive T cells, however, has not been shown. Similarly, vitiligo patients show increased PD-1 expression on CD8$^+$ T cells compared to healthy donor CD8$^+$ T cells (47), implying that PD-1 expression is due to excessive activation of autoreactive CD8$^+$ T cells. PD-1 expression on CD8$^+$ T cells appears to positively correlate with disease activity (47), suggesting a more vigorous attempt to control the inflammatory situation by increasing PD-1 expression. *In situ*, PD-1 is significantly expressed in marginal and lesional infiltrates when compared to non-lesional skin in patients with active non-segmental vitiligo (48). Because no CD4/PD-1 and CD8/PD-1 double staining was done, it remains unstudied which skin T cells express PD-1. Together, these results suggest a role for PD-1/PD-L1 in the lack of peripheral tolerance in vitiligo, providing a rationale to target this axis in vitiligo treatment.

Vitiligo is a polygenic disorder, implying simultaneous contributions of multiple genetic risk factors and environmental triggers. Large-scale genome-wide association studies have identified approximately 50 genetic loci that contribute to vitiligo risk, of which a large fraction encode proteins involved in immune regulation (49–52). To date, no single-nucleotide polymorphisms in the *PD-1* (*PDCD1*) or *PD-L1* (*CD274*) gene has been reported in human vitiligo. Nevertheless, *PD-1* polymorphisms that give a higher risk of developing other autoimmune disorders, among which rheumatoid arthritis and systemic lupus erythematosus, have been reported (53). Additionally, microsatellite polymorphisms in the *CTLA-4* gene, encoding the CTLA-4 immune checkpoint molecule, have been demonstrated in European-derived vitiligo patients (54). Likewise, a missense polymorphism in the *PTPN22* gene has been associated with susceptibility to autoimmune disorders, including vitiligo (55). Lymphoid protein tyrosine phosphatase (LYP), encoded by the *PTPN22* gene, is an important downregulator of T cell activation. Hypothetically, patients carrying a *PTPN22* polymorphism initially might be less sensitive to T cell inhibition (e.g., by PD-1/PD-L1 signaling), since the LYP missense polymorphism drastically reduce the binding of LYP to C-terminal Src kinase (CSK) *in vitro*. As a result downregulation of T cell activation is disrupted so that T cells lacking the LYP-CSK complex remain hyper-reactive.

Targeting PD-1/PD-L1 in Vitiligo

Therapeutically enhancing immune checkpoint signaling on self-reactive T cells, making them anergic, seems a promising therapeutic option for vitiligo. Maio et al. (2018) studied PD-1/PD-L1 signaling as a therapeutic target for vitiligo and showed that treatment with PD-L1 fusion protein reversed depigmentation in a Pmel-1 vitiligo mouse model (56). Around 60% of the original pigment loss was restored upon treatment. PD-L1 fusion protein reversed depigmentation in Pmel-1 vitiligo mice *via* a marked increase in regulatory T cells in the skin and a decrease in melanocyte-reactive T cells. Fortunately, no significant side effects were observed, among which skin oncogenesis, indicating that targeting the PD-1/PD-L1 axis can be effective as a treatment strategy for T cell-induced vitiligo. The suppression of depigmentation was relatively stable until 8 weeks after last treatment, but, none of the mice regained permanent repigmentation. This limited (long-term) repigmentation in Pmel-1 vitiligo mice might be due to loss of a pigment cell reservoir, suggesting that the presence of a viable melanocyte reservoir might be crucial for the efficacy of PD-L1 fusion protein and other immune suppressive therapies. More specifically, human vitiligo lesions in hairless skin and those containing white hair do not have a strong capacity for repigmentation (57). PD-1/PD-L1 therapy, therefore, might be most effective in an early or progressive stage of vitiligo, supported by the observation that disease duration negatively affects prognosis to treatment (58). Nevertheless, patients with long-duration vitiligo may repigment well, as long as their lesions contain pigmented hair. Patients with active vitiligo might therefore be the best responders to immunomodulating agents, whereas patients with long-duration disease might need additional melanocyte transplantation (57).

Concomitantly, UV phototherapy, especially narrow-band ultraviolet B (NB-UVB) therapy, might be necessary to obtain more complete repigmentation. Among current topical and systemic treatment modalities, NB-UVB phototherapy has emerged as one of the safest and most effective therapies in vitiligo (59). Therapeutically enhancing immune checkpoint signaling may induce peripheral tolerance, yet melanocytes might still need additional stimulation to restore skin pigmentation. At the same time, NB-UVB therapy likely affects PD-L1 expression. UV-B radiation was shown to induce PD-L1 expression in human melanoma cell lines in an NF-κB-dependent manner (60). Similarly, UV-B treatment induced NF-κB activation in human primary epidermal melanocytes, implying PD-L1 upregulation is a conserved stress response to UV exposure in human melanocytes and melanoma cells that can inhibit effector T cell activity. Altogether, combined use of

local agonistic PD-1/PD-L1 treatment and NB-UVB therapy seems promising in inducing local melanocyte tolerance and significant repigmentation.

Better understanding of the PD-1/PD-L1 may provide clues on how this pathway can most effectively be targeted in vitiligo. Topical treatment modalities, optionally with laser assisted delivery, or local injection are preferred over systemic therapy, partly to avoid systemic tolerance. Vitiligo patients show local autoreactivity resulting in depigmented skin patches, while tolerance to melanocyte antigens seems to predominate in other parts of the skin, leaving those pigmented. The feasibility of local vitiligo treatment, however, depends on the vitiligo disease extent. At the same time, as mentioned before, therapy is preferably given at an early disease stage, to acquire full melanocyte tolerance, long-term efficacy and possibly curation.

Local immunotherapy might decrease immunosurveillance, carrying a potential risk for oncogenesis. Vitiligo patients, however, have a 3-fold less risk of developing melanoma during life (19, 20). We, therefore, hypothesize that agonistic PD-1/PD-L1 therapy will increase the probability of melanoma development to the risk of healthy, control individuals. Nonetheless, clinicians should be aware of potential tolerance to, e.g., transformed melanocytes or keratinocytes

DISCUSSION

Mounting evidence has shown that signaling *via* the PD-1/PD-L1 axis is commonly involved in melanoma immune evasion and targeting these immune checkpoint molecules can reactivate potent effector immune responses against the tumor. The growing appreciation of similarities between vitiligo (autoimmunity) and melanoma (tumor immunity), has led to increased interest in PD-1/PD-L1 as promising targets for future immunotherapies in human vitiligo.

Although not fully confirmed so far, PD-1/PD-L1 seems to be involved in loss of peripheral tolerance in human vitiligo, with PD-1 being expressed on regulatory and $CD8^+$ T cells, and *in vivo* PD-L1 protein therapy reversing depigmentation in murine vitiligo. At the same time, however, PD-1/PD-L1 expression on other cell types, among which dendritic cells (DCs), B cells, keratinocytes and fibroblasts, remain understudied and might be relevant. Recently, efficacy of anti-PD-1/PD-L1 therapy in cancer was attributed to PD-L1 expression on DCs, rather than tumor cells (61–64). PD-1/PD-L1 blockade enhanced maturation of DC in tumor-draining lymph nodes and increased T cell priming (61, 62). In **Figure 1**, we propose a model of how PD-1/PD-L1 signaling in vitiligo maintains an inflammatory environment.

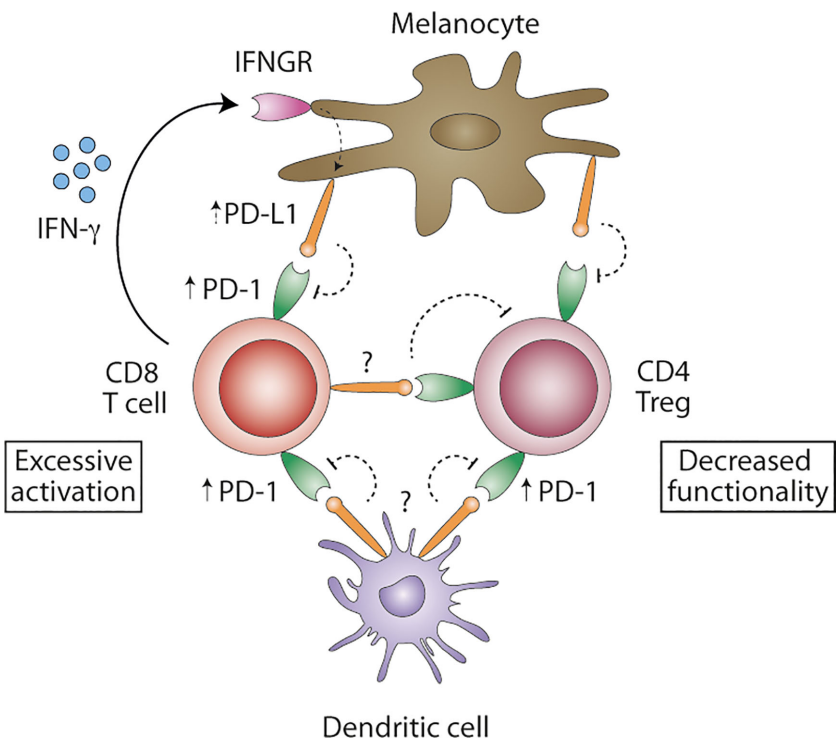


FIGURE 1 | Proposed model of PD-1/PD-L1 signaling in vitiligo. Blood regulatory $CD4^+$ and cytotoxic $CD8^+$ T cells show increased levels of PD-1 protein. IFN-γ-producing $CD8^+$ T cell are abundantly present in affected skin and may induce PD-L1 expression on melanocytes, albeit possibly ineffective in inhibiting autoreactive T cells. Consequently, melanocyte-reactive $CD8^+$ T cells remain activated. PD-L1 expression on DCs remains unstudied in vitiligo and might be important in effectively inhibiting autoreactive $CD8^+$ T cells. $PD\text{-}L1^+$ DC might inhibit not only T cell priming in the tumor-draining lymph nodes, but also T cell effector function. PD-L1 expression *in situ* remains unclear but might be expressed by melanocytes, DC or melanocyte-specific T cells, turning $PD\text{-}1^+$ regulatory $CD4^+$ T cells to functional exhaustion. Overcoming excessive activation of autoreactive $CD8^+$ T cells and decreased functionality of regulatory $CD4^+$ T cells in vitiligo might be achieved by interfering with PD-1/PD-L1 signaling.

Vitiligo patients show increased PD-1 expression on peripheral regulatory $CD4^+$ and cytotoxic $CD8^+$ T cells. Concomitantly, PD-1^+ mononuclear cells have been identified in (peri-)lesional vitiligo skin.

Though (peripheral) T cells have been demonstrated to be PD-1^+, PD-L1 expression remains largely understudied. PD-L1 can be expressed by melanocytes, especially in the case of inflammatory responses (43). IFN-γ-producing $CD8^+$ T cell are abundantly present in affected skin, but to date, (cytokine-induced) PD-L1 expression by melanocytes in (peri)lesional vitiligo skin remains unclear. If expressed, PD-$L1^+$ melanocytes seem to be ineffective in inhibiting melanocyte-specific T cells. Concomitantly, PD-L1 expression on myeloid cells, among which DC, in vitiligo skin has received no attention thus far. PD-$L1^+$ myeloid cells might be important in inhibiting autoreactive T cells as well. Future studies should clarify PD-L1 expression levels *in situ* to determine if these are diminished in human vitiligo and, thus, insufficiently inhibit autoreactive $CD8^+$ T cells. Finally, it has been hypothesized that PD-L1-expressing melanocyte-specific T cells cause exhaustion of PD-1^+ regulatory T cells, hence leading to uncontrolled autoimmunity (45). It is, therefore, important to study if PD-$L1^+$ cells decrease peripheral tolerance by inhibiting $CD4^+$ regulatory T cell function. Most importantly, however, are the PD-1 expression levels on skin-resident T cells, for skin autoimmunity appears locally and PD-L1-induced peripheral tolerance is only relevant when PD-1^+ T cells are present.

Given that PD-1 is expressed by both peripheral regulatory $CD4^+$ and cytotoxic $CD8^+$ T cells in vitiligo patients, we reasoned that agonistic PD-1/PD-L1 therapy might suppress melanocyte-reactive T cells, but concomitantly suppress regulatory T cells. Counterintuitively, injection of PD-L1 fusion protein in Pmel-1 vitiligo mice led to a marked enrichment of regulatory T cells in the skin and a reduction in effector T cells (56), implying PD-L1 negatively affects autoreactive T cells, but not regulatory T cells. PD-1 expression by regulatory T cells, however, was left unstudied in this mouse model. This suggests that effector T cells were the main PD-1^+ T cell population. Considering the presence of PD-1^+ $CD4^+$ regulatory T cells in blood of vitiligo patients, PD-L1 fusion protein might give different results in the human setting, justifying further research on the effect of increased PD-1/PD-L1 signaling on both T cell subsets to determine treatment outcome. Since topical or local treatment modalities are preferred, we hypothesize that PD-1 expression by skin-resident regulatory or memory T cells are most relevant for therapy outcome, rather than peripheral PD-1 expression.

With more knowledge becoming available on PD-1 and PD-L1 expression levels among different immune cell subsets, targeting this pathway might induce effective long-term melanocyte-specific tolerance in human vitiligo.

AUTHOR CONTRIBUTIONS

MW wrote the manuscript with input from all authors. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. *Lancet* (2015) 386(9988):74–84. doi: 10.1016/S0140-6736(14)60763-7
2. Palermo B, Campanelli R, Garbelli S, Mantovani S, Lantelme E, Brazzelli V, et al. Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. *J Invest Dermatol* (2001) 117(2):326–32. doi: 10.1046/j.1523-1747.2001.01408.x
3. Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res* (2003) 16(2):90–100. doi: 10.1034/j.1600-0749.2003.00023.x
4. 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(9):2220–32. doi: 10.1038/jid.2009.32
5. Kemp EH, Gavalas NG, Gawkrodger DJ, Weetman AP. Autoantibody responses to melanocytes in the depigmenting skin disease vitiligo. *Autoimmun Rev* (2007) 6(3):138–42. doi: 10.1016/j.autrev.2006.09.010
6. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-gamma for autoreactive CD8(+) T-cell accumulation in the skin. *J Invest Dermatol* (2012) 132(7):1869–76. doi: 10.1038/jid.2011.463
7. 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 (1):99–109. doi: 10.1111/j.1755-148X.2011.00920.x
8. Lin M, Zhang BX, Shen N, Dong XJ, Zhang C, Qi XY, et al. Regulatory T cells from active non-segmental vitiligo exhibit lower suppressive ability on CD8+ CLA+ T cells. *Eur J Dermatol* (2014) 24(6):676–82. doi: 10.1684/ejd.2014.2436
9. Dwivedi M, Kemp EH, Laddha NC, Mansuri MS, Weetman AP, Begum R. Regulatory T cells in vitiligo: Implications for pathogenesis and therapeutics. *Autoimmun Rev* (2015) 14(1):49–56. doi: 10.1016/j.autrev.2014.10.002
10. Cheuk S, Schlums H, Gallais Serezal I, Martini E, Chiang SC, Marquardt N, et al. CD49a Expression Defines Tissue-Resident CD8(+) T Cells Poised for Cytotoxic Function in Human Skin. *Immunity* (2017) 46(2):287–300. doi: 10.1016/j.immuni.2017.01.009
11. Boniface K, Jacquemin C, Darrigade AS, Dessarthe B, Martins C, Boukhedouni N, et al. Vitiligo Skin Is Imprinted with Resident Memory CD8 T Cells Expressing CXCR3. *J Invest Dermatol* (2018) 138(2):355–64. doi: 10.1016/j.jid.2017.08.038
12. Speeckaert R, van Geel N. Vitiligo: An Update on Pathophysiology and Treatment Options. *Am J Clin Dermatol* (2017) 18(6):733–44. doi: 10.1007/s40257-017-0298-5
13. Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, de Jong LA, Vyth-Dreese FA, et al. Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. *J Exp Med* (2003) 198 (4):569–80. doi: 10.1084/jem.20030590
14. Boasberg PD, Hoon DS, Piro LD, Martin MA, Fujimoto A, Kristedja TS, et al. Enhanced survival associated with vitiligo expression during maintenance biotherapy for metastatic melanoma. *J Invest Dermatol* (2006) 126(12):2658–63. doi: 10.1038/sj.jid.5700545
15. Gogas H, Ioannovich J, Dafni U, Stavropoulou-Giokas C, Frangia K, Tsoutsos D, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. *N Engl J Med* (2006) 354(7):709–18. doi: 10.1056/NEJMoa053007
16. Quaglino P, Marenco F, Osella-Abate S, Cappello N, Ortoncelli M, Salomone B, et al. Vitiligo is an independent favourable prognostic factor in stage III and IV

metastatic melanoma patients: results from a single-institution hospital-based observational cohort study. *Ann Oncol* (2010) 21(2):409–14. doi: 10.1093/annonc/mdp325

17. Teulings HE, Limpens J, Jansen SN, Zwinderman AH, Reitsma JB, Spuls PI, et al. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. *J Clin Oncol* (2015) 33(7):773–81. doi: 10.1200/JCO.2014.57.4756
18. Hua C, Boussemart L, Mateus C, Routier E, Boutros C, Cazenave H, et al. Association of Vitiligo With Tumor Response in Patients With Metastatic Melanoma Treated With Pembrolizumab. *JAMA Dermatol* (2016) 152(1):45–51. doi: 10.1001/jamadermatol.2015.2707
19. Teulings HE, Overkamp M, Ceylan E, Nieuweboer-Krobotova L, Bos JD, Nijsten T, et al. Decreased risk of melanoma and nonmelanoma skin cancer in patients with vitiligo: a survey among 1307 patients and their partners. *Br J Dermatol* (2013) 168(1):162–71. doi: 10.1111/bjd.12111
20. Paradisi A, Tabolli S, Didona B, Sobrino L, Russo N, Abeni D. Markedly reduced incidence of melanoma and nonmelanoma skin cancer in a nonconcurrent cohort of 10,040 patients with vitiligo. *J Am Acad Dermatol* (2014) 71(6):1110–6. doi: 10.1016/j.jaad.2014.07.050
21. Kim HS, Kim HJ, Hong ES, Kim KB, Lee JD, Kang TU, et al. The incidence and survival of melanoma and nonmelanoma skin cancer in patients with vitiligo: a nationwide population-based matched cohort study in Korea. *Br J Dermatol* (2020) 182(4):907–15. doi: 10.1111/bjd.18247
22. Teulings HE, Tjin EPM, Willemsen KJ, van der Kleij S, Ter Meulen S, Kemp EH, et al. Anti-Melanoma immunity and local regression of cutaneous metastases in melanoma patients treated with monobenzone and imiquimod; a phase 2 a trial. *Oncoimmunology* (2018) 7(4):e1419113. doi: 10.1080/2162402X.2017.1419113
23. Murakami N, Riella LV. Co-inhibitory pathways and their importance in immune regulation. *Transplantation* (2014) 98(1):3–14. doi: 10.1097/TP.0000000000000169
24. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* (2016) 16(5):275–87. doi: 10.1038/nrc.2016.36
25. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* (2002) 8(8):793–800. doi: 10.1038/nm730
26. Massi D, Brusa D, Merelli B, Ciano M, Audrito V, Serra S, et al. PD-L1 marks a subset of melanomas with a shorter overall survival and distinct genetic and morphological characteristics. *Ann Oncol* (2014) 25(12):2433–42. doi: 10.1093/annonc/mdu452
27. Capece D, Verzella D, Fischietti M, Zazzeroni F, Alesse E. Targeting costimulatory molecules to improve antitumor immunity. *J BioMed Biotechnol* (2012) 2012:926321. doi: 10.1155/2012/926321
28. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. *Nat Rev Clin Oncol* (2017) 14(8):463–82. doi: 10.1038/nrclinonc.2017.43
29. Wu X, Gu Z, Chen Y, Chen B, Chen W, Weng L, et al. Application of PD-1 Blockade in Cancer Immunotherapy. *Comput Struct Biotechnol J* (2019) 17:661–74. doi: 10.1016/j.csbj.2019.03.006
30. Belum VR, Benhuri B, Postow MA, Hellmann MD, Lesokhin AM, Segal NH, et al. Characterisation and management of dermatologic adverse events to agents targeting the PD-1 receptor. *Eur J Cancer* (2016) 60:12–25. doi: 10.1016/j.ejca.2016.02.010
31. Hwang SJE, Park JJW, Wakade D, Chou S, Byth K, Fernandez-Penas P. Cutaneous adverse events of anti-programmed death 1 antibodies combined with anti-cytotoxic T-lymphocyte-associated protein 4 therapy use in patients with metastatic melanoma. *Melanoma Res* (2019) 29(2):172–7. doi: 10.1097/CMR.0000000000000518
32. Rodriguez-Lomba E, Molina-Lopez I, Suarez-Fernandez R, Baniandres-Rodriguez O. Vitiligo-like lesions and immune checkpoint inhibition therapy: is it truly an adverse event exclusive to patients with melanoma? *Clin Exp Dermatol* (2018) 43(5):598–9. doi: 10.1111/ced.13382
33. Zarogoulidis P, Huang H, Tsiouda T, Sardeli C, Trakada G, Veletza L, et al. Immunotherapy "Shock" with vitiligo due to nivolumab administration as third line therapy in lung adenocarcinoma. *Respir Med Case Rep* (2017) 22:283–6. doi: 10.1016/j.rmcr.2017.10.006
34. Yin ES, Totonchy MB, Leventhal JS. Nivolumab-associated vitiligo-like depigmentation in a patient with acute myeloid leukemia: A novel finding. *JAAD Case Rep* (2017) 3(2):90–2. doi: 10.1016/j.jdcr.2016.10.008
35. Uenami T, Hosono Y, Ishijima M, Kanazu M, Akazawa Y, Yano Y, et al. Vitiligo in a patient with lung adenocarcinoma treated with nivolumab: A case report. *Lung Cancer* (2017) 109:42–4. doi: 10.1016/j.lungcan.2017.04.019
36. Nishino K, Ohe S, Kitamura M, Kunimasa K, Kimura M, Inoue T, et al. Nivolumab induced vitiligo-like lesions in a patient with metastatic squamous cell carcinoma of the lung. *J Thorac Dis* (2018) 10(6):E481–E4. doi: 10.21037/jtd.2018.05.104
37. Turner N, Leventhal J. Atezolizumab-associated vitiligo-like leukoderma in a patient with transitional cell carcinoma. *Int J Dermatol* (2018) 57(10):e90–e2. doi: 10.1111/ijd.14191
38. Hamid O, Molinero L, Bolen CR, Sosman JA, Munoz-Couselo E, Kluger HM, et al. Safety, Clinical Activity, and Biological Correlates of Response in Patients with Metastatic Melanoma: Results from a Phase I Trial of Atezolizumab. *Clin Cancer Res* (2019) 25(20):6061–72. doi: 10.1158/1078-0432.CCR-18-3488
39. Zamani MR, Aslani S, Salmaninejad A, Javan MR, Rezaei N. PD-1/PD-L and autoimmunity: A growing relationship. *Cell Immunol* (2016) 310:27–41. doi: 10.1016/j.cellimm.2016.09.009
40. Hirata S, Senju S, Matsuyoshi H, Fukuma D, Uemura Y, Nishimura Y. Prevention of experimental autoimmune encephalomyelitis by transfer of embryonic stem cell-derived dendritic cells expressing myelin oligodendrocyte glycoprotein peptide along with TRAIL or programmed death-1 ligand. *J Immunol* (2005) 174(4):1888–97. doi: 10.4049/jimmunol.174.4.1888
41. Ding H, Wu X, Wu J, Yagita H, He Y, Zhang J, et al. Delivering PD-1 inhibitory signal concomitant with blocking ICOS co-stimulation suppresses lupus-like syndrome in autoimmune BXSB mice. *Clin Immunol* (2006) 118(2-3):258–67. doi: 10.1016/j.clim.2005.10.017
42. Imai Y, Ayithan N, Wu X, Yuan Y, Wang L, Hwang ST. Cutting Edge: PD-1 Regulates Imiquimod-Induced Psoriasiform Dermatitis through Inhibition of IL-17A Expression by Innate gammadelta-Low T Cells. *J Immunol* (2015) 195(2):421–5. doi: 10.4049/jimmunol.1500448
43. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Sci Transl Med* (2012) 4(127):127ra37. doi: 10.1126/scitranslmed.3003689
44. Blank C, Kuball J, Voelkl S, Wiendl H, Becker B, Walter B, et al. Blockade of PD-L1 (B7-H1) augments human tumor-specific T cell responses in vitro. *Int J Cancer* (2006) 119(2):317–27. doi: 10.1002/ijc.21775
45. Tembhre MK, Parihar AS, Sharma VK, Sharma A, Chattopadhyay P, Gupta S. Alteration in regulatory T cells and programmed cell death 1-expressing regulatory T cells in active generalized vitiligo and their clinical correlation. *Br J Dermatol* (2015) 172(4):940–50. doi: 10.1111/bjd.13511
46. Franceschini D, Paroli M, Francavilla V, Videtta M, Morrone S, Labbadia G, et al. PD-L1 negatively regulates CD4+CD25+Foxp3+ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. *J Clin Invest* (2009) 119(3):551–64. doi: 10.1172/JCI36604
47. Rahimi A, Hossein-Nataj H, Hajheydari Z, Aryanian Z, Shayannia A, Ajami A, et al. Expression analysis of PD-1 and Tim-3 immune checkpoint receptors in patients with vitiligo; positive association with disease activity. *Exp Dermatol* (2019) 28(6):674–81. doi: 10.1111/exd.13952
48. Awad SS, Touni AA, Gabril MY. Expression of immune checkpoints in active nonsegmental vitiligo: a pilot study. *Int J Dermatol* (2020) 59(8):982–8. doi: 10.1111/ijd.14983
49. Jin Y, Birlea SA, Fain PR, Gowan K, Riccardi SL, Holland PJ, et al. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. *N Engl J Med* (2010) 362(18):1686–97. doi: 10.1056/NEJMoa0908547
50. Jin Y, Birlea SA, Fain PR, Mailloux CM, Riccardi SL, Gowan K, et al. Common variants in FOXP1 are associated with generalized vitiligo. *Nat Genet* (2010) 42(7):576–8. doi: 10.1038/ng.602
51. Jin Y, Birlea SA, Fain PR, Ferrara TM, Ben S, Riccardi SL, et al. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nat Genet* (2012) 44(6):676–80. doi: 10.1038/ng.2272
52. Jin Y, Andersen G, Yorgov D, Ferrara TM, Ben S, Brownson KM, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. *Nat Genet* (2016) 48(11):1418–24. doi: 10.1038/ng.3680

53. Gianchecchi E, Delfino DV, Fierabracci A. Recent insights into the role of the PD-1/PD-L1 pathway in immunological tolerance and autoimmunity. *Autoimmun Rev* (2013) 12(11):1091–100. doi: 10.1016/j.autrev.2013.05.003
54. Kemp EH, Ajjan RA, Waterman EA, Gawkrodger DJ, Cork MJ, Watson PF, et al. Analysis of a microsatellite polymorphism of the cytotoxic T-lymphocyte antigen-4 gene in patients with vitiligo. *Br J Dermatol* (1999) 140(1):73–8. doi: 10.1046/j.1365-2133.1999.02610.x
55. Canton I, Akhtar S, Gavalas NG, Gawkrodger DJ, Blomhoff A, Watson PF, et al. A single-nucleotide polymorphism in the gene encoding lymphoid protein tyrosine phosphatase (PTPN22) confers susceptibility to generalised vitiligo. *Genes Immun* (2005) 6(7):584–7. doi: 10.1038/sj.gene.6364243
56. Miao X, Xu R, Fan B, Chen J, Li X, Mao W, et al. PD-L1 reverses depigmentation in Pmel-1 vitiligo mice by increasing the abundance of Tregs in the skin. *Sci Rep* (2018) 8(1):1605. doi: 10.1038/s41598-018-19407-w
57. Frisoli ML, Harris JE. Vitiligo: Mechanistic insights lead to novel treatments. *J Allergy Clin Immunol* (2017) 140(3):654–62. doi: 10.1016/j.jaci.2017.07.011
58. Njoo MD, Spuls PI, Bos JD, Westerhof W, Bossuyt PM. Nonsurgical repigmentation therapies in vitiligo. Meta-analysis of the literature. *Arch Dermatol* (1998) 134(12):1532–40. doi: 10.1001/archderm.134.12.1532
59. Scherschun L, Kim JJ, Lim HW. Narrow-band ultraviolet B is a useful and well-tolerated treatment for vitiligo. *J Am Acad Dermatol* (2001) 44(6):999–1003. doi: 10.1067/mjd.2001.114752
60. Wang W, Chapman NM, Zhang B, Li M, Fan M, Laribee RN, et al. Upregulation of PD-L1 via HMGB1-Activated IRF3 and NF-kappaB Contributes to UV Radiation-Induced Immune Suppression. *Cancer Res* (2019) 79(11):2909–22. doi: 10.1158/0008-5472.CAN-18-3134
61. Mayoux M, Roller A, Pulko V, Sammicheli S, Chen S, Sum E, et al. Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy. *Sci Transl Med* (2020) 12(534):eaav7431. doi: 10.1126/scitranslmed.aav7431
62. Sun NY, Chen YL, Wu WY, Lin HW, Chiang YC, Chang CF, et al. Blockade of PD-L1 Enhances Cancer Immunotherapy by Regulating Dendritic Cell Maturation and Macrophage Polarization. *Cancers (Basel)* (2019) 11 (9):1400. doi: 10.3390/cancers11091400
63. Tang H, Liang Y, Anders RA, Taube JM, Qiu X, Mulgaonkar A, et al. PD-L1 on host cells is essential for PD-L1 blockade-mediated tumor regression. *J Clin Invest* (2018) 128(2):580–8. doi: 10.1172/JCI96061
64. Lin H, Wei S, Hurt EM, Green MD, Zhao L, Vatan L, et al. Host expression of PD-L1 determines efficacy of PD-L1 pathway blockade-mediated tumor regression. *J Clin Invest* (2018) 128(4):1708. doi: 10.1172/JCI120803

Conflict of Interest: CM was employed by ISA Pharmaceuticals.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 01 December 2020
doi: 10.3389/fimmu.2020.581433

Antigen Specificity Enhances Disease Control by Tregs in Vitiligo

Zhussipbek Mukhatayev[1,2,3,4], Emilia R. Dellacecca[1,2], Cormac Cosgrove[1,2], Rohan Shivde[1,2], Dinesh Jaishankar[1,2], Katherine Pontarolo-Maag[5], Jonathan M. Eby[5], Steven W. Henning[5], Yekaterina O. Ostapchuk[4], Kettil Cedercreutz[1], Alpamys Issanov[6], Shikhar Mehrotra[7], Andreas Overbeck[8], Richard P. Junghans[9], Joseph R. Leventhal[10] and I. Caroline Le Poole[1,2]**

[1] Department of Dermatology, Northwestern University, Chicago, IL, United States, [2] Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, United States, [3] Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan, [4] Laboratory of Molecular immunology and Immunobiotechnology, M.A. Aitkhozhin's Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan, [5] Oncology Research Institute, Loyola University, Maywood, IL, United States, [6] Department of Medicine, School of Medicine, Nazarbayev University, Nur-Sultan, Kazakhstan, [7] Department of Surgery, Medical University of South Carolina, Charleston, SC, United States, [8] Department for Surgery of Pigment Disorders, Lumiderm, Madrid, Spain, [9] Department of Hematology/Oncology, Boston University, Boston MA, United States, [10] Comprehensive Transplant Center, Northwestern Memorial Hospital, Chicago, IL, United States

OPEN ACCESS

Edited by:
Michele Marie Kosiewicz, University of Louisville, United States

Reviewed by:
Rasheedunnisa Begum, Maharaja Sayajirao University of Baroda, India
Jillian M. Richmond, University of Massachusetts Medical School, United States

***Correspondence:**
Zhussipbek Mukhatayev
zhussipbek.mukhatayev@nu.edu.kz
I. Caroline Le Poole
caroline.lepoole@northwestern.edu

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: 08 July 2020
Accepted: 27 October 2020
Published: 01 December 2020

Citation:
Mukhatayev Z, Dellacecca ER, Cosgrove C, Shivde R, Jaishankar D, Pontarolo-Maag K, Eby JM, Henning SW, Ostapchuk YO, Cedercreutz K, Issanov A, Mehrotra S, Overbeck A, Junghans RP, Leventhal JR and Le Poole IC (2020) Antigen Specificity Enhances Disease Control by Tregs in Vitiligo. Front. Immunol. 11:581433. doi: 10.3389/fimmu.2020.581433

Vitiligo is an autoimmune skin disease characterized by melanocyte destruction. Regulatory T cells (Tregs) are greatly reduced in vitiligo skin, and replenishing peripheral skin Tregs can provide protection against depigmentation. Ganglioside D3 (GD3) is overexpressed by perilesional epidermal cells, including melanocytes, which prompted us to generate GD3-reactive chimeric antigen receptor (CAR) Tregs to treat vitiligo. Mice received either untransduced Tregs or GD3-specific Tregs to test the hypothesis that antigen specificity contributes to reduced autoimmune reactivity *in vitro* and *in vivo*. CAR Tregs displayed increased IL-10 secretion in response to antigen, provided superior control of cytotoxicity towards melanocytes, and supported a significant delay in depigmentation compared to untransduced Tregs and vehicle control recipients in a TCR transgenic mouse model of spontaneous vitiligo. The latter findings were associated with a greater abundance of Tregs and melanocytes in treated mice versus both control groups. Our data support the concept that antigen-specific Tregs can be prepared, used, and stored for long-term control of progressive depigmentation.

Keywords: vitiligo, regulatory T cells, chimeric antigen receptor T cells, ganglioside D3, antigen-specific Treg, autoimmune diseases

INTRODUCTION

Vitiligo is an autoimmune disease wherein melanocytes are progressively destroyed, resulting in pale, white patches of skin (1, 2). The prevalence of vitiligo is 0.5–1%, and this disorder is associated with low self-esteem, psychological stress, and social stigma (3–5). Therapies currently available for vitiligo have limited efficacy, and a real need for new treatment strategies exists (6). Several factors contribute to the pathogenesis of vitiligo. Oxidative stress in melanocytes can trigger the release of inducible heat shock protein 70 (HSP70i) (7). HSP70i can directly activate innate immune cells and

chaperone melanocyte antigens for subsequent introduction to antigen presenting cells (APCs) (8, 9). In turn, these innate immune cells and APCs recruit auto-reactive T cells to mediate specific destruction of melanocytes in vitiligo (10). The loss of tolerance to self-antigens also involves reduced cutaneous regulatory T cell (Treg) activity (11, 12). Tregs are subsets of T cells responsible for peripheral tolerance *via* suppression of immune cells, including self-reactive, cytotoxic T cells, to maintain immune homeostasis (13). Paucity of and defects in Tregs have been reported in several autoimmune disorders (14–16). A local deficiency in Tregs was found in vitiligo skin within lesional, non-lesional, and perilesional sections, thereby suggesting a reduced ability of vitiligo patients to maintain peripheral immune homeostasis (17, 18). Others have reported that Tregs may be circulating in reduced number or exhibit reduced activity (16, 19). Limited Treg activity in vitiligo skin allows autoreactive, cytotoxic T cells to expand and eliminate melanocytes from the skin, facilitating progressive depigmentation (12).

To date, adoptive transfer of polyclonal Tregs has been used in clinical trials for several conditions wherein autologous cells were amplified *in vitro* and re-administered to patients, with limited success (20). In a mouse model of vitiligo, this approach was beneficial, by short term-maintenance of a favorable Treg to effector T cell ratio in this setting (21). Application of polyclonal Tregs can however impart systemic immunosuppression, *via* inadvertent dampening of responses to infection or malignancies, posing an important clinical consideration (22). Furthermore, generating sufficient numbers of polyclonal Tregs for clinical use can be challenging. Generic immunosuppression can present an issue that might be overcome by including antigen specificity to adoptively transferred cells. In transplant biology, antigen-specific Tregs are showing promise to provide local immunosuppression at the site of disease progression by targeting human leukocyte antigen (HLA) discrepancies between donor and recipient (23). Antigen-specific Tregs might offer significant advantages for the treatment of vitiligo as well.

Antigen-specific Tregs can be generated by introducing T-cell receptors (TCRs), but applications of these TCR-Tregs are limited by major histocompatibility complex (MHC)-restriction, not allowing all patients to benefit (24). Our study instead makes use of antigen-specific Tregs transduced to express a chimeric antigen receptor (CAR), which overcomes MHC-dependency. In CAR-T cell therapy, optimizing and selecting the correct CAR affinity and intracellular signaling domains is particularly important for the resulting therapeutic activity and cellular persistence of the Tregs. Since CARs are constructed using antibody variable regions, they hold higher affinity to their cognate antigen compared to TCRs (25). Also important is the identification of a cell surface antigen that is relatively abundant in the affected tissue under study and can be targeted by CAR Tregs. The concept is then that the selected surface antigen can serve as a target for antigen-specific CAR Tregs to selectively and locally suppress auto-reactive T cells in vitiligo (26). These results can be compared to those achieved using a tyrosinase-reactive TCR. The latter approach, tested in an immunodeficient melanoma model was the first to demonstrate activity for adoptively transferred, antigen-specific Tregs (27). Both the tyrosinase and HLA-A2 encoding-genes are associated with vitiligo disease development, rendering relevance to tyrosinase-reactive T cells, as well as to the associated HLA-A*0201 restriction (28). The same combination of TCR and MHC transgenes was used to generate the h3TA2 mouse model of vitiligo, used here to host our CAR transgenic Tregs (29). These mice develop rapid, continuous depigmentation with most differentiated pigment cell lost from the skin by 5 weeks of age (30). This aggressive model of disease sets a high bar for therapeutics to be effective and can provide a first estimate of efficacy for new therapeutics (30). The ganglioside D3 (GD3) antigen, meanwhile, holds relevance as GD3 expression is found in stressed melanocytes, and likely more prominently so in perilesional skin of vitiligo patients (31). This GD3 antigen is currently targeted for immunotherapy of melanoma as for its expression on membrane and accessibility to antibodies (32, 33). Besides, GD3 contributes to melanogenesis, cell growth, and cell dendricity (34, 35). To test the therapeutic potential of antigen-specific Tregs for the treatment of vitiligo, we isolated naïve T cells and polarized them towards Tregs before introducing a GD3-responsive CAR-encoding construct. Resulting cells were tested for cytokine secretion to relevant targets *in vitro*, and resulting cytotoxicity was measured. Finally, in a mouse model of progressive vitiligo, we have introduced the resulting CAR-transgenic Tregs to test their treatment potential *in vivo*. This includes evaluating depigmentation, and the persistence of Tregs and melanocytes in the skin. Adoptive transfer of antigen-specific Tregs may offer an exciting opportunity to halt depigmentation and to complement up-and-coming therapeutics such as modified inducible HSP70 to tolerize dendritic cells (DCs) and Janus kinase (JAK) inhibitors to mitigate T cell activation (36, 37).

MATERIALS AND METHODS

Study Design

The purpose of this study was to investigate the efficacy of GD3 CAR Tregs to halt progressing depigmentation in a mouse model of human vitiligo. We polarized and amplified Tregs *in vitro* before generating antigen-specific Tregs using a CAR construct to generate GD3 CAR Tregs with high transduction efficiencies. To define *in vitro* effects, we co-cultured human HLA-A2$^+$ melanocytes along with tyrosinase reactive effector T cells in the presence of untransduced Tregs and GD3 CAR Tregs. For *in vivo* efficacy, we adoptively transferred untransduced Tregs, GD3 CAR Tregs, and HBSS vehicle into a humanized mouse model prone to develop vitiligo, subjecting the animals to depigmentation analysis, while evaluating the local and distant effects of CAR Tregs by immunohistology and multiplex cytokine analysis. Human skin from vitiligo patients were stained for GD3 expression on lesional, perilesional, and non-lesional skin.

Sample sizes for *in vivo* experiments were determined based on statistical power calculations from previous studies and past experience with the h3TA2 mouse model of vitiligo. For *in vivo* studies, mice were randomly assigned to groups with regard to sex, and investigators were blinded to the experimental conditions and to the further analysis. All the experimental samples and animals were included in the analysis, with no exclusion of outliers. Sample sizes, replicates, and statistical methods are indicated in the results and in the figure legends.

Tissue Procurement, Cell Culture, and Reagents

Human perilesional skin tissue was obtained with informed consent from vitiligo patients attending the Dermatology clinic at Loyola University Medical Center in Maywood, IL (**Supplementary Table 1**). Studies were approved by the Institutional Review Board in adherence to principles described in the Declaration of Helsinki. Naïve mouse $CD4^+$ T cells and $CD4^+$ $FoxP3^+$ Tregs were cultured in RPMI media supplemented with 10% FBS, 1X non-essential amino acids (Corning), 50 U/ml Penicillin-Streptomycin (Thermo Fisher Scientific), 1 mM Sodium Pyruvate (Gibco Life Technologies), 10 mM HEPES (Gibco Life Technologies), and 50 μM β-Mercaptoethanol (Sigma Aldrich). Human melanocytes were cultured in Human Melanocyte Growth Supplement-2 (Thermo Fisher Scientific) added to Medium 254 (Thermo Fisher Scientific) with 10 mM L-Glutamine (Thermo Fisher Scientific) and 1X antibiotic-antimycotic (Thermo Fisher Scientific). Rabbit anti-GD3 CAR sera and stable GD3 CAR virus producing cells were generated as described (38). Stable GD3 CAR virus producing cells (VPCs) were maintained in the above described T cell medium for virus production.

Isolation of Naïve CD4+ T Cells and Polarization to CD4+FoxP3+ Tregs *In Vitro*

Naïve mouse $CD4^+$ T cells were isolated from spleens of 8–10 week-old B6.Cg-*Foxp3*^{tm2Tch}/J ("FoxP3 eGFP") reporter mice (Jackson Laboratories) using EasySep Mouse naive CD4+ T cell isolation kit (StemCell Technologies) following the manufacturer's protocol. These mice co-express enhanced green fluorescent protein (eGFP), which is restricted to the T cell lineage, primarily to the $CD4^+$ T cell population. Naïve $CD4^+$ T cells were polarized to $CD4^+$ $FoxP3^+$ using 30 ng/ml human transforming growth factor beta (TGF-β) (eBioscience) in the presence of Dynabeads™ Mouse T-Activator CD3/CD28 (Thermo Fisher Scientific) with a 1:1 bead to cell ratio and 300 international units/ml (IU/ml) of recombinant human interleukin 2 (rhIL-2) (NIH, Bethesda, MD) for 5 days. Human TGF-β was used to polarize murine Tregs as mouse and human TGF-β share 99% sequence homology with high cross-species activity (39, 40). Human IL-2 was used as human IL-2 efficiently stimulates mouse IL-2 receptor, whereas mouse IL-2 does not elicit efficient binding to human IL-2 receptors (41, 42).

Generation of GD3 CAR Transduced Mouse Tregs

Twenty-four well non-tissue culture plates were coated with 10 μg/ml retronectin (Takara Bio USA Inc.). The MFG retroviral vector-based second generation CAR construct (sFv-CD28/TCRζ) reactive to GD3 was generated as described (43). Conditioned medium supernatant from Phoenix E retroviral producer cells (43) consisting of GD3 CAR-encoding virus (80% confluent) (43, 44) was transferred to retronectin coated plates and centrifuged at 2,000xg. Supernatant was carefully removed and activated $CD4^+$ $FoxP3^+$ Tregs were transferred to the retronectin coated plates with additional viral supernatant, 5 μg/ml protamine sulfate (Sigma Aldrich) and 300 IU/mL rhIL2. Plates were centrifuged at 1,000xg, before an incubation with complete T cell culture medium and mouse T-Activator CD3/CD28 beads and rhIL-2 as above. The transduction was then repeated to increase the transduction efficiency. Transduced Tregs were reactivated with CD3/CD28 beads, 30 ng/ml human TGF-β and rhIL-2 for 2 days before flow analysis.

Flow Cytometry

Prior to surface staining, cells were incubated with mouse Fc Block (BioLegend) and LIVE/DEAD Fixable Near Infrared Dead Cell dye (Thermo Fisher Scientific) according to the manufacturer's instructions. Surface staining of directly labeled antibodies included BUV395-labeled anti-mouse CD3 clone 145-2C11 (BD Biosciences), and BV421-labeled anti-mouse CD4 clone GK1.5 (BioLegend). The eGFP marker expressed under the FoxP3 promoter in Treg reporter mice, as well as BB700- labeled rat anti-mouse CD25 clone PC61 (BD Biosciences) were used to identify Tregs. Unlabeled anti-GD3 CAR rabbit sera detected by anti-rabbit allophycocyanin (APC) (Invitrogen) antibodies were used to evaluate CAR expression by transgenic Tregs. Stained cells were analyzed using a BD FACSymphony flow cytometer and FlowJo v10.3.0 software (FlowJo LLC, OR, USA).

In Vitro Co-Culture Experiments

HLA-$A2^+$ melanocytes were identified by immunofluorescent staining using FITC-labeled BB7.2 to human HLA-A2 prior to *in vitro* co-culture experiments. Human HLA-$A2^+$ neonatal foreskin melanocytes (Mf0887, P6) and HLA-A2- abdominoplastic skin melanocytes (Ms18001, P6) were plated with tyrosinase reactive h3T effector T cells (Teffs) (29) and either untransduced or GD3 CAR-transduced suppressor Tregs at 10:1:1 effector to target to suppressor ratio for 36 h. Teff : Tregs ratio was used to mimic the natural occurrence of the T cell subsets as Tregs comprise 5–10% of the total T cell population Co-cultures were seeded in triplicates and incubated using IncuCyte® Caspase-3/7 Red Apoptosis Assay Reagent (Sartorius). Images were taken every 3 h, in triplicate, using the IncuCyte live-cell analysis system (Sartorius). Supernatants were saved for mouse IFN-γ (R&D systems, Minnesota, MN) and IL-10 ELISA assay (Mabtech AB, Stockholm, Sweden) performed according to the manufacturer's protocols. Cytotoxicity was examined by quantifying live cells

relative to control wells using Adobe Photoshop (Adobe Systems, San Jose, CA).

Adoptive Treg Transfer

Transgenic h3TA2 recipient mice with T cells expressing a TCR reactive to the human tyrosinase 368-376 (YMDTMSQV) epitope (21) were maintained under protocols approved by Northwestern University's Institutional Animal Care and Use Committee (IACUC) following guidelines for the care and use of laboratory animals as outlined by the US National Research Council. Mice were retro-orbitally administered $2x10^5$ untransduced Tregs/per animal (n = 11; 6♂, 5♀) or $2x10^5$ GD3 CAR Tregs/per animal (n = 11; 6♂, 5♀), or treated with vehicle (HBSS) alone (n = 12; 6♂, 6♀) four times, every two weeks, starting at 5 weeks of age. The number of adoptively transferred Tregs was identified to enable a comparison to our earlier studies (21), where the $2x10^5$ polyclonal Tregs controlled depigmentation in in the human Tyrosinase TCR Transgenic-HLA-A2 (h3TA2) mouse model between 3–9 weeks old mice. All groups received low dose of recombinant human IL-2 (3,000 IU) 3 times a week throughout the entire experiment to promote *in vivo* stimulation of adoptively transferred Tregs (45, 46). Animals were maintained for 15 weeks and humanely euthanized. Experiments were initiated at 6 different time points, including mice from different litters. Naïve T cells were polarized and transduced to generate CAR Treg for up to 4 mice/group at a time. Depigmentation was monitored for each experiment over time, with results pooled to further substantiate the results. Skin biopsies, spleen, brain, ileum, lymph nodes were maintained in optimal cutting temperature (OCT), and serum was stored for cytokine analysis.

Depigmentation Analysis

From 5 weeks to 15 weeks of age, mice were scanned weekly on a flatbed scanner (Hewlett-Packard, Palo Alto, CA) under isoflurane anesthesia. Using Adobe Photoshop software (Adobe Systems) ventral and dorsal luminosity was measured to calculate depigmentation, as previously described (47). Depigmentation was graphed over time, and statistical significance was determined by the time-adjusted area under the curve (AUC). Representing change in depigmentation from treatment initiation was calculated using the trapezoidal rule. No imputation was done for missing data, and the AUC for each mouse was divided by the total number of weeks of available data minus 1. The Wilcoxon Rank Sum (WRS) test was used to compare the time-adjusted AUC among groups.

Immunohistology

Mouse and human skin samples were frozen using OCT Compound (Sakura Finetek) on dry ice. Eight μm cryosections were cut (Leica). For FoxP3/CD3 staining, sections were paraformaldehyde-fixed and permeabilized using True-Nuclear Transcription factor buffer set (BioLegend). Sections were treated with SuperBlock (ScyTek Laboratories, Logan, UT). PE-labeled antibody 145-2C11 to mouse CD3ϵ (Biolegend) and Alexa Fluor 488-labeled antibody MF-14 to mouse FoxP3 (BioLegend) were used for double staining procedures, followed by 4′,6-diamidino-2-phenylindole (DAPI) (BD Biosciences) nuclear staining. For other tissue stainings, mouse and human skin sections were fixed in cold acetone. Mouse skin sections were blocked with SuperBlock and then incubated with either antibody H-90 to TRP-1 (Santa Cruz Biotechnology, Dallas, TX) followed by Alexa Fluor 555 labelled donkey anti-rabbit antibody (abcam), or PE-labeled MB3.6 to GD3 (Santa Cruz Biotechnology), or PE-labeled antibody YGITR 765 to Glucocorticoid-Induced TNF Receptor (GITR) (Biolegend), or AF488-labeled antibody B56 to Ki67 (BD Biosciences), all followed by DAPI nuclear staining. Human skin sections were blocked with 10% normal human serum (Gemini Bio Products, West Sacramento, CA) and then incubated with Ta99 to TRP-1 (BioLegend) or R24 to GD3 (Abcam, Cambridge, UK). Both were detected by an HRP-conjugated goat anti-mouse IgG antibody (Agilent Dako, Santa Clara, CA). These stainings were developed using AEC substrate (Abcam) and nuclei were subsequently detected by incubation in Mayer's hematoxylin (Sigma-Aldrich) and blued in Scott's tap water (Sigma-Aldrich). Slides were imaged on a Revolve microscope (Echo Laboratories). Cells were quantified using Adobe Photoshop software.

Cytokine Analysis

Included in cytokine analysis were supernatants from *in vitro* suppression assays (IncuCyte experiments), collected 36 h post-co-culture, and serum samples from HBSS vehicle (n = 11), untransduced (n = 10), and GD3 CAR Tregs (n = 9) treated mouse groups. Detection of murine Interferon gamma (IFN-γ), tumor necrosis factor (TNF-α), IL-4, and IL-10 was performed by using a custom made V-Plex panel for these mouse cytokines (Meso Scale Diagnostics, LLC) according to manufacturer's instructions. Data were acquired on MESO Quickplex SQ120 (Meso Scale Diagnostics, LLC) and analyzed using Prism version 8.3.0 (GraphPad Software).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad) and R-software. Data are presented as bars and dot plots with mean values ± standard deviation. The data were evaluated by one-way analysis of variance (ANOVA) analysis of variance accounting for different variances across the treatment groups, with post-hoc Tukey-Kramer comparisons. To determine statistical significance for immunosuppression *in vitro*, two-way ANOVAs were used with aligned rank transformation followed by multiple pairwise comparison testing using Tukey approach. For depigmentation, the time-adjusted AUC, representing change in depigmentation from treatment initiation, was calculated using the trapezoidal rule. No imputation was done for missing data, and the AUC for each mouse was divided by the total number of weeks of available data minus 1. The WRS test was used to compare the time-adjusted AUC among groups. Statistical significance is represented as *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, or **** $p < 0.0001$.

RESULTS

GD3 Is Expressed by Perilesional Epithelial Cells Including Melanocytes

Overexpression of O-acetylated GD3 has been reported for actively depigmenting vitiligo skin (31, 38). This prompted us to evaluate GD3 expression itself in skin biopsies from perilesional biopsies taken from actively depigmenting skin. Marked expression of GD3 was observed in human vitiligo perilesional epidermis (**Figure 1A**), while melanocytes are absent from the border biopsy section shown (**Figure 1B**). Vacuolization in **Figure 1A** and **1B** is frequently observed in vitiligo skin, and has been proposed as an indication of vitiligo by others (48, 49). Epidermal GD3 expression was not observed in healthy control skin (**Figure 1C**) whereas melanocytes are readily detectable in this tissue (**Figure 1D**). Similarly, GD3 expression was found in depigmenting h3TA2 mouse skin (**Figure 1E**).

High Viral Transduction of Tregs Was Achieved With GD3-Encoded CAR Construct

To generate therapeutic Tregs that will engage in suppressive activity where needed, we generated FoxP3$^+$CD4$^+$ T cells and transduced them to express a GD3-reactive CAR. In a representative example, approximately 1.5x10^6 naïve CD4$^+$ T cells were isolated from 3x10^8 splenocytes, maintained in presence of TGF-β, and successfully polarized and amplified to approximately 1.6x10^7 Tregs per donor mouse. TGF-β-polarized naïve CD4$^+$ T cells were retrovirally transduced and GD3 CAR expression was evaluated by flow cytometry. The gating strategy is shown in **Figure 2A**, where 86.6% of total CD4$^+$ T cells were successfully transduced with the GD3 CAR construct (**Figure 2B**). After further expansion, 64 ± 3.5% transduced cells were FoxP3$^+$ Tregs. From an initial pre-expansion and transduction pool of 4x10^6 FoxP3$^+$ Tregs, 2.1x10^7 GD3 CAR-expressing, FoxP3$^+$ Tregs were generated. The majority of resulting CAR transduced Tregs are expected to function as immunosuppressive T cells, and exert a local, immunosuppressive function. We next measured GD3 CAR Treg function *in vitro*.

Antigen Specificity Increases Immunosuppressive Cytokine Production

Production of representative cytokines IFN-γ, TNF-α, IL-4, and IL-10, relevant to immune activation or immunosuppression, was measured in co-cultures of GD3 CAR Tregs or untransduced Tregs with tyrosinase-reactive Teffs and their HLA-matched targets (1:10:1), measuring concentrations 42 h after cells were combined in culture in presence of IL-2 (**Figure 3**). Human melanocytes can be recognized by these Teffs (29). No significant differences in IFN-γ production were found in combinations that do or do not contain Tregs, suggesting that the latter had little influence on the production of this cytokine at this Treg to Teff ratio (**Figure 3A**). Significantly more TNF-α (**Figure 3B**, p = 0.0005), IL-4 (**Figure 3C**, p = 0.03), and IL-10 (**Figure 3D**, p = 0.0005) was produced in combinations with CAR Tregs, though overall IL-4 production remained consistently low. Importantly, increased IL-10 regulatory cytokine production was observed only in presence of cytotoxic T cells and HLA-matched human

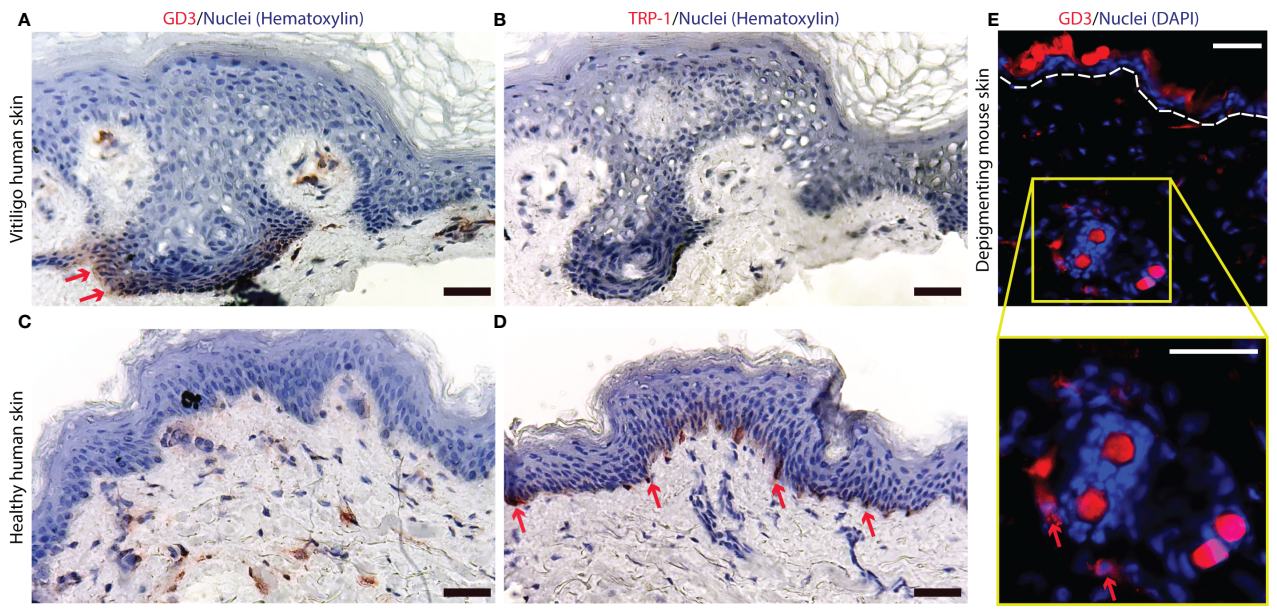


FIGURE 1 | Ganglioside D3 is expressed in depigmenting skin of human and mouse origin. **(A)** Expression of GD3 and **(B)** a lack of melanocytes, as represented by an absence of TRP-1+ staining, were found in human depigmenting epidermis from perilesional patient skin. Whereas **(C)** limited GD3 expression and **(D)** abundant TRP-1 expression are shown in healthy control skin. **(E)** In depigmenting mouse skin, GD3 expressing cells were observed around hair follicles and in proximity to the epidermis. Representative staining of GD3 expressing cells is shown in red with nuclei in blue, around autofluorescent mouse hairs (Scale bar = 50μm).

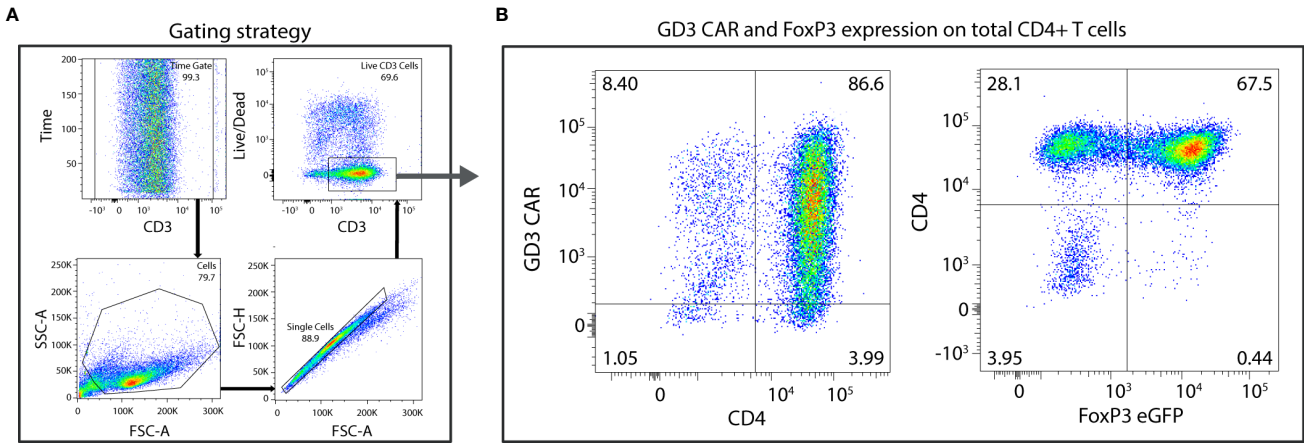


FIGURE 2 | High transduction efficiencies are observed for Tregs expressing the GD3 CAR. $CD4^+FoxP3^+$ cells, polarized from naïve $CD4^+$ T cells, were transduced using a GD3 CAR-encoding construct. **(A)** The gating strategy consists of a time gate followed by sequentially gating on lymphocytes, single cells, and live cells. **(B)** Eighty-six percent of total $CD4^+$ T cells were successfully transduced to express the GD3 CAR construct and 67% of that population express $FoxP3^+$.

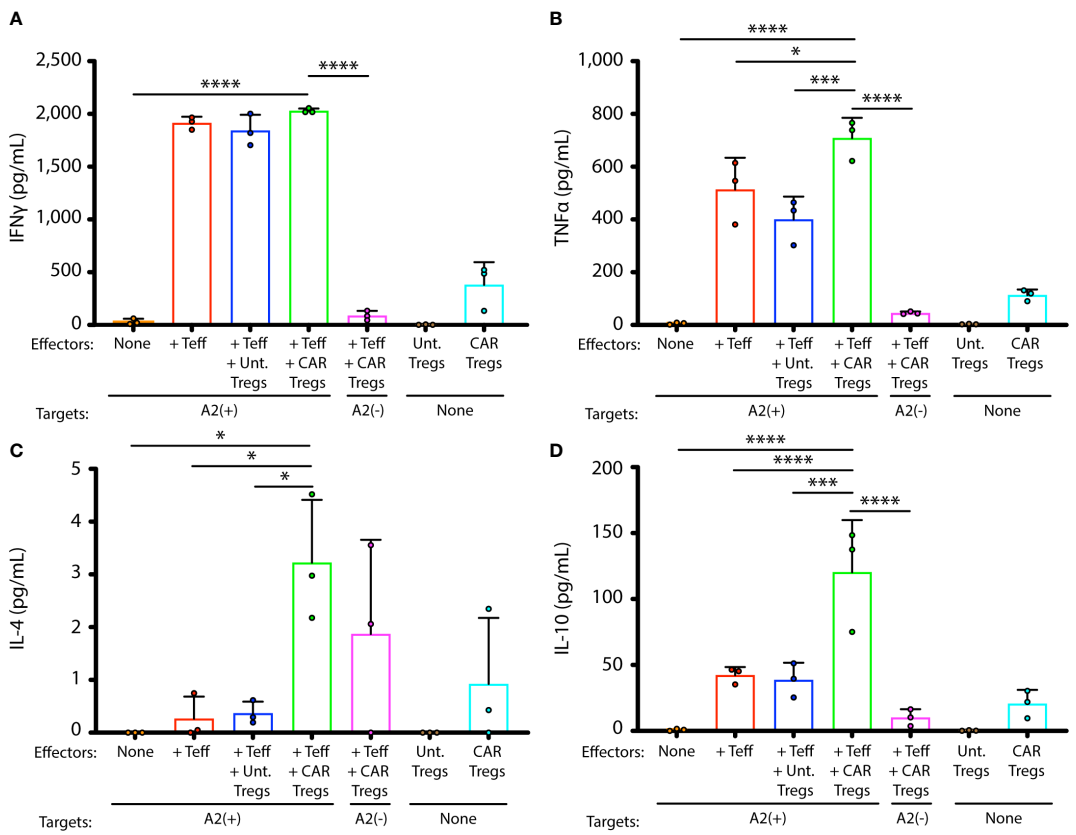


FIGURE 3 | GD3 CAR Tregs generate immunosuppressive cytokines in presence of activated T cells. Cytokines were measured in supernatants from cocultures of melanocyte targets and HLA-A2-restricted Teffs, in presence and absence of untransduced or CAR-transduced Tregs. Cytokine concentrations for each coculture, measured in triplicate cocultures, are shown for **(A)** IFN-γ, **(B)** TNF-α, **(C)** IL-4, and **(D)** IL-10. Statistical analysis was performed by a one-way ANOVA test followed by Tukey's post-hoc test for multiple comparisons. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

melanocytes. Taken together, the cytokine environment suggests a greater immunosuppressive ability in presence of antigen specific Tregs, stimulated by activated effector T cells. To measure whether this cytokine environment might translate to greater protection of melanocyte target cells from cell death *in vitro*, we next measured sustained target cell viability in these cocultures of melanocytes, Teff and Tregs.

Antigen Specificity Increases the Immunosuppressive Activity of Tregs *In Vitro*

Tregs suppress conventional T cells *via* cytokines, by cell-to-cell contact or through bystander effects (50). To measure the resulting suppressive activity, we evaluated sustained melanocyte viability in co-cultures of targets, Teffs, and Tregs *in vitro* for 36 h. **Figure 4A** shows the viability of targeted HLA-A2$^+$ human melanocytes in different combinations of targets, Teffs and Tregs 1:10:1. The number of viable targets increased slightly over time in absence of Teff cells. In comparison, 82.2% cytotoxicity ($p < 0.0001$) was observed in presence of effector T cells after 36 h. Untransduced Tregs offered 35.8% ($p = 0.02$) protection from cytotoxicity over time. A two-way ANOVA was performed with aligned rank transformation using R-software, and pairwise post-hoc multiple comparison testing according to Tukey to determine that in presence of CAR Tregs, cytotoxicity towards melanocytes was 62.0% prevented ($p = 0.0004$). Images representing each combination of cells including targets alone (**Figure 4B**), targets and Teff (**Figure 4C**), and the latter combination in presence of untransduced Tregs (**Figure 4D**) or CAR Tregs (**Figure 4E**) at different time points likewise reveal most inhibition of cytotoxicity in a combination that includes GD3 CAR Tregs. Thus, both untransduced Tregs and GD3 CAR Tregs offered significant protection of melanocyte viability. Importantly, the protection offered by GD3 CAR Tregs was significantly greater compared to untransduced Tregs ($p = 0.04$), demonstrating the added benefit of antigen specificity to enhance immunosuppression. Thus, we next explored the therapeutic effects of CAR Tregs *in vivo*.

Antigen-Specific Tregs Enhance Immunosuppression in h3TA2 Mice

To evaluate the suppressive activity of CAR Treg in a model of progressive depigmentation, we measured depigmentation in spontaneously depigmenting h3TA2 mice starting from 5 weeks of age. Depigmentation starts shortly after birth and the animals display half-maximum depigmentation within 23 weeks (51). Mice received adoptively transferred untransduced Tregs, transduced GD3 CAR Tregs or vehicle once every two weeks for 11 weeks as outlined in **Figure 5A**. Representative dorsal and ventral images of animals transfused with untransduced Tregs, GD3 CAR Tregs, or vehicle are shown in **Figure 5B**. The Wilcoxon rank sum (WRS) test was used to compare the time-adjusted area under the curve (AUC) among groups. Outcomes for both vehicle and untransduced Treg control groups did not differ (dorsal $p = 0.97$, ventral $p = 0.88$). Therefore, the vehicle and untransduced Treg groups were merged, and compared to the GD3 CAR Treg-treated group. In a one-sided t-approximation for

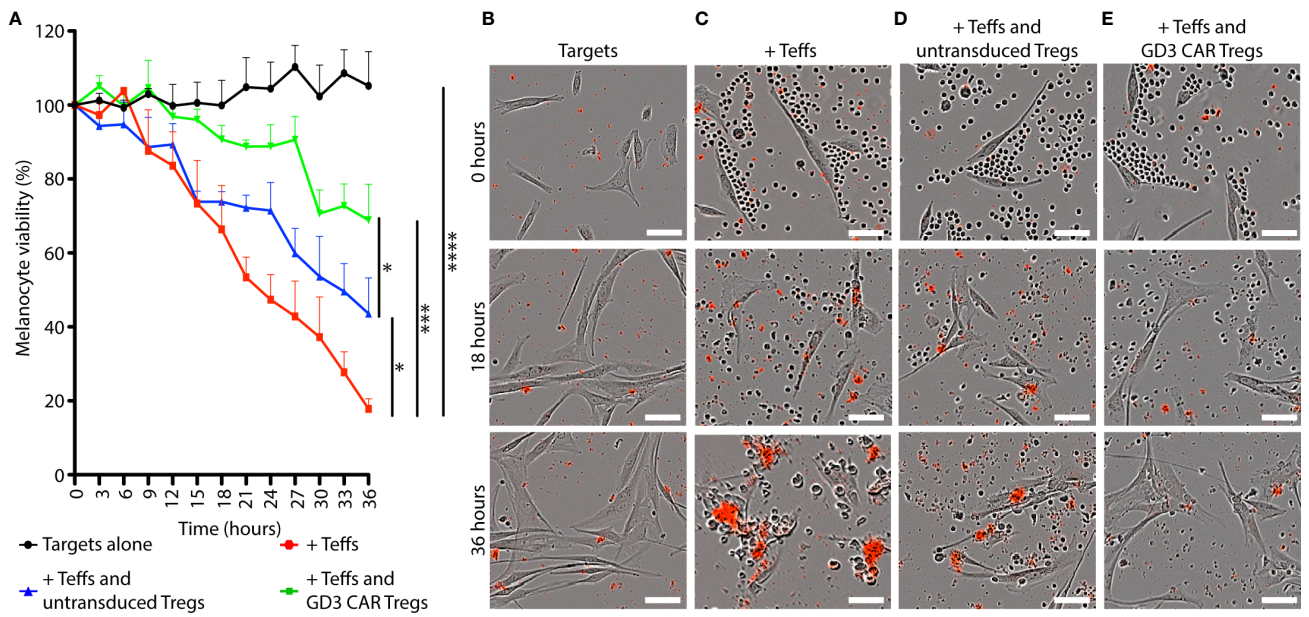


FIGURE 4 | GD3 CAR Tregs provide melanocytes with superior protection from T cell-mediated cytotoxicity *in vitro*. The immunosuppressive ability of GD3 CAR Tregs and untransduced Tregs was compared *in vitro*. **(A)** Viability of HLA-A2$^+$ human melanocytes (targets) in the presence or absence of murine Teffs and Tregs (1:10:1) is represented over time. Representative images of **(B)** HLA-A2$^+$ human melanocytes **(C)** combined with murine Teffs, and additionally with **(D)** murine untransduced Tregs, or **(E)** GD3 CAR Tregs. Dead cells are marked by a red precipitate formed by caspase activity. Statistical significance was determined by two-way ANOVA with aligned rank transformation followed by Tukey's pairwise multiple comparisons test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (Scale bar = 50μm).

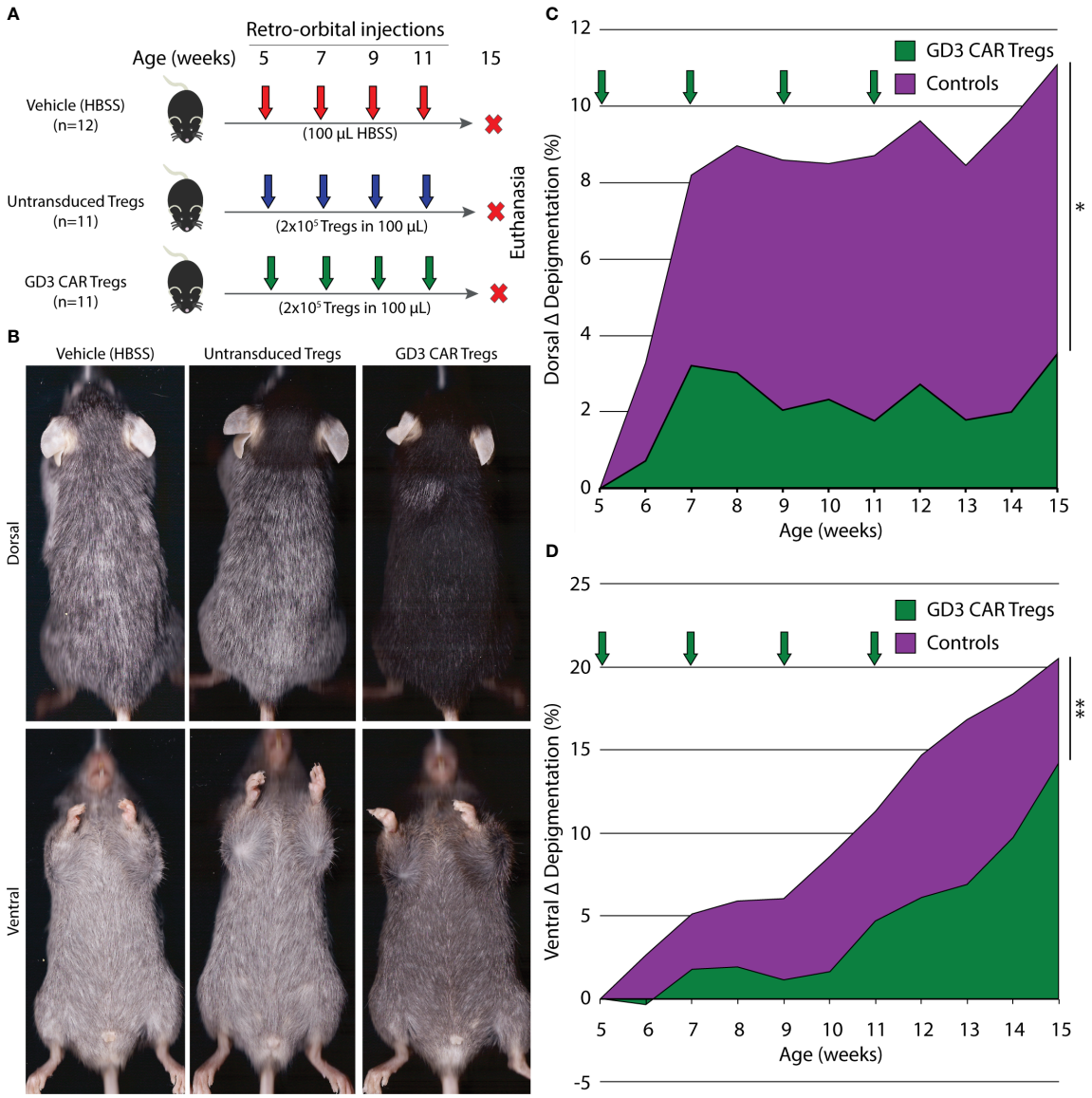


FIGURE 5 | GD3 CAR Tregs provide significant protection from depigmentation in vitiligo-prone mice. **(A)** Experimental outline initiated at 6 different time points showing vitiligo prone, h3TA2, mice treated with vehicle alone (n = 12), or by adoptive transfer of untransduced Tregs (n = 11) or GD3 CAR Tregs (n = 11). Adoptive transfer started at 5 weeks of age and continued biweekly until 11 weeks of age. Depigmentation was measured weekly from 5–15 weeks of age. **(B)** Representative dorsal and ventral scans of mice from the HBSS vehicle, untransduced Treg, and GD3 CAR Treg treated groups at 15 weeks of age. **(C)** Depigmentation quantified on dorsal and **(D)** ventral sides throughout the experiment. The Wilcoxon rank sum (WRS) test was used to compare the time-adjusted AUC among groups. Arrows: treatment times. *p < 0.05; **p < 0.01.

the WRS test, the AUC for dorsal depigmentation dropped by 73.0% (p = 0.028) for CAR Treg treated mice (n = 11) for the 15-week observation period. Ventral depigmentation occurs more rapidly and was evaluated separately. Here, depigmentation was significantly delayed among the CAR Treg treated group (n = 11) over the follow-up period (**Figure 5C**) resulting in a 60.5% reduction in the AUC (p = 0.006) among CAR Treg treated mice (**Figure 5D**). Individual dorsal and ventral depigmentation values for each mouse are shown in the supplementary data file (**Supplementary Figure 1**). The enhanced disease control by CAR Tregs might be due to local activation of suppressive activity by GD3 expression and the presence of activated Teff on site. To assess this, changes in serum cytokine content for IFN-γ, TNF-α, IL-4, and IL-10 were measured in serum samples from mice treated with vehicle alone (n = 11), untransduced Tregs (n = 10), or GD3 CAR Tregs (n = 9). Resulting cytokine levels were remarkably consistent among the groups at end point (**Supplementary Figure 2**). The results support the concept that Tregs, including CAR Tregs, may be preferentially activated on site in areas of immune activity. Adverse events were not observed

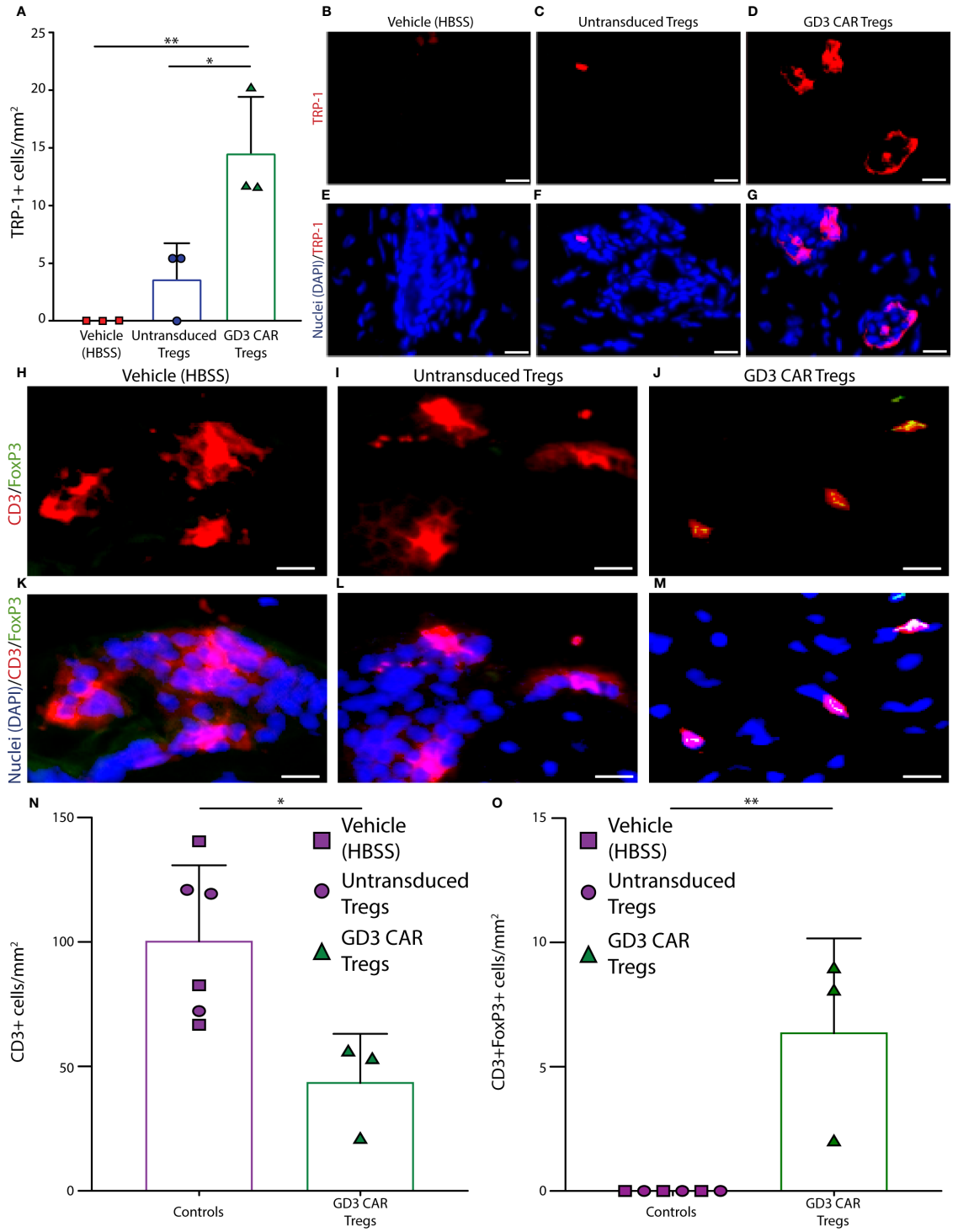


FIGURE 6 | Melanocytes are protected from h3T cytotoxic T cells in the presence of GD3 reactive CAR Tregs. Mouse skin was evaluated for melanocyte presence using antibodies to TRP-1. **(A)** Quantification of melanocytes, as well as accompanying representative images of TRP-1 staining from **(B)** HBSS vehicle, **(C)** untransduced Tregs, and **(D)** GD3 CAR Tregs treated mice (n = 3 per group), with **(E–G)** the respective overlay including DAPI nuclear staining in blue is shown. Mouse skin tissues were also evaluated for T cell infiltration using antibodies to CD3ϵ and FoxP3, and examples of staining in skin from **(H)** vehicle control, **(I)** untransduced Tregs, and **(J)** GD3 CAR Tregs administered mice are shown. Representative samples were used to quantify $CD3\epsilon^+$ T cells (red), $FoxP3^+$ cells (green), and double positive Tregs; **(K–M)** respective overlays with DAPI (blue) are also shown. Quantification of skin staining ± SD (*n* = 3 per group) for **(N)** T cells and **(O)** Tregs are shown, respectively. Statistical analysis was performed by non-parametric t tests. *p < 0.05, **p < 0.001 (Scale bar = 20 μm).

throughout the experiment, and no abnormalities were found during internal organ examination at euthanasia for mice from any groups. We next probed whether reduced depigmentation was accompanied by a sustained presence of melanocytes and changes in T cell populations.

Melanocytes Are Protected in the Presence of GD3 Reactive CAR Tregs

Mouse dorsal skin biopsies were evaluated for melanocyte abundance using antibodies to TRP-1, as shown in **Figure 6**. Melanocytes were quantified as shown in **Figure 6A**, where skin samples from vehicle treated mice (n = 3 per group) showed complete loss of melanocytes. Skin from untransduced Treg treated mice (n = 3 per group) displayed only a few remaining melanocytes, and a one-way ANOVA was performed followed by Tukey's post-hoc test to demonstrate that whereas skin from CAR Treg treated mice contained a significantly greater number of melanocytes compared to mice treated with untransduced Tregs ($p = 0.025$), and to vehicle treated controls ($p = 0.006$). Representative images of TRP-1 staining for vehicle-treated, untransduced Treg-treated, and CAR Treg-treated mice are shown in **Figures 6B-D**, and overlaid with DAPI nuclear staining in **Figures 6E–G**, respectively. Similar results were found when examining GD3 expression. Quantification of GD3 expressing cells revealed that mice transfused with CAR-Tregs maintained significantly more GD3 expressing cells than the vehicle HBSS-treated mice ($p = 0.003$) or mice transfused with untransduced Treg ($p = 0.003$) (**Supplementary Figure 3**). This observation supports the concept that GD3 expressing cells did not experience the cytotoxicity observed in vehicle-treated or untransduced Treg treated mice. This confirmatory melanocyte quantification mainly corresponds with *in vivo* data shown in **Figure 6**, demonstrating the improved suppressive ability of CAR Tregs. To explain the differences in pigmentation and melanocyte maintenance, we next compared these data to Treg infiltration in each treatment group.

CAR Tregs Gravitate Towards GD3 Expressing Cells in the Skin

To understand whether Treg activity is correlated to the abundance of immunosuppressive T cells on site, mouse skin tissues were evaluated for T cell infiltration using antibodies to CD3ϵ and FoxP3. Examples of skin from the vehicle control group, and samples from the mice treated with untransduced or CAR Treg-treated mice are also shown in **Figure 6**. Tregs were identified as $CD3\epsilon^+$ $FoxP3^+$ cells for the same groups in **Figures 6H–J**, respectively, overlaid with DAPI nuclear staining in **Figures 6K–M**. $CD3\epsilon^+$ cell and $CD3\epsilon^+/FoxP3^+$ Treg abundance was quantified as the mean ± SD (at n = 3 per group) for each treatment group. In a one-way ANOVA followed by Tukey's post-hoc test, the average number of infiltrating $CD3\epsilon^+$ T cells at end point was 2.3-fold greater ($p = 0.02$) in the control groups as compared to the CAR Treg treated group (**Figure 6N**). No (remaining) $CD3^+FoxP3^+$ Tregs were detected in either control group, whereas some $CD3\epsilon^+$ $FoxP3^+$ Tregs were still detectable in skin tissue from CAR Treg treated mice 10 weeks after adoptive transfer (**Figure 6O**). Evaluating Treg numbers by GITR-expression, an increase in Treg numbers at end point was again observed in skin from

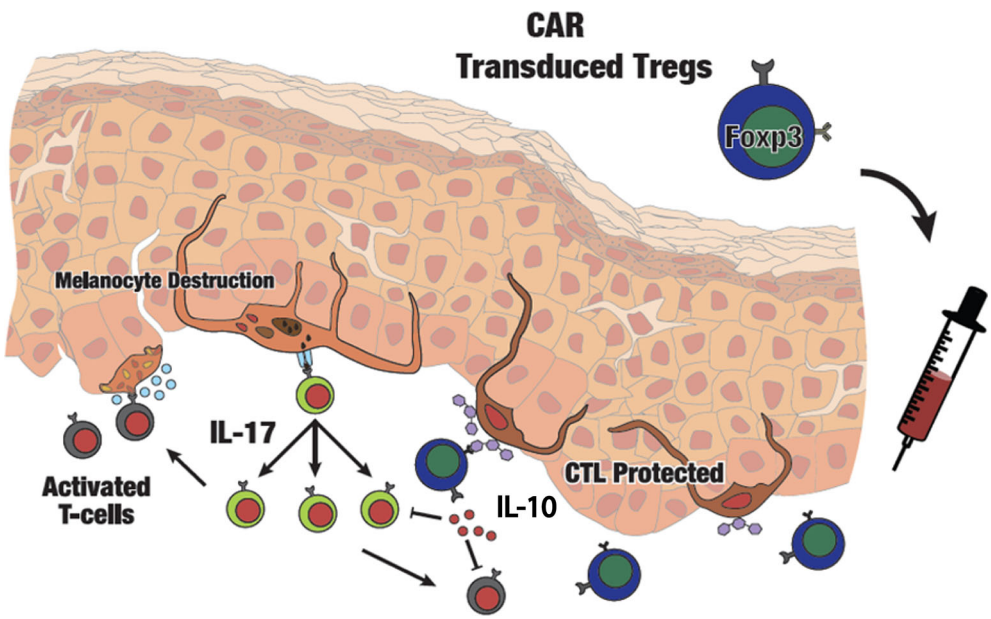


FIGURE 7 | Schematic presentation of adoptive transfer of CAR Tregs in vitiligo. Autoimmune melanocyte destruction is mediated by cytotoxic T cells, which are activated *via* self-antigens secreted by stressed melanocytes. Elevated IL-17 promotes an inflammatory environment in the skin. Infused GD3-specific CAR Tregs will infiltrate the skin and respond to GD3 expressed at the site of autoimmune activity, suppressing cytotoxic T cells on site and providing local immune tolerance in vitiligo perilesional skin.

CAR Treg treated mice compared to those treated with untransduced Tregs ($p = 0.0059$) or vehicle alone ($p = 0.0089$), yet there was no difference in abundance of proliferating $GITR^+Ki67^+$ cells among groups. This suggest that differences in Treg abundance may instead be defined by increased influx or decreased efflux of Tregs from the skin in CAR Treg treated mice (**Supplementary Figure 4**). Nevertheless, the increased abundance of Tregs in CAR Treg treated mice at end point may explain the improved suppressive activity by CAR Tregs and suggests that maintenance of a Treg presence on site is supported by local antigen recognition (**Figure 7**). In summary, the data show that antigen specificity prolonged the suppressive activity of adoptively transferred Tregs.

DISCUSSION

Here we describe the therapeutic potential of engineered GD3 CAR Tregs to provide antigen-specific immune tolerance for autoimmune vitiligo. In contrast to conventional immunosuppressive drugs, biologics, alkylating agents, and antimetabolites, Tregs can provide greater specificity with complex therapeutic benefits, and restore immune tolerance in various autoimmune diseases (20). Preclinical studies have provided promising results when using polyclonal $CD4^+CD25^+FoxP3^+$ Tregs to enforce immune tolerance in various mouse models, including vitiligo (21). A robust human Treg isolation protocol was established to sort Tregs with >90% purity, and an *ex vivo* expansion protocol was developed to acquire upwards of $3x10^9$ Tregs from a single donor, similar to the protocol used here (52). Overall, this and other phase I clinical studies provided answers to the isolation, and expansion that surrounded Treg immunotherapy (53–56). Yet the efficacy of polyclonal Treg transfer is not self-evident to date.

In vitiligo, some studies reported no significant differences in circulating Treg numbers (18, 57), whereas others report a difference in Treg abundance between vitiligo patient and healthy blood (15, 58). Whereas circulating Treg numbers may vary, more consistency is found among reports of a local deficiency of Tregs in patient skin, which might be a cause for uncontrolled peripheral immune responses and progressive depigmentation in vitiligo (12, 18, 57).

Adoptive transfer of islet-specific Tregs outperformed polyclonal Tregs for blocking type 1 diabetes progression compared to polyclonal Tregs (20, 59, 60). Unfortunately, diabetes is generally detected in patients when pancreas destruction is near complete, and other conditions may be more amenable to Treg based treatment in a clinical setting. Preclinical studies likewise suggest a superior efficacy of antigen-specific Tregs in transplantation procedures (61–64).

In the h3TA2 mouse model of vitiligo, new Teff are continuously attacking melanocytes, thus reflecting continuously active disease. In these mice, Tregs might be effective for the duration of treatment whereas depigmentation will return when adoptive transfer is halted. Indeed, adoptive Treg treatment is expected to be most efficacious during active disease. In human patients however, progressive disease periods are interspersed with periods of inactivity, providing melanocyte stem cells with an opportunity to differentiate and repopulate the depigmented lesions. Repigmentation can occur in patients treated with JAK inhibitors to suppress Teff activity, though supportive treatment by UV light may be required (65). For intermittent treatment of vitiligo, it will be beneficial to store autologous GD3 CAR Tregs for later use (66).

By virtue of their antigen specificity, these Tregs might provide better safety profiles and decrease the risk of generalized immunosuppression. This is supported by increased IL-10 secretion found where Tregs function in presence of activated Teff, as shown here. When comparing Tregs expressing first and second generation CARs, cells expressing a second-generation CAR with a 28ζ costimulatory domain the greatest amount of IL-10 (67). The CAR Treg construct used to transduce the Tregs included in our paper similarly showed significant IL-10 production upon activation through the CAR, which might explain the improved suppressive activity of these GD3 CAR Tregs compared to untransduced Tregs *in vitro*. Indeed, TCR and CAR transduction may also produce more potent and stable Tregs for *in vivo*, clinical use in vitiligo, and this condition holds an important advantage for investigating the superiority of antigen-specific Tregs by offering several target antigens associated with the condition (68). TNF-α was significantly elevated both in culture and in serum of experimental mice. Nevertheless, genetic ablation of TNF-α was not correlated with the development of vitiligo, and no difference in depigmentation was found when compared to wild type h3TA2 mice (21). In vitiligo, TNF-α plays a role in the development cytotoxic T cells (CTLs) and enhances expression of IFN-γ, which are implicated in imitation of vitiligo development (21, 69, 70). Recent studies reports that TNF-α might promote anti-inflammatory conditions *via* activation and induction of Treg proliferation *in vivo* (71–73). Thus, TNF-α can potentially be both destructive and protective in vitiligo, by promoting CTLs and stimulating Tregs, respectively. While TNF-α depletion halted the disease progression and promoted repigmentation in vitiligo, 18 of 5,928 patients developed vitiligo *de novo* when TNF-α inhibitors were administered for other autoimmune disorders. This leaves anti-TNF-α treatment option for vitiligo until side effects are fully averted (74).

We thus asked whether antigen-specific T regs might provide additional benefit for the treatment of the autoimmune disease vitiligo. This condition holds a complex etiology (2), with melanocyte loss as a common denominator and immune mechanisms held universally responsible for the spread of disease. TCR- and CAR-based Tregs possess different mechanistic and functional properties. Low-antigen expression levels are sufficient for TCR-based Tregs to become activated, whereas CAR-based Tregs require high density of antigens (75, 76) suggesting that TCRs or CARs could be selected based on antigen expression by target tissue. We performed repeat Treg injections, because the mice in our model exhibit chronically active disease and we do not yet know how long transgenic Tregs remain active on site. The long-term fate of adoptively

transferred Treg has yet to be established to better understand the need for repeated applications. Importantly, CARs are not subjected to HLA restriction and hold a higher affinity for their targets moieties (77). Thus, a CAR construct can find a universal application for patients with progressive disease.

We identified a potential target for antigen-specific Tregs in vitiligo. Our GD3 CAR Tregs protect melanocytes from T cell-mediated destruction in a mouse model of vitiligo, expressing a human TCR and matched human MHC, and capable of responding to human target cells. Importantly, the antigen of choice does not need to be expressed by target cells spared in the response themselves (78).

Though no side effects were apparent in our current studies during internal organ examination, some safety concerns remain, including the possible development of cytokine release syndrome (CRS) or neurotoxicity if transduced cells ultimately develop an effector profile (79). CRS is however more likely to develop when targeting liquid tumors than in solid tissues (80). To counter any potential side effects and promote safety, a construct that includes a caspase-based suicide gene can be incorporated in order to inactivate the GD3 CAR Tregs if necessary (81, 82).

A limitation to intravenous injection of antigen specific Tregs might be that these much-needed immunosuppressive cells display a paucity at the desired site (60). Should systemically applied Tregs not respond as required, local injection might be needed, or the CCR4 Treg homing receptor ligand CCL22, can be introduced where Tregs are needed to attract systemically applied Tregs (17). This leaves autoimmune diseases of the skin especially suited for adoptive treatment by antigen-specific Tregs when relevant antigens can be identified. In fact, GD3 might support keratinocyte proliferation while O-acetylated GD3 was overexpressed in psoriatic skin (83, 84). These findings suggest that GD3 CAR Tregs might temper lesions in the latter condition as well.

To date, only alloantigen-reactive Tregs are currently being tested to prevent rejection after organ transplantation in clinical trials (60). Here we prepared and expanded GD3 reactive CAR Tregs in amounts suited for adoptive cell transfer therapy in mice. The same can be pursued in patients, cryopreserving the therapeutic Tregs for future use (85–88). One of the challenges of adoptive transfer is the cost and scalability of the technique. This has prompted the concept of developing off-the-shelf "Universal CAR Tregs" readily adaptable for all patients. Versatility is provided by modules that bind both the universal CAR and the target cell (89). In summary, the data provided here support the use of antigen-specific CAR Tregs as an adoptive cell therapy for vitiligo, to control depigmentation and support immune tolerance in vitiligo.

Treg infusion has thus far been well tolerated in patients. The results from currently ongoing clinical trials can bring important insights regarding the optimal Treg dose, expected efficacy and any concern that may arise about possible side effects, and the treatment strategy can be further adjusted to support continued maintenance and memory formation (90), improved homing (91), and safety measures to eliminate adoptively transferred cells (92) as needed. The efficacy and specificity of Treg therapy for conditions other than vitiligo can be enhanced where antigens can be identified to serve as targets for engineered, antigen-specific Tregs. *In vivo* tracking will allow research groups to better understand the maintenance and memory-forming potential of antigen-specific Tregs. In conclusion, Treg-based therapy holds potential as a future therapy for vitiligo and for other autoimmune skin diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by Northwestern University's Institutional Animal Care and Use Committee. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Conceptualization: CLP and ZM. Methodology: ZM, CLP, CC, and DJ. Formal Analysis, ZM. Investigation, ZM, ERD, JME, and KP-M. Statistical analysis, AI and KC. Resources, RPJ, SM and AO. Writing—Original Draft, ZM. Writing—Review and Editing, ZM, CLP, ERD, YOO and JRL. Visualization, ZM, ERD, JME and SWH. Supervision, CLP. Project Administration, CLP. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported in part by NIH RO1s AR057643, CA191317, and by The LAM Foundation through an Established Investigator award to CLP. A foreign internship to ZM was supported by the Ministry of Education and Science of the Republic of Kazakhstan under the Ph.D. program at Al-Farabi Kazakh National University.

ACKNOWLEDGMENTS

We kindly acknowledge NCI Biological Resources Branch for providing rhIL-2 used for *in vivo* studies. We greatly appreciate the patients who provide informed consent to use the skin tissues for the study, and Northwestern University Skin Biology and Diseases Resource-Based Center (SBDRC) TEST IT core for the technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.581433/full#supplementary-material

REFERENCES

1. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. *Lancet (London England)* (2015) 386(9988):74–84. doi: 10.1016/s0140-6736(14)60763-7
2. Kundu RV, Mhlaba JM, Rangel SM, Le Poole IC. The convergence theory for vitiligo: A reappraisal. *Exp Dermatol* (2019) 28(6):647–55. doi: 10.1111/exd.13677
3. Henning SW, Jaishankar D, Barse LW, Dellacecca ER, Lancki N, Webb K, et al. The relationship between stress and vitiligo: Evaluating perceived stress and electronic medical record data. *PloS One* (2020) 15(1):e0227909. doi: 10.1371/journal.pone.0227909
4. Grimes PE, Miller MM. Vitiligo: Patient stories, self-esteem, and the psychological burden of disease. *Int J women's Dermatol* (2018) 4(1):32–7. doi: 10.1016/j.ijwd.2017.11.005
5. Osinubi O, Grainge MJ, Hong L, Ahmed A, Batchelor JM, Grindlay D, et al. The prevalence of psychological comorbidity in people with vitiligo: a systematic review and meta-analysis. *Br J Dermatol* (2018) 178(4):863–78. doi: 10.1111/bjd.16049
6. Frisoli ML, Essien K, Harris JE. Vitiligo: Mechanisms of Pathogenesis and Treatment. *Annu Rev Immunol* (2020) 38:621–48. doi: 10.1146/annurev-immunol-100919-023531
7. Mosenson JA, Flood K, Klarquist J, Eby JM, Koshoffer A, Boissy RE, et al. Preferential secretion of inducible HSP70 by vitiligo melanocytes under stress. *Pigm Cell melanoma Res* (2014) 27(2):209–20. doi: 10.1111/pcmr.12208
8. Tulic MK, Cavazza E, Cheli Y, Jacquel A, Luci C, Cardot-Leccia N, et al. Innate lymphocyte-induced CXCR3B-mediated melanocyte apoptosis is a potential initiator of T-cell autoreactivity in vitiligo. *Nat Commun* (2019) 10(1):2178. doi: 10.1038/s41467-019-09963-8
9. Jacquemin C, Rambert J, Guillet S, Thiolat D, Boukhedouni N, Doutre MS, et al. Heat shock protein 70 potentiates interferon alpha production by plasmacytoid dendritic cells: relevance for cutaneous lupus and vitiligo pathogenesis. *Br J Dermatol* (2017) 177(5):1367–75. doi: 10.1111/bjd.15550
10. Le Poole IC, Wankowicz-Kalinska A, van den Wijngaard RM, Nickoloff BJ, Das PK. Autoimmune aspects of depigmentation in vitiligo. *J Invest Dermatol Symp Proc* (2004) 9(1):68–72. doi: 10.1111/j.1087-0024.2004.00825.x
11. Bhardwaj S, Rani S, Kumaran MS, Bhatia A, Parsad D. Expression of Th17- and Treg-specific transcription factors in vitiligo patients. *Int J Dermatol* (2020) 59(4):474–81. doi: 10.1111/ijd.14766
12. Le Poole IC, Mehrotra S. Replenishing Regulatory T Cells to Halt Depigmentation in Vitiligo. *J Invest Dermatol Symp Proc* (2017) 18(2):S38–s45. doi: 10.1016/j.jisp.2016.10.023
13. Levings MK, Sangregorio R, Roncarolo MG. 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(11):1295–302. doi: 10.1084/jem.193.11.1295
14. Zhang X, Olsen N, Zheng SG. The progress and prospect of regulatory T cells in autoimmune diseases. *J Autoimmun* (2020) 111:102461. doi: 10.1016/j.jaut.2020.102461
15. 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. *Pigm Cell Melanoma Res* (2013) 26(4):586–91. doi: 10.1111/pcmr.12105
16. Giri PS, Dwivedi M, Laddha NC, Begum R, Bharti AH. Altered expression of nuclear factor of activated T cells, forkhead box P3, and immune-suppressive genes in regulatory T cells of generalized vitiligo patients. *Pigm Cell melanoma Res* (2020) 33(4):566–78. doi: 10.1111/pcmr.12862
17. Eby JM, Kang HK, Tully ST, Bindeman WE, Peiffer DS, Chatterjee S, et al. CCL22 to Activate Treg Migration and Suppress Depigmentation in Vitiligo. *J Invest Dermatol* (2015) 135(6):1574–80. doi: 10.1038/jid.2015.26
18. Klarquist J, Denman CJ, Hernandez C, Wainwright DA, Strickland FM, Overbeck A, et al. Reduced skin homing by functional Treg in vitiligo. *Pigm Cell melanoma Res* (2010) 23(2):276–86. doi: 10.1111/j.1755-148X.2010.00688.x
19. Hegab DS, Attia MA. Decreased Circulating T Regulatory Cells in Egyptian Patients with Nonsegmental Vitiligo: Correlation with Disease Activity. *Dermatol Res Pract* (2015) 2015:145409. doi: 10.1155/2015/145409
20. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation regulatory T cell therapy. *Nat Rev Drug Discovery* (2019) 18(10):749–69. doi: 10.1038/s41573-019-0041-4
21. 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(5):1285–94. doi: 10.1038/jid.2013.540
22. Serra P, Santamaria P. Nanoparticle-based autoimmune disease therapy. *Clin Immunol (Orlando Fla)* (2015) 160(1):3–13. doi: 10.1016/j.clim.2015.02.003
23. Mathew JM, Voss JH, McEwen ST, Konieczna I, Chakraborty A, Huang X, et al. Generation and Characterization of Alloantigen-Specific Regulatory T Cells For Clinical Transplant Tolerance. *Sci Rep* (2018) 8(1):1136. doi: 10.1038/s41598-018-19621-6
24. Dawson NAJ, Levings MK. Antigen-specific regulatory T cells: are police CARs the answer? *Trans Res J Lab Clin Med* (2017) 187:53–8. doi: 10.1016/j.trsl.2017.06.009
25. Oren R, Hod-Marco M, Haus-Cohen M, Thomas S, Blat D, Duvshani N, et al. Functional comparison of engineered T cells carrying a native TCR versus TCR-like antibody-based chimeric antigen receptors indicates affinity/avidity thresholds. *J Immunol* (2014) 193(11):5733–43. doi: 10.4049/jimmunol.1301769
26. Abbott RC, Cross RS, Jenkins MR. Finding the Keys to the CAR: Identifying Novel Target Antigens for T Cell Redirection Immunotherapies. *Int J Mol Sci* (2020) 21(2):515. doi: 10.3390/ijms21020515
27. 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(7):e11726. doi: 10.1371/journal.pone.0011726
28. Jin Y, Ferrara T, Gowan K, Holcomb C, Rastrou M, Erlich HA, et al. Next-generation DNA re-sequencing identifies common variants of TYR and HLA-A that modulate the risk of generalized vitiligo via antigen presentation. *J Invest Dermatol* (2012) 132(6):1730–3. doi: 10.1038/jid.2012.37
29. Mehrotra S, Al-Khami AA, Klarquist J, Husain S, Naga O, Eby JM, et al. A coreceptor-independent transgenic human TCR mediates anti-tumor and anti-self immunity in mice. *J Immunol (Baltimore Md 1950)* (2012) 189(4):1627–38. doi: 10.4049/jimmunol.1103271
30. Mosenson JA, Zloza A, Nieland JD, Garrett-Mayer E, Eby JM, Huelsmann EJ, et al. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. *Sci Trans Med* (2013) 5(174):174ra28. doi: 10.1126/scitranslmed.3005127
31. Le Poole IC, Stennett LS, Bonish BK, Dee L, Robinson JK, Hernandez C, et al. Expansion of vitiligo lesions is associated with reduced epidermal CDw60 expression and increased expression of HLA-DR in perilesional skin. *Br J Dermatol* (2003) 149(4):739–48. doi: 10.1046/j.1365-2133.2003.05539.x
32. Kemminer SE, Conradt HS, Nimtz M, Sagi D, Peter-Katalinić J, Diekmann O, et al. Production and molecular characterization of clinical phase i anti-melanoma mouse IgG3 monoclonal antibody R24. *Biotechnol Prog* (2001) 17(5):809–21. doi: 10.1021/bp010068y
33. Kniep B, Flegel WA, Northoff H, Rieber EP. CDw60 glycolipid antigens of human leukocytes: structural characterization and cellular distribution. *Blood* (1993) 82(6):1776–86. doi: 10.1182/blood.V82.6.1776.1776
34. Nakano J, Yasui H, Lloyd KO, Muto M. Biologic roles of gangliosides G(M3) and G(D3) in the attachment of human melanoma cells to extracellular matrix proteins. *J Invest Dermatol Symp Proc* (1999) 4(2):173–6. doi: 10.1038/sj.jidsp.5640204
35. Birklé S, Gao L, Zeng G, Yu RK. Down-regulation of GD3 ganglioside and its O-acetylated derivative by stable transfection with antisense vector against GD3-synthase gene expression in hamster melanoma cells: effects on cellular growth, melanogenesis, and dendricity. *J Neurochem* (2000) 74(2):547–54. doi: 10.1046/j.1471-4159.2000.740547.x
36. Karagaiah P, Valle Y, Sigova J, Zerbinati N, Vojvodic P, Parsad D, et al. Emerging drugs for the treatment of vitiligo. *Expert Opin emerging Drugs* (2020) 25(1):7–24. doi: 10.1080/14728214.2020.1712358
37. Henning SW, Fernandez MF, Mahon JP, Duff R, Azarafrooz F, Guevara-Patino JA, et al. HSP70iQ435A-Encoding DNA Repigments Vitiligo Lesions in Sinclair Swine. *J Invest Dermatol* (2018) 138(12):2531–9. doi: 10.1016/j.jid.2018.06.186
38. Wu X, Ragupathi G, Panageas K, Hong F, Livingston PO. Accelerated tumor growth mediated by sublytic levels of antibody-induced complement activation is associated with activation of the PI3K/AKT survival pathway. *Clin Cancer Res* (2013) 19(17):4728–39. doi: 10.1158/1078-0432.ccr-13-0088

39. Abnaof K, Mallela N, Walenda G, Meurer SK, Seré K, Lin Q, et al. TGF-β stimulation in human and murine cells reveals commonly affected biological processes and pathways at transcription level. *BMC Syst Biol* (2014) 8(1):55. doi: 10.1186/1752-0509-8-55
40. Tsang ML, Zhou L, Zheng BL, Wenker J, Fransen G, Humphrey J, et al. Characterization of recombinant soluble human transforming growth factor-beta receptor type II (rhTGF-beta sRII). *Cytokine* (1995) 7(5):389–97. doi: 10.1006/cyto.1995.0054
41. Arkin MR, Randal M, DeLano WL, Hyde J, Luong TN, Oslob JD, et al. Binding of small molecules to an adaptive protein–protein interface. *Proc Natl Acad Sci* (2003) 100(4):1603. doi: 10.1073/pnas.252756299
42. Arenas-Ramirez N, Woytschak J, Boyman O. Interleukin-2: Biology, Design and Application. *Trends Immunol* (2015) 36(12):763–77. doi: 10.1016/j.it.2015.10.003
43. Lo AS, Ma Q, Liu DL, Junghans RP. Anti-GD3 chimeric sFv-CD28/T-cell receptor zeta designer T cells for treatment of metastatic melanoma and other neuroectodermal tumors. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2010) 16(10):2769–80. doi: 10.1158/1078-0432.Ccr-10-0043
44. Yun CO, Nolan KF, Beecham EJ, Reisfeld RA, Junghans RP. Targeting of T lymphocytes to melanoma cells through chimeric anti-GD3 immunoglobulin T-cell receptors. *Neoplasia (New York NY)* (2000) 2(5):449–59. doi: 10.1038/sj.neo.7900108
45. Furlan SN, Singh K, Lopez C, Tkachev V, Hunt DJ, Hibbard J, et al. IL-2 enhances ex vivo–expanded regulatory T-cell persistence after adoptive transfer. *Blood Adv* (2020) 4(8):1594–605. doi: 10.1182/bloodadvances.2019001248
46. Wang J, Zhang S-X, Hao Y-F, Qiu M-T, Luo J, Li Y-Y, et al. The numbers of peripheral regulatory T cells are reduced in patients with psoriatic arthritis and are restored by low-dose interleukin-2. *Ther Adv chronic Dis* (2020) 11:2040622320916014–. doi: 10.1177/2040622320916014
47. Denman CJ, McCracken J, Hariharan V, Klarquist J, Oyarbide-Valencia K, Guevara-Patiño JA, et al. HSP70i Accelerates Depigmentation in a Mouse Model of Autoimmune Vitiligo. *J Invest Dermatol* (2008) 128(8):2041–8. doi: 10.1038/jid.2008.45
48. Faria AR, Tarlé RG, Dellatorre G, Mira MT. Castro CCSd. Vitiligo - Part 2 - classification, histopathology and treatment. *Anais Brasileiros Dermatol* (2014) 89:784–90. doi: 10.1590/abd1806-4841.20142717
49. Wagner RY, Luciani F, Cario-André M, Rubod A, Petit V, Benzekri L, et al. Altered E-Cadherin Levels and Distribution in Melanocytes Precede Clinical Manifestations of Vitiligo. *J Invest Dermatol* (2015) 135(7):1810–9. doi: 10.1038/jid.2015.25
50. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. *Front Immunol* (2012) 3:51:51. doi: 10.3389/fimmu.2012.00051
51. 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. *Pigm Cell melanoma Res* (2014) 27 (6):1075–85. doi: 10.1111/pcmr.12284
52. Putnam AL, Brusko TM, Lee MR, Liu W, Szot GL, Ghosh T, et al. Expansion of human regulatory T-cells from patients with type 1 diabetes. *Diabetes* (2009) 58(3):652–62. doi: 10.2337/db08-1168
53. Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci Transl Med* (2015) 7(315):315ra189. doi: 10.1126/scitranslmed.aad4134
54. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Techmanska I, Juscinska J, et al. Administration of CD4+CD25highCD127-regulatory T cells preserves β-cell function in type 1 diabetes in children. *Diabetes Care* (2012) 35(9):1817–20. doi: 10.2337/dc12-0038
55. Marek-Trzonkowska N, Myśliwec M, Siebert J, Trzonkowski P. Clinical application of regulatory T cells in type 1 diabetes. *Pediatr Diabetes* (2013) 14(5):322–32. doi: 10.1111/pedi.12029
56. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Derkowska I, Juscinska J, et al. Therapy of type 1 diabetes with CD4(+) CD25(high)CD127-regulatory T cells prolongs survival of pancreatic islets - results of one year follow-up. *Clin Immunol* (2014) 153(1):23–30. doi: 10.1016/j.clim.2014.03.016
57. 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. *Pigm Cell Melanoma Res* (2012) 25(1):99–109. doi: 10.1111/j.1755-148X.2011.00920.x
58. 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(5):e37513. doi: 10.1371/journal.pone.0037513
59. Raffin C, Vo LT, Bluestone JA. T(reg) cell-based therapies: challenges and perspectives. *Nat Rev Immunol* (2020) 20(3):158–72. doi: 10.1038/s41577-019-0232-6
60. Bluestone JA, Tang Q. T(reg) cells-the next frontier of cell therapy. *Science* (2018) 362(6411):154–5. doi: 10.1126/science.aau2688
61. Trenado A, Charlotte F, Fisson S, Yagello M, Klatzmann D, Salomon BL, et al. Recipient-type specific CD4+CD25+ regulatory T cells favor immune reconstitution and control graft-versus-host disease while maintaining graft-versus-leukemia. *J Clin Invest* (2003) 112(11):1688–96. doi: 10.1172/jci17702
62. Golshayan D, Jiang S, Tsang J, Garin MI, Mottet C, Lechler RI. In vitro-expanded donor alloantigen-specific CD4+CD25+ regulatory T cells promote experimental transplantation tolerance. *Blood* (2007) 109(2):827–35. doi: 10.1182/blood-2006-05-025460
63. Sagoo P, Ali N, Garg G, Nestle FO, Lechler RI, Lombardi G. Human regulatory T cells with alloantigen specificity are more potent inhibitors of alloimmune skin graft damage than polyclonal regulatory T cells. *Sci Transl Med* (2011) 3(83):83ra42. doi: 10.1126/scitranslmed.3002076
64. Noyan F, Lee YS, Hardtke-Wolenski M, Knoefel AK, Taubert R, Baron U, et al. Donor-specific regulatory T cells generated on donor B cells are superior to CD4+CD25high cells in controlling alloimmune responses in humanized mice. *Transplant Proc* (2013) 45(5):1832–7. doi: 10.1016/j.transproceed.2013.01.073
65. Liu LY, Strassner JP, Refat MA, Harris JE, King BA. Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. *J Am Acad Dermatol* (2017) 77(4):675–82.e1. doi: 10.1016/j.jaad.2017.05.043
66. MacDonald KN, Ivison S, Hippen KL, Hoeppli RE, Hall M, Zheng G, et al. Cryopreservation timing is a critical process parameter in a thymic regulatory T-cell therapy manufacturing protocol. *Cytotherapy* (2019) 21(12):1216–33. doi: 10.1016/j.jcyt.2019.10.011
67. Boroughs AC, Larson RC, Choi BD, Bouffard AA, Riley LS, Schiferle E, et al. Chimeric antigen receptor costimulation domains modulate human regulatory T cell function. *JCI Insight* (2019) 4(8):e126194. doi: 10.1172/jci.insight.126194
68. Le Poole IC, Luiten RM. Autoimmune etiology of generalized vitiligo. *Curr Dir Autoimmun* (2008) 10:227–43. doi: 10.1159/000131485
69. Ranges GE, Figari IS, Espevik T, Palladino MAJr. Inhibition of cytotoxic T cell development by transforming growth factor beta and reversal by recombinant tumor necrosis factor alpha. *J Exp Med* (1987) 166(4):991–8. doi: 10.1084/jem.166.4.991
70. Scheurich P, Thoma B, Ucer U, Pfizenmaier K. Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-alpha: induction of TNF receptors on human T cells and TNF-alpha-mediated enhancement of T cell responses. *J Immunol* (1987) 138(6):1786–90.
71. Grinberg-Bleyer Y, Saadoun D, Baeyens A, Billiard F, Goldstein JD, Grégoire S, et al. Pathogenic T cells have a paradoxical protective effect in murine autoimmune diabetes by boosting Tregs. *J Clin Invest* (2010) 120(12):4558–68. doi: 10.1172/jci42945
72. Biton J, Semerano L, Delavallée L, Lemeiter D, Laborie M, Grouard-Vogel G, et al. Interplay between TNF and regulatory T cells in a TNF-driven murine model of arthritis. *J Immunol* (2011) 186(7):3899–910. doi: 10.4049/jimmunol.1003372
73. Biton J, Boissier MC, Bessis N. TNFα: activator or inhibitor of regulatory T cells? *Joint Bone Spine* (2012) 79(2):119–23. doi: 10.1016/j.jbspin.2011.09.017
74. Webb KC, Tung R, Winterfield LS, Gottlieb AB, Eby JM, Henning SW, et al. Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. *Br J Dermatol* (2015) 173(3):641–50. doi: 10.1111/bjd.14016
75. Watanabe K, Terakura S, Martens AC, van Meerten T, Uchiyama S, Imai M, et al. Target antigen density governs the efficacy of anti-CD20-CD28-CD3 ζ chimeric antigen receptor-modified effector CD8+ T cells. *J Immunol* (2015) 194(3):911–20. doi: 10.4049/jimmunol.1402346
76. Walker AJ, Majzner RG, Zhang L, Wanhainen K, Long AH, Nguyen SM, et al. Tumor Antigen and Receptor Densities Regulate Efficacy of a Chimeric

Antigen Receptor Targeting Anaplastic Lymphoma Kinase. *Mol Ther* (2017) 25(9):2189–201. doi: 10.1016/j.ymthe.2017.06.008
77. Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: Chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol Rev* (2014) 257(1):83–90. doi: 10.1111/imr.12125
78. Raffin C, Zhou Y, Piccoli L, Lanzavecchia A, Sadelain M, Bluestone JA. Development of citrullinated-vimentin-specific CAR for targeting Tregs to treat autoimmune rheumatoid arthritis. *J Immunol* (2018) 200:176.
79. Hua J, Inomata T, Chen Y, Foulsham W, Stevenson W, Shiang T, et al. Pathological conversion of regulatory T cells is associated with loss of allotolerance. *Sci Rep* (2018) 8(1):7059. doi: 10.1038/s41598-018-25384-x
80. Junghans RP. The challenges of solid tumor for designer CAR-T therapies: a 25-year perspective. *Cancer Gene Ther* (2017) 24(3):89–99. doi: 10.1038/cgt.2016.82
81. Jones BS, Lamb LS, Goldman F, Di Stasi A. Improving the safety of cell therapy products by suicide gene transfer. *Front Pharmacol* (2014) 5:254:254. doi: 10.3389/fphar.2014.00254
82. Rossignoli F, Grisendi G, Spano C, Golinelli G, Recchia A, Rovesti G, et al. Inducible Caspase9-mediated suicide gene for MSC-based cancer gene therapy. *Cancer Gene Ther* (2019) 26(1-2):11–6. doi: 10.1038/s41417-018-0034-1
83. Paller AS, Arnsmeier SL, Alvarez-Franco M, Bremer EG. Ganglioside GM3 inhibits the proliferation of cultured keratinocytes. *J Invest Dermatol* (1993) 100(6):841–5. doi: 10.1111/1523-1747.ep12476755
84. Huang BB, Bonish BK, Chaturvedi V, Qin JZ, Nickoloff BJ. Keratinocyte CDw60 expression is modulated by both a Th-1 type cytokine IFN-gamma and Th-2 cytokines IL-4 and IL-13: relevance to psoriasis. *J Invest Dermatol* (2001) 116(2):305–12. doi: 10.1046/j.1523-1747.2001.01242.x
85. Safinia N, Vaikunthanathan T, Fraser H, Thirkell S, Lowe K, Blackmore L, et al. Successful expansion of functional and stable regulatory T cells for immunotherapy in liver transplantation. *Oncotarget* (2016) 7(7):7563–77. doi: 10.18632/oncotarget.6927
86. Hippen KL, Merkel SC, Schirm DK, Sieben CM, Sumstad D, Kadidlo DM, et al. Massive ex vivo expansion of human natural regulatory T cells (T(regs)) with minimal loss of in vivo functional activity. *Sci Transl Med* (2011) 3 (83):83ra41. doi: 10.1126/scitranslmed.3001809
87. Weiner J, Duran-Struuck R, Zitsman J, Buhler L, Sondermeijer H, McMurchy AN, et al. Restimulation After Cryopreservation and Thawing Preserves the Phenotype and Function of Expanded Baboon Regulatory T Cells. *Transplant Direct* (2015) 1(1):1–7. doi: 10.1097/txd.0000000000000511
88. Guo H, Zhang H, Lu L, Ezzelarab MB, Thomson AW. Generation, cryopreservation, function and in vivo persistence of ex vivo expanded cynomolgus monkey regulatory T cells. *Cell Immunol* (2015) 295(1):19–28. doi: 10.1016/j.cellimm.2015.02.006
89. Koristka S, Kegler A, Bergmann R, Arndt C, Feldmann A, Albert S, et al. Engrafting human regulatory T cells with a flexible modular chimeric antigen receptor technology. *J Autoimmun* (2018) 90:116–31. doi: 10.1016/j.jaut.2018.02.006
90. Rost F, Lambert K, Rakebrandt N, Joller N. Preceding Viral Infections Do Not Imprint Long-Term Changes in Regulatory T Cell Function. *Sci Rep* (2020) 10 (1):8350. doi: 10.1038/s41598-020-65212-9
91. Bonacina F, Martini E, Svecla M, Nour J, Cremonesi M, Beretta G, et al. Adoptive transfer of CX3CR1 transduced-T regulatory cells improves homing to the atherosclerotic plaques and dampens atherosclerosis progression. *Cardiovasc Res* (2020). doi: 10.1093/cvr/cvaa264
92. Boursier G, Siri A, de Boysson H. [Use of regulatory T cells in cellular therapies in autoimmune diseases]. *Med Sci M/S* (2012) 28(8-9):757–63. doi: 10.1051/medsci/2012288018

Conflict of Interest: United States and PCT international patent (Utility&PCT, serial No.17/072,939&PCT/US2020/056104) filed as "Materials and methods for treating vitiligo" on 10/16/2020.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 15 December 2020
doi: 10.3389/fimmu.2020.590558

Case Series: Gene Expression Analysis in Canine Vogt-Koyanagi-Harada/Uveodermatologic Syndrome and Vitiligo Reveals Conserved Immunopathogenesis Pathways Between Dog and Human Autoimmune Pigmentary Disorders

Ista A. Egbeto[1,2], Colton J. Garelli[1], Cesar Piedra-Mora[3], Neil B. Wong[1], Clement N. David[4], Nicholas A. Robinson[3†] and Jillian M. Richmond[1]*

[1] Department of Dermatology, UMass Medical School, Worcester, MA, United States, [2] Tufts University School of Medicine, Boston, MA, United States, [3] Pathology Department, Tufts Cummings School of Veterinary Medicine, Grafton, MA, United States, [4] Nanostring Technologies, Seattle, WA, United States

Vogt-Koyanagi-Harada syndrome (VKH) and vitiligo are autoimmune diseases that target melanocytes. VKH affects several organs such as the skin, hair follicle, eyes, ears, and meninges, whereas vitiligo is often limited to the skin and mucosa. Many studies have identified immune genes, pathways and cells that drive the pathogeneses of VKH and vitiligo, including interleukins, chemokines, cytotoxic T-cells, and other leukocytes. Here, we present case studies of 2 canines with VKH and 1 with vitiligo, which occurred spontaneously in client-owned companion dogs. We performed comparative transcriptomics and immunohistochemistry studies on lesional skin biopsies from these cases in order to determine if the immunopathogenesis of autoimmune responses against melanocytes are conserved. In dogs, we found enrichment of T cell gene signatures, with upregulation of IFNG, TNF, PRF1, IL15, CTSW, CXCL10, and CCL5 in both VKH and vitiligo in dogs compared to healthy controls. Similar findings were reported in humans, suggesting that these genes play a role in the pathogenesis of spontaneous VKH and vitiligo. T cell-associated genes, including FOXP3 and TBX21, were enriched, while IGFBP5, FOXO1, and PECAM1 were decreased compared to healthy controls. Further, we identified TGFB3, SFRP2, and CXCL7 as additional potential drivers of autoimmune pigmentary disorders. Future studies exploring the immunopathogenesis of spontaneous autoimmunity will expand our understanding of these disorders, and will be useful in developing targeted therapies, repurposing drugs for veterinary and human medicine, and predicting disease prognosis and treatment response.

Keywords: vitiligo, Vogt-Koyanagi-Harada disease, immunopathogenesis, transcriptomics, comparative immunology, canine (dog)

OPEN ACCESS

Edited by:
Isabelle Caroline Le Poole, Northwestern University, United States

Reviewed by:
Mrinal K. Sarkar, University of Michigan, United States
Peizeng Yang, First Affiliated Hospital of Chongqing Medical University, China
Shikhar Mehrotra, Medical University of South Carolina, United States

****Correspondence:***
Jillian M. Richmond
jillian.richmond@umassmed.edu

†Present address:
Nicholas A. Robinson, Bluebird Bio, Cambridge, MA, United States

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *01 August 2020*
Accepted: *02 November 2020*
Published: *15 December 2020*

Citation:
Egbeto IA, Garelli CJ, Piedra-Mora C, Wong NB, David CN, Robinson NA and Richmond JM (2020) Case Series: Gene Expression Analysis in Canine Vogt-Koyanagi-Harada/Uveodermatologic Syndrome and Vitiligo Reveals Conserved Immunopathogenesis Pathways Between Dog and Human Autoimmune Pigmentary Disorders. Front. Immunol. 11:590558. doi: 10.3389/fimmu.2020.590558

INTRODUCTION

Autoimmune pigmentary disorders include vitiligo and Vogt-Koyanagi-Harada (VKH) syndrome, which are caused by the destruction of melanocytes (1, 2). These diseases are mediated by T-cells that target melanocyte self-antigens, including tyrosinase, tyrosinase-related proteins 1 & 2 (TRP1/2), gp100/Pmel-17, and melan-a/MART-1 (3–9). Vitiligo is characterized by loss of melanocytes in the skin and mucosa (1). VKH is more extensive and includes the skin, mucosa, eyes, ears, and meninges, resulting in chronic uveitis, alopecia, vitiligo, poliosis, and irritation of the meninges (10). The current hypothesis in the field is that VKH represents an exacerbated reaction by melanocytes and their precursors as compared to vitiligo.

VKH is usually manifested during the third decade of life and is present in all ethnic groups across the world. The prevalence is higher in groups with darker skin tones and in Asians (11). VKH syndrome is thought to be associated with HLA-DRB1*0405 (12), and polymorphisms in immune genes (13). Patients often present with bilateral uveitis that is often preceded by a mild prodromal illness, along with auditory and neurological symptoms. Isolated ocular involvement in the initial phases of the disease is also common. The choroid is the primary site of inflammation, with the iris and ciliary body also affected. VKH syndrome is classified into three different categories:

1. Complete VKH- vitiligo is associated with complete VKH. Manifestations of complete VKH includes diffuse choroiditis affecting the eyes bilaterally and causing retinal detachments. Other signs include tinnitus, alopecia, neck stiffness, poliosis, and vitiligo.
2. Incomplete VKH- these patients present with similar ocular symptoms as patients with complete VKH, but they do not have both neurological and skin symptoms.
3. Probable VKH- these patients have similar ocular symptoms as patients with VKH, and they tend to have neurologic and auditory manifestations or dermatologic signs, but not both (10).

Several cytokines and chemokines contribute to the development of vitiligo and VKH, including type 1 responses (IFNγ, CXCL9/10/11, IL-12, TNF) type 17 responses (IL-17, CCL20, IL-23) and IL-2, which supports T cell growth and survival (13–19). Some studies have demonstrated that patients with VKH that have vitiligo have a predominance of CD4+ T-cell lymphocytes and an imbalanced ratio of CD4+/CD8+ T-cells (10), though others have demonstrated a CD8+ cytotoxic T cell preponderance (15). These CD8+ T cells react to melanocyte antigens and exhibit markers of skin resident memory T cells (20, 21).

Vitiligo and VKH in canines share similar clinical characteristics with human vitiligo and VKH [reviewed in (22)]. Dogs that have VKH present similarly to humans with incomplete VKH, exhibiting panuveitis and bilateral retinal detachment. Here, we analyzed lesional skin tissue from two dogs with VKH and one dog with vitiligo who presented to community veterinary clinics and were biopsied for diagnostic purposes. We performed transcriptomic and immunohistochemistry analysis on lesional skin tissue to examine features of immunopathogenesis of pigmentary disorders that are conserved during spontaneous disease in dogs versus humans. Our findings support the IFNγ-CXCR3 axis as a prominent feature in canine autoimmune pigmentary disorders. We also identified TGFB3, SFRP2 and CXCL7 as other potential drivers of immunopathogenesis.

CASE PRESENTATIONS

Case 1

A 2.5-year-old male Bernese Mountain dog that presented with a 6-month history of loss of pigmentation on his nose with periocular erythema, seborrhea, and crusts around eyes, on the nose, back, and tail. These symptoms were resolved with three months of steroid therapy. Two punch biopsies were performed, and histopathology revealed a lichenoid inflammation that multifocally obscured the dermo-epidermal junction (interface inflammation). The inflammation is predominantly composed of macrophages and fewer lymphocytes and plasma cells. There was significant pigmentary incontinence in the areas of inflammation with macrophages containing fine, dust-like, granular melanin pigment. The epidermis was moderately hyperplastic and hyperkeratotic. All the above findings were consistent with VKH-like syndrome.

Case 2

A 4-year-old neutered male Siberian Husky dog that presented with a 2-month history of changes of pigmentation and pruritus of the nasal planum, muzzle, periocular skin, and oral mucous membranes. The dog developed blepharospasm, iridal color change, and pupillary miosis in the left eye. A punch biopsy in this dog revealed similar histopathological findings as in case 1 that were all consistent with VKH-like syndrome.

Case 3

A 1.5-year-old, neutered male Rottweiler-Labrador mixed dog presented with 1-month history of pigment changes of the nose and haircoat. On physical examination the dog had bilaterally symmetrical areas of leukotrichia interspersed with pigmented (black) hair and no evidence of erythema, alopecia, or crusting. Histopathology patchy and sharply demarcated loss of melanocytes in the epidermis and follicular epithelium. Multifocally, the epidermis and follicular epithelium, particularly in samples from the planum nasale, there is a mild, perivascular lymphocytic infiltrate with frequent migration of lymphocytes into the epidermis. Some hair shafts contained minimal pigment. In light of the clinical and histopathological findings, a diagnosis of vitiligo was made.

RESULTS

Gene Expression Analysis From Case Series Reveals Both New and Previously Identified Genes Relevant to Anti-Melanocyte Autoimmunity

RNA was extracted from two VKH cases, one vitiligo case, and five healthy control leg margins and was analyzed with a custom

Nanostring probeset (H&E from cases presented in **Figure 1A**). Agglomerative clustering of the entire 160 codeset revealed that the three pigmentary disorder cases grouped together apart from healthy controls (**Figure S1**). To understand the biology of VKH and vitiligo in dogs, we performed more detailed analyses on subsets of genes based on known cellular functions. First, we examined genes related to skin and hair biology, as well as neuroendocrine function in the skin (see **Figure S2** for complete gene set analyses). Tyrosinase (TYR) transcript was expressed in the healthy controls and the vitiligo case, while the mean trended lower in the VKH cases but showed no statistical difference from controls (**Figure 1B**).IGFBP5, which is known to mediate fibrosis (23), and FOXO1, which is associated with adipogenesis (24), were significantly downregulated in lesional skin (**Figure 1B**). These genes have not previously been associated with vitiligo or VKH, thus their functions in disease warrant further investigation. TGFB3, a growth factor and immune modulator, was significantly upregulated in both VKH and vitiligo (**Figure 1B**). Profilaggrin (gene name FLG), a protein hormone that promotes hair growth, and SFRP2, a soluble regulator of WNT signaling, were significantly upregulated in VKH but not vitiligo (**Figure 1B**). WNT signaling has previously been reported to be disrupted in lesional vitiligo skin (25), though its role in VKH is still unclear. Other skin-relevant transcripts including involucrin (IVL), LORICRIN, EDA, EDAR, KIT, WIF1, DNMT1, RXRG, VDR, TGFB1/2, and DCT showed no statistical differences from controls (**Figure S2**). The neuroendocrine gene CYP1B1 was significantly downregulated in cases versus controls, though this contrasts with a previous report describing upregulation in vitiligo blood samples (Dey-Rao and Sinha 2017). KRT10, a marker of basal keratinocytes, was significantly higher and PECAM1, a marker of endothelial cells, was significantly lower in cases versus healthy controls, which may reflect the site of biopsy (e.g. healthy leg margin versus nose for cases; **Figure S2**).

Next, we used Nanostring Advanced Analysis software to quantify cell types across our samples. We found that, similar to humans (5, 26), T-cells are the predominant immune cells found in VKH and vitiligo compared to healthy controls (**Figures 2A, B**). We examined the T-cell-associated transcription factors FOXP3 and TBX21 (Tbet), which are the master regulators in Tregs and Th1/Tc1 cells respectively, and found they were induced in VKH and vitiligo compared to controls (**Figure 2C**). Th1 cells, cytotoxic cells, exhausted CD8 T-cells and NK cells gene expression signatures were increased in VKH and vitiligo compared to healthy controls (**Figure 2D**). Neutrophil and B cell signatures were also increased in lesional skin, whereas macrophage and dendritic cells (DC) scores were equivalent across all 3 conditions. However, there was an increase in CD103+ dermal DC scores, which are known to cross-present antigens during antiviral and anti-tumor immune responses (27, 28). The full analysis of the canine immune cell panel genes is presented in **Figure S3**.

We next analyzed cytokines and chemokines. We found statistically significant induction of IFNG, ISG15, TNF, IL12, PRF1, and IL15 cytokine expression in dogs with VKH compared

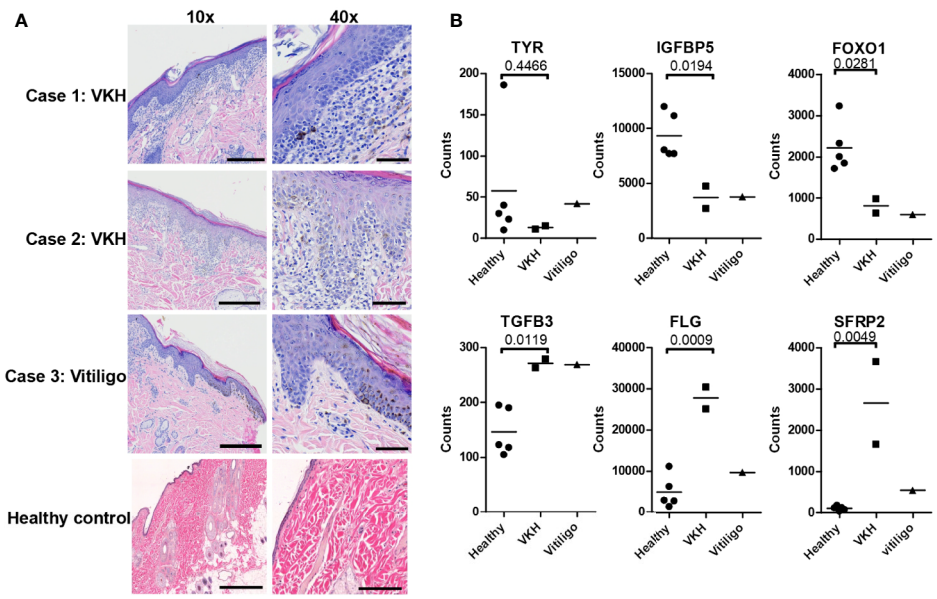


FIGURE 1 | Examination of histopathology, skin, and hair gene expression patterns in VKH and vitiligo in dogs reveals marked infiltration with changes in specific genes. **(A)** Histopathologic examination of skin tissue samples. Hematoxylin and eosin (H&E) staining, magnifications 10x and 40x (scale bar = 300 microns 10x, scale bar = 60 microns 40x). There is marked inflammation at the dermo-epidermal junction (interface dermatitis) in VKH (cases 1 and 2) and epidermal hyperplasia in vitiligo (case 3), with pigment incontinence noted in all cases. **(B)** Relative mRNA expression of skin and hair-related gene transcripts in the cases compared to 5 healthy controls (leg margins). TGFB3, FLG, and SFRP2 were significantly increased, and TYR, IGFBP5, and FOXO1 were decreased; n = 2 VKH, 1 vitiligo, and 5 healthy controls; *p* values from two-tailed t tests healthy vs VKH: TYR p = 0.4466, IGFBP5 p = 0.0194, FOXO1 p = 0.0281, TGFB3 p = 0.0119, FLG p = 0.0009, SFRP2 p = 0.0049.

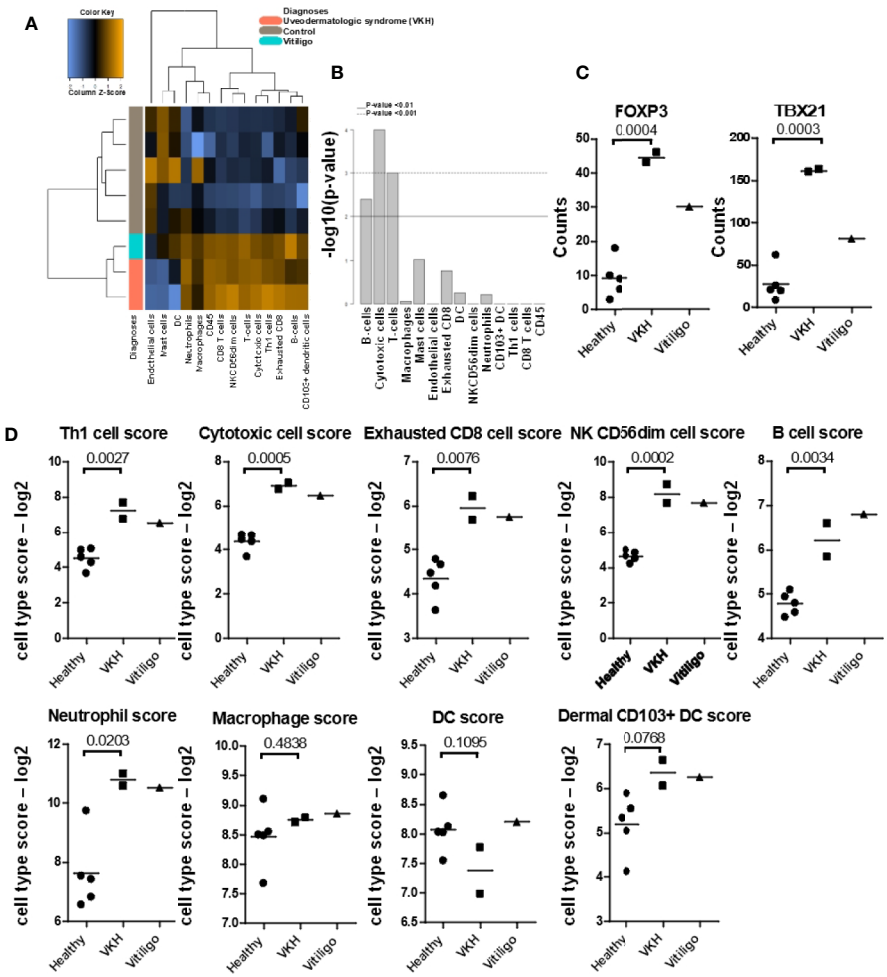


FIGURE 2 | Quantification of cell types and mRNA expression of T-cell-associated transcription factors FOXP3 and TBX21/Tbet reveals cytotoxic and Th1 responses. **(A)** Nanostring advanced cell type analysis heat map representing color-coded expression levels of differentially expressed genes from the indicated leukocyte populations. Cases clustered together apart from healthy controls. **(B)** Bar plots of p-values for cell type enrichment analysis revealed that cytotoxic T-cells, B-cells, and cytokines are the predominant cells in VKH samples. **(C)** FOXP3 and TBX21 were significantly increased in VKH samples as compared to healthy controls; *p* values from two-tailed t tests FOXP3 p = 0.0004, TBX21 p = 0.0003. **(D)** Cell type scores from Nanostring advanced analysis demonstrating significant increases in Th1 cells, cytotoxic cells, exhausted CD8s, NK CD56dim cells, B cells, and neutrophils in VKH cases. Macrophage and DC scores remained unchanged, whereas dermal CD103+ dendritic cell scores were significantly increased as compared to healthy controls. n = 2 VKH, 1 vitiligo, and 5 healthy controls; *p* values from two-tailed t tests: Th1 p = 0.0027, Cytotoxic p = 0.0005, exhausted CD8 p = 0.0076, NK CD56dim p = 0.0002, B cell p = 0.0034, neutrophil p = 0.0203, macrophage p = 0.4838, DC p = 0.1095, dermal CD103+ DC p = 0.0768.

to healthy controls (**Figure 3A**). The vitiligo case exhibited even higher ISG15 and PRF1, while IFNG, TNF, IL12, and IL15 levels were closer to those in healthy controls. All of these genes have been reported to be induced in immune-mediated pigmentary disorders (21, 29–31). There was a trend towards increased CD215, a receptor chain for IL15 that has previously been shown to be upregulated on keratinocytes from lesional skin (21), in the cases. IL21, which has previously been reported to be upregulated in the Smyth chicken line, another model of spontaneous vitiligo (32), was unchanged in our samples. However, the IL21R was highly upregulated in lesional skin in the VKH and vitiligo dogs. The full analyses of the immune disease related genes, interferons and granzymes, and interleukins/cytokines are presented in **Figure S4**.

Mouse and human studies have shown that the chemokines CCL5 and CXCL10 are expressed in the skin during vitiligo (14, 15, 33, 34). We found significant upregulation of CCL5 and CXCL10, as well as CXCL7 (gene name PPBP) in canine VKH and vitiligo. CX3CL1 was significantly upregulated in VKH only, and there was a similar, though not significant, trend in CXCL11 upregulation. CCL2 (**Figure S3**) and CXCL12 (**Figure 3A**) were significantly downregulated in both conditions compared to healthy controls. The full analysis of chemokine expression is presented in **Figure S5**.

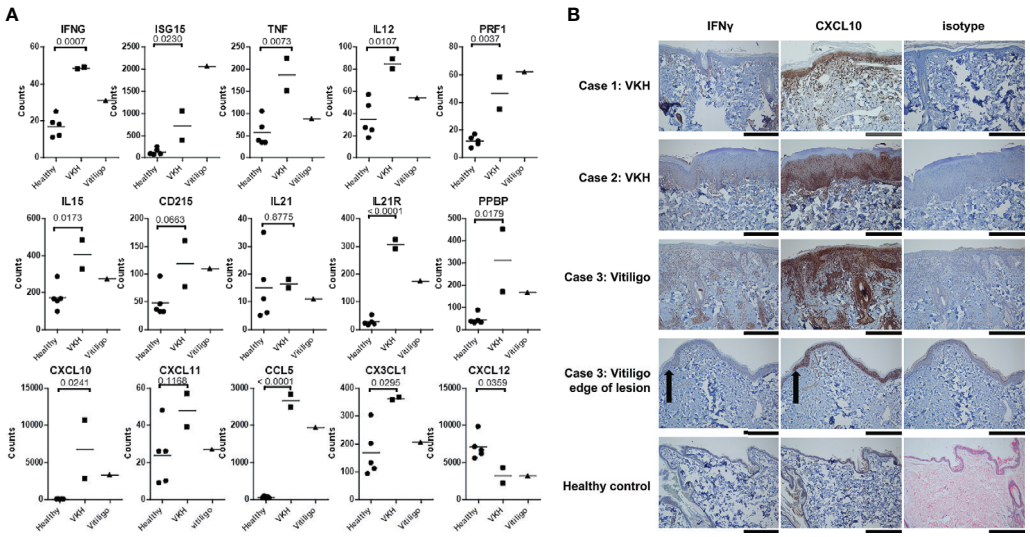


FIGURE 3 | Many cytokines and chemokines previously associated with human VKH and vitiligo are enriched in canine lesional skin. **(A)** Relative mRNA expression of IFNG, ISG15, TNF, IL12, PRF1, IL15, IL21R, CXCL7, CXCL10, CCL5, and CX3CL1 were statistically significantly higher in VKH samples as compared to healthy controls. CXCL12 mRNA expression was significantly lower in VKH samples compared to healthy controls, CD215 and CXCL11 were trending higher (though they did not reach statistical significance), and IL21 was not significantly different. n = 2 VKH, 1 vitiligo, and 5 healthy controls; *p* values from two-tailed t tests healthy vs VKH: IFNG p = 0.0007, ISG15 p = 0.023, TNF p = 0.0073, IL12 p = 0.0107, PRF1 p = 0.0037, IL15 p = 0.0173, CD215 p = 0.0663, IL21 p = 0.8775, IL21R p < 0.0001, CXCL7 p = 0.0179, CXCL10 p = 0.0241, CXCL11 p = 0.1168, CCL5 p < 0.0001, CX3CL1 p = 0.0295, CXCL12 p = 0.0359. **(B)** Immunohistochemistry stains for IFN-γ and CXCL10 expression in lesional skin biopsies from dogs demonstrating increased protein levels in cases as compared to isotype and healthy controls (black scale bars = 300 microns 10x, grey scale bar = 150 microns 20x, black arrow case 3 indicates edge of lesion). n = 2 VKH, 1 vitiligo, and 5 healthy controls.

IFNγ and CXCL10 Protein Expression Are Increased in Lesional Skin of VKH and Vitiligo Compared to Healthy Controls

To confirm that IFNG and CXCL10, two well-characterized drivers of anti-melanocyte responses, are expressed at the protein level in lesional skin of VKH and vitiligo canines, we performed immunohistochemistry. These cytokines were highly expressed in tissue as compared to isotype and healthy controls, with higher CXCL10 expression consistent with its role in amplifying IFNγ signals (**Figure 3B**). Taken together, these results characterize immune and skin gene expression in canine VKH and vitiligo, identify novel potential drivers of disease, and reveal conserved immunopathogeneses between human and canine spontaneous disease.

DISCUSSION

The pathogeneses of vitiligo and VKH are complex and not fully defined. To better understand driving factors of the autoimmune response in spontaneous autoimmune pigmentary disorders, we performed this retrospective comparative immunology case study to examine transcriptomics and immunohistochemistry of many known, and several unknown, genes central to vitiligo and VKH pathogeneses. In our study, we found increased T cell responses, type 1 cytokines, chemokines, and memory T cell responses in VKH dogs compared to healthy controls. These genes have been established as drivers of human vitiligo immunopathogenesis, particularly the IFNG cytokine signaling pathway (29, 35, 36) and subsequent CXCR3 ligand expression following activation of Janus Kinases (JAK) 1 and 2 (14, 37–40). CCR5 and CXCR3 expressing leukocytes are recruited to the skin following their ligands CCL5 and CXCL10, respectively (15, 33, 39). IL15 promotes development of skin resident memory T cells (41), and a recent studies in a vitiligo mouse model and human tissues revealed that the IL15 receptor is important for autoimmune memory in vitiligo (21). Other groups have reported that IL10, IL13, IL17A, and IL21 are increased in vitiligo (42–45), though we did not observe this in our case series study. Further, it is unclear if these cytokine profiles are pertinent to specific clinical subtypes of disease, such as inflammatory vitiligo, or if they are upregulated at specific phases of disease or as a result of concurrent autoimmunity or infections.

Like vitiligo, VKH appears to be driven by type 1 T cell responses (46, 47), though eye involvement reveals Th17 signatures (48). CX3CL1 was upregulated specifically in VKH skin in our case studies. Fractalkine has only been explored in ocular disease, warranting further investigation in cutaneous VKH, especially to understand differences in the biology versus vitiligo (49).

We examined skin-specific genes that are associated with skin and hair biology, including TYR, IGFBP5, FOXO1, FLG, WIF1, and TGFB3. Of these genes, TGFB3 and FLG were found to be significantly upregulated, whereas IGFBP5, FOXO1 and WIF1

were downregulated. In contrast to our findings of increased TGFB3 ligand, polymorphisms in its receptor were not found to be associated with VKH disease in a Chinese Han population (50). The disparity in TGFB3 expression could be explained by differences in dog versus human VKH, the stage at which the skin biopsies have been studied, and/or differences in the biology of the ligand versus the receptor. For example, TGFB3 (along with IL10) is expressed in the resolution phase of VKH and may play a relevant role in controlling the disease (51). The upregulation of FLG in VKH and not in vitiligo was an unexpected finding. We hypothesize that increased FLG expression in VKH dogs suggests a process of chronic inflammation in hair follicles, which is lacking in vitiligo. These differences may also be due to dog versus human pathogenesis, or perhaps due to disease stage/duration. We suggest further studies are needed to determine the roles of TGFB3 and FLG in VKH and vitiligo.

IGFBP5, FOXO1, and WIF1 downregulation may indicate a loss of tolerance mechanisms or melanocyte regenerative capacity in the skin in canine VKH and vitiligo. IGFBP5 acts as a tumor suppressor in human melanoma cells (52), which is interesting given the hypothesis that vitiligo exists on an "immune spectrum" with melanoma (53). FOXO1 represses TBX21-mediated effector functions to promote memory CD8+ T cell formation and Treg function; thus, a loss of FOXO1 may drive continued T cell effector function in the skin during melanocyte autoimmunity (54–56). WIF1 promotes melanogenesis in normal human melanocytes (57), though it is not yet clear what impact a loss of expression would have on vitiligo or VKH.

We also investigated genes involved in T-cell and Treg regulation and function: PPARG, FOXO3A, TBX21, and FOXP3. PPARG, which activates growth of melanocytes through apoptosis (58) and promotes T cell differentiation and survival (59, 60), was decreased in our cases compared to healthy controls. FOXO3 is a transcription factor that is an important regulator of the magnitude of CD8 T cell memory (61). FOXO3A polymorphisms have been associated with oxidative stress and altered Treg function in vitiligo patients (62, 63). Thus, decreases in PPARG and FOXO3 may indicate reduced ability of Tregs to function to suppress autoimmune responses of T cells against melanocytes. FOXP3 and TBX21 were increased in our cases compared to healthy controls, which correlates with data revealing increased expression of these transcription factors in VKH patients during an active uveitis episode (64). Together, our data support enhanced effector T cell populations and reduced Treg function present in the skin of canine vitiligo and VKH patients.

TYR, which we hypothesized would be significantly downregulated in VKH due to melanocyte loss, was not statistically different from the healthy controls. Notably, a study investigating tyrosinase gene family loci in VKH in Japanese patients using single microsatellite marker analysis and haplotype analysis did not find an association between TYR loci and VKH syndrome (65). This suggests that perhaps immature melanocytes or melanocyte precursors are preferentially targeted in VKH. Vitiligo was found to be associated with the major alleles of SNPs in the *TYR* region, particularly rs1393350 and the R402Q SNP rs1126809 (66). This may suggest a difference in pathogenesis between vitiligo and VKH involving tyrosinase as an autoantigen.

Our chemokine analysis revealed indicators of active, though not very early, disease in the dogs as evidenced by high CCL5 and FOXP3 (transiently upregulated in activated T-cells, allowing for the development of peripheral/induced regulatory T-cells) and low CXCL12 (67). We also identified PPBP/CXCL7 as a significantly upregulated chemokine in the skin. CXCL7 was previously reported to be upregulated in the serum of vitiligo patients, though little is known about its role in skin homing, warranting further study (68).

Our immune cell signature findings are strongly supported by previous studies that vitiligo and VKH are mediated by T-cells, particularly CD8 cytotoxic T-cells (69). Neutrophils are understudied in the skin in vitiligo and VKH, in part because they comprise a small fraction of the infiltrate and are not a prominent component. The increase in neutrophils we observed in these cases may be due to scratching behavior in the dogs, which is known to induce neutrophil recruitment to the skin (70). Nevertheless, blood neutrophils can contribute to ROS generation (71) and were reported to be elevated in the peripheral blood of patients with generalized vitiligo (72). We also observed increased B-cell signatures. It has been reported that in Akita dogs with VKH-like syndrome, cutaneous lesions are mediated by T cells and macrophages and ocular lesions are mediated primarily by B cells and macrophages (73). Autoantibodies against melanocytes have been identified in vitiligo and VKH, though it is still unclear if they are biomarkers or drive pathogenesis (74, 75). In lupus, another autoimmune disease, B cells have been postulated to serve as antigen presenting cells (76). Future studies will need to be conducted to determine the precise role of B cells and neutrophils in vitiligo and VKH.

In contrast to increases in other immune cell signatures, we found similar levels of macrophage and dendritic cell gene signatures in lesional skin tissue. Previous studies have noted the presence of macrophages in vitiligo lesions (6); thus a limitation of our data is that we did not examine localization of these cells within the tissue. It could be that, while total numbers of antigen presenting cell populations remain largely unchanged, that their localization within the tissue promotes lymphoid aggregates.

Limitations in our study include the small sample of genes analyzed (160 genes), and small sample size (we only had one vitiligo dog sample, thus preventing us from including the vitiligo sample in our statistical analyses). Due to the small group size of our VKH sample data, the t tests we performed may exhibit type I or type II errors which are dependent upon the variance of gene expression data within the groups (77). Nevertheless, several genes that we identified as differentially expressed between healthy and VKH have been previously published in human literature as DEGs for the condition. Future studies would include a larger scale comparative analyses and whole genome sequencing to better understand the influence of genetic factors on the pathogenesis of VKH disease and vitiligo.

In summary, our data support the hypothesis that T cell responses, type 1 cytokines, memory T cell responses, and chemokines drive immunopathogenesis of spontaneous VKH and vitiligo in both dogs and humans. Future studies expanding our understanding of spontaneous autoimmunity will be useful for providing a better understanding of autoimmune diseases and will pave the way for drug repurposing in human and veterinary medicine. For example, JAK inhibitors induce re-pigmentation in patients with vitiligo (78–81). Oclacitinib, a veterinary JAK inhibitor currently marketed for allergy and itch relief (82–85), could potentially be repurposed to treat vitiligo and VKH in dogs. Drugs that induce or worsen vitiligo could serve as a novel therapeutic approach to melanoma. A canine case report found a link between skin depigmentation and toceranib phosphate, a tyrosine kinase inhibitor (86), and the Flk-1 tyrosine kinase inhibitor SU5416 showed efficacy in phase II clinical trials for advanced melanoma (87). Future studies examining the mechanistic involvement of the gene targets we have identified in our case series for treatment of autoimmune pigmentary disorders or melanoma are warranted.

MATERIALS AND METHODS

Clinical Samples

Skin biopsies from the biorepository at Tufts Cummings School (NR) were selected based on pathology reports and H&E sections were reexamined by a board-certified veterinary pathologist to confirm diagnoses and absence of obvious infectious disease. Healthy control samples were obtained from leg margin biopsies from amputations. Two VKH and one vitiligo biopsy sample were obtained from shave and/or punch biopsies of male dogs as noted in the case presentation section. Of note, vitiligo has equal sex bias in dogs, whereas VKH is almost twice as likely to occur in male dogs (22). Samples were deposited with written owner consent in the Tufts biobank at the time the veterinary patients were seen at the hospital, spanning the years 2011–2019.

Isolation of RNA From FFPE Blocks

30 μm curls were cut from the blocks and stored in Eppendorf tubes at ambient temperature. RNA was isolated using the Qiagen FFPE RNeasy kit per the manufacturer directions. Briefly, razor blades were treated with RNase, excess paraffin was removed, and tissues were sliced into thin strips (5 μm) to create more surface area prior to incubation with deparaffinization solution (Qiagen). The protocol was followed and RNA was quantified using a nanodrop.

Nanostring Cartridge and Processing

A custom Nanostring canine gene panel of 160 genes including cytokine, chemokine, and immune genes, as well as skin and immune cell specific transcripts was created. We used B2M, RPL13A, CCZ1, and HPRT as housekeeping genes for this study. RNA was hybridized using a BioRad C1000 touch machine, and samples were loaded into Nanostring cartridges and analyzed with a Sprint machine. Gene expression data are deposited on GEO under Accession # GSE154024.

nSolver Analysis

NanoString's software, nSolver was used for all NanoString analysis. Raw counts were plotted with GraphPad Prism. Advanced analysis was used for the "cell Type Score", which is a summary statistic of the expression of the marker genes for each cell type. It is the geometric mean of the log2-transformed normalized counts for each set of marker genes. These scores were validated against FACS and IHC, and are a robust method of quantifying relative cell type abundance (88).

IHC

IHC was performed on 5 μm sections using rabbit-anti-canine CXCL10, IFNγ (US Biological) or isotype control (Biolegend) at 1:100 dilution using a Dako automated slide staining machine. All sections were counterstained with hematoxylin. H&E images were taken using an Olympus BX51 microscope with Nikon NIS Elements software version 3.10, and IHC images were taken using an Olympus BX40 microscope with cellSens Entry software version 1.14.

Statistics

To assess the statistical significance of our results, we performed two-way ANOVA with Bonferroni posttests between healthy controls and VKH cases on sets of defined genes (skin & hair, neuroendocrine, immune disease related, interleukins/cytokines, CC chemokines, CXC chemokines, immune cell panel genes, and housekeeping genes) to observe obvious differences across the dataset. We next performed two-tailed t-tests of VKH vs healthy using GraphPad Prism software version 5 to examine potential differences in previously identified genes pertinent to VKH pathogenesis (De Winter 2013). A statistically significant difference was considered as $p < 0.05$.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found on Gene Expression Omnibus (GEO) Database under Accession # GSE154024.

ETHICS STATEMENT

The animal study was reviewed and approved by Cummings School of Veterinary Medicine at Tufts University IACUC. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: JMR. Methodology: NAR, JMR. Software: CND, CJG. Validation: CP-M, JMR. Formal analysis: IAE, NBW, JMR. Investigation: CJG, CP-M, NAR, JMR, CND. Resources: NAR, JMR. Data curation: NAR, CP-M, CJG, JMR. Writing—original draft: IAE. Writing—review and editing: all authors. Visualization: IAE, CP-M, NBW, JMR. Supervision:

JMR, NAR. Project administration: JMR. Funding acquisition: JMR. All authors contributed to the article and approved the submitted version.

FUNDING

JMR is supported by a Calder Research Scholar Award in Vitiligo/Pigment Cell Disorders from the American Skin Association, a Women's Health Career Development Award from the Dermatology Foundation, and a Target Identification in Lupus Award from the Lupus Research Alliance.

ACKNOWLEDGMENTS

We thank Yu Liu from UMass, and Linda Wrijil and Gina Scariglia from Tufts for technical assistance. We thank the Shim lab at UMass for use of their microscope. Immunohistochemistry studies were performed in collaboration with the UMass DERC morphology core. The Nanostring Sprint machine is maintained by the Silverman lab in the UMass Department of Medicine.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.590558/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | Heat map showing the expression patterns of the 160 gene Nanostring panel in cases and healthy controls. Purple represents upregulation and orange and yellow represent downregulation of gene expression. Color density is associated with the level of gene expression. Dendrogram indicates clustering/relatedness of samples, which segregated based on disease status.

SUPPLEMENTARY FIGURE 2 | Expression of skin, hair, and neuroendocrine genes in VKH and vitiligo cases compared to healthy controls. The relative expression of **(A)** highly expressed skin and hair genes, **(B)** lower level transcript genes, and **(C)** neuroendocrine genes was evaluated by NanoString's nSolver. n = 2 VKH, 1 vitiligo, and 5 healthy controls; *p* values from two-way ANOVA with Bonferroni post tests for healthy vs VKH: KRT10 $p < 0.001$, PECAM1 $p < 0.001$, SFRP2 $p < 0.01$, IGFBP5 $p < 0.001$, CYP1B1 $p < 0.001$.

SUPPLEMENTARY FIGURE 3 | Expression of immune disease related genes in the autoimmune pigmentary cases versus healthy controls. **(A)** Immune related genes and **(B)** Interferons and granzymes expressed in skin. n = 2 VKH, 1 vitiligo, and 5 healthy controls; *p* values from two-way ANOVA with Bonferroni post tests for healthy vs VKH: SERPINB2 $p < 0.05$, VGLL3 $p < 0.01$, FAS $p < 0.001$, CTSW $p < 0.01$.

SUPPLEMENTARY FIGURE 4 | Expression of interleukins/cytokines and chemokines in cases versus healthy controls. **(A)** Interleukins/cytokines, **(B)** CC chemokines, and **(C)** CXC chemokines in skin biopsies of VKH, vitiligo, and healthy samples. n = 2 VKH, 1 vitiligo, and 5 healthy controls; *p* values from two-way ANOVA with Bonferroni post tests for healthy vs VKH: IL6 $p < 0.001$, CCL2 $p < 0.01$, CCL5 $p < 0.01$, CXCL10 $p < 0.01$, CXCL14 $p < 0.001$.

SUPPLEMENTARY FIGURE 5 | Expression of canine immune cell genes and housekeeping genes in cases versus healthy controls. **(A)** Highly expressed and **(B)** lower expressed canine cell panel genes. **(C)** Housekeeping genes in biopsies from cases and healthy controls. n = 2 VKH, 1 vitiligo, and 5 healthy controls; *p* values from two-way ANOVA with Bonferroni post tests for healthy vs VKH: PECAM1 $p < 0.01$, S100A12 $p < 0.001$, BLK $p < 0.01$, CD244 $p < 0.001$, CD6 $p < 0.001$, CD84 $p < 0.001$, GZMA $p < 0.001$, GZMB $p < 0.001$, IL21R $p < 0.001$, KLRD1 $p < 0.001$, KLRK1 $p < 0.001$, SH2D1A $p < 0.001$, TBX21 $p < 0.001$, TNFRSF17 $p < 0.01$, B2M $p < 0.001$.

REFERENCES

1. Rodrigues M, Ezzedine K, Hamzavi I, Pandya AG, Harris JE. New discoveries in the pathogenesis and classification of vitiligo. *J Am Acad Dermatol* (2017) 77:1–13. doi: 10.1016/j.jaad.2016.10.048
2. Baltmr A, Lightman S, Tomkins-Netzer O. Vogt-Koyanagi-Harada syndrome - current perspectives. *Clin Ophthalmol* (2016) 10:2345–61. doi: 10.2147/OPTH.S94866
3. Palermo B, Campanelli R, Garbelli S, Mantovani S, Lantelme E, Brazzelli V, et al. Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. *J Invest Dermatol* (2001) 117:326–32. doi: 10.1046/j.1523-1747.2001.01408.x
4. Sugita S, Takase H, Taguchi C, Imai Y, Kamoi K, Kawaguchi T, et al. Ocular infiltrating CD4+ T cells from patients with Vogt-Koyanagi-Harada disease recognize human melanocyte antigens. *Invest Ophthalmol Vis Sci* (2006) 47:2547–54. doi: 10.1167/iovs.05-1547
5. van den Boorn JG, Konijnenberg D, Dellemijn TAM, van der Veen JP, Bos JD, Melief CJM, et al. Autoimmune Destruction of Skin Melanocytes by Perilesional T Cells from Vitiligo Patients. *J Invest Dermatol* (2009) 129:2220–32. doi: 10.1038/jid.2009.32
6. Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J Pathol* (1996) 148:1219–28.
7. Maezawa N, Yano A. Two distinct cytotoxic T lymphocyte subpopulations in patients with Vogt-Koyanagi-Harada disease that recognize human melanoma cells. *Microbiol Immunol* (1984) 28:219–31. doi: 10.1111/j.1348-0421.1984.tb00673.x
8. Yamaki K, Gocho K, Hayakawa K, Kondo I, Sakuragi S. Tyrosinase family proteins are antigens specific to Vogt-Koyanagi-Harada disease. *J Immunol* (2000) 165:7323–9. doi: 10.4049/jimmunol.165.12.7323
9. Hayakawa K, Yamaki K, Takiyama N, Kotani T, Itoh N. Identification of Dog VKH Disease and Experimental Model of VKH in Dog(2002). Available at: https://iovs.arvojournals.org/article.aspx?articleid=2418842 (Accessed June 29, 2020).
10. Patil YB, Garg R, Rajguru JP, Sirsalmath M, Bevinakatti VA, Kumar M, et al. Vogt-Koyanagi-Harada (VKH) syndrome: A new perspective for healthcare professionals. *J Family Med Prim Care* (2020) 9:31–5. doi: 10.4103/jfmpc.jfmpc_787_19
11. Lavezzo MM, Sakata VM, Morita C, Rodriguez EEC, Abdallah SF, da Silva FTG, et al. Vogt-Koyanagi-Harada disease: review of a rare autoimmune disease targeting antigens of melanocytes. *Orphanet J Rare Dis* (2016) 11:29. doi: 10.1186/s13023-016-0412-4
12. Shindo Y, Inoko H, Yamamoto T, Ohno S. HLA-DRB1 typing of Vogt-Koyanagi-Harada's disease by PCR-RFLP and the strong association with DRB1*0405 and DRB1*0410. *Br J Ophthalmol* (1994) 78:223–6. doi: 10.1136/bjo.78.3.223
13. Ng JY, Luk FO, Lai TY, Pang C-P. Influence of molecular genetics in Vogt-Koyanagi-Harada disease. *J Ophthalmic Inflamm Infect* (2014) 4:20. doi: 10.1186/s12348-014-0020-1
14. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su M-W, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci Transl Med* (2014) 6:223ra23–223ra23. doi: 10.1126/scitranslmed.3007811
15. Strassner JP, Rashighi M, Ahmed Refat M, Richmond JM, Harris JE. Suction blistering the lesional skin of vitiligo patients reveals useful biomarkers of disease activity. *J Am Acad Dermatol* (2017) 76:847–55.e5. doi: 10.1016/j.jaad.2016.12.021
16. Sushama S, Dixit N, Gautam RK, Arora P, Khurana A, Anubhuti A. Cytokine profile (IL-2, IL-6, IL-17, IL-22, and TNF-α) in vitiligo-New insight into pathogenesis of disease. *J Cosmet Dermatol* (2019) 18:337–41. doi: 10.1111/jocd.12517

17. Gomes IA, de Carvalho FO, de Menezes AF, Almeida FM, Shanmugam S, de Souza Siqueira Quintans J, et al. The role of interleukins in vitiligo: a systematic review. *J Eur Acad Dermatol Venereol* (2018) 32:2097–111. doi: 10.1111/jdv.15016
18. Singh S, Singh U, Pandey SS. Serum concentration of IL-6, IL-2, TNF-α, and IFNγ in Vitiligo patients. *Indian J Dermatol* (2012) 57:12–4. doi: 10.4103/0019-5154.92668
19. Zhang L, Kang Y, Chen S, Wang L, Jiang M, Xiang L. Circulating CCL20: A potential biomarker for active vitiligo together with the number of Th1/17 cells. *J Dermatol Sci* (2019) 93:92–100. doi: 10.1016/j.jdermsci.2018.12.005
20. Cheuk S, Schlums H, Gallais Sérézal I, Martini E, Chiang SC, Marquardt N, et al. CD49a Expression Defines Tissue-Resident CD8+ T Cells Poised for Cytotoxic Function in Human Skin. *Immunity* (2017) 46:287–300. doi: 10.1016/j.immuni.2017.01.009
21. Richmond JM, Strassner JP, Zapata LJr, Garg M, Riding RL, Refat MA, et al. Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo. *Sci Transl Med* (2018) 10(450):eaam7710. doi: 10.1126/scitranslmed.aam7710
22. Tham HL, Linder KE, Olivry T. Autoimmune diseases affecting skin melanocytes in dogs, cats and horses: vitiligo and the uveodermatological syndrome: a comprehensive review. *BMC Vet Res* (2019) 15:251. doi: 10.1186/s12917-019-2003-9
23. Nguyen X-X, Muhammad L, Nietert PJ, Feghali-Bostwick C. IGFBP-5 Promotes Fibrosis via Increasing Its Own Expression and That of Other Pro-fibrotic Mediators. *Front Endocrinol* (2018) 9:601. doi: 10.3389/fendo.2018.00601
24. Nakae J, Kitamura T, Kitamura Y, Biggs WH3, Arden KC, Accili D. The forkhead transcription factor Foxo1 regulates adipocyte differentiation. *Dev Cell* (2003) 4:119–29. doi: 10.1016/s1534-5807(02)00401-x
25. Regazzetti C, Joly F, Marty C, Rivier M, Mehul B, Reiniche P, et al. Transcriptional Analysis of Vitiligo Skin Reveals the Alteration of WNT Pathway: A Promising Target for Repigmenting Vitiligo Patients. *J Invest Dermatol* (2015) 135:3105–14. doi: 10.1038/jid.2015.335
26. Gocho K, Kondo I, Yamaki K. Identification of autoreactive T cells in Vogt-Koyanagi-Harada disease. *Invest Ophthalmol Vis Sci* (2001) 42:2004–9.
27. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. *Cancer Cell* (2017) 31:711–23. doi: 10.1016/j.ccell.2017.04.003
28. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 Deficiency Reveals a Critical Role for CD8α+ Dendritic Cells in Cytotoxic T Cell Immunity. *Science* (2008) 322:1097–100. doi: 10.1126/science.1164206
29. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive $CD8^+$ T-cell accumulation in the skin. *J Invest Dermatol* (2012) 132:1869–76. doi: 10.1038/jid.2011.463
30. Gholijani N, Yazdani M-R, Dastgheib L. Predominant role of innate pro-inflammatory cytokines in vitiligo disease. *Arch Dermatol Res* (2020) 312:123–31. doi: 10.1007/s00403-019-01996-9
31. Dey-Rao R, Sinha AA. Vitiligo blood transcriptomics provides new insights into disease mechanisms and identifies potential novel therapeutic targets. *BMC Genomics* (2017) 18:109. doi: 10.1186/s12864-017-3510-3
32. 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. doi: 10.1038/jid.2011.377
33. Richmond JM, Bangari DS, Essien KI, Currimbhoy SD, Groom JR, Pandya AG, et al. Keratinocyte-Derived Chemokines Orchestrate T-Cell Positioning in the Epidermis during Vitiligo and May Serve as Biomarkers of Disease. *J Invest Dermatol* (2017) 137:350–8. doi: 10.1016/j.jid.2016.09.016
34. Wang XX, Wang QQ, Wu JQ, Jiang M, Chen L, Zhang CF, et al. Increased expression of CXCR 3 and its ligands in patients with vitiligo and CXCL 10 as a potential clinical marker for vitiligo. *Br J Dermatol* (2016) 174:1318–26. doi: 10.1111/bjd.14416
35. Yang L, Wei Y, Sun Y, Shi W, Yang J, Zhu L, et al. Interferon-gamma Inhibits Melanogenesis and Induces Apoptosis in Melanocytes: A Pivotal Role of CD8+ Cytotoxic T Lymphocytes in Vitiligo. *Acta Derm Venereol* (2015) 95:664–70. doi: 10.2340/00015555-2080
36. Karam RA, Zidan HE, Khater MH. Genetic variants of interferon-gamma and its mRNA expression and inflammatory parameters in the pathogenesis of vitiligo. *Biochem Cell Biol* (2017) 95:474–81. doi: 10.1139/bcb-2016-0228
37. Richmond JM, Masterjohn E, Chu R, Tedstone J, Youd ME, Harris JE. CXCR3 Depleting Antibodies Prevent and Reverse Vitiligo in Mice. *J Invest Dermatol* (2017) 137:982–5. doi: 10.1016/j.jid.2016.10.048
38. Boniface K, Jacquemin C, Darrigade A-S, Dessarthe B, Martins C, Boukhedouni N, et al. Vitiligo Skin Is Imprinted with Resident Memory CD8 T Cells Expressing CXCR3. *J Invest Dermatol* (2018) 138:355–64. doi: 10.1016/j.jid.2017.08.038
39. Gregg RK, Nichols L, Chen Y, Lu B, Engelhard VH. Mechanisms of spatial and temporal development of autoimmune vitiligo in tyrosinase-specific TCR transgenic mice. *J Immunol* (2010) 184:1909–17. doi: 10.4049/jimmunol.0902778
40. Yang L, Yang S, Lei J, Hu W, Chen R, Lin F, et al. Role of chemokines and the corresponding receptors in vitiligo: A pilot study. *J Dermatol* (2018) 45:31–8. doi: 10.1111/1346-8138.14004
41. Mackay LK, Rahimpour A, Ma JZ, Collins N, Stock AT, Hafon M-L, et al. The developmental pathway for CD103+CD8+ tissue-resident memory T cells of skin. *Nat Immunol* (2013) 14:1294. doi: 10.1038/ni.2744
42. Bassiouny D, Shaker O. Role of interleukin-17 in the pathogenesis of vitiligo. *Clin Exp Dermatol* (2011) 36:292–7. doi: 10.1111/j.1365-2230.2010.03972.x
43. Kotobuki Y, Tanemura A, Yang L, Itoi S, Wataya-Kaneda M, Murota H, et al. Dysregulation of melanocyte function by Th17-related cytokines: significance of Th17 cell infiltration in autoimmune vitiligo vulgaris. *Pigment Cell Melanoma Res* (2012) 25:219–30. doi: 10.1111/j.1755-148X.2011.00945.x
44. 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. doi: 10.1016/j.cca.2013.05.005
45. Zhou L, Shi Y-L, Li K, Hamzavi I, Gao T-W, Huggins RH, et al. Increased circulating Th17 cells and elevated serum levels of TGF-beta and IL-21 are correlated with human non-segmental vitiligo development. *Pigment Cell Melanoma Res* (2015) 28:324–9. doi: 10.1111/pcmr.12355
46. Imai Y, Sugita M, Nakamura S, Toriyama S, Ohno S. Cytokine production and helper T cell subsets in Vogt-Koyanagi-Harada's disease. *Curr Eye Res* (2001) 22:312–8. doi: 10.1076/ceyr.22.4.312.5510
47. Huang Y, Yu H, Cao Q, Deng J, Huang X, Kijlstra A, et al. The Association of Chemokine Gene Polymorphisms with VKH and Behcet's Disease in a Chinese Han Population. *BioMed Res Int* (2017) 2017:1274960. doi: 10.1155/2017/1274960
48. Wang C, Tian Y, Lei B, Xiao X, Ye Z, Li F, et al. Decreased IL-27 expression in association with an increased Th17 response in Vogt-Koyanagi-Harada disease. *Invest Ophthalmol Vis Sci* (2012) 53:4668–75. doi: 10.1167/iovs.12-9863
49. El-Asrar AMA, Berghmans N, Al-Obeidan SA, Gikandi PW, Opdenakker G, Van Damme J, et al. Differential CXC and CX3C Chemokine Expression Profiles in Aqueous Humor of Patients With Specific Endogenous Uveitic Entities. *Invest Ophthalmol Vis Sci* (2018) 59:2222–8. doi: 10.1167/iovs.17-23225
50. Chen Y, Yang P, Li F, Hou S, Jiang Z, Shu Q, et al. Association analysis of TGFBR3 gene with Vogt-Koyanagi-Harada disease and Behcet's disease in the Chinese Han population. *Curr Eye Res* (2012) 37:312–7. doi: 10.3109/02713683.2011.635398
51. Commodaro AG, Peron JPS, Genre J, Arslanian C, Sanches L, Muccioli C, et al. IL-10 and TGF-β Immunoregulatory Cytokines rather than Natural Regulatory T Cells are Associated with the Resolution Phase of Vogt-Koyanagi-Harada (VKH) Syndrome. *Scand J Immunol* (2010) 72:31–7. doi: 10.1111/j.1365-3083.2010.02401.x
52. Wang J, Ding N, Li Y, Cheng H, Wang D, Yang Q, et al. Insulin-like growth factor binding protein 5 (IGFBP5) functions as a tumor suppressor in human melanoma cells. *Oncotarget* (2015) 6:20636–49. doi: 10.18632/oncotarget.4114
53. Spritz RA. The genetics of generalized vitiligo: autoimmune pathways and an inverse relationship with malignant melanoma. *Genome Med* (2010) 2:78. doi: 10.1186/gm199
54. Rao RR, Li Q, Bupp MRG, Shrikant PA. Transcription Factor Foxo1 Represses T-bet-Mediated Effector Functions and Promotes Memory CD8+ T Cell Differentiation. *Immunity* (2012) 36:374–87. doi: 10.1016/j.immuni.2012.01.015
55. Ouyang W, Beckett O, Flavell RA, Li MO. An essential role of the Forkhead-box transcription factor Foxo1 in control of T cell homeostasis and tolerance. *Immunity* (2009) 30:358–71. doi: 10.1016/j.immuni.2009.02.003
56. Ouyang W, Liao W, Luo CT, Yin N, Huse M, Kim MV, et al. Novel Foxo1-dependent transcriptional programs control T reg cell function. *Nature* (2012) 491:554–9. doi: 10.1038/nature11581

57. Park TJ, Kim M, Kim H, Park SY, Park K-C, Ortonne J-P, et al. Wnt inhibitory factor (WIF)-1 promotes melanogenesis in normal human melanocytes. *Pigment Cell Melanoma Res* (2014) 27:72–81. doi: 10.1111/pcmr.12168
58. Kang HY, Lee JY, Lee JS, Choi YM. Peroxisome proliferator-activated receptors-gamma activator, ciglitazone, inhibits human melanocyte growth through induction of apoptosis. *Arch Dermatol Res* (2006) 297:472–6. doi: 10.1007/s00403-006-0646-4
59. Wohlfert EA, Nichols FC, Nevius E, Clark RB. Peroxisome proliferator-activated receptor γ (PPARγ) and immunoregulation: enhancement of regulatory T cells through PPARγ-dependent and-independent mechanisms. *J Immunol* (2007) 178:4129–35. doi: 10.4049/jimmunol.178.7.4129
60. Yang XY, Wang LH, Mihalic K, Xiao W, Chen T, Li P, et al. Interleukin (IL)-4 indirectly suppresses IL-2 production by human T lymphocytes via peroxisome proliferator-activated receptor γ activated by macrophage-derived 12/15-lipoxygenase ligands. *J Biol Chem* (2002) 277:3973–8. doi: 10.1074/jbc.M105619200
61. Sullivan JA, Kim EH, Plisch EH, Peng SL, Suresh M. FOXO3 regulates CD8 T cell memory by T cell-intrinsic mechanisms. *PloS Pathog* (2012) 8:e1002533. doi: 10.1371/journal.ppat.1002533
62. Ozel Turkcu U, Solak Tekin N, Gokdogan Edgunlu T, Karakas Celik S, Oner S. The association of FOXO3A gene polymorphisms with serum FOXO3A levels and oxidative stress markers in vitiligo patients. *Gene* (2014) 536:129–34. doi: 10.1016/j.gene.2013.11.055
63. Giri PS, Dwivedi M, Laddha NC, Begum R, Bharti AH. Altered expression of nuclear factor of activated T cells, forkhead box P3, and immune-suppressive genes in regulatory T cells of generalized vitiligo patients. *Pigment Cell Melanoma Res* (2020) 33(4):566–78. doi: 10.1111/pcmr.12862
64. Li B, Yang P, Zhou H, Huang X, Jin H, Chu L, et al. Upregulation of T-bet expression in peripheral blood mononuclear cells during Vogt-Koyanagi-Harada disease. *Br J Ophthalmol* (2005) 89:1410–2. doi: 10.1136/bjo.2005.074062
65. Horie Y, Takemoto Y, Miyazaki A, Namba K, Kase S, Yoshida K, et al. Tyrosinase gene family and Vogt-Koyanagi-Harada disease in Japanese patients. *Mol Vis* (2006) 12:1601–5.
66. Jin Y, Birlea SA, Fain PR, Gowan K, Riccardi SL, Holland PJ, et al. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. *N Engl J Med* (2010) 362:1686–97. doi: 10.1056/NEJMoa0908547
67. Rezk AF, Kemp DM, El-Domyati M, El-Din WH, Lee JB, Uitto J, et al. Misbalanced CXCL12 and CCL5 Chemotactic Signals in Vitiligo Onset and Progression. *J Invest Dermatol* (2017) 137:1126–34. doi: 10.1016/j.jid.2016.12.028
68. Liang L, Li Y, Tian X, Zhou J, Zhong L. Comprehensive lipidomic, metabolomic and proteomic profiling reveals the role of immune system in vitiligo. *Clin Exp Dermatol* (2019) 44:e216–23. doi: 10.1111/ced.13961
69. Boniface K, Seneschal J, Picardo M, Taïeb A. Vitiligo: Focus on Clinical Aspects, Immunopathogenesis, and Therapy. *Clin Rev Allergy Immunol* (2018) 54:52–67. doi: 10.1007/s12016-017-8622-7
70. Walsh CM, Hill RZ, Schwendinger-Schreck J, Deguine J, Brock EC, Kucirek N, et al. Neutrophils promote CXCR3-dependent itch in the development of atopic dermatitis. *Elife* (2019) 8:e48448. doi: 10.7554/eLife.48448
71. Mitra S, De Sarkar S, Pradhan A, Pati AK, Pradhan R, Mondal D, et al. Levels of oxidative damage and proinflammatory cytokines are enhanced in patients with active vitiligo. *Free Radic Res* (2017) 51:986–94. doi: 10.1080/10715762.2017.1402303
72. Solak B, Dikicier BS, Cosansu NC, Erdem T. Neutrophil to lymphocyte ratio in patients with vitiligo. *Postepy Dermatol Alergol* (2017) 34:468–70. doi: 10.5114/ada.2017.71114
73. Carter WJ, Crispin SM, Gould DJ, Day MJ. An immunohistochemical study of uveodermatologic syndrome in two Japanese Akita dogs. *Vet Ophthalmol* (2005) 8:17–24. doi: 10.1111/j.1463-5224.2005.04059.x
74. Kemp EH, Waterman EA, Gawkrodger DJ, Watson PF, Weetman AP. Autoantibodies to tyrosinase-related protein-1 detected in the sera of vitiligo patients using a quantitative radiobinding assay. *Br J Dermatol* (1998) 139:798–805. doi: 10.1046/j.1365-2133.1998.02503.x
75. Kemp EH, Gawkrodger DJ, Watson PF, Weetman AP. Autoantibodies to human melanocyte-specific protein pmel17 in the sera of vitiligo patients: a sensitive and quantitative radioimmunoassay (RIA). *Clin Exp Immunol* (1998) 114:333–8. doi: 10.1046/j.1365-2249.1998.00746.x
76. Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. *J Exp Med* (1999) 189:1639–48. doi: 10.1084/jem.189.10.1639
77. De Winter JCF. Using the Student's t-test with extremely small sample sizes. *Pract Assess Res Eval* (2013) 18:10.
78. Craiglow BG, King BA. Tofacitinib Citrate for the Treatment of Vitiligo: A Pathogenesis-Directed Therapy. *JAMA Dermatol* (2015) 151:1110–2. doi: 10.1001/jamadermatol.2015.1520
79. Liu LY, Strassner JP, Refat MA, Harris JE, King BA. Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. *J Am Acad Dermatol* (2017) 77:675–82. doi: 10.1016/j.jaad.2017.05.043
80. Harris JE, Rashighi M, Nguyen N, Jabbari A, Ulerio G, Clynes R, et al. Rapid skin repigmentation on oral ruxolitinib in a patient with coexistent vitiligo and alopecia areata (AA). *J Am Acad Dermatol* (2016) 74:370–1. doi: 10.1016/j.jaad.2015.09.073
81. Rothstein B, Joshipura D, Saraiya A, Abdat R, Ashkar H, Turkowski Y, et al. Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib. *J Am Acad Dermatol* (2017) 76:1054–60. doi: 10.1016/j.jaad.2017.02.049
82. Gonzales AJ, Bowman JW, Fici GJ, Zhang M, Mann DW, Mitton-Fry M. Oclacitinib (APOQUEL®) is a novel Janus kinase inhibitor with activity against cytokines involved in allergy. *J Vet Pharmacol Ther* (2014) 37:317–24. doi: 10.1111/jvp.12101
83. Cosgrove SB, Wren JA, Cleaver DM, Martin DD, Walsh KF, Harfst JA, et al. Efficacy and safety of oclacitinib for the control of pruritus and associated skin lesions in dogs with canine allergic dermatitis. *Vet Dermatol* (2013) 24:479–e114. doi: 10.1111/vde.12047
84. Little PR, King VL, Davis KR, Cosgrove SB, Stegemann MR. A blinded, randomized clinical trial comparing the efficacy and safety of oclacitinib and ciclosporin for the control of atopic dermatitis in client-owned dogs. *Vet Dermatol* (2015) 26:23–e8. doi: 10.1111/vde.12186
85. Cosgrove SB, Wren JA, Cleaver DM, Walsh KF, Follis SI, King VI, et al. A blinded, randomized, placebo-controlled trial of the efficacy and safety of the J anus kinase inhibitor oclacitinib (A poquel®) in client-owned dogs with atopic dermatitis. *Vet Dermatol* (2013) 24:587–e142. doi: 10.1111/vde.12088
86. Cavalcanti JVJ, Hasbach A, Barnes K, Dange RB, Patterson J, Saavedra PV. Skin depigmentation associated with toceranib phosphate in a dog. *Vet Dermatol* (2017) 28:400–e95. doi: 10.1111/vde.12422
87. Peterson AC, Swiger S, Stadler WM, Medved M, Karczmar G, Gajewski TF. Phase II study of the Flk-1 tyrosine kinase inhibitor SU5416 in advanced melanoma. *Clin Cancer Res* (2004) 10:4048–54. doi: 10.1158/1078-0432.CCR-03-0766
88. Danaher P, Warren S, Dennis L, D'Amico L, White A, Disis ML, et al. Gene expression markers of Tumor Infiltrating Leukocytes. *J Immunother Cancer* (2017) 5:18. doi: 10.1186/s40425-017-0215-8

Conflict of Interest: JMR is an inventor on patent application #15/851,651, "Anti-human CXCR3 antibodies for the Treatment of Vitiligo" which covers targeting CXCR3 for the treatment of vitiligo; and on patent #62489191, "Diagnosis and Treatment of Vitiligo" which covers targeting IL-15 and Trm for the treatment of vitiligo. CND is an employee of Nanostring Technologies.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 04 February 2021
doi: 10.3389/fimmu.2020.624566

A Concise Review on the Role of Endoplasmic Reticulum Stress in the Development of Autoimmunity in Vitiligo Pathogenesis

Shahnawaz D. Jadeja, Jay M. Mayatra, Jayvadan Vaishnav, Nirali Shukla and Rasheedunnisa Begum *

Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, India

Vitiligo is characterized by circumscribed depigmented macules in the skin resulting due to the autoimmune destruction of melanocytes from the epidermis. Both humoral as well as cell-mediated autoimmune responses are involved in melanocyte destruction. Several studies including ours have established that oxidative stress is involved in vitiligo onset, while autoimmunity contributes to the disease progression. However, the underlying mechanism involved in programing the onset and progression of the disease remains a conundrum. Based on several direct and indirect evidences, we suggested that endoplasmic reticulum (ER) stress might act as a connecting link between oxidative stress and autoimmunity in vitiligo pathogenesis. Oxidative stress disrupts cellular redox potential that extends to the ER causing the accumulation of misfolded proteins, which activates the unfolded protein response (UPR). The primary aim of UPR is to resolve the stress and restore cellular homeostasis for cell survival. Growing evidences suggest a vital role of UPR in immune regulation. Moreover, defective UPR has been implicated in the development of autoimmunity in several autoimmune disorders. ER stress-activated UPR plays an essential role in the regulation and maintenance of innate as well as adaptive immunity, and a defective UPR may result in systemic/tissue level/organ-specific autoimmunity. This review emphasizes on understanding the role of ER stress-induced UPR in the development of systemic and tissue level autoimmunity in vitiligo pathogenesis and its therapeutics.

Keywords: endoplasmic reticulum, unfolded protein response, vitiligo, melanocytes, autoimmunity

OPEN ACCESS

Edited by:
Isabelle Caroline Le Poole, Northwestern University, United States

Reviewed by:
Jillian M. Richmond, University of Massachusetts Medical School, United States
Mauro Picardo, San Gallicano Hospital, Italy

***Correspondence:**
Rasheedunnisa Begum
rasheedunnisab@yahoo.co.in

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *31 October 2020*
Accepted: *22 December 2020*
Published: *04 February 2021*

Citation:
Jadeja SD, Mayatra JM, Vaishnav J, Shukla N and Begum R (2021) A Concise Review on the Role of Endoplasmic Reticulum Stress in the Development of Autoimmunity in Vitiligo Pathogenesis. Front. Immunol. 11:624566. doi: 10.3389/fimmu.2020.624566

INTRODUCTION

Extensive research over the years established that a complex interaction between genetic, environmental, biochemical, and immunological factors collectively generate a microenvironment favoring melanocyte loss in vitiligo (1–3). The complex genetics of vitiligo involves multiple susceptibility loci, incomplete penetrance, and genetic heterogeneity with gene-gene and gene-environment interactions and altered miRNA expression (**Table S1**) (4–6). Accumulation of oxidative stress due to defective recycling of tetrahydrobiopterin, mitochondrial impairment

(**Table S2**), and compromised antioxidant system are reported in vitiligo patients (7–11). This accumulated oxidative stress might result in DNA damage, lipid and protein peroxidation, neoantigen formation, and may affect normal melanogenesis in melanocytes (12). Moreover, both humoral and cellular autoimmunity, altered $CD4^+/CD8^+$ T cell ratio, decreased regulatory T cells (Tregs) function, presence of autoreactive anti-melanocyte $CD8^+$ T cells in both blood and skin, as well as imbalance of pro- and anti-inflammatory cytokine levels are reported to be involved in vitiligo pathogenesis (2, 13–20). Our extensive population based studies indicated impeded redox and immune homeostasis in the skin and blood of vitiligo patients from Gujarat population (2, 14, 17, 21–41). Hence, based on our observations, we proposed that oxidative stress triggers vitiligo onset, while autoimmunity contributes to the disease progression (2). Despite extensive research, the exact mechanism which connects the triggering factors with the disease progression is still obscure. Investigating the connecting link between the factors involved in onset and progression of vitiligo may enhance our understanding of its pathomechanisms and thereby open new avenues for development of novel therapeutic strategies.

It has been reported that melanocytes from vitiligo patients had dilated endoplasmic reticulum (ER) as compared to healthy melanocytes (42). The accumulation of misfolded proteins in the ER lumen and its dilation are the characteristics of ER stress. Excessive load of protein folding in ER may also generate oxidative stress (43). Several studies suggested the generation of ROS during normal protein folding process in ER and oxidation of ER proteins under oxidative stress led to the accumulation of misfolded proteins (44, 45). Interestingly, vitiligo patients are reported to have significantly elevated homocysteine levels which may induce oxidative stress, ER stress, and expression of pro-inflammatory cytokines (28, 46, 47). Unfolded protein response (UPR) upon ER stress is also known to regulate the innate immune response in different ways (48). Based on several direct and indirect evidences, earlier we speculated that ER stress could be a major link between oxidative stress and autoimmunity, which might play a key role in the onset and exacerbation of vitiligo (49). In this review, we will emphasize on the potential role of ER stress in the development of autoimmune/inflammatory responses in vitiligo.

UNDERSTANDING ER STRESS-INDUCED UPR

The ER is an active intracellular organelle with different functions like protein folding and maturation within the eukaryotic cell, essential for cellular homeostasis, proteostasis, cellular development, and stress responsiveness (50). Aberrations in protein folding may result in an imbalance leading to the accumulation of misfolded proteins in the ER, which is known as ER stress. To combat ER stress, the cell activates UPR which may alleviate ER stress through global translation attenuation, induction of chaperones, degradation of misfolded proteins by ER-associated degradation (ERAD), and apoptosis (51). The accumulation of misfolded proteins increases the production of BiP/GRP78 (78-kDa glucose-regulated protein) (52). GRP78 forms dynamic stability between the nascent polypeptides (unfolded proteins) and intra-luminal domains of the three ER stress sensors *viz.* inositol-requiring enzyme 1α (IRE1α), PKR like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (53–56). In non-stress conditions, all three sensors are primarily bound with GRP78, which helps to maintain its inactive state. The fate of the stressed cell towards survival or death depends upon the interplay among these three major arms of the UPR signaling pathways (57, 58) (**Figure 1**).

ROLE OF UPR IN INFLAMMATION AND IMMUNE REGULATION

The UPR has emerged as a hallmark of several diseases including inflammatory bowel disease, arthritis, neurodegenerative diseases, diabetes mellitus, stroke, and cancer (58–60). UPR plays a vital role in inflammation, mainly regulated by nuclear factor kappaB (NF-κB) and activator protein 1 (AP-1) (61–63). NF-κB regulates the expression of various genes including those encoding cytokines, chemokines, and *also* participates in inflammasome regulation. All three pathways can activate NF-κB independently, but IRE1α plays an essential role in inflammatory pathways (64). IRE1 interaction with TRAF2 (TNF Receptor Associated Factor 2) in response to ER stress leads to the recruitment of IκB kinase (IKK) which phosphorylates and subsequently degrades IκB (65), thereby activating NF-κB. PERK-eIF2α signaling pathway halts overall protein synthesis. Thus, NF-κB to IκB ratio in cell increases due to IκB's shorter half-life than NF-κB, which subsequently favors NF-κB dependent transcription (66, 67). Activated IRE1 also interacts with TRAF2 and ASK1 that further activates the JNK in addition to the activation of NF-κB and leading to the AP1 activation (68–70). Genes transcribed by AP1 include cytokines such as tumor necrosis factor (TNF), keratinocyte growth factor (KGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL8, IL-1 receptor antagonists, and fibroblast growth factor receptor 1, implying that AP1 also plays a crucial role in the regulation of cytokine receptors (71).

All three major arms of UPR *viz.* PERK, ATF6, and IRE1 have a central role in immune regulation. In PERK signaling, ATF4 activates *IL6* transcription in macrophages (72). Further, phosphorylation of eIF2α upon activation of PERK signaling allows selective translation of mRNAs bearing upstream open reading frames (uORFs) in their 5′-untranslated regions (5′-UTR), which might act as novel antigens for MHC-I presentation (73). IRE1α undergoes phosphorylation by signals downstream to Toll-like receptors (TLRs). Phosphorylated IRE1α induces unspliced *XBP1u* mRNA splicing resulting in an active transcription factor, spliced XBP1 (XBP1s), which activates the

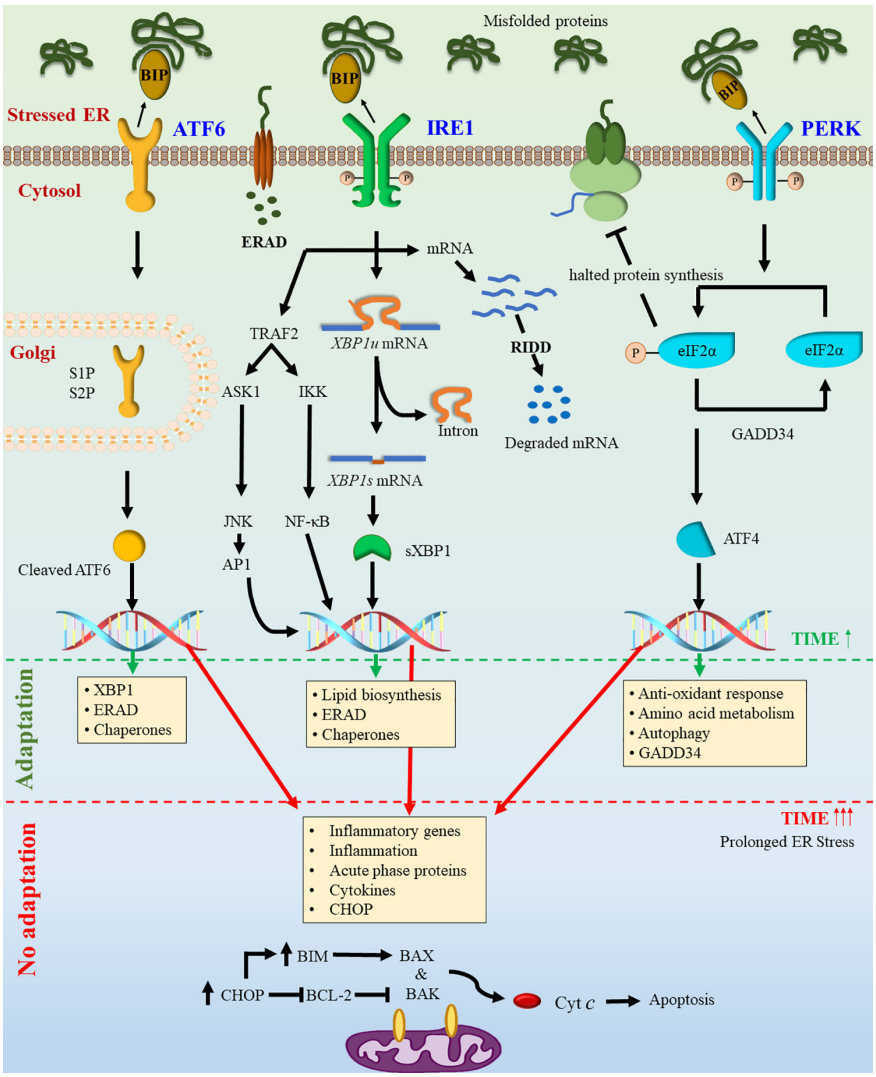


FIGURE 1 | Activation of unfolded protein response. Due to stress conditions, unfolded protein levels increase in the ER lumen. The dissociation of GRP78 from transmembrane sensors PERK, ATF6, and IRE1 leads to the activation of UPR signaling. Activation of IRE1 and PERK results in their oligomerization and transphosphorylation. Active IRE1 triggers the unconventional splicing of *XBP1u* mRNA resulting in the translation of an active transcription factor sXBP1. The active IRE1 can also interact with JNK and TRAF2 and thereby activating downstream signaling. The activation of ATF6 leads to its translocation to the Golgi and its proteolytic cleavage, resulting in a transcriptionally active form. Activation of PERK triggers phosphorylation of eIF2α leading to global translational attenuation and favoring translation of ATF4. Activation of all three pathways activate downstream transcriptional machinery resulting in expression of target genes to overcome the stress conditions. Persistent and excessive ER stress may lead to activation of mitochondria mediated cell death pathway.

production of pro-inflammatory cytokines in macrophages (74). IRE1α/XBP1s also contributes to homeostasis and survival of CD8α$^+$ conventional dendritic cells (DCs) (75). Furthermore, it has also been reported that XBP1s may regulate the expression of *TNF* and *IL6* in macrophages (74). Notably, transcriptional targets of ATF6 include XBP1 and thus ATF6 is also recognized as a regulator of the IRE1/XBP1 axis (76–78). Interestingly, it has been reported that cleaved ATF6 can act as an enhancer and increase the CREBH-mediated (cAMP response element-binding protein H) acute inflammatory response, indicating a link between ATF6 and inflammation (79).

IMPLICATIONS OF LOCALIZED AND PERIPHERAL ER STRESS IN VITILIGO

ER Stress in the Skin Microenvironment

In the skin, ER stress may be induced by various endogenous as well as exogenous stressors such as UV irradiation, trauma, and chemical stressors (**Table S3**). Interestingly, chemical stressors including phenolic derivatives such as rhododendrol, hydroquinone, MBEH (mono benzyl ether of hydroquinone), and 4-TBP (4-tertiary butyl phenol) present in the cosmetic skin whitening agents have been identified to induce UPR mediated

melanocyte death (80–84). Importantly, physiological ER stress is required for the maintenance of normal biological functions including keratinocyte differentiation in the skin (85). ER stress-signaled UPR was found to be activated during epidermal keratinocyte differentiation (85–87). Expression of UPR activation markers such as sXBP1, CHOP, and GRP78 is increased in the undifferentiated/proliferative stage of keratinocytes during their differentiation (85, 88). Furthermore, $CD8^+$ T cells are found to be essential effectors of melanocyte destruction in vitiligo patients (89, 90). The recruitment of $CD8^+$ T cells to skin lesions is carried out by the IFN-γ-mediated T cell chemokine receptor, C-X-C motif chemokine receptor 3 (CXCR3), and its ligands CXCL9, CXCL10, and CXCL11, which are found to be abundant in skin biopsy specimens from vitiligo patients (91). The blockade of this pathway mitigated the vitiligo in mice as well as in human subjects (92, 93). IRE1α/sXBP1 signaling in stressed keratinocytes augmented the levels of CXCL16, which is involved in $CD8^+$ T cell recruitment to skin lesions (94).

ER Stress in Peripheral System

Peripheral blood mononuclear cells (PBMCs) play a critical role in immune response, metabolism, and communication with other cells. PBMCs of vitiligo patients were reported to have metabolic deregulations and oxidative stress, similar to those found in melanocytes and the lesional epidermis (95–97). Histological studies have demonstrated that infiltration of $CD8^+$ T cells occurs surrounding the vitiligo lesions (98–101). Hence, the role of ER stress in the regulation of the peripheral immune system may be interesting in understanding vitiligo pathogenesis. The UPR signaling is involved in the differentiation, proliferation, and homeostasis of both B and T cells. In the presence of a differentiation stimulus, both B and T cells increase GRP78 protein levels, initiate *XBP1* splicing, and induce ATF6 signaling (102–105). The inhibition of GRP78, ATF6, or XBP1 greatly reduces plasma cell differentiation and their efficacy upon maturation (102, 106). Cell fate determines whether UPR signaling is maintained for example, early B cells exhibit active UPR signaling, but it is absent in mature B cells. Similarly, $CD4^-$/$CD8^-$ progenitor T cells do not exhibit UPR, but greatly increase UPR during maturation as $CD4^+$/$CD8^+$ T cells. Upon differentiation to $CD4^+$ T cells, the UPR is once again repressed (103). $CD8^+$ T cells play a major role in anti-melanocyte autoimmunity in vitiligo. Infection of mice with lymphocytic choriomeningitis virus (LCMV) resulted in the upregulation of spliced and unspliced XBP1 that further enhanced differentiation of $CD8^+$ T cells (104). ER stress chaperone, GRP78 also plays an essential role in the regulation of granzyme B in $CD8^+$ T cells and $CD8^+$ intraepithelial lymphocytes. $CD8^+$ T cells of heterozygous GRP78 mouse model had reduced granzyme B secretion and cytotoxicity. This granzyme B deficiency was due to a reduction in IL-2 mediated proliferation, as exogenous IL-2 helped to partially restore granzyme B expression (107).

ER stress is also implicated in the regulation of Treg cells. Human Treg clones had elevated IL-10 production when treated with thapsigargin, an activator of ER stress and UPR, in an eIF2α phosphorylation-dependent manner (108). Loss of ATF4 led to a modest increase in *FOXP3* mRNA expression in mouse $CD4^+$ cells differentiated under T regulatory conditions in a high oxidizing environment (109). Recently, decreased levels of NFATs and FOXP3 are reported in Tregs of generalized vitiligo patients which may impair Treg cell function along with reduced IL10 and CTLA4 levels (18–20).

PLAUSIBLE INVOLVEMENT OF ER STRESS IN VITILIGO AUTOIMMUNITY

The ER stress may contribute to the development of autoimmunity through the recognition of misfolded proteins by autoreactive immune cells. Release of neo-autoantigens and UPR-related autoantigens by stressed cells, subsequently provoke autoimmunity. ER stress may indirectly contribute to autoimmunity through impairment of immune-tolerance mechanisms in cells with an abnormal UPR and conferring resistance to UPR mediated apoptosis in autoreactive cells by upregulating ERAD-associated proteins (48). Under certain pathophysiological conditions, several ER chaperones are translocated to the cell surface or released in extracellular space, which may serve as damage-associated molecular patterns (DAMPS) and attract the innate immune system to target "abnormal" cells for phagocytosis leading to subsequent activation of adaptive immunity (110). These phenomena have been established in various autoimmune disorders such as type I diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) (111, 112). Interestingly, one of the essential ER chaperones, GRP78 has immunomodulatory functions upon cell surface translocation. Vig et al. (112) have demonstrated that sGRP78 serves as a pro-apoptotic signaling receptor in beta cells and postulated that inflamed beta cells set up a self-destructing feedback loop through the combined surface translocation and secretion of GRP78. These findings suggest an important role of surface translocated GRP78 in autoimmune destruction of target cells. Though the role of sGRP78 is not yet established in melanocyte destruction, a few studies on other chaperones have encouraged researchers to hypothesize its role in melanocyte destruction in vitiligo. Kroll et al. (113) have observed that 4-tertiary butyl-phenol (4-TBP) induced expression and release of HSP70 by PIG3V melanocytes (immortalized vitiligo melanocytes). Further, it induced expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on the membrane and activated DC effector functions towards the stressed melanocytes. Interestingly, they observed increased expression of TRAIL and CD11c+ dendritic cell infiltration in the perilesional skin of vitiligo patients. This suggested that HSP70 release by stressed melanocytes may facilitate DC activation leading to melanocyte destruction in vitiligo (113). In another exciting study, Zhang et al. have reported oxidative stress-induced translocation of calreticulin (CRT) on melanocyte surface (114). They observed that CRT surface translocation (sCRT) on melanocytes induced expression of pro-inflammatory cytokines such as IL-6 and TNF-a by human PBMCs *in vitro*. Elevated sCRT was concordant with decreased membrane CD47 expression; CD47 acts as a "don't eat

me" signal in contrast to "eat me" signal of CRT, resulting in immunogenic cell death (114, 115). Moreover, a positive correlation of plasma CRT levels was observed with the area affected and the activity of the disease in vitiligo patients suggesting CRT's role in vitiligo pathogenesis (114). These studies led us to postulate the potential role of ER stress response proteins in the initial development of autoimmune response against stressed melanocytes.

FUTURE PROSPECTS AND TRANSLATIONAL RELEVANCE OF ER STRESS IN VITILIGO

As per the recent understanding, it is clear that the ER stress is at the verge of oxidative stress and inflammatory/immunoregulatory response in the cell, making it an ideal therapeutic target. However, the core UPR signaling involved in melanocyte biology and vitiligo pathomechanism is not much explored. A few studies demonstrate that therapeutic agents modulating ER stress can be promising for vitiligo treatment. Zhu et al. (116) have reported that Baicalin attenuated the progression and reduced the area of depigmentation in the C57BL/6 mouse model of vitiligo. Furthermore, they observed that Baicalin stimulated the proliferation of melanocytes in depigmented skin, which further led to a decrease in $CD8^+$ T cell infiltration and the expression of CXCL10 and CXCR3 in mice skin. Interestingly, they also observed significantly decreased levels of IL-6, TNF-α, IFN-γ, and IL-13 in sera of vitiligo mice models (116). Baicalin is an active ingredient of *S. baicalensis*, which is reported to protect cardio-myocytes and chondrocytes from ER stress-induced apoptosis (117, 118). Bilobalide is one of the active components of *G. biloba* extract. Lu et al. reported that pre-treatment with bilobalide could protect melanocytes from oxidative damage by inhibiting H_2O_2 induced cytotoxicity. It also inhibited eIF2α phosphorylation and downregulated CHOP expression (119). However, the exact mechanism of ER stress modulation by these herbal extracts is not clear. Apart from these, therapeutic strategies aiming to improve protein-folding capacity during ER stress might also be promising. Chemical chaperones such as Tauro-ursodeoxycholic acid (TUDCA) and 4–phenyl butyrate (PBA) can improve protein folding in the ER. Success in the alleviation of ER stress-induced hyperglycemia, restoration of insulin sensitivity, and fatty liver disease amelioration was observed upon TUDCA and 4-PBA treatments in obese mice (120). Cao et al. have reported that TUDCA and 4-PBA decreased ER stress in the intestinal epithelium leading to reduced dextran sodium sulfate (DSS) induced colitis severity (121). Moreover, it was found that 4-PBA leads to a decrease in lipopolysaccharide (LPS)-induced lung inflammation through modulating ER stress, NF–κB, and hypoxia-inducing factor 1α (HIF1α) signaling (122). Nevertheless, further studies to understand the molecular mechanism of ER stress signaling in melanocytes, neighboring keratinocytes, and circulatory as well as infiltrated immune cells are warranted for the development of novel targeted and personalized ER stress modulating therapeutics for vitiligo.

CONCLUSIONS

Over the decades, the role of UPR in the pathogenesis of various autoimmune disorders is well established. However, its role in anti-melanocyte autoimmunity in vitiligo is yet to be unraveled.

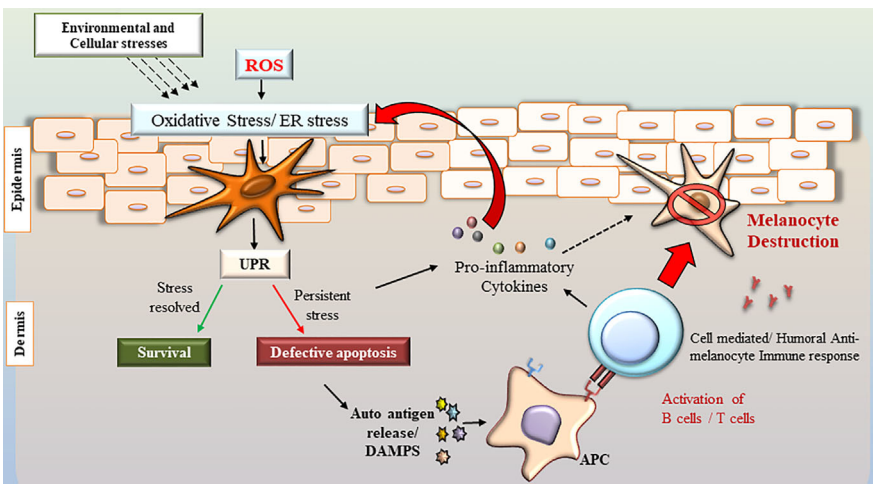


FIGURE 2 | Role of ER stress, oxidative stress, inflammation, and autoimmunity in vitiligo pathogenesis. Various exogenous and endogenous stressors in the skin result in oxidative stress and ER stress. ER stress activates the UPR signaling to resolve the stress. However, prolonged ER stress and defective UPR may lead to activation of inflammatory transcriptional program and release of proinflammatory cytokines, which generates further ER stress and oxidative stress. Further, the defective apoptosis of melanocytes might result in the release of misfolded/unfolded proteins that can potentially act as autoantigens and might be identified as damage-associated molecular patterns by the immune cells. The antigen presenting cells (APC) may process and present the altered proteins/peptides generating novel epitopes, which in turn will activate target B and T cells, resulting in an anti-melanocyte autoimmune response.

Although extensive research has been done to decipher the conundrum of the underlying molecular mechanisms of melanocyte destruction, the role of UPR in vitiligo still remains an enigma. Several studies have uncovered essential direct and indirect mechanistic links that established cross-talk among oxidative stress, ER stress, and autoimmunity, which appears to be crucial in vitiligo pathogenesis (**Figure 2**). A wide range of studies has demonstrated that ER stress-activated UPR plays an essential role in the regulation and maintenance of innate as well as adaptive immunity. Though the role of ER stress in affecting immunity at systemic as well as tissue level is not well understood, a defective UPR might result in organ-specific autoimmunity. Since the immune response is a multi-step process, depending on the microenvironment of the cell, UPR can promote cell survival or death. This review suggests that the UPR is orchestrating the cell fate differently in the active participating immune cells and the target melanocytes. The genetic predisposition and the microenvironment of the target tissue play a major role in deciding the cell fate. Thus, further studies deciphering the tissue/cell type-specific UPR and developing UPR modulating strategies accordingly are warranted. Future research work in this direction will be promising in the development of novel immunotherapeutics for vitiligo.

AUTHOR CONTRIBUTIONS

SJ and RB developed the concept. SJ, JM, JV, and NS performed a literature survey contributed to manuscript writing. RB contributed to the critical revision and approval of the article. All authors contributed to the article and approved the submitted version.

FUNDING

RB thanks financial support from the Science & Engineering Research Board (SERB), Government of India (Grant No. EMR/2016/001565) and SJ thanks the University Grants Commission (UGC) for Senior Research Fellowship.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.624566/full#supplementary-material

REFERENCES

1. Shajil EM, Chatterjee S, Agrawal D, Bagchi T, Begum R. Vitiligo: Pathomechanisms and genetic polymorphism of susceptible genes. *Indian J Exp Biol* (2006) 44:526–39.
2. Laddha NC, Dwivedi M, Mansuri MS. Vitiligo: Interplay between oxidative stress and immune system. *Exp Dermatol* (2013a) 22:245–50. doi: 10.1111/exd.12103
3. Kundu RV, Mhlaba JM, Rangel SM, Le Poole IC. The Convergence Theory For Vitiligo: A Reappraisal. *Exp Dermatol* (2019) 28:647–55. doi: 10.1111/exd.13677
4. Zhang XJ, Chen JJ, Liu JB. The genetic concept of vitiligo. *J Dermatol Sci* (2005) 39:137–46. doi: 10.1016/j.jdermsci.2005.06.004
5. Mansuri MS, Singh M, Dwivedi M, Laddha NC, Marfatia YS, Begum R. miRNA profiling revealed differentially expressed miRNA signatures from skin of non-segmental vitiligo patients. *Br J Dermatol* (2014a) 1:1263–7. doi: 10.1186/1755-8166-7-S1-P118
6. Mansuri MS, Singh M, Begum R. miRNA signatures and transcriptional regulation of their target genes in vitiligo. *J Dermatol Sci* (2016a) 84:50–8. doi: 10.1016/j.jdermsci.2016.07.003
7. Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. *J Invest Dermatol* (1991) 97:1081–5. doi: 10.1111/1523-1747.ep12492612
8. Schallreuter KU, Wood JM, Ziegler I. Defective tetrahydrobiopterin and catecholamine biosynthesis in the depigmentation disorder vitiligo. *BBA - Mol Basis Dis* (1994a) 1226:181–92. doi: 10.1016/0925-4439(94)90027-2
9. Schallreuter KU, Wood JM, Pittelkow MR. Regulation of melanin biosynthesis in the human epidermis by tetrahydrobiopterin. *Science* (1994b) 11(263):1444–6. doi: 10.1126/science.8128228
10. Dell'Anna ML, Maresca V, Briganti S. Mitochondrial impairment in peripheral blood mononuclear cells during the active phase of vitiligo. *J Invest Dermatol* (2001) 117:908–13. doi: 10.1046/j.0022-202X.2001.01459.x
11. Shajil EM, Begum R. Antioxidant status of segmental and non-segmental vitiligo. *Pigment Cell Res* (2006) 19:179–80. doi: 10.1111/j.1600-0749.2006.00299.x
12. Westerhof W, D'Ischia M. Vitiligo puzzle: The pieces fall in place. *Pigment Cell Res* (2007) 20:345–59. doi: 10.1111/j.1600-0749.2007.00399.x
13. Luiten RM, Van Den Boorn JG, Konijnenberg D. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. *J Invest Dermatol* (2009) 129:2220–32. doi: 10.1038/jid.2009.32
14. 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* (2013a) 26(4):586–91. doi: 10.1111/pcmr.12105
15. Dwivedi M, Laddha NC, Weetman AP, Begum R, Kemp H. Vitiligo-A Complex Autoimmune Skin Depigmenting. Autoimmune Diseases. In: K Chatzidionysiou, ed. *Autoimmunity - Pathogenesis, Clinical Aspects and Therapy of Specific Autoimmune Diseases*. Intech- open science/open minds (2015a). p. 153. doi: 10.5772/59762
16. Dwivedi M, Kemp EH, Laddha NC, Mansuri MS, Weetman AP, Begum R. Regulatory T cells in Vitiligo: Implications for pathogenesis and therapeutics. *Autoimmun Rev* (2015b) 14:49–56. doi: 10.1016/j.autrev.2014.10.002
17. Singh M, Kotnis A, Jadeja SD, et al. Cytokines: the yin and yang of vitiligo pathogenesis. *Expert Rev Clin Immunol* (2018a) 15(2):177–88. doi: 10.1080/1744666X.2019.1550358
18. Giri PS, Dwivedi M, Laddha NC, Begum R, Bharti AH. Altered Expression of Nuclear Factor of Activated T Cells, Forkhead Box P3, and Immune-Suppressive Genes in Regulatory T Cells of Generalized Vitiligo Patients. *Pigment Cell Melanoma Res* (2020a) 33:566–78. doi: 10.1111/pcmr.12862
19. Giri PS, Dwivedi M, Begum R. Decreased suppression of CD^{8+} and CD^{4+} T cells by peripheral Regulatory T cells in Generalized Vitiligo due to reduced NFATC1 and FOXP3 proteins. *Exp Dermatol* (2020b) 29:759–75. doi: 10.1111/exd.14157
20. Giri PS, Patel S Begum R, Dwivedi M. Association of FOXP3 and GAGE10 Promoter Polymorphisms and Decreased FOXP3 Expression in Regulatory T cells with Susceptibility to Generalized Vitiligo in Gujarat Population. *Gene* (2020c) 09:145295. doi: 10.1016/j.gene.2020.145295
21. Birlea SA, Ahmad FJ, Uddin RM, Ahmad S, Pal SS, Begum R, et al. Association of Generalized Vitiligo with MHC Class II Loci in Patients from the Indian Subcontinent. *J Invest Dermatol* (2013) 133:1369–72. doi: 10.1038/jid.2012.501
22. 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. doi: 10.1111/j.1755-148X.2011.00892.x
23. Dwivedi M, Laddha NC, Begum R. Correlation of increased MYG1 expression and its promoter polymorphism with disease progression and higher susceptibility in vitiligo patients. *J Dermatol Sci* (2013b) 71:195–202. doi: 10.1016/j.jdermsci.2013.04.026

24. Dwivedi M, Laddha NC, Shah K, Shah BJ, Begum R. Involvement of interferon-gamma genetic variants and intercellular adhesion molecule-1 in onset and progression of generalized Vitiligo. *J Interferon Cytokine Res* (2013c) 33:646–59. doi: 10.1089/jir.2012.0171
25. Dwivedi M, Laddha NC, Mansuri MS, Marfatia YS, Begum R. Association of NLRP1 genetic variants and mRNA overexpression with generalized vitiligo and disease activity in a Gujarat population. *Br J Dermatol* (2013d) 169:1114–25. doi: 10.1111/bjd.12467
26. Imran M, Laddha NC, Dwivedi M, Mansuri MS, Singh J, Rani R, et al. Interleukin- 4 genetic variants correlate with its transcript and protein levels in patients with vitiligo. *Br J Dermatol* (2012) 1:314–23. doi: 10.1111/j.1365-2133.2012.11000.x
27. Jadeja SD, Mansuri MS, Singh M, Dwivedi M, Laddha NC, Begum R. A case-control study on association of proteasome subunit beta 8 (PSMB8) and transporter associated with antigen processing 1 (TAP1) polymorphisms and their transcript levels in vitiligo from Gujarat. *PLoS One* (2017) 10(12): e0180958. doi: 10.1371/journal.pone.0180958
28. Jadeja SD, Mansuri MS, Singh M, Patel H, Marfatia YS, Begum R. Association of elevated homocysteine levels and Methylenetetrahydrofolate reductase (MTHFR) 1298 A>C polymorphism with Vitiligo susceptibility in Gujarat. *J Dermatol Sci* (2018) 90:112–22. doi: 10.1016/j.jdermsci.2018.01.003
29. Laddha NC, Dwivedi M, Shajil EM, Prajapati H, Marfatia YS, Begum R. Association of PTPN22 1858C/T polymorphism with vitiligo susceptibility in Gujarat population. *J Dermatol Sci* (2008) 49:260–2. doi: 10.1016/j.jdermsci.2007.10.002
30. Laddha NC, Dwivedi M, Begum R. Increased Tumor Necrosis Factor (TNF)-α and Its Promoter Polymorphisms Correlate with Disease Progression and Higher Susceptibility towards Vitiligo. *PLoS One* (2012) 7:e52298. doi: 10.1371/journal.pone.0052298
31. Laddha NC, Dwivedi M, Gani AR. Involvement of superoxide dismutase isoenzymes and their genetic variants in progression of and higher susceptibility to vitiligo. *Free Radic Biol Med* (2013b) 65:1110–25. doi: 10.1016/j.freeradbiomed.2013.08.189
32. Laddha NC, Dwivedi M, Gani AR. Tumor Necrosis Factor B (TNFB) Genetic Variants and Its Increased Expression Are Associated with Vitiligo Susceptibility. *PLoS One* (2013c) 8:1–11. doi: 10.1371/journal.pone.0081736
33. Laddha NC, Dwivedi M, Mansuri MS. Role of oxidative stress and autoimmunity in onset and progression of vitiligo. *Exp Dermatol* (2014a) 23:352–3. doi: 10.1111/exd.12372
34. Laddha NC, Dwivedi M, Mansuri MS, Singh M, Patel HH, Agarwal N, et al. Association of Neuropeptide Y (NPY), Interleukin-1B (IL1B) Genetic Variants and Correlation of IL1B Transcript Levels with Vitiligo Susceptibility. *PLoS One* (2014b) 15(9):e107020. doi: 10.1371/journal.pone.0107020
35. Mansuri MS, Laddha NC, Dwivedi M, Patel D, Alex T, Singh M, et al. Genetic variations (Arg5Pro and Leu6Pro) modulate the structure and activity of GPX1 and genetic risk for vitiligo. *Exp Dermatol* (2016b) 25:654–7. doi: 10.1111/exd.13007
36. Mansuri MS, Jadeja SD, Singh M. The catalase gene promoter and 5′-untranslated region variants lead to altered gene expression and enzyme activity in vitiligo. *Br J Dermatol* (2017) 177:1590–600. doi: 10.1111/bjd.15681
37. Mansuri MS, Singh M, Jadeja SD, Begum R. Association of glucose 6-phosphate dehydrogenase (G6PD) 3'UTR polymorphism with vitiligo and in vitro studies on G6PD inhibition in melanocytes. *J Dermatol Sci* (2019) 93:133–5. doi: 10.1016/j.jdermsci.2018.12.001
38. Shajil EM, Laddha NC, Chatterjee S, Gani AR, Malek RA, Shah BJ, et al. Association of catalase T/C exon 9 and glutathione peroxidase codon 200 polymorphisms in relation to their activities and oxidative stress with vitiligo susceptibility in Gujarat population. *Pigment Cell Res* (2007) 20:405–7. doi: 10.1111/j.1600-0749.2007.00406.x
39. Singh A, Sharma P, Kar HK, Sharma VK, Tembhre MK, Gupta S, et al. HLA alleles and amino-acid signatures of the peptide-binding pockets of HLA molecules in vitiligo. *J Invest Dermatol* (2012) 132:124–34. doi: 10.1038/jid.2011.240
40. Singh M, Mansuri MS, Jadeja SD, Marfatia YS, Begum R. Association of Interleukin 1 Receptor Antagonist (IL1RN) intron 2 VNTR polymorphism with vitiligo susceptibility in Gujarat population. *Ind J Dermatol Venereol Leprol* (2018b) 84:285–91. doi: 10.4103/ijdvl.IJDVL_1_17
41. Singh M, Jadeja SD, Vaishnav J, Mansuri MS, Shah C, Mayatra JM, et al. Investigation of the Role of Interleukin 6 in Vitiligo Pathogenesis. *Immunol Invest* (2020) 30:1–8. doi: 10.1080/08820139.2020.1813756
42. Boissy RE, Liu YY, Medrano EE, Nordlund JJ. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. *J Invest Dermatol* (1991) 97:395–404. doi: 10.1111/1523-1747.ep12480976
43. Tu BP, Weissman JS. Oxidative protein folding in eukaryotes: Mechanisms and consequences. *J Cell Biol* (2004) 164:341–6. doi: 10.1083/jcb.200311055
44. Van der Vlies D, Makkinje M, Jansens A. Oxidation of ER resident proteins upon oxidative stress: Effects of altering cellular redox/antioxidant status and implications for protein maturation. *Antioxid Redox Signal* (2003) 5:381–7. doi: 10.1089/152308603768295113
45. Malhotra JD, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid Redox Signal* (2007) 9:2277–93. doi: 10.1089/ars.2007.1782
46. Anbar T, Zuel-Fakkar NM, Matta MF, Arbab MMI. Elevated homocysteine levels in suction-induced blister fluid of active vitiligo lesions. *Eur J Dermatol* (2016) 26:64–7. doi: 10.1684/ejd.2015.2682
47. Tsai TY, Kuo CY, Huang YC. Serum homocysteine, folate, and vitamin B 12 levels in patients with vitiligo and their potential roles as disease activity biomarkers: A systematic review and meta-analysis. *J Am Acad Dermatol* (2019) 80:646–54.e5. doi: 10.1016/j.jaad.2018.08.029
48. Todd DJ, Lee AH, Glimcher LH. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* (2008) 8:663–74. doi: 10.1038/nri2359
49. Mansuri MS, Singh M, Jadeja SD, Gani AR, Patel R, Dwivedi M. Could ER Stress Be A Major Link Between Oxidative Stress And Autoimmunity In Vitiligo? *J Pigment Disord* (2014b) 01:123. doi: 10.4172/2376-0427.1000123
50. Ramirez MU, Hernandez SR, Soto-Pantoja DR, Cook KL. Endoplasmic reticulum stress pathway, the unfolded protein response, modulates immune function in the tumor microenvironment to impact tumor progression and therapeutic response. *Int J Mol Sci* (2020) 25(21):169. doi: 10.3390/ijms21010169
51. Schuck S, Prinz WA, Thorn KS, Voss C, Walter P. Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. *J Cell Biol* (2009) 16(187):525–36. doi: 10.1083/jcb.200907074
52. Kozutsumi Y, Segal M, Normington K. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature* (1988) 332:462–4. doi: 10.1038/332462a0
53. Wang XZ, Harding HP, Zhang Y. Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J* (1998) 17:5708–17. doi: 10.1093/emboj/17.19.5708
54. Haze K, Yoshida H, Yanagi H. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* (1999) 10:3787–99. doi: 10.1091/mbc.10.11.3787
55. Huang G, Yao J, Zeng W. ER stress disrupts Ca2+-signaling complexes and Ca2+ regulation in secretory and muscle cells from PERK-knockout mice. *J Cell Sci* (2006) 119:153–61. doi: 10.1242/jcs.02731
56. Mu TW, Ong DS, Wang YJ, Balch WE, Yates JR3, Segatori L, et al. Chemical and biological approaches synergize to ameliorate protein-folding diseases. *Cell* (2008) 5(134):769–81. doi: 10.1016/j.cell.2008.06.037
57. Chen X, Shen J, Prywes R. The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the er to the Golgi. *J Biol Chem* (2002) 277:13045–52. doi: 10.1074/jbc.M110636200
58. Oakes SA, Papa FR. The Role of Endoplasmic Reticulum Stress in Human Pathology. *Annu Rev Pathol Mech Dis* (2015) 10:173–94. doi: 10.1146/annurev-pathol-012513-104649
59. Bettigole SE, Glimcher LH. Endoplasmic reticulum stress in immunity. *Annu Rev Immunol* (2015) 33:107–38. doi: 10.1146/annurev-immunol-032414-112116
60. Grootjans J, Kaser A, Kaufman RJ, Blumberg RS. The unfolded protein response in immunity and inflammation. *Nat Rev Immunol* (2016) 16:469–84. doi: 10.1038/nri.2016.62

61. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol* (2008) 8:923–34. doi: 10.1038/nri2449
62. Walter P, Ron D. The unfolded protein response: From stress pathway to homeostatic regulation. *Science* (2011) 25(334):1081–6. doi: 10.1126/science.1209038
63. Verfaillie T, Garg AD, Agostinis P. Targeting ER stress induced apoptosis and inflammation in cancer. *Cancer Lett* (2013) 332:249–64. doi: 10.1016/j.canlet.2010.07.016
64. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. *Nature* (2008) 454(7203):455–62. doi: 10.1038/nature07203
65. Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1α-mediated NF-κB activation and down-regulation of TRAF2 expression. *Mol Cell Biol* (2006) 26(8):3071–84. doi: 10.1128/MCB.26.8.3071-3084.2006
66. Deng J, Lu PD, Zhang Y. Translational Repression Mediates Activation of Nuclear Factor Kappa B by Phosphorylated Translation Initiation Factor 2. *Mol Cell Biol* (2004) 24:10161–8. doi: 10.1128/mcb.24.23.10161-10168.2004
67. Tam AB, Mercado EL, Hoffmann A, Niwa M. ER Stress Activates NF-κB by Integrating Functions of Basal IKK Activity, IRE1 and PERK. *PLoS One* (2012) 7:e45078. doi: 10.1371/journal.pone.0045078
68. Luo D, He Y, Zhang H, Yu L, Chen H, Xu Z, et al. AIP1 is critical in transducing IRE1-mediated endoplasmic reticulum stress response. *J Biol Chem* (2018) 2(283):11905–12. doi: 10.1074/jbc.M710557200
69. Urano F, Wang XZ, Bertolotti A. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* (2000) 287:664–6. doi: 10.1126/science.287.5453.664
70. Eferl R, Wagner EF. AP-1: A double-edged sword in tumorigenesis. *Nat Rev Cancer* (2003) 3:859–68. doi: 10.1038/nrc1209
71. Angel P, Szabowski A, Schorpp-Kistner M. Function and regulation of AP-1 subunits in skin physiology and pathology. *Oncogene* (2001) 20:2413–23. doi: 10.1038/sj.onc.1204380
72. Iwasaki Y, Suganami T, Hachiya R, Shirakawa I, Kim-Saijo M, Tanaka M, et al. Activating transcription factor 4 links metabolic stress to interleukin-6 expression in macrophages. *Diabetes* (2014) 63:152–61. doi: 10.2337/db13-0757
73. Starck SR, Tsai JC, Chen K. Translation from the 5' untranslated region shapes the integrated stress response. *Science* (2016) 29(351):aad3867. doi: 10.1126/science.aad3867
74. Martinon F, Chen X, Lee AH, Glimcher LH. TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat Immunol* (2010) 11:411–8. doi: 10.1038/ni.1857
75. Osorio F, Tavernier SJ, Hoffmann E. The unfolded-protein-response sensor IRE-1α regulates the function of CD8α+ dendritic cells. *Nat Immunol* (2014) 15:248–57. doi: 10.1038/ni.2808
76. Yoshida H, Matsui T, Yamamoto A. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* (2001) 107:881–91. doi: 10.1016/S0092-8674(01)00611-0
77. Lee K, Tirasophon W, Shen X. IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev* (2002) 15(16):452–66. doi: 10.1101/gad.964702
78. Yoshida H, Okada T, Haze K. ATF6 Activated by Proteolysis Binds in the Presence of NF-Y (CBF) Directly to the cis-Acting Element Responsible for the Mammalian Unfolded Protein Response. *Mol Cell Biol* (2000) 20:6755–67. doi: 10.1128/mcb.20.18.6755-6767.2000
79. Zhang K, Shen X, Wu J. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* (2006) 10 (124):587–99. doi: 10.1016/j.cell.2005.11.040
80. Harris JE. Chemical-Induced Vitiligo. *Dermatol Clin* (2017) 35:151–61. doi: 10.1016/j.det.2016.11.006
81. Sasaki M, Kondo M, Sato K, Umeda M, Kawabata K, Takahashi Y, et al. Rhododendrol, a depigmentation-inducing phenolic compound, exerts melanocyte cytotoxicity via a tyrosinase-dependent mechanism. *Pigment Cell Melanoma Res* (2014) 27:754–63. doi: 10.1111/pcmr.12269
82. Toosi S, Orlow SJ, Manga P. Vitiligo-inducing phenols activate the unfolded protein response in melanocytes resulting in upregulation of IL6 and IL8. *J Invest Dermatol* (2012) 132:2601–9. doi: 10.1038/jid.2012.181
83. Tokura Y, Fujiyama T, Ikeya S, Tatsuno K, Aoshima M, Kasuya A, et al. Biochemical, cytological, and immunological mechanisms of rhododendrol-induced leukoderma. *J Dermatol Sci* (2015) 77:146–9. doi: 10.1016/j.jdermsci.2015.02.001
84. Tsutsumi R, Sugita K, Abe Y, Hozumi Y, Suzuki T, Yamada N, et al. Leukoderma induced by rhododendrol is different from leukoderma of vitiligo in pathogenesis: A novel comparative morphological study. *J Cutan Pathol* (2019) 46:123–9. doi: 10.1111/cup.13396
85. Saheki Y, De Camilli P. Endoplasmic Reticulum–Plasma Membrane Contact Sites. *Annu Rev Biochem* (2017) 20(86):659–84. doi: 10.1146/annurev-biochem-061516-044932
86. Celli A, Sanchez S, Behne M. The epidermal Ca2+ gradient: Measurement using the phasor representation of fluorescent lifetime imaging. *Biophys J* (2010) 3(98):911–21. doi: 10.1016/j.bpj.2009.10.055
87. Celli A, MacKenzie DS, Crumrine DS. Endoplasmic reticulum Ca2+ depletion activates XBP1 and controls terminal differentiation in keratinocytes and epidermis. *Br J Dermatol* (2011) 164:16–25. doi: 10.1111/j.1365-2133.2010.10046.x
88. Phillips MJ, Voeltz GK. Structure and function of ER membrane contact sites with other organelles. *Nat Rev Mol Cell Biol* (2016) 17:69–82. doi: 10.1038/nrm.2015.8
89. Oyarbide-Valencia K, van den Boorn JG, Denman CJ. Therapeutic implications of autoimmune vitiligo T cells. *Autoimmun Rev* (2006) 5:486–92. doi: 10.1016/j.autrev.2006.03.012
90. Han D, Lerner AG, Walle LV. IRE1 a Kinase Activation Modes Control Alternate Endoribonuclease Outputs to Determine Divergent Cell Fates. *Cell* (2009) 138:562–75. doi: 10.1016/j.cell.2009.07.017
91. Ferrari SM, Fallahi P, Santaguida G. Circulating CXCL10 is increased in non-segmental vitiligo, in presence or absence of autoimmune thyroiditis. *Autoimmun Rev* (2017) 16:946–50. doi: 10.1016/j.autrev.2017.07.006
92. Harris JE, Harris TH, Weninger W. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive CD8+ T-cell accumulation in the skin. *J Invest Dermatol* (2012) 132:1869–76. doi: 10.1038/jid.2011.463
93. Wang XX, Wang QQ, Wu JQ. Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo. *Br J Dermatol* (2016) 174:1318–26. doi: 10.1111/bjd.14416
94. Riedhammer C, Weissert R. Antigen presentation, autoantigens, and immune regulation in multiple sclerosis and other autoimmune diseases. *Front Immunol* (2015) 6:322. doi: 10.3389/fimmu.2015.00322
95. Dell'Anna ML, Urbanelli S, Mastrofrancesco A, Camera E, Iacovelli P, Leone G, et al. Alterations of Mitochondria in Peripheral Blood Mononuclear Cells of Vitiligo Patients. *Pigment Cell Res* (2003) 16:553–9. doi: 10.1034/j.1600-0749.2003.00087.x
96. Giovannelli L, Bellandi S, Pitozzi V, Fabbri P, Dolara P, Moretti S. Increased Oxidative DNA Damage in Mononuclear Leukocytes in Vitiligo. *Mutat Res* (2004) 22(556):101–6. doi: 10.1016/j.mrfmmm.2004.07.005
97. Schallreuter KU, Moore J, Wood JM, Beazley WD, Peters EM, Marles LK, et al. Epidermal H2O2 accumulation alters tetrahydrobiopterin (6BH4) recycling in vitiligo: identification of a general mechanism in regulation of all 6BH4-dependent processes? *J Invest Dermatol* (2001) 116(1):167–74. doi: 10.1046/j.1523-1747.2001.00220.x
98. Badri A, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG. An Immunohistological Study of Cutaneous Lymphocytes in Vitiligo. *J Pathol* (1993) 170:149–55. doi: 10.1002/path.1711700209
99. Wankowicz-Kalinska A, Le Poole IC, van den Wijngaard R, Storkus WJ, Das PK. Melanocyte-Specific Immune Response in Melanoma and Vitiligo: Two Faces of the Same Coin? *Pigment Cell Res* (2003a) 16:254–60. doi: 10.1034/j.1600-0749.2003.00038.x
100. Wańkowicz-Kalińska 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* (2003b) 83:683–95. doi: 10.1097/01.LAB.0000069521.42488.1B
101. Wijngaard RM, Aten J, Scheepmaker A, Le Poole IC, Tigges AJ, Westerhof W, et al. Expression and Modulation of Apoptosis Regulatory Molecules in Human Melanocytes: Significance in Vitiligo. *Br J Dermatol* (2000) 143:573–81. doi: 10.1046/j.1365-2133.2000.03712.x

102. Gass JN, Gifford NM, Brewer JW. Activation of an Unfolded Protein Response during Differentiation of Antibody-Secreting B Cells. *J Biol Chem* (2002) 277:49047–54. doi: 10.1074/jbc.M205011200
103. Brunsing R, Omori SA, Weber F, Bicknell A, Friend L, Rickert R, et al. B- and T-Cell Development Both Involve Activity of the Unfolded Protein Response Pathway. *J Biol Chem* (2008) 283:17954–61. doi: 10.1074/jbc.M801395200
104. Kamimura D, Bevan MJ. Endoplasmic Reticulum Stress Regulator XBP-1 Contributes to Effector CD8+T Cell Differentiation during Acute Infection. *J Immunol* (2008) 15(181):5433–41. doi: 10.4049/jimmunol.181.8.5433
105. Iwakoshi NN, Lee AH, Glimcher LH. The X-box binding protein-1 transcription factor is required for plasma cell differentiation and the unfolded protein response. *Immunol Rev* (2003) 194:29–38. doi: 10.1034/j.1600-065x.2003.00057.x
106. Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, Gravallese EM, et al. Plasma Cell Differentiation Requires the Transcription Factor XBP-1. *Nature* (2001) 412(6844):300–7. doi: 10.1038/35085509
107. Chang JS, Ocvirk S, Berger E, Kisling S, Binder U, Skerra A, et al. Endoplasmic reticulum stress response promotes cytotoxic phenotype of CD8αβ+ intraepithelial lymphocytes in a mouse model for Crohn's disease-like ileitis. *J Immunol* (2012) 189:1510–20. doi: 10.4049/jimmunol.1200166
108. Franco A, Almanza G, Burns JC, Wheeler M, Zanetti M. Endoplasmic Reticulum Stress Drives a Regulatory Phenotype in Human T-Cell Clones. *Cell Immunol* (2010) 266:1–6. doi: 10.1016/j.cellimm.2010.09.006
109. Yang X, Xia R, Yue C, Zhai W, Du W, Yang Q, et al. ATF4 regulates CD4+ T cell immune responses through metabolic reprogramming. *Cell Rep* (2018) 23(6):1754–66.
110. Wiersma VR, Michalak M, Abdullah TM. Mechanisms of Translocation of ER Chaperones to the Cell Surface and Immunomodulatory Roles in Cancer and Autoimmunity. *Front Oncol* (2015) 5:7. doi: 10.3389/fonc.2015.00007
111. Morito D, Nagata K. ER Stress Proteins in Autoimmune and Inflammatory Diseases. *Front Immunol* (2012) 15:2012.00048(3):48. doi: 10.3389/fimmu.2012.00048
112. Vig S, Buitinga M, Rondas D. Cytokine-induced translocation of GRP78 to the plasma membrane triggers a pro-apoptotic feedback loop in pancreatic beta cells. *Cell Death Dis* (2019) 5(10):309. doi: 10.1038/s41419-019-1518-0
113. Kroll TM, Bommiasamy H, Boissy RE. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: Relevance to vitiligo. *J Invest Dermatol* (2005) 124:798–806. doi: 10.1111/j.0022-202X.2005.23653.x
114. Zhang Y, Liu L, Jin L. Oxidative stress-induced calreticulin expression and translocation: New insights into the destruction of melanocytes. *J Invest Dermatol* (2014) 134:183–91. doi: 10.1038/jid.2013.268
115. Gardai SJ, McPhillips KA, Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, et al. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on sthe phagocyte. *Cell* (2005) 123:321–34. doi: 10.1016/j.cell.2005.08.032
116. Zhu Y, Zhong L, Peng J. The therapeutic effects of baicalin on vitiligo mice. *Biol Pharm Bull* (2019) 42:1450–5. doi: 10.1248/bpb.b19-00319
117. Shen M, Wang L, Yang G. Baicalin Protects the Cardiomyocytes from ER Stress-Induced Apoptosis: Inhibition of CHOP through Induction of Endothelial Nitric Oxide Synthase. *PLoS One* (2014) 9:e88389. doi: 10.1371/journal.pone.0088389
118. Cao J, Zhang Y, Wang T, Li B. Endoplasmic Reticulum Stress Is Involved in Baicalin Protection on Chondrocytes From Patients With Osteoarthritis. *Dose Response* (2018) 16:155932581881063. doi: 10.1177/1559325818810636
119. Lu L, Wang S, Fu L. Bilobalide protection of normal human melanocytes from hydrogen peroxide-induced oxidative damage via promotion of antioxidase expression and inhibition of endoplasmic reticulum stress. *Clin Exp Dermatol* (2016) 41:64–73. doi: 10.1111/ced.12664
120. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* (2006) 25(313):1137–40. doi: 10.1126/science.1128294
121. Cao SS, Zimmermann EM, Chuang BM, Song B, Nwokoye A, Wilkinson JE, et al. The unfolded protein response and chemical chaperones reduce protein misfolding and colitis in mice. *Gastroenterology* (2013) 1(144):989–1000. doi: 10.1053/j.gastro.2013.01.023
122. Kim HJ, Jeong JS, Kim SR, Park SY, Chae HJ, Lee YC. Inhibition of endoplasmic reticulum stress alleviates lipopolysaccharide-induced lung inflammation through modulation of NF-κB/HIF-1α signaling pathway. *Sci Rep* (2013) 28(3):1–0. doi: 10.1038/srep01142

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 26 February 2021
doi: 10.3389/fimmu.2021.624131

The Role of NKG2D in Vitiligo

Lourdes Plaza-Rojas[1] *and José A. Guevara-Patiño*[2]*

[1] Department of Cancer Biology, Loyola University Chicago, Chicago, IL, United States, [2] Department of Surgery, Loyola University Chicago, Chicago, IL, United States

Vitiligo is an acquired multifactorial disease that affects melanocytes and results in skin depigmentation. In this review, we examine the role of cells stress and self-reactive T cells responses. Given the canonical and non-canonical functions of NKG2D, such as authenticating stressed target and enhance TCR signaling, we examine how melanocyte stress leads to the expression of ligands that are recognized by the activating receptor NKG2D, and how its signaling results in the turning of T cells against self (melanocyte suicide by proxy). We also discuss how this initiation phase is followed by T cell perpetuation, as NKG2D signaling results in self-sustained long-lasting T cells, with improved cytolytic properties.

Keywords: vitiligo, NKG2D, T cells, horror autotoxicus, Hsp70, oxidative stress

OPEN ACCESS

Edited by:
Thierry Passeron,
Université Côte d'Azur, France

Reviewed by:
Jillian M. Richmond,
University of Massachusetts Medical School, United States
Richard David Leslie,
Queen Mary University of London, United Kingdom

***Correspondence:**
José A. Guevara-Patiño
jaguevara@luc.edu

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *30 October 2020*
Accepted: *02 February 2021*
Published: *26 February 2021*

Citation:
Plaza-Rojas L and Guevara-Patiño JA (2021) The Role of NKG2D in Vitiligo. Front. Immunol. 12:624131. doi: 10.3389/fimmu.2021.624131

INTRODUCTION

At the end of the eighteenth century, Paul Ehrlich introduced the concept of horror autotoxicus, which describes the body's innate aversion to immunological self-destruction. This model remains valid, as the immune system is entrusted with protecting the host against infections while avoiding autoimmunity. It is well-accepted that the killing of target cells by T cells occurs through recognition of the peptide-MHC Class I complex via the T cell receptor (TCR). In the thymus, T cells undergo positive and negative selection based on the TCR signal strength (1). T cells that recognize self-antigens with strong affinity undergo negative selection. In contrast, T cells that weakly respond to self-antigens undergo positive selection and populate the periphery as mature T cells. Thus, while self-reactive T cells are present in the periphery, their TCR interactions with peptide-MHC Class I complex are insufficient to mediate activation. Moreover, additional peripheral mechanisms are also in place to prevent their activation, namely regulatory T cells (Tregs) (2, 3).

Under certain pathophysiological conditions, T cells are responsible for several autoimmune disorders, including vitiligo. Vitiligo is an autoimmune disease characterized by the progressive loss of skin pigmentation as a result of melanocyte destruction. In this context, T cells are known to target and kill melanocytes, the pigment-producing cells in the skin. Vitiligo is the most common skin pigmentation disorder, affecting 0.1–2% of the population worldwide (4, 5), with no sex bias (6–9). While it can affect people at all ages, vitiligo appears more frequently before 20 years of age and its early onset (during childhood) is associated with hereditary disease (5, 7, 9–11). Vitiligo is characterized by white lesions on the skin, and the associated psychological and social effects make patients more prone to depression and low self-esteem (12). Regrettably, there are currently no FDA-approved treatments for vitiligo (13). Although great progress has been made throughout the past decades in elucidating the mechanisms that cause this disease, it remains incurable.

While T cells turn against melanocytes for reasons that remain largely unknown, recent studies suggest that CD8 T cells kill melanocytes through the recognition of stressed/damaged cells via Natural Killer Group 2D (NKG2D). NKG2D is a type II transmembrane receptor encoded by the

killer cell lectin-like receptor subfamily K member 1 (Klrk1) gene (14, 15) expressed by CD8 T cells, NK cells, NKT cells, and a subset of γδ T cells, and by CD4 T cells in some pathologies (16, 17). Although NKG2D is expressed in both NK and CD8 T cells, the mechanisms of action differ. In NK cells, NKG2D signaling is sufficient to mediate direct killing of target cells (18). However, in CD8 T cells, NKG2D requires the concurrent activation of the TCR. In this case, NKG2D enhances TCR activation and thus, T cell function (18–20).

A number of elements are thought to contribute to the initiation of vitiligo, including genetic predisposition (21, 22), reduced proliferative capacity of melanocytes, increased oxidative stress in melanocytes, the subsequent expression of "danger" signals, reduced presence and function of Tregs, and increased activation of a self-reactive T cell repertoire (7, 23, 24). Moreover, there is evidence of an association between early inflammatory events (e.g., exposure to UV light, a bleaching agent, phenols, or trauma) and vitiligo (25, 26). We believe this is of significance, as the expression of NKG2D ligands (NKG2DL) is upregulated in response to cell stress (insult), rendering these cells visible to NKG2D-expressing immune cells (14, 18–20, 27). The ligands for NKG2D are composed of a variety of stress molecules, including the major histocompatibility complex (MHC) class-I-related chain (MIC) A/B and ULBP binding proteins 1–6 in humans (16) and members of the Rae-1 and H60a-c families in mice (28, 29). Importantly, NKG2DL expression on the cell surface is rigidly controlled. In contrast to physiological conditions in which few or no NKG2DL are expressed, their expression is induced under stress conditions, such as infection and transformation (28, 29). Of significance, proinflammatory signals such as IFN-α (30) and TLR4 and TLR7/8 signaling (28), as well as the ataxia telangiectasia mutated and Rad3-related (ATM/ATR) DNA damage response pathway also result in surface expression of these ligands (20, 27, 31, 32). Thus, the canonical function of NKG2D is to authenticate the stressed/damaged feature of the target cells. This cell authentication occurs in part by favoring the TCR signaling.

Notably, studies from our group have established in animal models that NKG2D signaling also leads to the development of long-lasting CD8 T cells with enhanced cytolytic function (33–35). NKG2DL upregulation and NKG2D signaling activation in effector CD8 T cells play a role in the onset or development of some autoimmune diseases, including vitiligo, rheumatoid arthritis, celiac disease, type 1 diabetes, alopecia areata, systemic lupus erythematosus (SLE), among others (36–41). Moreover, inhibiting NKG2D engagement can prevent inflammation and disease development in some models of type 1 diabetes (42), vitíligo (30), and other inflammatory diseases (16).

Here, we describe the relationship between multiple factors that can lead to vitiligo development with a main focus on the involvement of CD8 T cells and NKG2D signaling.

MELANIN SYNTHESIS, UV EXPOSURE, AND OXIDATIVE STRESS

While multiple factors may induce the development of vitiligo, a dysfunction of the redox balance has been largely observed both systemically and in active lesions of vitiligo patients, causing an excessive accumulation of reactive oxygen species (ROS) and inducing cellular stress (43–45). Oxidative stress can arise when molecular oxygen (O_2) is converted into oxygen radicals and the production of these species surpasses the anti-oxidant capacity of the cell, causing oxidation of cellular structures and DNA and potentially leading to cell death (46–49). A number of pathologies are associated with excessive ROS generation, which can also lead to inflammation, particularly in autoimmune diseases (50). Given the effects of ROS on inflammation as well as the active role of immune cells in the development of vitiligo, a relationship between oxidative stress and the immune system is apparent in the onset of the disease.

Melanocytes are derived from the neural crest and their main function is to produce the pigment melanin, which is responsible for skin and eye color (51). Keratinocytes, the most abundant cell type in the epidermis, regulate melanocytic functions, including proliferation, melanogenesis, and differentiation (52). Melanocytes and keratinocytes are arranged in epidermal melanin units, in which 1 melanocyte is surrounded by 36 keratinocytes. While its synthesis occurs in melanocytes, melanin is transported to the surrounding keratinocytes (53). This pigment is generated from the oxidation of tyrosine in a multistep reaction during which free radicals may arise (54). These reactions are highly regulated and carried on by a multienzyme complex unique to melanocytes, including the enzymes tyrosinase, TYRP1 and TYRP2 (55).

Exposure to UV light and its absorption by melanocytes causes photo-oxidation of melanin, generating superoxide radicals (54), which in turn induce melanin biosynthesis (56). Melanin creates a supranuclear cap to protect DNA and prevent or reduce its damage (57) and it has also been shown to act as an antioxidant and a free radical scavenger (58–60). However, not only does melanin biosynthesis consist of several oxidation reactions, it also requires energy production via mitochondrial respiration (61). Even though ROS generation has an important role in cell signaling, increased or altered mitochondrial activity may lead to excessive ROS production (62) that results in detrimental effects and apoptosis, as is the case in vitiliginous melanocytes. Although oxidative stress has been observed in both melanocytes and keratinocytes, the latter are less likely to undergo apoptosis. Instead, keratinocytes become senescent in response to UV irradiation (63, 64), and exposure to ROS induces the production of inflammatory cytokines by keratinocytes (65, 66).

Despite an increased O_2 consumption rate, mitochondrial respiration and energy production is impaired, while ROS production is increased in vitiliginous melanocytes (67). Higher levels of ROS peroxynitrite and H_2O_2 are found in the skin of vitiligo patients, in concert with lower concentration of antioxidants and reducing enzymes (43, 68–70). High concentrations of H_2O_2 disrupt melanin synthesis by inhibiting tyrosinase and dihydropteridine reductase (71). Calreticulin, an endoplasmic reticulum (ER) protein that regulates Ca^{2+} homeostasis and signaling, is also modulated by H_2O_2, which increases calreticulin expression and translocation to the cell surface of melanocytes. This is associated with higher melanocyte apoptosis and production of pro-inflammatory cytokines IL-6 and TNF-α in

vitiligo (72). As will be further discussed, pro-inflammatory cytokines have an important role in the vitiligo-associated immune response.

High ROS can also induce ER oxidation; in particular, H_2O_2 can interfere with the ion channel TRPM2, resulting in higher Ca^{2+} influx into the cell and the mitochondria (73). Increased mitochondrial Ca^{2+} concentration ($[Ca^{2+}]$), in addition to augmented ROS production, induces a reduction of the mitochondrial membrane potential ($\Delta\Psi m$) in melanocytes and the circulating mononuclear cells of vitiligo patients (44, 72). While melanin is known to chelate Ca^{2+} and control intracellular $[Ca^{2+}]$, protecting the cell from DNA damage caused by ROS or UV rays, eventually, these alterations may lead to cytochrome c release and apoptosis (74, 75). In the ER, ROS induces accumulation of dysfunctional and unfolded proteins, triggering the unfolded protein response (UPR) within vitiliginous melanocytes, potentially leading to cell death (76). Moreover, aberrant self-proteins, perceived as foreign antigens, can elicit immune responses (77). Therefore, oxidative stress in vitiligo skin is likely to precede the autoimmune response. In particular, NKG2DL expression can be triggered by ROS in multiple cell types and in cancer (78–83). NKG2D-mediated activation of CD8 T cells may thus play a significant role in the development of vitiligo, as discussed below.

NKG2D, STRESS SIGNALS, AND T CELLS

NKG2D is an activating receptor that is expressed by many cytotoxic lymphocytes and binds to a variety of ligands (i.e., MICA and Rae-1 in human and mouse, respectively) expressed on stressed cells. Studies including ours have shown that the function of these ligands is to enable stressed cell recognition and destruction in a suicide by proxy mechanism, facilitated by immune cells expressing NKG2D (34, 84, 85). Interestingly, increased expression of NKG2DL has been shown in response to multiple insults, including oxidative cell stress (86, 87). Moreover, studies have shown an association between CD8 T cells expressing NKG2D and various autoimmune diseases, including skin conditions such as alopecia areata (88–90), vitiligo, pancreatitis, diabetes (91), and celiac disease (39, 92). For example, in type 1 diabetes, NKG2D engagement is necessary for the development of the disease (42) and destruction of β-cells in the pancreas by CD8 T cells has been compared to melanocyte death in skin patches of vitiligo patients (93, 94).

In a mouse model of vitiligo, we show that engagement of NKG2D results in exacerbation of CD8 T cell-mediated vitiligo (34). Consistent with this, melanocyte-reactive T cells are further activated and drawn to stressed melanocytes that express NKG2DL (19). Moreover, NKG2D-expressing T cells are enriched in vitiligo patients (30). Thus, understanding the mechanisms that regulate NKG2DL expression on stressed melanocytes can lead to development of therapeutic approaches targeting the interactions of $NKG2D^+$ self-reactive CD8 T cells with melanocytes in vitiligo.

The expression of NKG2DL is upregulated in response to cell stress, rendering stressed or altered cells visible to NKG2D-expressing CD8 T cells. Studies have shown that stress signals induce the expression of NKG2DL on most cell types, including melanocytes (30). Increased expression of such signals in healthy tissue may induce the destruction and inflammation associated with autoimmune diseases (36, 37, 39, 41, 42, 88, 95–97). In fact, melanocyte-reactive T cells, normally suppressed by Tregs, have been found in circulating blood of individuals that do not exhibit vitiligo (98). While such self-reactive cytolytic T cells can be exploited for anti-tumor therapy, loss of functional Treg cells can result in the onset of vitiligo (98, 99).

NKG2DL expression on melanocytes may also drive the onset of the disease. This was demonstrated in a mouse model which revealed that NKG2DL expression on target cells is sufficient to induce vitiligo (34). Accordingly, melanocyte-reactive T cells also have a higher NKG2D expression. In particular, a subset of tissue-resident memory CD8 T (T_{RM}) cells populating lesion areas in the skin of vitiligo patients was shown to have increased NKG2D levels and to be responsible for the increased production of IFN-γ and TNF-α (30). This T_{RM} cell activation was mediated not only by IL-15, a key cytokine in memory formation, but also by skin dendritic cells (DCs) expressing human NKG2DL MICA/B (30). Engagement of NKG2D on CD8 T cells in combination with IL-15 stimulation can trigger the killing of target cells, a feature of autoimmune diseases (39, 84, 95, 100–102). While NKG2D plays an important role in the effector phase of T cells, we have shown that engagement of this receptor also has long-term effects, including the promotion of memory formation (35). In the mentioned study, we show that signaling through NKG2D mediates a process that we termed memory certification. We found that temporary blockade of NKG2D signaling during the effector phase resulted in the formation of highly defective memory CD8 T cells characterized by altered expression of the ribosomal protein S6 and epigenetic modifiers, suggesting modifications in the T cell translational machinery and epigenetic programming. Based on these data, we concluded that NKG2D signaling during this initial effector phase, poises NKG2D engaged cells with a certification or molecular accreditation that results in their optimal development as memory T cells. This process of certification guarantees that the memory compartment is populated with CD8 T cells that have demonstrated their ability to kill the correct targets through a two-step process that utilizes the TCR and NKG2D signaling. Interestingly, in this system, NKG2D-certtified memory T cells will be able to kill melanocytes independently of NKG2D, thus resulting in the destruction of healthy melanocytes. We postulate that melanocyte-reactive CD8 T cells that receive NKG2D signaling during the killing phase will also persist as memory cells and potentially enrich the skin TRM population in vitiligo patients.

Heat shock protein are stress-inducible chaperones that protect cells from undergoing apoptosis through several mechanisms, including the binding and renaturing or degradation of misfolded proteins during the stress response (103). In particular, Hsp70 expression in tumor cells has been shown to elicit both innate and adaptive immune responses (104–108). In vitiligo, stressed melanocytes increase the expression of Hsp70, which binds and transports potentially immunogenic antigens to the MHC complex, allowing for their presentation on the cell surface to cytotoxic T cells (109). Moreover, free Hsp70 also triggers an immune response by interacting with DC surface receptors (107, 110, 111), inducing the expression of human

NKG2DL MICA (107). This acts as a danger signal, rendering stressed cells visible to both NK and CD8 T cells expressing NKG2D. Hsp70 expression can be induced not only by physical and chemical stress, but also in physiological conditions, such as under high ROS production, as seen in vitiligo and other pathologies (110, 112, 113). Expression of both inducible HSP70 and NKG2DL is controlled by the transcriptional activator heath shock factor 1 (HSF1) (83). In response to cell stress, HSF1 is released in the cytoplasm. Following nuclear translocation, HSF1 binds to the promoter regions of both HSP70i and NKG2DL, initiating their transcription. These observations raise a novel paradox: while the canonical function of intracellular HSP70i is cytoprotectant, expression of NKG2DL results in cell suicide by proxy. While it is not known how melanocytes survive or render themselves visible for killing after cell stress, we believe that focusing on answering this question may in turn help identify actionable therapies in vitiligo.

Environmental insults can trigger inflammation and result in the onset of vitiligo, especially in adulthood (25, 26). For example, monobenzone, a skin bleaching agent is used to complete skin depigmentation in vitiligo patients. Monobenzone induces ROS generation and oxidative stress in the skin (114), further contributing to the autoimmune response in vitiligo. In addition, monobenzone is converted into a reactive quinone by tyrosinase (114); these quinone products bind covalently to proteins in melanocytes, forming aberrant antigens targeted by T cells (115). Therefore, monobenzone induces a systemic T cell response, effectively targeting all melanocytes in the skin.

THE INFLAMMATORY MICROENVIRONMENT IN VITILIGO

Cytokines have a crucial role in regulating immune responses. High levels of cytokines are often found in vitiligo patients, including cytokines corresponding to Th1, Th17, and innate cell responses (116). Interestingly, keratinocytes in vitiligo lesions aberrantly produce IL-1, IL-6, and TNF-α, which inhibit melanocyte function (65, 66) and elicit an inflammatory response.

The production of pro-inflammatory cytokines allows for enhanced T cell recruitment and activation, resulting in increased presence of activated CD8 T cells in vitiligo lesions (13, 117–122). Produced by multiple immune cell types, IFN-γ is associated with vitiligo. IFN-γ promotes the production of other cytokines and chemokines, and the recruitment of T cells as well as other types of immune cells (123). Studies show that IFN-γ directly induces melanocyte apoptosis (124, 125) and its signaling enhances CD8 T cell function and expansion (126). Notably, we and others have demonstrated that NKG2D signaling upregulates IFN-γ production (18, 33). IFN-γ also promotes the expression of MHC Class I and therefore antigen presentation for CD8 T cells. IFN-γ is also responsible for stimulating the production of CXCL9 and CXCL10 in keratinocytes, which engages the chemokine receptor CXCR3 on melanocytes and triggers apoptosis, further contributing to the disease (127).

TNF-α is also produced at high levels in vitiligo (128). While its role as an apoptotic mediator has been shown in multiple cell types (129), in vitiligo, TNF-α inhibits melanogenesis by activating the transcription factor NF-κB (130). In addition, it induces the expression of ICAM-1 on melanocytes (131), allowing for the attachment of T cells and triggering cytotoxicity (132). Moreover, TNF-α inhibits tyrosinase and Trp1 activity, both essential for melanin synthesis (61). ROS production is also increased by TNF-α, further promoting stress signals in vitiligo melanocytes.

While keratinocytes can contribute to the production of inflammatory factors, melanocyte-reactive CD8 T cells are sufficient to initiate the development of vitiligo, as it has been shown that perilesional T cells from vitiligo patients can recognize and kill melanocytes in healthy skin (122). Activated self-reactive T cells remain in the skin and differentiate into T_{RM} cells. This differentiation is mediated by IL-15 derived from keratinocytes (133). Blockade of IL-15Rβ (CD122) reduces IFN-γ production and can eliminate skin T_{RM} and reverse vitiligo (133).

Another indicator of an existing melanocyte-reactive T cell population is the development of vitiligo in melanoma patients who receive immunotherapy (134–140). In patients without the right stimuli, these T cells do not mount a response, but can potentially be activated for the treatment of cancer. This observation also indicates the possibility to treat melanoma with agents known to trigger vitiligo, such as skin bleaching agents (114, 115).

In addition to a high production of pro-inflammatory cytokines, there is a corresponding reduction in suppressive cytokines in the skin of vitiligo patients. Tregs maintain immune tolerance by controlling self-reactive cytotoxic T cells. In healthy adults, Tregs constitute about half of the CD4 T cell population in the skin (141). In vitiligo, however, a much lower percentage of Tregs is often found surrounding lesions (23), which has been associated with reduced levels of Treg growth and differentiation factors TGF-β and IL-10 (142, 143). In particular, low TGF-β concentrations in both serum and skin have been found to correlate with skin depigmentation in vitiligo (142, 144). In addition, there is an association between reduced expression of CCL22, a skin-homing chemokine, and Treg infiltration in vitiligo skin, suggesting impaired migration of Tregs to the skin in vitiligo patients (24). These events can thus result in the alteration of the Treg/cytotoxic T cell ratio, impaired Treg differentiation, and increased inflammation in vitiligo.

In summation, the imbalance of pro-inflammatory and suppressive cytokines further contributes to disease progression. The interplay of these messengers, the immune system, and the affected skin cells is being actively investigated to identify novel therapeutic avenues.

GENETIC AND TRANSCRIPTIONAL ALTERATIONS IN VITILIGO

Genetic predisposition is heavily linked to an overreactive immune system and patients often exhibit more than one autoimmune disease. While the inflammation and stress

observed in vitiligo patients can have a variety of causes, including environmental, there is also a high risk of vitiligo heritability, with about 8% of patients having at least one affected relative (7). Gene analysis studies have revealed a higher expression of Class II MHC haplotypes HLA-DR and HLA-DQ in Caucasian patients resulting from a polymorphism in a super-enhancer within the Class II region (6, 145). Moreover, there is a correlation in the expression of such MHC haplotypes and a higher production of pro-inflammatory cytokines IFN-γ and IL-1β (6). In addition, mutations affecting other loci implicated in immune regulation have been associated with vitiligo and other autoimmune diseases. These genes include those encoding IL-2Rα (146), the TCR signaling regulator UBASH3A (146), and CCL20 receptor CCR6 (147), among others (21). While these reports indicate that there is an association between immune regulation and the onset of autoimmunity and vitiligo, there is also evidence that some polymorphisms lead to reactivity specifically against melanocytes. These include peptides derived from tyrosinase, the transmembrane protein OCA2, and melanocortin 1 receptor (MC1R), all of which are naturally processed and presented as antigens by Class I MHC molecules (5, 148, 149). This, in combination with higher MHC expression, increases the potential for the presentation of melanocyte-specific antigens and for self-reactive T cells targeting these cells.

Reduced function and number of the tolerogenic Tregs has also been associated with the development of vitiligo. Genetic polymorphisms in Foxp3, a transcription factor critical for Treg differentiation, and transcription factor FoxP1 (147), also critical for Treg homeostasis (150, 151), have been associated with Treg dysfunction in vitiligo (23, 152–154). Similarly, variants in the genes encoding TGF-β receptor II (155) and IL-10 (156) may affect Treg-mediated immune suppression in vitiligo. As mentioned, both TGF-β and IL-10 are cytokines essential for Treg differentiation and development (157–159).

Additionally, transcriptional analyses have demonstrated altered expression of factors involved in proliferation, apoptosis and regeneration of melanocytes (160). This is supported by the observation that vitiliginous melanocytes display poor proliferative capacity and cannot be sub-cultured *in vitro* (161, 162).

Aberrant expression of miRNAs has also been observed in vitiligo (163–165). In particular, miRNA-211 is highly expressed in healthy melanocytes, but its expression is severely reduced in non-pigmented melanoma and in the vitiligo cell line PIG3V (22). miRNA-211 target genes have functions involved in cell respiration and metabolism, including mitochondrial complexes I, II, and IV. In consequence, reduced miRNA-211 expression in vitiligo is associated with increased O_2 consumption and

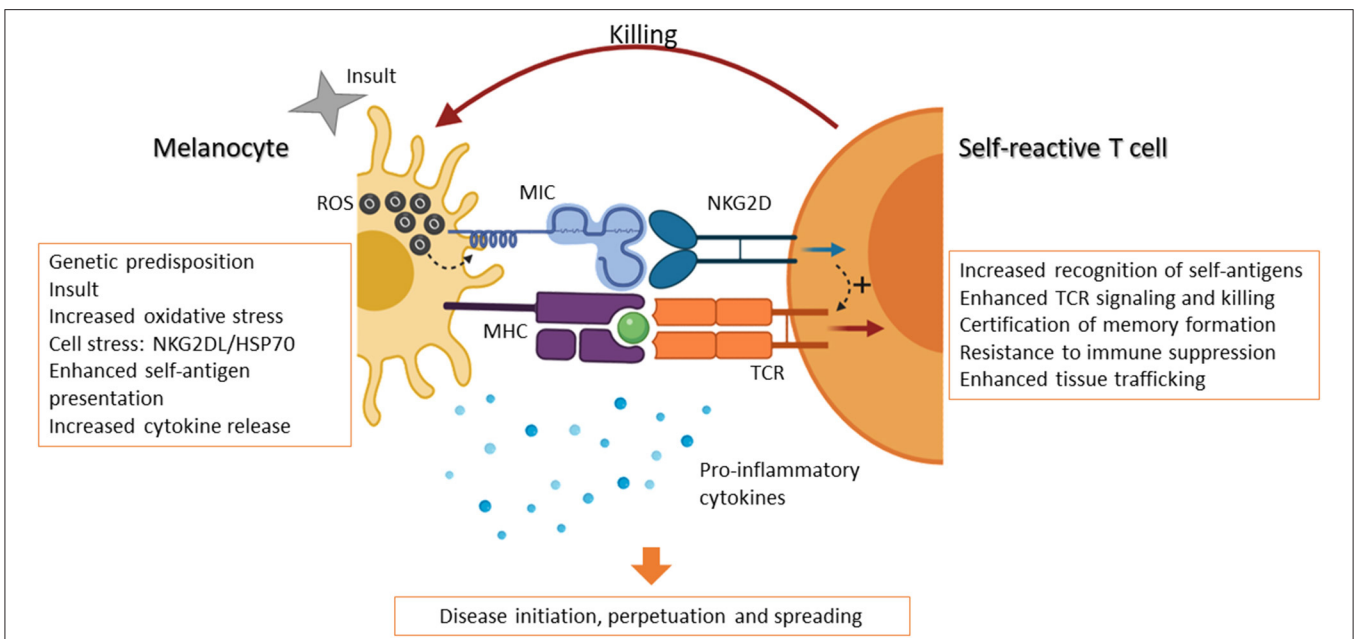


FIGURE 1 | NKG2D-expressing T cells initiate and perpetuate vitiligo. The occurrence of vitiligo can be associated with genetic predisposition and hereditary disease, which in turned is linked to an overreactive immune system. Expression of certain MHC variants, for example, can lead to increased self-antigen presentation. However, other contributing factors can trigger the onset of the disease. An inflammatory event, or insult, may lead to increased ROS production and oxidative stress in melanocytes and their surroundings. In response to this, the production of cytokines by immune cells and keratinocytes can further induce inflammation and an immune response. Melanocytes in turn express "danger" signals, including NKG2D ligands (e.g., MICA/B in humans) and chaperone proteins, such as hsp70. Self-reactive cytolytic T cells are thus able to recognize melanocytes through the TCR and become activated via the engagement of NKG2D, whose signaling further enhances the downstream effects of TCR signaling, including cytotoxicity. Moreover, NKG2D activation certifies these self-reactive T cells to become memory cells and renders them more resistant to immune suppression. These certified T cells have enhanced local trafficking and are able to continue killing melanocytes. These events, in combination with reduced suppression by dysfunctional Treg cells, result in the onset, perpetuation, and spreading of vitiligo. Created with BioRender.com.

may partially explain the oxidative stress observed in patients (22, 166).

CONCLUSIONS

Vitiligo is a complex disease with multiple causal factors, including genetic predisposition, enhanced self-antigen presentation, and increased pro-inflammatory signals. Inflammation is known to induce DNA and cell damage and increased ROS production, triggering the expression of NKG2DL on melanocytes. It is well-accepted that the interaction between the autoantigen:MHC-I complex and the self-reactive TCR on CD8 T cells is weak. However, under stress and inflammation, the recognition of both autoantigen and NKG2DL by NKG2D-expressing T cells causes the reduction of the T cell activating threshold (**Figure 1**). In this scenario, the expression of NKG2D ligands on stressed melanocytes is sensed by the immune system as an instruction for destruction (suicide by proxy). This initiation phase is followed by the perpetuation phase: once T cells have killed stressed melanocytes via engagement of both the TCR and NKG2D, these T cells are endowed with a transcriptional program that enables them to become long-lasting, with enhanced cytolytic properties as well as independence from immunological help. Evidence suggests that these NKG2D-engaged T cells are then responsible for the killing of healthy melanocytes in an NKG2D-independent mechanism, thus perpetuating the disease.

AUTHOR CONTRIBUTIONS

JG-P and LP-R prepared and wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the NIH R01CA188395, R01CA250514, and the generous gift from the Volo Family Foundation.

REFERENCES

1. Daniels MA, Teixeiro E, Gill J, Hausmann B, Roubaty D, Holmberg K, et al. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature.* (2006) 444:724–9. doi: 10.1038/nature05269
2. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4 + CD25 high regulatory cells in human peripheral blood. *J Immunol.* (2001) 167:1245–53. doi: 10.4049/jimmunol.167.3.1245
3. Barthlott T, Moncrieffe H, Veldhoen M, Atkins CJ, Christensen J, O'Garra A, et al. CD25+CD4+ T cells compete with naive CD4+ T cells for IL-2 and exploit it for the induction of IL-10 production. *Int Immunol.* (2005) 17:279–88. doi: 10.1093/intimm/dxh207
4. Zhang Y, Cai Y, Shi M, Jiang S, Cui S, Wu Y, et al. The prevalence of vitiligo: a meta-analysis. *PLoS ONE.* (2016) 11:163806. doi: 10.1371/journal.pone.0163806
5. Jin Y, Birlea SA, Fain PR, Ferrara TM, Ben S, Riccardi SL, et al. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nat Genet.* (2012) 44:676–80. doi: 10.1038/ng.2272
6. Cavalli G, Hayashi M, Jin Y, Yorgov D, Santorico SA, Holcomb C, et al. MHC class II super-enhancer increases surface expression of HLA-DR and HLA-DQ and affects cytokine production in autoimmune vitiligo. *Proc Natl Acad Sci USA.* (2016) 113:1363–8. doi: 10.1073/pnas.15234 82113
7. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res.* (2003) 16:208–14. doi: 10.1034/j.1600-0749.2003.00032.x
8. Bergqvist C, Ezzedine K. Vitiligo: a review. *Dermatology.* (2020) 236:571–92. doi: 10.1159/000506103
9. Ezzedine K, Eleftheriadou V, Whitton M, Van Geel N. Vitiligo. *Lancet.* (2015) 386:74–84. doi: 10.1016/S0140-6736(14)60763-7
10. Skov J, Eriksson D, Kuja-Halkola R, Höijer J, Gudbjörnsdottir S, Svensson AM, et al. Co-aggregation and heritability of organ-specific autoimmunity: a population-based twin study. *Eur J Endocrinol.* (2020) 182:473. doi: 10.1530/EJE-20-0049
11. Handa S, Kaur I. Vitiligo: clinical findings in 1436 patients. *J Dermatol.* (1999) 26:653–7. doi: 10.1111/j.1346-8138.1999.tb02067.x
12. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CCE, et al. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res.* (2012) 25:E1–13. doi: 10.1111/j.1755-148X.2012.00997.x
13. Ezzedine K, Sheth V, Rodrigues M, Eleftheriadou V, Harris JE, Hamzavi IH, et al. Vitiligo is not a cosmetic disease. *J Am Acad Dermatol.* (2015) 73:883–5. doi: 10.1016/j.jaad.2015.07.039
14. Houchins JP, Yabe T, McSherry C, Bach FH. DNA sequence analysis of NKG2, a family of related cDNA clones encoding type II integral membrane proteins on human natural killer cells. *J Exp Med.* (1991) 173:1017–20. doi: 10.1084/jem.173.4.1017
15. Brown MG, Fulmek S, Matsumoto K, Robert C, Lyons PA, Levy ER, et al. A 2-Mb YAC contig and physical map of the natural killer gene complex on mouse chromosome 6. *Genomics.* (1997) 42:16–25. doi: 10.1006/geno.1997.4721
16. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol.* (2003) 3:781–90. doi: 10.1038/nri1199
17. Sáez-Borderías A, Gumá M, Angulo A, Bellosillo B, Pende D, López-Botet M. Expression and function of NKG2D in CD4+ T cells specific for human cytomegalovirus. *Eur J Immunol.* (2006) 36:3198–206. doi: 10.1002/eji.200636682
18. Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity.* (2002) 17:19–29. doi: 10.1016/S1074-7613(02)00333-3
19. Bauer S, Groh V, Wu J, Steinle a, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science.* (1999) 285:727–9. doi: 10.1126/science.285.5428.727
20. Diefenbach A, Jamieson AM, Liu SD, Shastri N, Raulet DH. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. *Nat Immunol.* (2000) 1:119–26. doi: 10.1038/77793
21. Jin Y, Andersen G, Yorgov D, Ferrara TM, Ben S, Brownson KM, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk loci and highlight key pathways and regulatory variants. *Nat Genet.* (2016) 48:1418–24. doi: 10.1038/ng.3680
22. Sahoo A, Lee B, Boniface K, Seneschal J, Sahoo SK, Seki T, et al. MicroRNA-211 regulates oxidative phosphorylation and energy metabolism in human vitiligo. *J Invest Dermatol.* (2017) 137:1965–74. doi: 10.1016/j.jid.2017.04.025
23. Dwivedi M, Kemp EH, Laddha NC, Mansuri MS, Weetman AP, Begum R. Regulatory T cells in vitiligo: implications for pathogenesis and therapeutics. *Autoimmun Rev.* (2015) 14:49–56. doi: 10.1016/j.autrev.2014.10.002
24. Klarquist J, Denman CJ, Hernandez C, Wainwright DJ, Strickland FM, Overbeck A, et al. Reduced skin homing by functional Treg in vitiligo. *Pigment Cell Melanoma Res.* (2010) 23:276–86. doi: 10.1111/j.1755-148X.2010.00688.x

25. O'Malley MA, Mathias CGT, Priddy M, Molina D, Grote AA, Halperin WE. Occupational vitiligo due to unsuspected presence of phenolic antioxidant byproducts in commercial bulk rubber. *J Occup Med.* (1988) 30:512–6. doi: 10.1097/00043764-198806000-00014
26. Le Poole IC, Wańkowicz-Kalińska A, Van Den Wijngaard RMJGJ, Nickoloff BJ, Das PK. Autoimmune aspects of depigmentation in vitiligo. *J Investig Dermatol Symp Proc.* (2004) 9:68–72. doi: 10.1111/j.1087-0024.2004.00825.x
27. Cerwenka A, Bakker ABH, McClanahan T, Wagner J, Wu J, Phillips JH, et al. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity.* (2000) 12:721–7. doi: 10.1016/S1074-7613(00)80222-8
28. Cerwenka A, Baron JL, Lanier LL. Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor *in vivo*. *Proc Natl Acad Sci USA.* (2001) 98:11521–6. doi: 10.1073/pnas.201238598
29. O'Callaghan CA, Cerwenka A, Willcox BE, Lanier LL, Bjorkman PJ. Molecular competition for NKG2D: H60 and RAE1 compete unequally for NKG2D with dominance of H60. *Immunity.* (2001) 15:201–11. doi: 10.1016/S1074-7613(01)00187-X
30. Jacquemin C, Martins C, Lucchese F, Thiolat D, Taieb A, Seneschal J, et al. NKG2D defines a subset of skin effector memory CD8 T cells with proinflammatory functions in vitiligo. *J Invest Dermatol.* (2020) 140:1143–53.e5. doi: 10.1016/j.jid.2019.11.013
31. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature.* (2005) 436:1186–90. doi: 10.1038/nature03884
32. Cosman D, Müllberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity.* (2001) 14:123–33. doi: 10.1016/S1074-7613(01)00095-4
33. Zloza A, Kohlhapp FJ, Lyons GE, Schenkel JM, Moore TV, Lacek AT, et al. NKG2D signaling on $CD8^+$ T cells represses T-bet and rescues $CD4^-$ unhelped $CD8^+$ T cell memory recall but not effector responses. *Nat Med.* (2012) 18:422–8. doi: 10.1038/nm.2683
34. Zloza A, Lyons GE, Chlewicki LK, Kohlhapp FJ, O'Sullivan JA, Lacek AT, et al. Engagement of NK receptor NKG2D, but not 2B4, results in self-reactive $CD8^+$ T cells and autoimmune vitiligo. *Autoimmunity.* (2011) 44:599–606. doi: 10.3109/08916934.2011.593599
35. Perez C, Prajapati K, Burke B, Plaza-Rojas L, Zeleznik-Le NJ, Guevara-Patino JA. NKG2D signaling certifies effector CD8 T cells for memory formation. *J Immunother Cancer.* (2019) 7:48. doi: 10.1186/s40425-019-0531-2
36. Groh V, Brühl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc Natl Acad Sci USA.* (2003) 100:9452–7. doi: 10.1073/pnas.1632807100
37. Andersson AK, Sumariwalla PF, McCann FE, Amjadi P, Chang C, McNamee K, et al. Blockade of NKG2D ameliorates disease in mice with collagen-induced arthritis: a potential pathogenic role in chronic inflammatory arthritis. *Arthritis Rheum.* (2011) 63:2617–29. doi: 10.1002/art.30460
38. Antonangeli F, Soriani A, Cerboni C, Sciumè G, Santoni A. How mucosal epithelia deal with stress: role of NKG2D/NKG2D ligands during inflammation. *Front Immunol.* (2017) 8:1583. doi: 10.3389/fimmu.2017.01583
39. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz T, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity.* (2004) 21:357–66. doi: 10.1016/J.IMMUNI.2004.06.020
40. Ma H, Lu Y, Lowe K, van der Meijden-Erkelens L, Wasserfall C, Atkinson MA, et al. Regulated hAAT expression from a novel rAAV vector and its application in the prevention of type 1 diabetes. *J Clin Med.* (2019) 8:1321. doi: 10.3390/jcm8091321
41. Qin H, Lee IF, Panagiotopoulos C, Wang X, Chu AD, Utz PJ, et al. Natural killer cells from children with type 1 diabetes have defects in NKG2D-dependent function and signaling. *Diabetes.* (2011) 60:857–66. doi: 10.2337/db09-1706
42. Ogasawara K, Hamerman JA, Ehrlich LR, Bour-Jordan H, Santamaria P, Bluestone JA, et al. NKG2D blockade prevents autoimmune diabetes in NOD mice. *Immunity.* (2004) 20:757–67. doi: 10.1016/j.immuni.2004.05.008
43. Schallreuter KU, Moore J, Wood JM, Beazley WD, Gaze DC, Tobin DJ, et al. *In vivo* and *in vitro* evidence for hydrogen peroxide (H2O2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase. *J Investig Dermatol Symp Proc.* (1999) 4:91–6. doi: 10.1038/sj.jidsp.5640189
44. Dell'Anna ML, Urbanelli S, Mastrofrancesco A, Camera E, Iacovelli P, Leone G, et al. Alterations of mitochondria in peripheral blood mononuclear cells of vitiligo patients. *Pigment Cell Res.* (2003) 16:553–9. doi: 10.1034/j.1600-0749.2003.00087.x
45. Morrone A, Picardo M, De Luca C, Terminali O, Passi S, Ippolito F. Catecholamines and vitiligo. *Pigment Cell Res.* (1992) 5:65–9. doi: 10.1111/j.1600-0749.1992.tb00003.x
46. Bhattacharyya S, Saha J. Tumour, oxidative stress and host T cell response: cementing the dominance. *Scand J Immunol.* (2015) 82:477–88. doi: 10.1111/sji.12350
47. Bickers DR, Athar M. Oxidative stress in the pathogenesis of skin disease. *J Invest Dermatol.* (2006) 126:2565–75. doi: 10.1038/sj.jid.5700340
48. Bossi O, Gartsbein M, Leitges M, Kuroki T, Grossman S, Tennenbaum T. UV irradiation increases ROS production via PKCδ signaling in primary murine fibroblasts. *J Cell Biochem.* (2008) 105:194–207. doi: 10.1002/jcb.21817
49. Rinnerthaler M, Bischof J, Streubel M, Trost A, Richter K. Oxidative stress in aging human skin. *Biomolecules.* (2015) 5:545–89. doi: 10.3390/biom5020545
50. Di Dalmazi G, Hirshberg J, Lyle D, Freij JB, Caturegli P. Reactive oxygen species in organ-specific autoimmunity. *Autoimmun Highlights.* (2016) 7:11. doi: 10.1007/s13317-016-0083-0
51. Nordlund JJ, Abdel-Malek ZA, Boissy RE, Rheins LA. Pigment cell biology: an historical review. *J Invest Dermatol.* (1989) 92:S53–60. doi: 10.1038/jid.1989.33
52. Hirobe T. Structure and function of melanocytes: microscopic morphology and cell biology of mouse melanocytes in the epidermis and hair follicle. *Histol Histopathol.* (1995) 10:223–37.
53. Nordlund JJ. The melanocyte and the epidermal melanin unit: an expanded concept. *Dermatol Clin.* (2007) 25:271–81. doi: 10.1016/j.det.2007.04.001
54. Riley PA. Melanin. *Int J Biochem Cell Biol.* (1997) 29:1235–9. doi: 10.1016/S1357-2725(97)00013-7
55. D'Mello SAN, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling pathways in melanogenesis. *Int J Mol Sci.* (2016) 17:1144. doi: 10.3390/ijms17071144
56. Slominski A, Tobin DJ, Shibahara S, Wortsman J. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol Rev.* (2004) 84:1155–228. doi: 10.1152/physrev.00044.2003
57. Kobayashi N, Nakagawa A, Muramatsu T, Yamashina Y, Shirai T, Hashimoto MW, et al. Supranuclear melanin caps reduce ultraviolet induced DNA photoproducts in human epidermis. *J Invest Dermatol.* (1998) 110:806–10. doi: 10.1046/j.1523-1747.1998.00178.x
58. Song X, Mosby N, Yang J, Xu A, Abdel-Malek Z, Kadekaro AL. α-MSH activates immediate defense responses to UV-induced oxidative stress in human melanocytes. *Pigment Cell Melanoma Res.* (2009) 22:809–18. doi: 10.1111/j.1755-148X.2009.00615.x
59. Hoogduijn M, Cemeli E, Ross K, Anderson D, Thody A, Wood J. Melanin protects melanocytes and keratinocytes against H_2O_2-induced DNA strand breaks through its ability to bind Ca^{2+}. *Exp Cell Res.* (2004) 294:60–7. doi: 10.1016/j.yexcr.2003.11.007
60. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem Photobiol.* (2008) 84:539–49. doi: 10.1111/j.1751-1097.2007.00226.x
61. Koga S, Nakano M, Tero-Kubota S. Generation of superoxide during the enzymatic action of tyrosinase. *Arch Biochem Biophys.* (1992) 292:570–5. doi: 10.1016/0003-9861(92)90032-R
62. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* (2009) 417:1–13. doi: 10.1042/BJ20081386
63. Lewis DA, Travers JB, Somani AK, Spandau DF. The IGF-1/IGF-1R signaling axis in the skin: a new role for the dermis in aging-associated skin cancer. *Oncogene.* (2010) 29:1475–85. doi: 10.1038/onc.2009.440
64. Passi S, Grandinetti M, Maggio F, Stancato A, Luca C. Epidermal oxidative stress in vitiligo. *Pigment Cell Res.* (1998) 11:81–5. doi: 10.1111/j.1600-0749.1998.tb00714.x

65. Luger TA. Epidermal cytokines. *Acta Derm Venereol Suppl.* (1989) 151:61–76. doi: 10.1007/978-3-642-83360-1_71
66. Swope VB, Abdel-Malek Z, Kassem LM, Nordlund JJ. Interleukins 1α and 6 and tumor necrosis factor-α are paracrine inhibitors of human melanocyte proliferation and melanogenesis. *J Invest Dermatol.* (1991) 96:180–5. doi: 10.1111/1523-1747.ep12460991
67. Dell'Anna ML, Ottaviani M, Kovacs D, Mirabilii S, Brown DA, Cota C, et al. Energetic mitochondrial failing in vitiligo and possible rescue by cardiolipin. *Sci Rep.* (2017) 7:6237. doi: 10.1038/s41598-017-13961-5
68. Salem MMAEL, Shalbaf M, Gibbons NCJ, Chavan B, Thornton JM, Schallreuter KU. Enhanced DNA binding capacity on up-regulated epidermal wild-type p53 in vitiligo by H_2O_2 -mediated oxidation: a possible repair mechanism for DNA damage. *FASEB J.* (2009) 23:3790–807. doi: 10.1096/fj.09-132621
69. Schallreuter KU, Salem MAEL, Holtz S, Panske A. Basic evidence for epidermal H2H2/ONOO–mediated oxidation/nitration in segmental vitiligo is supported by repigmentation of skin and eyelashes after reduction of epidermal H2H2 with topical NB-UVB-activated pseudocatalase PC-KUS. *FASEB J.* (2013) 27:3113–22. doi: 10.1096/fj.12-226779
70. Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. *J Invest Dermatol.* (1991) 97:1081–5. doi: 10.1111/1523-1747.ep12492612
71. Hasse S, Gibbons NCJ, Rokos H, Marles LK, Schallreuter KU. Perturbed 6-Tetrahydrobiopterin recycling via decreased dihydropteridine reductase in vitiligo: more evidence for H_2O_2 stress. *J Invest Dermatol.* (2004) 122:307–13. doi: 10.1046/j.0022-202X.2004.22230.x
72. Zhang Y, Liu L, Jin L, Yi X, Dang E, Yang Y, et al. Oxidative stress-induced calreticulin expression and translocation: new insights into the destruction of melanocytes. *J Invest Dermatol.* (2014) 134:183–91. doi: 10.1038/jid.2013.268
73. Kang P, Zhang W, Chen X, Yi X, Song P, Chang Y, et al. TRPM2 mediates mitochondria-dependent apoptosis of melanocytes under oxidative stress. *Free Radic Biol Med.* (2018) 126:259–68. doi: 10.1016/j.freeradbiomed.2018.08.022
74. Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis.* (2000) 5:415–8. doi: 10.1023/A:1009616228304
75. Liu B, Jian Z, Li Q, Li K, Wang Z, Liu L, et al. Baicalein protects human melanocytes from H_2O_2-induced apoptosis via inhibiting mitochondria-dependent caspase activation and the p38 MAPK pathway. *Free Radic Biol Med.* (2012) 53:183–93. doi: 10.1016/j.freeradbiomed.2012.04.015
76. Kroll TM, Bommiasamy H, Boissy RE, Hernandez C, Nickoloff BJ, Mestril R, et al. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: Relevance to vitiligo. *J Invest Dermatol.* (2005) 124:798–806. doi: 10.1111/j.0022-202X.2005.23653.x
77. Xie H, Zhou F, Liu L, Zhu G, Li Q, Li C, et al. Vitiligo: how do oxidative stress-induced autoantigens trigger autoimmunity? *J Dermatol Sci.* (2016) 81:3–9. doi: 10.1016/j.jdermsci.2015.09.003
78. Eagle R, Jafferji I, Barrow A. Beyond stressed self: evidence for NKG2D ligand expression on healthy cells. *Curr Immunol Rev.* (2009) 5:22–34. doi: 10.2174/157339509787314369
79. Yamamoto K, Fujiyama Y, Andoh A, Bamba T, Okabe H. Oxidative stress increases MICA and MICB gene expression in the human colon carcinoma cell line (CaCo-2). *Biochim Biophys Acta - Gen Subj.* (2001) 1526:10–12. doi: 10.1016/S0304-4165(01)00099-X
80. Hedlund M, Nagaeva O, Kargl D, Baranov V, Mincheva-Nilsson L. Thermal- and oxidative stress causes enhanced release of NKG2D ligand-bearing immunosuppressive exosomes in leukemia/lymphoma T and B cells. *PLoS ONE.* (2011) 6:16899. doi: 10.1371/journal.pone.0016899
81. Siemens DR, Hu N, Sheikhi AK, Chung E, Frederiksen LJ, Pross H, et al. Hypoxia increases tumor cell shedding of MHC class I chain-related molecule: role of nitric oxide. *Cancer Res.* (2008) 68:4746–53. doi: 10.1158/0008-5472.CAN-08-0054
82. Barsoum IB, Hamilton TK, Li X, Cotechini T, Miles EA, Siemens DR, et al. Hypoxia induces escape from innate immunity in cancer cells via increased expression of ADAM10: role of nitric oxide. *Cancer Res.* (2011) 71:7433–41. doi: 10.1158/0008-5472.CAN-11-2104
83. Venkataraman GM, Suciu D, Groh V, Boss JM, Spies T. Promoter region architecture and transcriptional regulation of the genes for the MHC class i-related chain A and B ligands of NKG2D. *J Immunol.* (2007) 178:961–9. doi: 10.4049/jimmunol.178.2.961
84. Van Belle TL, von Herrath MG. The role of the activating receptor NKG2D in autoimmunity. *Mol Immunol.* (2009) 47:8–11. doi: 10.1016/j.molimm.2009.02.023
85. Raulet DH. Interplay of natural killer cells and their receptors with the adaptive immune response. *Nat Immunol.* (2004) 5:996–1002. doi: 10.1038/ni1114
86. Kraetzel K, Stoelcker B, Eissner G, Multhoff G, Pfeifer M, Holler E, et al. NKG2D-dependent effector function of bronchial epithelium-activated alloreactive T-cells. *Eur Respir J.* (2008) 32:563–70. doi: 10.1183/09031936.00096407
87. Borchers MT, Harris NL, Wesselkamper SC, Vitucci M, Cosman D. NKG2D ligands are expressed on stressed human airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol.* (2006) 291:L222–31. doi: 10.1152/ajplung.00327.2005
88. Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. *Nature.* (2010) 466:113–7. doi: 10.1038/nature09114
89. Xing L, Dai Z, Jabbari A, Cerise JE, Higgins CA, Gong W, et al. Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition. *Nat Med.* (2014) 20:1043–9. doi: 10.1038/nm.3645
90. Dai Z, Xing L, Cerise J, Wang EHC, Jabbari A, de Jong A, et al. CXCR3 blockade inhibits T cell migration into the skin and prevents development of alopecia areata. *J Immunol.* (2016) 197:1089–99. doi: 10.4049/jimmunol.1501798
91. Markiewicz MA, Wise EL, Buchwald ZS, Pinto AK, Zafirova B, Polic B, et al. RAE1ε ligand expressed on pancreatic islets recruits NKG2D receptor-expressing cytotoxic T cells independent of T cell receptor recognition. *Immunity.* (2012) 36:132–41. doi: 10.1016/j.immuni.2011.11.014
92. Tang F, Sally B, Lesko K, Discepolo V, Abadie V, Ciszewski C, et al. Cysteinyl leukotrienes mediate lymphokine killer activity induced by NKG2D and IL-15 in cytotoxic T cells during celiac disease. *J Exp Med.* (2015) 212:1487–95. doi: 10.1084/jem.20150303
93. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. *Clin Exp Immunol.* (2009) 155:173–81. doi: 10.1111/j.1365-2249.2008.03860.x
94. Eisenbarth GS. Banting lecture 2009: an unfinished journey: molecular pathogenesis to prevention of type 1A diabetes. *Diabetes.* (2010) 59:759–74. doi: 10.2337/db09-1855
95. Hüe S, Mention JJ, Monteiro RC, Zhang SL, Cellier C, Schmitz J, et al. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity.* (2004) 21:367–77. doi: 10.1016/j.immuni.2004.06.018
96. Allez M, Tieng V, Nakazawa A, Treton X, Pacault V, Dulphy N, et al. CD4+NKG2D+ T cells in Crohn's disease mediate inflammatory and cytotoxic responses through MICA interactions. *Gastroenterology.* (2007) 132:2346–58. doi: 10.1053/j.gastro.2007.03.025
97. Van Belle TL, Ling E, Haase C, Bresson D, Ursø B, Von Herrath MG. NKG2D blockade facilitates diabetes prevention by antigen-specific Tregs in a virus-induced model of diabetes. *J Autoimmun.* (2013) 40:66–73. doi: 10.1016/j.jaut.2012.08.001
98. Maeda Y, Nishikawa H, Sugiyama D, Ha D, Hamaguchi M, Saito T, et al. Detection of self-reactive $CD8^+$ T cells with an anergic phenotype in healthy individuals. *Science.* (2014) 346:1536–40. doi: 10.1126/science.aaa1292
99. Richards DM, Ruggiero E, Hofer A-C, Sefrin JP, Schmidt M, von Kalle C, et al. The contained self-reactive peripheral T cell repertoire: size, diversity, and cellular composition. *J Immunol.* (2015) 195:2067–79. doi: 10.4049/jimmunol.1500880
100. Ruck T, Bittner S, Afzali AM, Göbel K, Glumm S, Kraft P, et al. The NKG2D - IL-15 signaling pathway contributes to T-cell mediated pathology in inflammatory myopathies. *Oncotarget.* (2015) 6:43230–43. doi: 10.18632/oncotarget.6462
101. Groh V, Smythe K, Dai Z, Spies T. Fas ligand-mediated paracrine T cell regulation by the receptor NKG2D in tumor immunity. *Nat Immunol.* (2006) 7:755–62. doi: 10.1038/ni1350
102. Saikali P, Antel JP, Pittet CL, Newcombe J, Arbour N. Contribution of astrocyte-derived IL-15 to CD8 t cell effector functions in multiple sclerosis. *J Immunol.* (2010) 185:5693–703. doi: 10.4049/jimmunol.1002188

103. Beere HM. Death versus survival: Functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J Clin Invest.* (2005) 115:2633–9. doi: 10.1172/JCI26471
104. Melcher A, Todryk S, Hardwick N, Ford M, Jacobson M, Vile RG. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. *Nat Med.* (1998) 4:581–7. doi: 10.1038/nm0598-581
105. Clark PR, Ménoret A. The inducible Hsp70 as a marker of tumor immunogenicity. *Cell Stress Chaperones.* (2001) 6:121–5. doi: 10.1379/1466-1268(2001) 006<0121:TIHAAM>2.0.CO;2
106. Massa C, Guiducci C, Arioli I, Parenza M, Colombo MP, Melani C. Enhanced efficacy of tumor cell vaccines transfected with secretable hsp70. *Cancer Res.* (2004) 64:1502–8. doi: 10.1158/0008-5472.CAN-03-2936
107. Elsner L, Muppala V, Gehrmann M, Lozano J, Malzahn D, Bickeböller H, et al. The heat shock protein HSP70 promotes mouse NK cell activity against tumors that express inducible NKG2D ligands. *J Immunol.* (2007) 179:5523–33. doi: 10.4049/jimmunol.179.8.5523
108. Srivastava P. Roles of heat-shock proteins in innate and adaptive immunity. *Nat Rev Immunol.* (2002) 2:185–94. doi: 10.1038/nri749
109. Castellino F, Boucher PE, Eichelberg K, Mayhew M, Rothman JE, Houghton AN, et al. Receptor-mediated uptake of antigen/heat shock protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. *J Exp Med.* (2000) 191:1957–64. doi: 10.1084/jem.191.11.1957
110. Qiao Y, Liu B, Li Z. Activation of NK cells by extracellular heat shock protein 70 through induction of NKG2D ligands on dendritic cells. *Cancer Immun.* (2008) 8:12. Available online at: https://cancerimmunolres.aacrjournals.org/content/canimmarch/8/1/12.short
111. Multhoff G, Mizzen L, Winchester CC, Milner CM, Wenk S, Eissner G, et al. Heat shock protein 70 (Hsp70) stimulates proliferation and cytolytic activity of natural killer cells. *Exp Hematol.* (1999) 27:1627–36. doi: 10.1016/S0301-472X(99)00104-6
112. Jacquemin C, Rambert J, Guillet S, Thiolat D, Boukhedouni N, Doutre M -S, et al. Heat shock protein 70 potentiates interferon alpha production by plasmacytoid dendritic cells: relevance for cutaneous lupus and vitiligo pathogenesis. *Br J Dermatol.* (2017) 177:1367–75. doi: 10.1111/bjd.15550
113. Peraldi M-N, Berrou J, Dulphy N, Seidowsky A, Haas P, Boissel N, et al. Oxidative stress mediates a reduced expression of the activating receptor NKG2D in NK cells from end-stage renal disease patients. *J Immunol.* (2009) 182:1696–705. doi: 10.4049/jimmunol.182.3.1696
114. van den Boorn JG, Melief CJ, Luiten RM. Monobenzone-induced depigmentation: from enzymatic blockade to autoimmunity. *Pigment Cell Melanoma Res.* (2011) 24:673–9. doi: 10.1111/j.1755-148X.2011.00878.x
115. Ewens S, Wulferink M, Goebel C, Gleichmann E. T cell-dependent immune reactions to reactive benzene metabolites in mice. *Arch Toxicol.* (1999) 73:159–67. doi: 10.1007/s002040050601
116. Gholijani N, Yazdani MR, Dastgheib L. Predominant role of innate pro-inflammatory cytokines in vitiligo disease. *Arch Dermatol Res.* (2020) 312:123–31. doi: 10.1007/s00403-019-01996-9
117. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive $CD8^+$ T-cell accumulation in the skin. *J Invest Dermatol.* (2012) 132:1869–76. doi: 10.1038/jid.2011.463
118. Engelhorn ME, Guevara-Patiño JA, Noffz G, Hooper AT, Lou O, Gold JS, et al. Autoimmunity and tumor immunity induced by immune responses to mutations in self. *Nat Med.* (2006) 12:198–206. doi: 10.1038/nm1363
119. Gregg RK, Nichols L, Chen Y, Lu B, Engelhard VH. Mechanisms of spatial and temporal development of autoimmune vitiligo in tyrosinase-specific TCR transgenic mice. *J Immunol.* (2010) 184:1909–17. doi: 10.4049/jimmunol.0902778
120. Harris JE. Cellular stress and innate inflammation in organ-specific autoimmunity: lessons learned from vitiligo. *Immunol Rev.* (2016) 269:11–25. doi: 10.1111/imr.12369
121. 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. doi: 10.1126/scitranslmed.3005127
122. Luiten RM, Van Den Boorn JG, Konijnenberg D, Dellemijn TAM, Van Der Veen JPW, Bos JD, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. *J Invest Dermatol.* (2009) 129:2220–32. doi: 10.1038/jid.2009.32
123. Richmond JM, Bangari DS, Essien KI, Currimbhoy SD, Groom JR, Pandya AG, et al. Keratinocyte-derived chemokines orchestrate T-cell positioning in the epidermis during vitiligo and may serve as biomarkers of disease. *J Invest Dermatol.* (2017) 137:350–8. doi: 10.1016/j.jid.2016.09.016
124. Rashighi M, Harris JE. Interfering with the IFN-γ/CXCL10 pathway to develop new targeted treatments for vitiligo. *Ann Transl Med.* (2015) 3:343. doi: 10.3978/j.issn.2305-5839.2015.11.36
125. Yang L, Wei Y, Sun Y, Shi W, Yang J, Zhu L, et al. Interferon-gamma inhibits melanogenesis and induces apoptosis in melanocytes: a pivotal role of $CD8^+$ cytotoxic T lymphocytes in vitiligo. *Acta Derm Venereol.* (2015) 95:664–70. doi: 10.2340/00015555-2080
126. Curtsinger JM, Agarwal P, Lins DC, Mescher MF. Autocrine IFN-γ promotes naive CD8 T cell differentiation and synergizes with IFN-α to stimulate strong function. *J Immunol.* (2012) 189:659–68. doi: 10.4049/jimmunol.1102727
127. Tulic MK, Cavazza E, Cheli Y, Jacquel A, Luci C, Cardot-Leccia N, et al. Innate lymphocyte-induced CXCR3B-mediated melanocyte apoptosis is a potential initiator of T-cell autoreactivity in vitiligo. *Nat Commun.* (2019) 10:2178. doi: 10.1038/s41467-019-09963-8
128. Morelli JG, Norris DA. Influence of inflammatory mediators and cytokines on human melanocyte function. *J Invest Dermatol.* (1993) (Suppl. 2):191–5S: doi: 10.1038/jid.1993.75
129. Rath PC, Aggarwal BB. TNF-induced signaling in apoptosis. *J Clin Immunol.* (1999) 19:350–64. doi: 10.1023/a:1020546615229
130. Englaro W, Bahadoran P, Bertolotto C, Buscà R, Dérijard B, Livolsi A, et al. Tumor necrosis factor alpha-mediated inhibition of melanogenesis is dependent on nuclear factor kappa B activation. *Oncogene.* (1999) 18:1553–9. doi: 10.1038/sj.onc.1202446
131. Yang X, Yan L, Ha DA, Qu L, Liu L, Tao YU. Changes in sICAM-1 and GM-CSF levels in skin tissue fluid and expression of IL-6, IL-17 and TNF-α in blood of patients with vitiligo. *Exp Ther Med.* (2019) 17:408–12. doi: 10.3892/etm.2018.6937
132. Sharma CK, Sharma M, Prasad K. Involvement of different genes expressions during immunological and inflammatory responses in vitiligo. *Crit Rev Eukaryot Gene Expr.* (2017) 27:277–87. doi: 10.1615/CritRevEukaryotGeneExpr.2017019558
133. Richmond JM, Strassner JP, Zapata LZ, Garg M, Riding RL, Refat MA, et al. Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo. *Sci Transl Med.* (2018) 10:7710. doi: 10.1126/scitranslmed.aam7710
134. Burdick KH, Hawk WA. Vitiligo in a case of vaccinia virus-treated melanoma. *Cancer.* (1964) 17:708–12. doi: 10.1002/1097-0142(196406)17:6<708::AID-CNCR2820170604>3.0.CO;2-3
135. Kaercher K. Systematized depigmentation in melanoma treated with x-irradiation. *Dermatologica.* (1960) 120:255–63.
136. Smith JL, Stehlin JS. Spontaneous regression of primary malignant melanomas with regional metastases. *Cancer.* (1965) 18:1399–415. doi: 10.1002/1097-0142(196511)18:11<1399::AID-CNCR2820181104>3.0.CO;2-R
137. Nordlund JJ, Kirkwood JM, Forget BM, Milton G, Albert DM, Lerner AB. Vitiligo in patients with metastatic melanoma: a good prognostic sign. *J Am Acad Dermatol.* (1983) 9:689–96. doi: 10.1016/S0190-9622(83)70182-9
138. Bystryn J, Rigel D, Friedman R, Kopf A. Prognostic significance of hypopigmentation in malignant melanoma. *Arch Dermatol.* (1987) 123:1053–5.
139. Quaglino P, Marenco F, Osella-Abate S, Cappello N, Ortoncelli M, Salomone B, et al. Vitiligo is an independent favourable prognostic factor in stage III and IV metastatic melanoma patients: results from a single-institution hospital-based observational cohort study. *Ann Oncol.* (2010) 21:409–14. doi: 10.1093/annonc/mdp325
140. Teulings HE, Tjin EPM, Willemsen KJ, van der Kleij S, Meulen S ter, Kemp EH, et al. Anti-Melanoma immunity and local regression of cutaneous metastases in melanoma patients treated with monobenzone and imiquimod; a phase 2 a trial. *Oncoimmunology.* (2018) 7:e1419113. doi: 10.1080/2162402X.2017.1419113

141. Scharschmidt TC, Vasquez KS, Truong HA, Gearty SV, Pauli ML, Nosbaum A, et al. A Wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. *Immunity.* (2015) 43:1011–21. doi: 10.1016/j.immuni.2015.10.016
142. 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. doi: 10.1016/j.cca.2013.05.005
143. Dwivedi M, Laddha NC, Shah K, Shah BJ, Begum R. Involvement of interferon-gamma genetic variants and intercellular adhesion molecule-1 in onset and progression of generalized vitiligo. *J Interf Cytokine Res.* (2013) 33:646–59. doi: 10.1089/jir.2012.0171
144. 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 Dermatology.* (2009) 60:256–60. doi: 10.1016/j.jaad.2008.09.048
145. Jin Y, Roberts GHL, Ferrara TM, Ben S, van Geel N, Wolkerstorfer A, et al. Early-onset autoimmune vitiligo associated with an enhancer variant haplotype that upregulates class II HLA expression. *Nat Commun.* (2019) 10:1–10. doi: 10.1038/s41467-019-08337-4
146. Jin Y, Birlea SA, Fain PR, Gowan K, Riccardi SL, Holland PJ, et al. Variant of *TYR* and autoimmunity susceptibility loci in generalized vitiligo. *N Engl J Med.* (2010) 362:1686–97. doi: 10.1056/NEJMoa0908547
147. Jin Y, Birlea SA, Fain PR, Mailloux CM, Riccardi SL, Gowan K, et al. Common variants in FOXP1 are associated with generalized vitiligo. *Nat Genet.* (2010) 42:576–8. doi: 10.1038/ng.602
148. Touloukian CE, Leitner WW, Robbins PF, Rosenberg SA, Restifo NP. Mining the melanosome for tumor vaccine targets: P.polypeptide is a novel tumor-associated antigen. *Cancer Res.* (2001) 61:8100–4. Available online at: https://cancerres.aacrjournals.org/content/61/22/8100.article-info
149. Skipper JCA, Hendrickson RC, Gulden PH, Brichard V, Van Pel A, Chen Y, et al. An HLA-A2-restricted tyrosinase antigen on melanoma cells results from posttranslational modification and suggests a novel pathway for processing of membrane proteins. *J Exp Med.* (1996) 183:527–34. doi: 10.1084/jem.183.2.527
150. Konopacki C, Pritykin Y, Rubtsov Y, Leslie CS, Rudensky AY. Transcription factor Foxp1 regulates Foxp3 chromatin binding and coordinates regulatory T cell function. *Nat Immunol.* (2019) 20:232–42. doi: 10.1038/s41590-018-0291-z
151. Ren J, Han L, Tang J, Liu Y, Deng X, Liu Q, et al. Foxp1 is critical for the maintenance of regulatory T-cell homeostasis and suppressive function. *PLoS Biol.* (2019) 17:e3000270. doi: 10.1371/journal.pbio.3000270
152. Song P, Wang X-W, Li H-X, 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. doi: 10.1111/bjd.12377
153. 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. doi: 10.1016/j.jaad.2013.01.035
154. 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. doi: 10.1038/jid.2010.337
155. Yun JY, Uhm YK, Kim HJ, Lim SH, Chung JH, Shin MK, et al. Transforming growth factor beta receptor II (TGFBR2) polymorphisms and the association with nonsegmental vitiligo in the Korean population. *Int J Immunogenet.* (2010) 37:289–91. doi: 10.1111/j.1744-313X.2010.00923.x
156. Abanmi A, Al Harthi F, Zouman A, Kudwah A, Al Jamal M, Arfin M, et al. Association of interleukin-10 gene promoter polymorphisms in Saudi patients with vitiligo. *Dis Markers.* (2008) 24:51–7. doi: 10.1155/2008/210609
157. 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. doi: 10.4049/jimmunol.169.8.4183
158. Hsu P, Santner-Nanan B, Hu M, Skarratt K, Lee CH, Stormon M, et al. IL-10 potentiates differentiation of human induced regulatory T cells via STAT3 and Foxo1. *J Immunol.* (2015) 195:3665–74. doi: 10.4049/jimmunol.1402898
159. Liu Y, Zhang P, Li J, Kulkarni AB, Perruche S, Chen WJ. A critical function for TGF-β signaling in the development of natural $CD4^+CD25^+Foxp3^+$ regulatory T cells. *Nat Immunol.* (2008) 9:632–40. doi: 10.1038/ni.1607
160. Dey-Rao R, Sinha AA. Vitiligo blood transcriptomics provides new insights into disease mechanisms and identifies potential novel therapeutic targets. *BMC Genomics.* (2017) 18:109. doi: 10.1186/s12864-017-3510-3
161. Puri N, Mojamdar M, Ramaiah A. *In vitro* growth characteristics of melanocytes obtained from adult normal and vitiligo subjects. *J Invest Dermatol.* (1987) 88:434–8. doi: 10.1111/1523-1747.ep12469795
162. Goldstein NB, Koster MI, Hoaglin LG, Spoelstra NS, Kechris KJ, Robinson SE, et al. Narrow band ultraviolet b treatment for human vitiligo is associated with proliferation, migration, and differentiation of melanocyte precursors. *J Invest Dermatol.* (2015) 135:2068–76. doi: 10.1038/jid.2015.126
163. Mansuri MS, Singh M, Dwivedi M, Laddha NC, Marfatia YS, Begum R. MicroRNA profiling reveals differentially expressed microRNA signatures from the skin of patients with nonsegmental vitiligo. *Br J Dermatol.* (2014) 171:1263–7. doi: 10.1111/bjd.13109
164. Shi YL, Weiland M, Li J, Hamzavi I, Henderson M, Huggins RH, et al. MicroRNA expression profiling identifies potential serum biomarkers for non-segmental vitiligo. *Pigment Cell Melanoma Res.* (2013) 26:418–21. doi: 10.1111/pcmr.12086
165. Shi YL, Weiland M, Lim HW, Mi QS, Zhou L. Serum miRNA expression profiles change in autoimmune vitiligo in mice. *Exp Dermatol.* (2014) 23:140–2. doi: 10.1111/exd.12319
166. Spencer JD, Gibbons NCJ, Rokos H, Peters EMJ, Wood JM, Schallreuter KU. Oxidative stress via hydrogen peroxide affects proopiomelanocortin peptides directly in the epidermis of patients with vitiligo. *J Invest Dermatol.* (2007) 127:411–20. doi: 10.1038/sj.jid.5700538

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 02 March 2021
doi: 10.3389/fimmu.2021.624517

Translational Research in Vitiligo

Erica L. Katz and John E. Harris *

Department of Medicine, Division of Dermatology, University of Massachusetts Medical School, Worcester, MA, United States

Vitiligo is a disease of the skin characterized by the appearance of white spots. Significant progress has been made in understanding vitiligo pathogenesis over the past 30 years, but only through perseverance, collaboration, and open-minded discussion. Early hypotheses considered roles for innervation, microvascular anomalies, oxidative stress, defects in melanocyte adhesion, autoimmunity, somatic mosaicism, and genetics. Because theories about pathogenesis drive experimental design, focus, and even therapeutic approach, it is important to consider their impact on our current understanding about vitiligo. Animal models allow researchers to perform mechanistic studies, and the development of improved patient sample collection methods provides a platform for translational studies in vitiligo that can also be applied to understand other autoimmune diseases that are more difficult to study in human samples. Here we discuss the history of vitiligo translational research, recent advances, and their implications for new treatment approaches.

Keywords: vitiligo, translational research, autoimmunity, melanocyte oxidative stress, genetics

OPEN ACCESS

Edited by:
Antonio Serrano, Research Institute Hospital 12 de Octubre, Spain

Reviewed by:
Silvia Moretti, University of Florence, Italy
Rasheedunnisa Begum, Maharaja Sayajirao University of Baroda, India

***Correspondence:**
John E. Harris
john.harris@umassmed.edu

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *31 October 2020*
Accepted: *14 January 2021*
Published: *02 March 2021*

Citation:
Katz EL and Harris JE (2021) Translational Research in Vitiligo. Front. Immunol. 12:624517. doi: 10.3389/fimmu.2021.624517

INTRODUCTION: EARLY RESEARCH IN VITILIGO

Focused research in vitiligo has surged over the past 30 years. This is revealed by the exponential increase in scientific papers published with vitiligo in the title (**Figure 1**) and significant progress in developing new targeted treatment approaches. However, the first documentation of vitiligo and its treatment exists from 3,500 years ago. These ancient texts describe the appearance of vitiligo, emphasize the social stigma associated with the disease, and list treatment approaches used at the time. Vitiligo patients still experience the social stigmas documented in ancient Indian and Buddhist writings, including the restriction of marriage (1). Most of the treatments noted in these ancient texts, including the application of elephant stool or ingestion of cobra bones, are not currently utilized. However, the basis of one remedy is still used today. According to the Atharvaveda, an ancient Indian medical text written in ~1400 B.C., patients with vitiligo were instructed to chew on black seeds from the Bavachi (*Psoralea corylifolia*) plant, which was later determined to be a source of psoralen. They then sat in the afternoon sun until they began to sweat profusely, which led to blistering sunburns of their exposed skin. While this treatment is unacceptable by today's standards, it would have likely been effective, as evidenced by its continued use in ancient times and its later rediscovery in the mid 1900's as topical or oral psoralen derivatives in combination with UVA light treatment, currently known as (PUVA) (2).

Vitiligo has been discussed in modern medical literature for over one hundred years. These early records were primarily case studies characterizing clinical observations and their correlation with other diseases. Vitiligo was described as white patchy depigmentation of the skin beginning as small macules that gradually increased and coalesced into larger patches over time, frequently sparing the

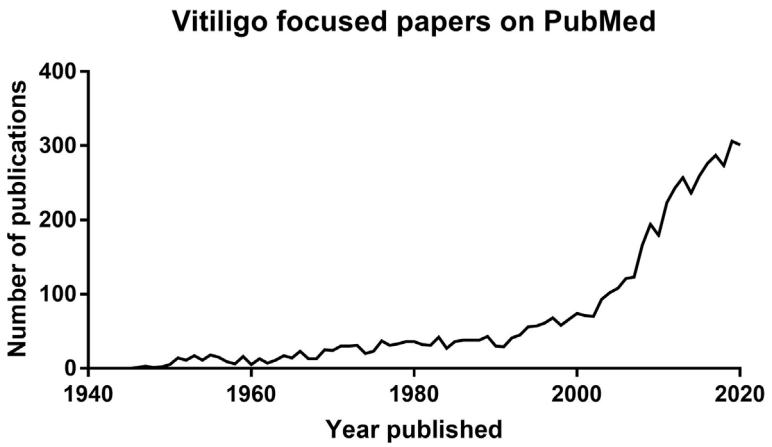


FIGURE 1 | Graph showing the exponential increase of publications available on Pubmed with titles including vitiligo published over time.

hair pigment (3). Authors noted that patients with vitiligo exhibited a higher incidence of other autoimmune diseases, such as alopecia areata, type 1 diabetes, and Addison's Disease (4). The documented therapeutic approaches for treating vitiligo included arsenic, sulfur, mercury, antimony, and sulphuric acid, which are thankfully not used any longer due to limited efficacy and the potential for severe side effects (5). Sulfuric acid was used to burn the skin at borders of the lesions to make them less distinct, and therefore less obvious to the observer. The tolerance of these toxic approaches highlights the motivation of patients to treat their disease.

It was not until the 1940s when more in-depth research studies were performed. Haxthausen in 1947 reported that grafting vitiligo lesional skin onto non-lesional sites led to varying degrees of repigmentation of the lesional skin, while transplanting nonlesional skin onto a lesional area led to the graft depigmentation. He hypothesized that the local environment, which might be determined by the nervous system, influenced the pigmentation state of the graft (6). Comel, in 1948, reported similar results with pedicled and tubed flaps. The lesional flaps repigmented, while the nonlesional flaps attached to lesional skin depigmented (7). Conversely, Spencer and Tolmach in 1951 reported that graft transplantation between lesional and nonlesional skin did not lead to the repigmentation of lesional grafts or depigmentation of nonlesional grafts, but reported extension of depigmentation around the lesional skin graft (8). A possible reason for the conflicting results is the different methodologies used for the skin grafts. Haxthausen used thin split thickness grafts, referred to as a Thiersch graft, while Spencer and Tolmach used full thickness skin grafts. Although these were small case studies that showed mixed results, they suggested that the environment in which the graft is transplanted is an important factor. It wasn't until 1964 when Behl applied skin grafts as a large scale treatment option for vitiligo (9), utilizing the Thiersch grafting technique similar to the Haxthausen study and limiting the variability in repigmentation and relapse by restricting the procedure to only those with stable disease.

PATHOGENESIS OF VITILIGO

There are a number of hypotheses offered to address the pathogenesis of vitiligo. These hypotheses consider roles for innervation, microvascular anomalies, melanocyte degeneration from oxidative stress, defects in melanocyte adhesion, autoimmunity, somatic mosaicism, and genetic influences (1, 10–15) (**Figures 2A–G**). Some have strong evidence to support them, while others are relatively unsupported but continue to be discussed. We will outline these hypotheses and the evidence offered to support them below.

Neural Theory

The hypothesis that innervation influences depigmentation, often labeled the "neural hypothesis", is primarily based on a few largely unsupported observations: 1) Segmental vitiligo is unilateral, and therefore mistakenly labeled as dermatomal; however, disease is rarely limited to a single dermatome, and often crosses many dermatomes (11, 16–18). 2) Lerner hypothesized that vitiligo is caused by the increase of neuropeptides released by neurons and that this decreases melanocyte melanin production (19). Catecholamines have also been reported to be elevated in the urine of vitiligo patients, leading some to assume this came from dysfunctional neurons (20–22). However, others dispute these results and note catecholamines are also produced by melanocytes (23, 24). Thus, these studies do not implicate nerves in disease pathogenesis. 3) There are case reports of vitiligo preferentially affecting limbs that had been traumatically denervated, but the opposite is also true, that denervated limbs have been reported to repigment or be spared from vitiligo altogether (19, 25, 26). In most cases, denervation does not affect vitiligo. Thus, these discordant case reports are more likely chance observations than actual indications of disease pathogenesis. 4) Sweating and vasoconstriction have been reported to be altered in lesions (27, 28). However, many reports are contradictory. Some reports claim an increase in

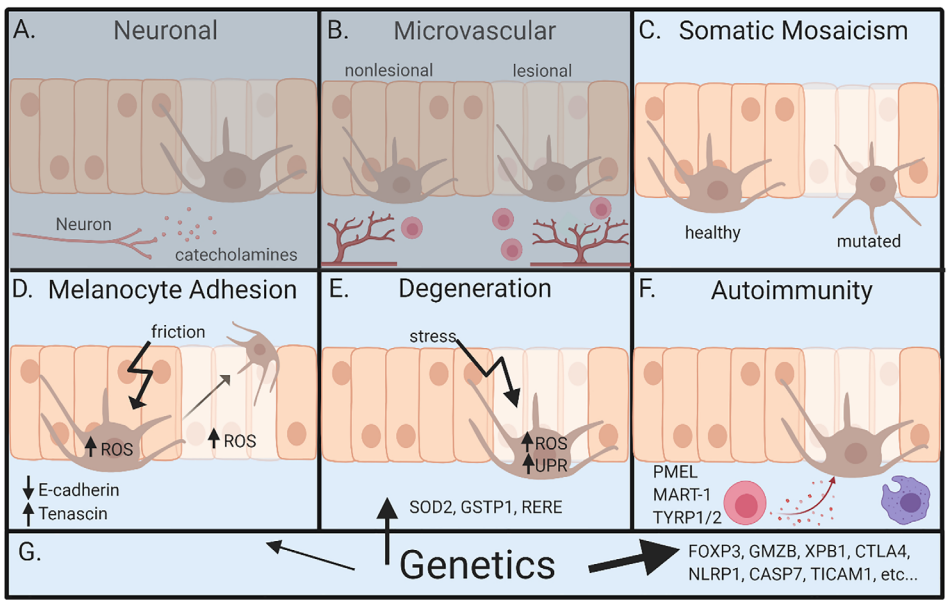


FIGURE 2 | Overview of pathogenesis for vitiligo. **(A)** Neuronal involvement – neurons within the skin release neuropeptides like catecholamines, which act on melanocytes and lead to depigmentation. **(B)** Microvascular theory – vitiligo lesions have increased blood flow during segmental vitiligo, which allows for increased infiltration of lymphocytes that results in autoimmune attack of melanocytes. **(C)** Somatic mosaicism – depigmentation develops because a somatic mutation in melanocytes leads to genetically distinct populations that are susceptible to autoimmune attack. **(D)** Melanocyte adhesion – friction or oxidative stress in melanocytes or keratinocytes leads to melanocyte loss because of reduced adhesion to the skin. **(E)** Degenerative theory – depigmentation occurs because of intrinsic melanocyte defects, such as increased susceptibility to environmental stressors and dysregulation of reactive oxidative species (ROS). **(F)** Autoimmunity theory – autoreactive immune cells attack and kill melanocytes, ultimately leading to depigmentation. **(G)** Genetics – underlies all pathways leading to vitiligo. Genetic studies most clearly implicate autoimmunity, but also melanocyte contributions. Neuronal and microvascular theories are least supported, represented by **(A, B)** being grayed. Figure created in BioRender.com.

cholinergic influence in all vitiligo lesions, while others report this observation occurs in segmental lesions but not bilateral lesions. Data on the level of sympathetic tone is also conflicting, as increased sympathetic tone has been observed by some groups while others observed decreased sympathetic tone (27–34). Since these studies are largely inconclusive, it is premature to suggest they provide any insight into vitiligo pathogenesis. 5) Stress or trauma appears to exacerbate vitiligo (19). This is often thought to be due to neurohormonal mechanisms, however there is currently no evidence to support neuronal involvement. Many autoimmune conditions are exacerbated by stress, an observation that has not implicated neurons in their pathogenesis. 6) Animal studies reveal neural control of pigmentation, particularly in fish (33, 35). However, there is no evidence for this in mammals, especially humans (36). Thus, we suggest future discussions defer mention of the "neural hypothesis", at least until there is solid evidence available to support it.

Microvascular Theory

The microvascular theory suggests there is an increased blood flow into lesional skin during segmental vitiligo. The use of iontophoresis and laser Doppler flowmetry demonstrated up to triple the blood flow in lesional skin of segmental vitiligo patients compared to healthy, but not within nonsegmental lesional skin (37). The unilateral presentation was proposed to be from the activation and expansion of the autoreactive T cells in the regional lymph nodes that then exit into the blood circulation. An increased blood flow may then escalate the migration of lymphocytes, specifically melanocyte-specific cytotoxic T cells because of their expression of cutaneous homing receptors, to the lesion for the destruction of melanocytes (13, 38–40). However, a contradictory observation is that when vitiligo develops in melanoma patients as the result of a vaccine injected for the expansion of melanocyte-specific T cells, disease is typically reported to develop in a nonsegmental pattern. If the microvascular theory were true, then it would be expected to develop in a segmental pattern originating in the region of the injection site (12). Furthermore, the microvascular theory is often grouped closely with the neural theory because it has been proposed that damaged sympathetic nerves leads to the increased blood flow due to the vasodilation of the skin blood vessels (41). The lack of real evidence for this theory supports discarding it until further studies provide support.

Degenerative Theory

The degenerative theory focused on the idea that there is an intrinsic defect within melanocytes that leads to their loss within the skin, thereby causing depigmentation. This theory arose from

observations that melanocytes from vitiligo patients are difficult to culture and proliferate much slower than melanocytes from healthy individuals (42). Additionally, dysregulation of reactive oxidative species (ROS) and increased susceptibility to chemicals that induce ROS have been reported in melanocytes from patients with vitiligo (43, 44). Catalase is an enzyme that converts hydrogen peroxide, a source of ROS, into water, and expression of this enzyme is reportedly reduced in vitiligo lesional skin (45). Attempts to mitigate oxidative stress in melanocytes using a topical psuedocatalase cream does not seem to have an impact on disease, but use in combinational treatments with calcium and UVB light treatments have been reported to result in effective repigmentation (46, 47). However, Gilhar et al. demonstrated that melanocytes are functional within lesions because human vitiligo skin grafted onto nude mice fully repigmented and the number of melanocytes within the graft increased (48). Therefore, although there is clear evidence indicating melanocytes are abnormal in lesional skin, the idea that vitiligo exclusively develops from intrinsic melanocyte degeneration is unlikely because of the disputing studies supporting the fact that melanocyte defects are not sufficient to cause disease.

Melanocyte Adhesion Theory

The melanocyte adhesion theory suggests that melanocytes lose or have decreased adhesion to the skin and are therefore easily eliminated or otherwise lost during oxidative stress or mechanical pressure, such as friction from clothing (49). Some studies report a decreased expression of the adhesion molecule E-cadherin on melanocytes, while another reported no significant differences in expression of adhesion molecules between healthy, nonlesional, and lesional skin but elevated expression of an "anti-adhesion molecule" tenascin in vitiligo lesional and nonlesional skin, which might lead to the loss of melanocytes within the skin (50, 51). More *in vivo* data is needed to determine the role of melanocyte adhesion defects during melanocyte loss, such as whether adhesion molecules knocked out in melanocytes in mouse models lead to increased disease severity. Keratinocytes may also play a role in the loss of melanocytes. Keratinocytes and melanocytes comprise a "epidermal-melanin unit" and maintain homeostasis in the epidermis through paracrine signaling of growth and survival factors (52). Studies report that keratinocytes within vitiligo perilesional and lesional skin have dysregulation of oxidative stress (52). For example, keratinocytes have mitochondrial abnormalities evident from increased ROS production, lower production of antioxidants, and expression of apoptotic proteins (53, 54). Keratinocytes clearly have a role in pathogenesis, as their production of CXCL9 and CXCL10 recruits autoreactive CD8+ T cells to the skin (55). Therefore, it is conceivable that intrinsic defects within keratinocytes may play contribute to abnormalities in melanocyte adhesion during vitiligo pathogenesis. The current available data is intriguing and may support a role in vitiligo pathogenesis. The loss of melanocyte adhesion to keratinocytes might be a cause or effect of autoimmune inflammation. For example, it may trigger autoimmunity by a large release of autoantigens, or intrinsic defects in handling stress or autoimmune attack itself might lead to reduced adhesion (56).

Autoimmunity Theory

The autoimmunity theory offered that self-reactive immune cells attack melanocytes, which then leads to their death and loss from the skin, resulting in depigmentation. This was one of the earliest theories, since initial case studies noted the coincident onset of vitiligo with other autoimmune diseases, thus implicating autoimmunity by association. In the late 1970s, a study revealed the presence of autoantibodies against melanocytes only in vitiligo patients and not in healthy controls using immunofluorescence complement fixation tests (57). This supported the notion that autoimmunity played a functional role in disease because it indicated activation of adaptive immunity specifically against melanocytes. Later studies revealed that autoantibodies could lead to the destruction of melanocytes *in vivo* by grafting human skin onto nude mice and subsequently injecting purified IgG antibodies from vitiligo patients (58). This resulted in a decrease of melanocytes in the grafts on mice injected with vitiligo patient IgG compared to healthy patient control IgG (58). It was later determined that autoantibodies are likely not the main drivers of disease because autoantibody levels do not correlate with disease severity in vitiligo patients and autoantibodies are equally distributed throughout the body, rather than concentrated in lesions (59).

While autoantibodies indicated an autoimmune response against melanocytes in vitiligo, it became apparent that melanocyte damage results from another mechanism. Early immunohistochemical (IHC) studies noted the decrease of melanocytes within lesional skin and the presence of immune cells in the upper dermis and epidermis (33, 60). It was then reported that CD8+ T cells infiltrate the skin and are positioned within close proximity to dying melanocytes (39, 61). Van den Boorn et al. determined that the immune system, and specifically CD8+ T cells, were both necessary and sufficient for melanocyte destruction in the skin of vitiligo patients by incubating T cells isolated from perilesional skin with nonlesional skin explants from the same donor, which resulted in melanocyte death (62). Furthermore, autoimmunity is supported by genome-wide association (GWA) studies that revealed genes associated with vitiligo risk are predominately immune-related, including genes associated with both adaptive and innate immune responses (63).

Melanocytes during vitiligo appear to be abnormal at baseline, producing heat shock proteins to help manage cellular stress, which activate the unfolded protein stress response. These abnormalities may lead to the release of damage-associated molecular patterns (DAMPs), which signal through pattern recognition receptors (PRRs) like TLR2, TLR4, and NLRP inflammasomes (64). Vitiligo-inducing chemicals like phenols initiate the unfolded protein response (UPR) in melanocytes, which results in the production of IL-6 and IL-8 (65), two cytokines that promote immune cell recruitment. Additional studies report infiltration of vitiligo lesional skin by

innate immune cells, including inflammatory dendritic cells, macrophages, and natural killer (NK) cells (39, 66, 67). Yu et al. reported an increase of NK cells in vitiligo nonlesional skin, suggesting a role in the onset of disease (67). These studies suggest that melanocytes may initiate the innate immune response through the recruitment of innate immune cells from inflammatory signals and the release of DAMPs that are able to signal through pathogen recognition receptors (PRRs) on these cells. Additional studies will be important to further support these observations and enhance our understanding how the innate immune system bridges melanocyte stress and adaptive autoimmunity in vitiligo.

Genetics

Genetics clearly influences the risk of developing vitiligo, although should not be described as a theory in itself. Indeed, genetic variation influences all pathways in the body, including immunology, melanocyte stress and adhesion, etc. Several epidemiological and familial studies addressing the incidence of vitiligo report that vitiligo affects 1%–2% of the overall population and that there is a higher risk for people who have first-degree relatives (FDR) with disease (68–71). Many of these studies were limited due to the low number of participants, although a recent study by Kim et al. incorporated a large number of individuals in a Korean population, providing significant power in their analysis. Their study calculated incidence risk ratio (IRR) for FDRs and found that siblings of vitiligo patients have a higher incidence of disease, with a larger increase if the sibling is a twin (69, 71, 72). Unlike previous studies, Kim et al. also reported a higher IRR for offspring that have a mother with vitiligo compared to a father (71). Although this increased risk could be from shared environmental risks, another possibility is the inheritance of mitochondrial DNA that is only obtainable maternally. Overall, homogeneity of the study population provided strong, powered data about vitiligo heritability, but more studies will be needed to determine whether these observations can be applied to other ethnicities and mixed populations.

Recent GWA studies revealed small nucleotide polymorphisms (SNPs) in many immune-related and melanocyte specific genes (63). The identification of melanocyte-specific genes (OCA2-HERC2, PMEL) through GWAS support a role for melanocyte involvement, while genes involving stress and apoptosis (SOD2, GSTP1, RERE, XPB1) support a role for melanocyte stress as well (68, 73, 74). The autoimmunity theory is validated by GWA study hits, as well with the melanocyte-specific genes are often autoantigens (PMEL) and immune-related genes (FOXP3, GMZB, XPB1). While these SNPs have provided significant insight, functional genomic studies are needed to understand the full relationship SNPs have on disease pathogenesis. However, it is difficult to identify gene target of SNPs because most occur in noncoding regions that likely regulate gene transcription, which may be present at a significant distance from their target genes. Recent three-dimensional (3D) genome analysis studies have reported that SNPs can influence genes that are linearly distant because of higher-order chromatin structures allow interactions of distant genome regions (75–77).

While genetics clearly plays a role in vitiligo, environmental exposures, and stochastic events factor into pathogenesis as well. This is indicated by the fact that coincident rates for identical twins to develop vitiligo is only 23%–26%, rather than the expected 100% if pathogenesis was only influenced by genetics (69, 71). Chemicals in permanent hair dyes and cosmetics, as well as chemicals like 4-TBP and monobenyzl ether of hydroquinone, induce and exacerbate vitiligo (78–80). This is likely through the induction of cellular stress pathways within melanocytes when they attempt to convert the chemical into melanin, as the chemicals are very similar in structure to the amino acid tyrosine, the building block of melanin synthesis. Additionally, stochastic events within the immune system may result in the develop of vitiligo because T cell receptors and antibodies are generated through random recombination of existing genes. Thus, a single panel of genetic factors as found in identical twins could result in very different immune responses through stochastic events. Therefore, a combination of genetic predisposition, environmental exposures, and stochastic events contribute to the risk of developing vitiligo.

SEGMENTAL VITILIGO

While vitiligo is typically bilateral and symmetric, segmental vitiligo is a subtype of the disease that presents as unilateral depigmentation that does not cross the midline. Historically, the segmental subtype has been misnamed "dermatomal vitiligo" because its unilateral presentation was reminiscent of shingles, which affects the skin in a distribution overlying a single sensory nerve, which also localizes unilaterally. However, segmental vitiligo rarely, if ever, follows a single dermatome, but frequently involves multiple dermatomes and even runs perpendicular to dermatomes, indicating that this disease is not related to single nerves (12). Because of its unilateral nature and dermatomal misnomer, many believed that segmental vitiligo may have a separate and distinct pathogenesis, hypothesizing that nerves might play a role in this subtype of disease. However, more recent studies have shed light on mechanisms that lead to segmental vitiligo, including strong evidence that it is also driven by autoimmunity (40, 62).

In fact, there may be an important role for somatic mosaicism in the pathogenesis of segmental vitiligo. Somatic mosaicism occurs when there are two genetically distinct populations of cells arising from a mutation that appears within the zygote during development. A post-zygotic mutation occurring in an embryonic melanocyte can be passed on to daughter cells that then migrate ventrally from the neural crest (81). The somatic mosaicism theory suggests that a somatic mutation creates susceptible melanocytes by inducing melanocyte stress or increasing the expression of melanocyte specific markers (12), which then become the targets for autoimmune attack that is limited to the mutant population of cells (82). The ventral migration of these melanocytes during development without

crossing the midline may lead to the unilateral distribution of disease, characteristic of segmental vitiligo (11). Future studies will be important to test this hypothesis directly.

HYPOTHESES INFLUENCE RESEARCH QUESTIONS AND DATA INTERPRETATION

Hypotheses that address the pathogenesis of disease influence experimental design, as well as data interpretation, which can be either beneficial or detrimental to progress in understanding disease, as this natural bias can focus investigation leading to accelerated discovery, or negatively influence data interpretation within a narrow framework. This has been done in some respect with the "neural hypothesis". As discussed above, the characteristic unilateral nature of segmental vitiligo for years was misinterpreted as supportive of neural involvement, despite the fact that lesions did not follow specific dermatome patterns at all (11). In addition, the presence of elevated catecholamines in the blood and urine of vitiligo patients were interpreted to indicate support for the neural hypothesis, suggesting that these catecholamines emanated from dysfunctional nerves (21). An alternative interpretation proposed that the increased catecholamines were a product of melanocyte destruction. This interpretation led to studies that observed that melanocytes also produce catecholamines, thus providing new insight about melanocyte biology during disease and further refutes the neural hypothesis (23, 24). Discussions and new interpretations when exploring both proposed hypotheses about the presence of catecholamines and nerve mapping helped drive focus to more promising pathogenesis ideas.

Another example highlighting different data interpretations driving experimental approaches is the observation that immune cells infiltrate vitiligo lesions. Immunohistochemical studies revealed infiltrating immune cells within the upper dermis and epidermis of vitiligo lesions (33, 60). It was then identified these immune infiltrates were predominantly CD8+ T cells and macrophages, which were positioned within close proximity to dying melanocytes (39, 61). Some interpreted these immune cells as simply "cleaning up" after melanocyte degeneration, which led to exploration of the role of macrophage clearance of debris in promoting repigmentation (83). Alternatively, researchers hypothesized immune infiltration into the epidermis supported a direct autoimmune attack against melanocytes. This led to additional characterization of the infiltrating T cells, which reported a higher frequency of melanocyte specific CD8+ T cells in the circulation of vitiligo patients expressing the skin homing receptor cutaneous leukocyte-associated antigen (CLA) (38, 84, 85). Further studies on T cells isolated directly from lesional skin revealed that cytotoxic CD8+ T cells selectively kill melanocytes, further validating the autoimmune theory (62).

In addition, increased production of heat shock proteins (HSPs) was observed in melanocytes from patients with vitiligo. One interpretation of this data in the context of the degenerative theory is that the elevated HSPs are simply a result of increased melanocyte stress from intrinsic defects in regulating ROS production. This led to the exploration of melanocyte ROS and HSP production during normal cellular processes and under environmental stressors (65, 86–88). Another interpretation is that the increased production of HSPs by melanocytes acts as danger signals that induce inflammation, leading to autoimmunity. With this in mind, studies indicate that HSP70i is elevated in human melanocytes during chemical-induced vitiligo, has the capacity to activate skin dendritic cells, and is required for the induction of vitiligo in mouse models (66, 89).

In fact, both possibilities may be true. Melanocyte intrinsic defects regulating ROS production may play in a role in activating autoimmunity. The interplay of endoplasmic reticulum (ER) stress and ROS production have been implicated in the development of multiple autoimmune diseases, including vitiligo (90–92). Melanocytes within vitiligo skin show signs of oxidative and ER stress, including elevated ROS, a dilated ER, as well as gene expression consistent with UPR activation, including X-box protein 1 (XBP1), a transcription factor that is needed to produce cytokines involved in innate immune cell recruitment (65, 87). Vitiligo-inducing phenols induce expression of XBP1 in melanocytes (93), providing a feasible connection between melanocyte oxidative and ER stress to the immune response. Furthermore, these stress pathways within the melanocytes lead to the production of HSPs to help mitigate this stress, which are then secreted from the melanocyte or released during cell death to signal through TLRs and other PRRs to activate the immune response. Exploring multiple hypotheses, such as melanocyte stress, innate immune activation, and adaptive autoimmunity through complementary approaches will give a better understanding of the complex cell interactions and mechanisms at play during vitiligo pathogenesis.

ADDRESSING EXISTING HYPOTHESES IN FUTURE RESEARCH

To further explore existing hypotheses and to better understand how they interface to cause vitiligo, genetic and functional analyses comparing lesional, nonlesional, and healthy skin will be needed. Tracing mutations within melanocyte lineages will interrogate somatic mosaicism development and progression. Performing single cell RNA sequencing and functional genomics techniques like Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) can guide functional studies addressing the mutations found in lineage tracing to determine their direct role on melanocyte homeostasis and function.

The results of genetic studies have launched many experiments. Since many GWAS hits are in both melanocyte and immune related genes, the identified genomic risk alleles have guided studies that address both the degenerative and autoimmunity theories. Studies to dissect intrinsic pathways within melanocytes have provided insight about stress

responses, ROS production, metabolism, and adhesion. These insights have led to new questions that have yet to be answered, such as understanding the interface between melanocytes and inflammation. For example, future studies may dissect how melanocyte stress and inflammation influence each other during disease initiation and progression. Stressed melanocytes may induce local inflammation that then initiates and perpetuates autoimmunity in vitiligo. Inflammation could be also a stressor to melanocytes, and since melanocytes are defective in vitiligo this stress may weaken the melanocytes further, exacerbating autoimmune attack.

Additionally, the interplay of inflammation and melanocyte adhesion should be pursued. Skin inflammation could be reducing melanocyte adhesion or melanocytes that lose adhesion could be triggering inflammation and breaking tolerance. One reason for these unanswered questions is that cell interactions are not as easily studied in cell culture because multiple cell types interact within the tissue within a 3-dimensional (3D) environment. 3D skin models and animal models could be useful tools for exploring these questions. These models will also be helpful in working to define the complex, multicellular interactions that characterize autoimmunity within the skin. Unanswered questions include the mechanism of autoimmune melanocyte killing, tolerance mechanisms for suppression or prevention of disease, and whether other immune cells besides autoreactive CD8+ T cells contribute to melanocyte death.

These questions will be elucidated by creatively utilizing developing technologies and tools. Interest in understanding these interactions has supported the advancement and utilization of translational research tools such as flow cytometry, cell sorting, and live *in vivo* imaging. As these technologies develop, opportunities to explore these cellular interactions will expand.

TRANSLATIONAL TOOLS FOR VITILIGO RESEARCH

Rubio et al. defined translational research as the merging of basic, clinical, and population research, which continuously build on each other (94). The multi-directionality of translational research is advantageous because it aligns mechanistic studies in a model system with clinical observations and studies on human tissue. Thus, translational research leverages the best of both basic and clinical approaches, understanding the mechanism of disease while maintaining physiologic relevance (**Figure 3**).

Vitiligo is uniquely suited to translational research because of its high prevalence in the population, opportunity for visual evaluation of disease progression, and ready access to the target tissue. Procedures for obtaining patient skin biopsies are less invasive than in other organs, such as the intestines where endoscopic procedures are required or pancreas and brain where samples can only be safely obtained postmortem. Tissue samples are critically important for understanding human disease pathogenesis, because other sources of patient tissue such as the blood is *at best* a diluted sample of disease-causing factors in the tissues, and *at worst* may be specifically depleted of these factors.

Traditional methods used to sample the skin include punch or shave biopsies, which yield small pieces of solid tissue for analysis. These biopsies can be analyzed using histology,

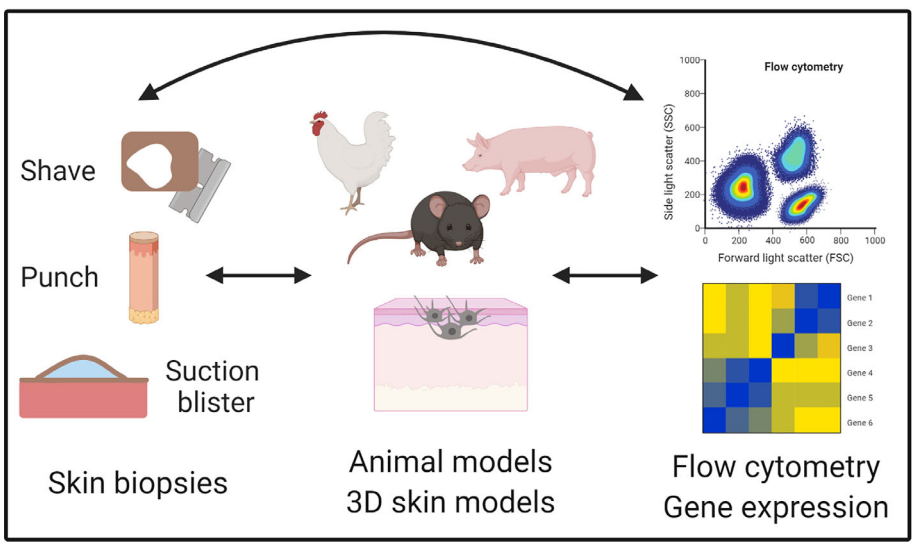


FIGURE 3 | Translational tools to study vitiligo. Vitiligo is uniquely positioned for translational studies because of ready access to the skin and multiple collection techniques for human skin samples such as shave, punch, and suction blister biopsies. These biopsies can then be used for the identification and characterization of cell populations on the single cell level with technologies like flow cytometry and single cell RNA sequencing. Animal models are available for studying initiating factors and adaptive immune responses, as well as cell culture techniques like 3D skin models that have been useful for elucidating mechanisms during vitiligo pathogenesis. Figure created in BioRender.com.

immunohistochemistry, and immunofluorescence to determine protein expression, which enables positional information that indicates spacial relationships among cells. Tissue can be homogenized for RNA or protein quantification; however, this eliminates information on individual cells or their position within tissues. Finally, solid tissue can be enzymatically digested to yield single cells for single cell analyses such as flow cytometry, RNA sequencing, and others. Disadvantages to these approaches include pain, need for anesthesia, scarring of the sampled site, and requirement for sutures in some cases. In addition, immune cells infiltrating vitiligo lesions can be difficult to isolate, since they are frequently present in a perilesional location, somewhere beyond the lesional border. This is because these cells migrate through the skin outward from the lesion center while melanocyte loss requires epidermal turnover to observe clinically, so that the true lesion edge is located beyond the visible border. Thus, it can be very difficult to determine where the biopsy should be taken in order to "catch" migratory immune cells.

The use of suction blistering on human volunteers was first described by Kiistala and Mustakallio in 1964 for the purpose of separating the epidermis and dermis (95). Originally, suction blister roofs had been used for obtaining cells for culture, quantifying bacteria on the epidermis, and studying epidermal permeability and biochemical properties (96–98). The time blisters took to form on various skin disease states compared to healthy could also be used to categorize skin diseases and their severity, especially blistering diseases like pemphigus vulgaris (99). Blister fluid was utilized for to analyze immune cells in the skin, such as cell recruitment during allergic inflammation because an influx of immune cells into the blister occurred over multiple days after induction of the blister (100). Suction blistering later was used for epidermal grafting, using cells from both the blister roofs and fluid, to treat stable vitiligo lesions (101). More recently, this technique has been used in other contexts to identify cell populations, protein levels of soluble receptors, and secreted growth factors and cytokines (102–104).

The suction blistering approach is less invasive, not painful, does not typically scar, and does not require anesthesia or sutures. It is accomplished through application of negative pressure by a suction chamber, often with the addition of gentle heating (99). While a single punch biopsy collects 4-6 mm of skin, suction blistering can yield many more total cells because the interstitial skin fluid that forms the blister (up to 10mm) is drawn from an even larger area and each subject can contribute multiple blisters, we frequently acquire up to 10 per subject. We optimized this technique to sample the involved and uninvolved skin of subjects with vitiligo as well as healthy controls, and found that the extracted blister fluid contains multiple cell types that can be characterized by flow cytometry, as well as proteins that can be measured with ELISA (104). Thus, it is an efficient technique to isolate migratory immune cells from vitiligo lesions as well as soluble proteins from interstitial fluid. However, blisters do not preserve the architecture of the skin, and thus positional information is lost. In addition, it may preferentially sample the epidermis and upper dermis, potentially missing cells (particularly structural cells like fibroblasts and endothelial cells) and fluid from the deeper dermis (105, 106). Since vitiligo consists of melanocyte destruction at the basal epidermis, the blistering approach is well-suited for translational studies in vitiligo.

Once single cells have been obtained from skin through suction blistering or enzymatic digestion of solid tissue biopsies, several techniques can be used for analysis of the cells (**Table 1**). Flow cytometry has enabled the identification and characterization of cell phenotypes on the protein level in vitiligo patients. It does not only distinguish the number of cells that are expressing a marker of interest but can also measure the amount of protein expressed by each cell as mean fluorescence intensity (MFI). In addition, since vitiligo has known autoantigens, the use of tetramers and pentamers have been valuable tools for the detection and characterization of circulating autoreactive cells in peripheral blood and skin. Flow cytometry has limitations though, as many transcription factors and intracellular proteins are difficult to detect due to the lack of available antibodies or sufficient permeabilization methods, and some receptors are quickly internalized so protein expression by flow cytometry may not always be an accurate assessment.

Single cell RNA sequencing can provide valuable insights at the single cell level (107). This approach enables researchers to identify unknown immune cell interactions through signaling networks with ligand/receptor pairing, unlike flow cytometry, which detects a small set of previously selected target proteins. It also can use gene expression within individual cells to discover new cell subtypes, which is not possible through bulk tissue gene expression analysis. However, not all changes on the RNA transcript level are reflected at the protein level. Further regulation can occur post-transcriptionally and post-translationally, which can affect whether a protein is available or functional. Therefore, validation studies must be done to determine whether RNA expression changes are reflected at the protein level.

Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) is a technique to assess genome-wide chromatin accessibility by probing open chromatin with hyperactive transposases that are pre-loaded with sequencing adaptors (108). This technique can be performed in bulk sequencing and on single cells (109). If done at the single cell level, cell specific transcription factors, binding sites and changes in cell-to-cell DNA accessibility variability within different disease states can be explored (110). These epigenetic approaches could be applied to understanding vitiligo pathogenesis, as already described for Systemic Lupus Erythematosus (SLE) (111). However, open chromatin does not always lead to higher expression of genes or their expression as proteins, and thus further studies are required to validate findings at this level, similar to RNA sequencing.

Interstitial fluid, which cannot be obtained using conventional biopsy approaches, can be used to analyze soluble factors that might contribute to disease pathogenesis. For example, we used ELISA to determine that CXCL9 was a sensitive and specific biomarker of disease activity in vitiligo lesions (104). Metabolomics has been performed on interstitial skin fluid, which could be used to understand changes in metabolic processes within nonlesional and lesional skin (112). While soluble factors in fluid cannot be

measured at the single cell level, when combined with single cell techniques like scRNA-seq, pathways can be inferred, and new hypotheses developed. These tools will provide new insight into vitiligo pathogenesis and potentially fast track the discovery of new pathways and mechanisms involved in vitiligo, in hopes of developing better and durable therapeutic options. All these techniques would provide a wealth of new and exciting insights into vitiligo pathogenesis. While current techniques using suction blister fluid lack spatial information, emerging techniques seek to examine this, including spatial transcriptomics like Slide-seq and RNA seqFISH+, as well as spatial mass spectrometry imaging (113, 114).

All biopsy techniques allow for the isolation of cells for culture. While healthy melanocytes can be cultured, melanocytes from vitiligo lesions were difficult to establish *in vitro* because they had a long lag time for growth and could not be passaged (42, 115). In the early 1990's, cell culture techniques improved to enable the culture of both healthy and vitiligo melanocytes by the addition of growth factors, such as fibroblast growth factor (bFGF), phorbol 12-myristate 13-acetate (PMA), and bovine pituitary extract (BPE) (115, 116). This has supported investigation of melanocyte biology and autoimmune killing in co-culture experiments. There are limitations to what can studied in 2D cell culture however, and so the future development of re-creating the skin environment *in vitro* with 3D skin cultures may help further dissect mechanisms of autoimmunity from more physiologically relevant interactions (117–119). This has been used by Van den Boorn et al., who cultured T cells isolated from punch biopsies with additional biopsies from normal skin from the same patient (62). Boukhedouni et al. also used 3D skin model to study the effect of TNFα on melanocyte adhesions (56).

Translational research covers a vast range of experimental designs. While many only consider experiments containing human samples and clinical studies to be translational, others consider animal models that parallel human disease to be translational work as well. It is easy to recognize the direct impact for patients when working with patient samples, but these studies tend to lack mechanistic insights in this context, which basic research is better positioned to address. Therefore, the development and use of animal models has helped to add mechanistic understanding to clinical observations. Animal models of vitiligo have been extensively reviewed by Essien and Harris (120). Models of vitiligo include both spontaneous

TABLE 1 | Table comparing the advantages and disadvantages of translational tools available to study vitiligo.

Translational tools to study vitiligo			
Technique		**Advantages**	**Limitations**
Tissue acquision methods	Punch Biopsy	Maintains architecture; used for histology, IHC, IF, obtaining cells for cell culture	Can cause pain; scarring of a sample site; requires anesthesia and sutures or gelfoam
	Shave Biopsy	Maintains architecture; used for histology, IHC, IF. obtaining cells for cell culture	Can cause pain; scarring of a sample site; requires anesthesia
	Suction Blister	Less invasive; not painful; non- scarring; collects more cells than conventional biopsies; provides interstitial skin fluid for analysis of soluble molecules	Samples epidermis and upper dermis; missing deeper, structural cells
Assay methods	Flow cytometry	Identification and characterization of single cell protein expression	Dependent on availability and quality of antibodies; limited number of proteins can be analyzed; lacks spatial information
	Histology/ Immunohistochemistry/ Immunofluorescence	Identifies immune infiltration and protein expression with spacial information is maintained	Requires optimization of staining and imaging; dependent on availability and quality of antibodies; limited number of proteins can be analyzed; less quantifiable than single-cell methods
	Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq)	Determine epigenetic changes between disease states	Epigenetic changes do not always lead to protein expression; lacks spatial information; expensive
	Single cell RNA sequencing (scRNA-seq)	Identification and characterization of single cell transcripts; does not require prior knowledge of genes of interest; no antibody optimization	Not all transcript changes reflect protein expression; lacks spatial information; expensive
	Metabolomics	Understand changes in metabolic processes between disease states	Unable to identify which cells are producing which metabolites
	Spatial mass spectrometry imaging	Identify which cell types are producing which proteins and metabolites with spatial resolution	Not as quantitative as standard metabolomics
	Enzyme-linked immunosorbent assay (ELISA)	Identify and measure soluble proteins	Does not identify which cell types produce which proteins
	Slide-seq/ RNA seqFISH+	Transcriptomics with spatial information	Fewer targets can be analyzed
	2D cell culture	Can characterize primary cells, including cell-cell interactions through co-culture	Missing physiologically relevent conditions, including 3D interactions, air-tissue interface
	3D cell culture	Modeling of 3D skin envirionment and cellular interactions; may be useful for modeling autoimmune cell-cell interactions	More complex methods; does not involve all cell interactions
	Animal models	Models in vivo disease; parallels to human disease; useful for complex cellular interactions and mechanistic studies	Costly to maintain; can be complex methods; some human pathways not present in animal models

and induced systems, and spontaneous models include Smyth line chickens, Sinclair swine, Gray-allele horses, and a variety of mouse models (121–125). These are useful for studying disease initiation but can be costly to maintain and may be more difficult to study because of limited reagents available that are compatible with these species. Inducible models are helpful to study disease progression after onset and include the mouse model developed by Harris et al. that parallels human disease by developing patches of skin depigmentation without involving hair, and thus has the potential to repigment with treatment. Like humans, this model is mediated by CD8+ T cells and exhibits IFN-γ driven disease with a similar Type 1 cytokine signature (126–128). Animal models are most useful for focused studies that probe specific questions for which the model is optimized to answer. For example, questions about disease initiation, including genetic influences, should be studied in spontaneous models, models designed for this purpose, or directly in humans, since many induced models bypass this step of pathogenesis.

LESSONS LEARNED FROM TRANSLATIONAL RESEARCH

The long history of studying vitiligo using translational methods has enabled us to develop a more complete picture about disease pathogenesis, which has been more difficult to achieve in diseases that target less-accessible tissues. Translational studies have been great assets for understanding the mechanisms of disease, which can then promote the development of promising new treatments (**Figure 4**). For instance, patient biopsies revealed that there is an influx of melanocyte specific CD8+ T cells into lesional skin, which produce IFNγ and IFNγ-induced genes, and express NKG2D (39, 62, 127, 129–131). One study reported type I IFN expression within lesions using CXCL9 and MxA as surrogate markers induced by IFNs, however these can be induced by IFNγ as well. Mouse studies do not support a role for type I IFNs in vitiligo, as IFNα receptor (IFNaR) knockout mice still develop vitiligo to the same extent (132). Thus, while IFNγ clearly plays an important role in vitiligo pathogenesis, it is still unclear whether other IFNs contribute to disease.

Infiltrating CD8+ T cells express CXCR3 at high levels within lesions, and expression of CXCR3 ligands are characteristic of vitiligo lesions (104, 127, 133, 134). Supporting data using mouse models reveal that CXCR3 and CXCR3 ligands are expressed within lesional vitiligo skin in mouse models, and it is required for recruitment of T cells during disease progression and maintenance (127, 135, 136). Mouse models also made it possible to determine that keratinocytes respond to IFNγ production from T cells and secrete CXCL10 to recruit melanocyte-reactive CD8+ T cells to the skin (55). These discoveries provided the rationale to test JAK inhibitors, which block IFNγ signaling, as treatments for vitiligo and eventually led to a successful Phase 2 clinical trial to test topical ruxolitinib as a novel treatment for the disease (137–143).

However, conventional treatments (144, 145) and even JAK inhibitors (137, 139) do not appear to provide durable responses following treatment, as disease relapses within previous lesions once they are stopped. Translational studies revealed that autoreactive CD8+ T cells form tissue resident memory cells (Trm) in vitiligo lesional skin (133, 146, 147). Mouse models confirmed the presence of Trm in vitiligo lesions and revealed that they are responsible for maintenance and relapse of disease after stopping treatment (146, 148–150). Additional studies determined that melanocyte-specific autoreactive Trm require IL-15, and that targeting IL-15 signaling eliminates Trm from the skin and results in durable repigmentation in mice (146). IL-15 expression is elevated in vitiligo lesional skin and may be induced by oxidative stress in keratinocytes (151). Therefore, targeting IL-15 may provide a durable treatment option for vitiligo, and clinical trials to test this hypothesis are imminent (152).

As mentioned above, increased expression of HSP70i has been reported in vitiligo lesions, and activates skin dendritic cells that could prime autoreactive T cells against melanocytes (89). A mouse model required HSP70i for vitiligo initiation and when inhibited, disease is prevented (66, 89). Additionally, melanocytes produce HSP70 in response to oxidative stress, which may provide a link between cellular stress in melanocytes and inflammation that initiates autoimmunity in vitiligo (92, 153). Therefore, melanocyte stress could be mitigated by targeting this pathway and thus provide a new opportunity for treatment.

Regazzetti et al. reported a decreased expression of WNT signaling components in melanocytes when exposed to oxidative stress. The authors went on to demonstrate that treatment with a chemical WNT agonist can promote melanoblast differentiation in vitiligo skin, suggesting that activating WNT signaling in melanocytes might promote their regeneration (154). While that study did not show whether this differentiation fully progresses to a mature functional melanocyte, Yamada et al. reported that WNT signaling is critical in melanocyte stem cell differentiation and that WNTs are secreted predominantly by keratinocytes and melanocytes (154, 155). Furthermore, WNT signaling regulates E-cadherin, an important adhesion molecule for melanocytes, through the availability of β-catenin (156). Wagner et al. reported that there is a reduction of E-cadherin expression on melanocytes in vitiligo skin (51). Together these studies suggest that a decrease in WNT signaling might impair melanocyte adhesion. Additionally, Boukhedouni *et al.* reported that the type 1 cytokines IFNγ and TNFα impair melanocyte adhesion from the epidermis by inhibiting melanocyte E-cadherin expression and inducing keratinocyte release of protease MMP-9 (56). MMP-9 cleaves E-cadherin complexes, which results in melanocyte detachment from the epidermis. This study also reported that when type 1 cytokines were inhibited with JAK inhibitors, or when MMP-9 activity was inhibited, fewer melanocytes detached (56). Therefore, anti-inflammatory treatments like JAK inhibitors may also stabilize melanocytes and potentially could become more durable if used in conjunction with WNT agonists or inhibitors of detachment to promote melanocyte regeneration and maintenance.

Some have suggested that activating immune checkpoint inhibitors, which are receptors expressed on the surface of T

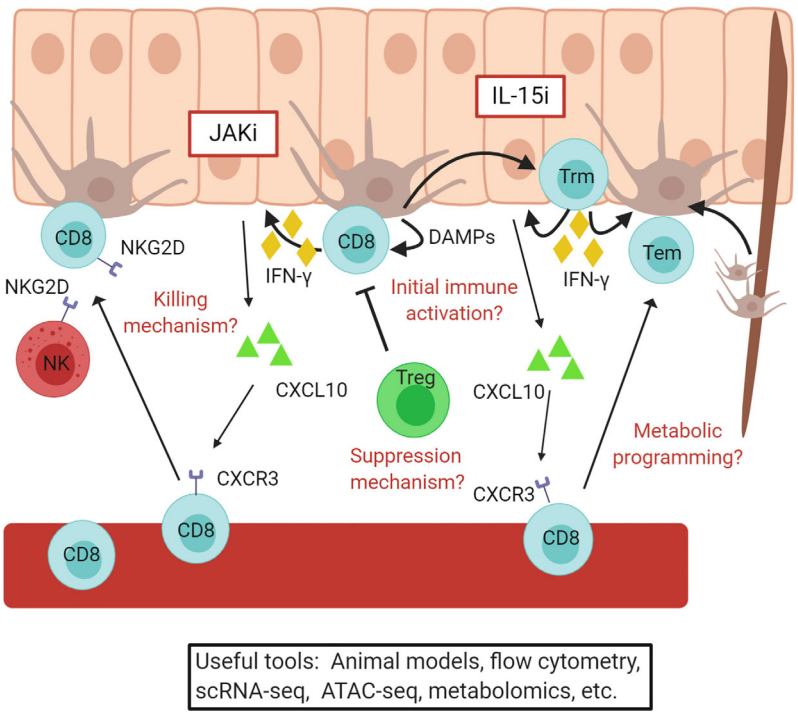


FIGURE 4 | Vitiligo pathogenesis revealed through translational research. A simple overview of the current understanding of vitiligo pathogenesis. Autoreactive CD8+ T cells are recruited to the skin by CXCL10 produced by keratinocytes and kill melanocytes. These cells convert to resident memory T cells, which maintain vitiligo and require IL-15 signaling in the skin. JAKi and IL15i, outlined in red, may be effective treatments for vitiligo and are currently being tested in clinical trials. Some questions that remain include how melanocyte abnormalities initiate autoimmunity, the mechanism by which CD8+ T cells kill melanocytes, how Tregs suppress disease, and how changes in metabolism effect disease activity. Existing and developing translational tools will help to answer these and other questions. Figure created in BioRender.com.

cells that negatively regulate their activity, might be an effective therapeutic target for vitiligo (157, 158). Blocking cutaneous lymphocyte antigen-4 (CTLA-4) and program cell death protein 1 (PD-1) for melanoma treatment induce and exacerbate vitiligo, presumably by removing negative regulation that prevents autoimmune activation of these cells (159–162). Decreased CTLA-4 mRNA expression has been reported in whole blood of vitiligo patients, and PD-1 expression on CD8+ T cells is positively correlated with disease activity, suggesting increasing activation (163, 164). PD-L1 inhibits cytokine production and is associated with maintaining peripheral tolerance (165, 166). Miao et al. reported that the injection of a PD-L1 fusion protein into a mouse model of vitiligo led to reduced disease severity and an increased Treg to effector T cell ratio (167). However, PD-1 is also a marker for T cell exhaustion and its upregulation was reported on regulatory T cells in the peripheral blood of vitiligo patients (168). Theoretically, PD-L1 may negatively regulate Tregs similarly to effector T cells, however neutralization of PD-L1 *in vitro* led to an expansion of human Tregs isolated from chronically infected HCV patients (169). Targeting these or other negative stimulatory molecules may limit the autoreactive CD8+ T cell activity, which may allow for Tregs to recover to reinstate tolerance, but further investigation is needed.

There have been recent advancements in Treg-based therapeutics. Tregs appear to actively prevent vitiligo in healthy subjects and may be impaired in those with the disease (170). Many report possible defects in Tregs in vitiligo patients, although they do not necessarily agree whether the defects are in total Treg number, ability to migrate to the skin, or suppressive function (168, 171–176). Expansion of Tregs and adoptive transfer of these cells into patients have promising results in mitigating some autoimmune diseases and transplantation (177, 178). Selective expansion of antigen-specific Tregs reportedly controls inflammation in mouse studies to a greater extent than administration of polyclonal Tregs (179–181). Chimeric antigen receptor (CAR)-Tregs are being developed as treatment options for autoimmunity and GVHD. CAR-Tregs allow for a more stable, antigen-specific Treg population that can be engineered to migrate to the site of inflammation, however more improvements are needed as off-target immunosuppression can result in susceptibility to infections and cancer development (177, 182, 183). Chatterjee et al. reported that adoptively transferred Tregs into a spontaneous vitiligo mouse model reduced disease onset. In addition, treating these mice with rapamycin limited disease progression and increased the number of circulating Tregs (184). Further investigation will be important to optimize these therapies and reduce risks for the treatment of vitiligo.

USING VITILIGO TO UNDERSTAND ORGAN-SPECIFIC AUTOIMMUNITY

Progress made through translational research in vitiligo may facilitate progress in other diseases that are more difficult to study because they are less common and/or because target tissues are less accessible for translational research. Diseases in which CD8+ T cells target a single cell type resulting in loss of that cell type likely share a similar pathogenesis with vitiligo (92). Examples include type 1 diabetes (T1D) within the pancreas, Hashimoto's thyroiditis in the thyroid, Addison's disease in the adrenal cortex, and multiple sclerosis (MS) in the brain. In support of this hypothesis, GWA studies reveal common risk SNPs (92, 185–188). In addition, studies have implicated CD8+ T cells in Hashimoto's thyroiditis, MS, and Addison's Disease, similar to vitiligo (189–192), as well as an important role for the IFN-γ chemokine axis during pathogenesis. IFN-γ and CXCL10 are expressed in human islets to recruit lymphocytes in T1D, autoreactive cells produce IFN-γ in Addison's Disease, and IFN-γ level positively correlates with disease severity in Hashimoto's thyroiditis (193–196). IFN-γ is also involved in MS, although its role is still not fully understood (197). Furthermore, targeting IL-15 in a NOD mouse model reduced disease severity, suggesting IL-15 may play a role in T1D pathogenesis (198). Hence, vitiligo pathogenesis can be used as a guide for these diseases to launch more targeted investigations, as well as repurposing treatments. Narrowing the potential areas for investigation can help accelerate the understanding of pathogenesis for these autoimmune diseases.

CONCLUSION

Vitiligo is an organ-specific autoimmune disease that results in white patchy skin depigmentation. Since the beginning of modern vitiligo research over 40 years ago, translational work has been at the forefront of vitiligo studies because of the simple, non-invasive collection of patient skin samples. These observations have led to clinical studies and a successful clinical trial that are likely to change how we manage patients in the clinic. In addition to sample collection, the discussion, collaboration, and open-minded consideration among researchers have made vitiligo one of the best understood autoimmune diseases. Independent, parallel studies addressing both the degenerative and autoimmune theories of pathogenesis have led to a broader understanding of vitiligo pathogenesis by preventing narrow of experimental focus. This has not only helped our understanding of vitiligo but has also provided a valuable platform to study organ-specific autoimmunity in general. The knowledge gained about vitiligo may be beneficial for those who study similar autoimmune diseases that do not have ready access to tissues for translational studies. Our understanding for vitiligo and other autoimmune disease will improve as more discussions, collaborations, and technology continue to advance. These advancements will be continuously applied toward new therapeutic development to help patients.

AUTHOR CONTRIBUTIONS

Wrote the paper: EK and JEH. All authors contributed to the article and approved the submitted version.

FUNDING

"NIH R33/R61 AR073042", "NIH 5 R01 AR069114", "Hartford Foundation", and "Vitiligo Research Fund supported by many gifts of all sizes".

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.624517/full#supplementary-material

Supplementary Table 1 | Table describing key papers showcasing the inconsistencies within the neuronal hypothesis. Green indicates studies supporting the neuronal hypothesis, while red indicates studies not supporting the neuronal hypothesis.

REFERENCES

1. Frisoli ML, Essien K, Harris JE. Vitiligo: Mechanisms of Pathogenesis and Treatment. *Annu Rev Immunol* (2020) 38. doi: 10.1146/annurev-immunol-100919-023531
2. Lerner AB, Denton CR, Fitzpatrick TB. Clinical and experimental studies with 8-methoxypsoralen in vitiligo. *J Invest Dermatol* (1953) 20(4):299–314. doi: 10.1038/jid.1953.36
3. Harrington AW. Vitiligo: With Notes of Four Cases. *Glasgow Med J* (1918) 90(2):87–90.
4. Burgess N. A Case of Vitiligo with Addison's Disease. *Proc R Soc Med* (1926) 19:38–40. doi: 10.1177/003591572601900348
5. Brito PS. On leucoderm, vitiligo, ven kuttam (tamil) or cabbare (singhalese) and several new methods of treatment. *Br Med J* (1885) 1(2369):824–35. doi: 10.1136/bmj.1.1269.834
6. Falabella R. History and Chronology of Development of Surgical Therapies for Vitiligo. In: *Surgical Management of Vitiligo*. Oxford, UK: Blackwell Publishing Ltd (2007). p. 40–8. doi: 10.1002/9780470987568.ch5
7. Comèl M. Modificazioni delle alterazioni cutanee della vitiligo e della sclerodermia in zone di trapianto cutaneo. *Dermatology* (1948) 96:366–72. doi: 10.1159/000256228
8. Spencer GA, Tolmach JA. Exchange Grafts in Vitiligo. *J Invest Dermatol* (1952) 19:1–5. doi: 10.1038/jid.1952.59
9. Behl P. Treatment of vitiligo with homologous thin Thiersch grafts. *Curr Med Pr* (1964) 8:21.
10. Taïeb A, Morice-Picard F, Jouary T, Ezzedine K, Cario-André M, Gauthier Y. Segmental vitiligo as the possible expression of cutaneous somatic mosaicism: Implications for common non-segmental vitiligo. *Pigment Cell Melanoma Res* (2008) 21(6):646–52. doi: 10.1111/j.1755-148X.2008.00511.x

11. Van Geel N, Speeckaert R, Melsens E, Toelle SP, Speeckaert M, De Schepper S, et al. The distribution pattern of segmental vitiligo: Clues for somatic mosaicism. *Br J Dermatol* (2013) 168(1):56–64. doi: 10.1111/bjd.12013
12. van Geel N, Mollet I, Brochez L, Dutré M, De Schepper S, Verhaeghe E, et al. New insights in segmental vitiligo: case report and review of theories. *Br J Dermatol* (2012) 166:240–6. doi: 10.1111/j.1365-2133.2011.10650.x
13. Westerhof W, d'Ischia M. Vitiligo puzzle: the pieces fall in place. *Pigment Cell Res* (2007) 20(5):345–59. doi: 10.1111/j.1600-0749.2007.00399.x
14. Gauthier Y, Andre MC, Taïeb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? *Pigment Cell Res* (2003) 16 (4):322–32. doi: 10.1034/j.1600-0749.2003.00070.x
15. Speeckaert R, Dugardin J, Lambert J, Lapeere H, Verhaeghe E, Speeckaert MM, et al. Critical appraisal of the oxidative stress pathway in vitiligo: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol* (2018) 32(7):1089–98. doi: 10.1111/jdv.14792
16. Van Geel N, De Lille S, Vandenhaute S, Gauthier Y, Mollet I, Brochez L, et al. Different phenotypes of segmental vitiligo based on a clinical observational study. *J Eur Acad Dermatol Venereol* (2011) 25(6):673–8. doi: 10.1111/j.1468-3083.2010.03847.x
17. Zaima H, Koga M. Clinical course of 44 cases of localized type vitiligo. *J Dermatol* (2002) 29(1):15–19. doi: 10.1111/j.1346-8138.2002.tb00158.x
18. Scholtz JR, Williamson C. Vitiligo in (apparent) dermatomal distribution. *AMA Arch Dermatol Syphilol* (1951) 64(3):366. doi: 10.1001/archderm.1951.01570090113017
19. Lerner AB. Part V: Clinical Applications of Psoralens, and Related Materials: Vitiligo11From the Section of Dermatology, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut. *J Invest Dermatol* (1959) 32:285–308. doi: 10.1038/jid.1959.49
20. Basnet B, Bhushan A, Khan R, Kumar G, Sharma VK, Sharma A, et al. Plasma & urinary catecholamines & urinary vanillylmandelic acid levels in patients with generalized vitiligo. *Indian J Med Res* (2018) 147(4):384. doi: 10.4103/ijmr.IJMR_657_16
21. Morrone A, Picardo M, De Luca C, Terminali O, Passi S, Ippolito F. Catecholamines and Vitiligo. *Pigment Cell Res* (1992) 5(2):65–69. doi: 10.1111/j.1600-0749.1992.tb00003.x
22. Al'abadie MSK, Senior HJ, Bleehen SS, Gawkrodger DJ. Neuropeptide and neuronal marker studies in vitiligo. *Br J Dermatol* (1994) 131(2):160–5. doi: 10.1111/j.1365-2133.1994.tb08486.x
23. Eisenhofer G, Tian H, Holmes C, Matsunaga J, Roffler-Tarlov S, Hearing VJ. Tyrosinase: a developmentally specific major determinant of peripheral dopamine. *FASEB J* (2003) 17:1248–55. doi: 10.1096/fj.02-0736com
24. Orecchia G, Frattini P, Cucchi ML, Santagostino G. Normal-Range Plasma Catecholamines in Patients with Generalized and Acrofacial Vitiligo: Preliminary Report. *Dermatology* (1994) 189:350–3. doi: 10.1159/000246877
25. Nellhaus G. Acquired unilateral vitiligo and poliosis of the head and subacute encephalitis with partial recovery. *Neurology* (1970) 20(10):965. doi: 10.1212/wnl.20.10.965
26. Tremiteria S. Vitiligo from spinal anesthesia. *Rinazcenca Med* (1927) 4:107–8.
27. Chanco Turner ML, Lerner AB. Physiologic Changes In Vitiligo. *Arch Dermatol* (1965) 91(4):390. doi: 10.1001/archderm.1965.01600100106027
28. Sharquie KE, Assaf F. Sweating in vitiligo in relation to electrical skin resistance. *Clin Exp Dermatol* (1985) 10:598–9. doi: 10.1111/j.1365-2230.1985.tb00632.x
29. Gokhale BB, Mehta LN, Damle PS. Skin resistance to electric current and its correlation with sweat gland histology in vitiligo. *Indian J Med Res* (1977) 66 (5):859–64.
30. Elwary SMA, Headley K, Schallreuter KU. Calcium homeostasis influences epidermal sweating in patients with vitiligo. *Br J Dermatol* (1997) 137(1):81–85. doi: 10.1111/j.1365-2133.1997.tb03704.x
31. Kumakiri M, Kimura T, Miura Y, Tagawa Y. Vitiligo with an inflammatory erythema in Vogt-Koyanagi-Harada disease: demonstration of filamentous masses and amyloid deposits. *J Cutan Pathol* (1982) 9(4):258–66. doi: 10.1111/j.1600-0560.1982.tb01061.x
32. Morohashi M, Hashimoto K, Goodman TF, Newton DE, Rist T. Ultrastructural Studies of Vitiligo, Vogt-Koyanagi Syndrome, and Incontinentia Pigmenti Achromians. *Arch Dermatol* (1977) 113(6):755. doi: 10.1001/archderm.1977.01640060051004
33. Gopinathan T. A study of the lesion of vitiligo. *Arch Dermatol* (1965) 91:397–404. doi: 10.1001/archderm.91.4.397
34. Breathnach AS, Bor S, Wyllie LM. Electron microscopy of peripheral nerve terminals and marginal melanocytes in vitiligo. *J Invest Dermatol* (1966) 47 (2):125–40. doi: 10.1038/jid.1966.117
35. Kawauchi T, Chihama K, Nabeshima YI, Hoshino M. Cdk5 phosphorylates and stabilizes p27kip1 contributing to actin organization and cortical neuronal migration. *Nat Cell Biol* (2006) 8(1):17–26. doi: 10.1038/ncb1338
36. Ortonne J-P, Mosher DB, Fitzpatrick TB, Ortonne J-P, Mosher DB, Fitzpatrick TB. Skin Color and the Melanin Pigmentary System. In *Vitiligo and Other Hypomelanoses of Hair and Skin*. Boston, MA: Springer (1983) doi: 10.1007/978-1-4615-9272-3_1
37. Wu CS, Yu HS, Chang HR, Yu CL, Yu CL, Wu BN. Cutaneous blood flow and adrenoceptor response increase in segmental-type vitiligo lesions. *J Dermatol Sci* (2000) 23(1):53–62. doi: 10.1016/S0923-1811(99)00090-0
38. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* (1990) 136(5):1053–68.
39. van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W, Das P. Local Immune Response in Skin of Generalized Vitiligo Patients. *Lab Investig* (2000) 80:1299–309. doi: 10.1038/labinvest.3780138
40. Van Geel NAC, Mollet IG, De Schepper S, Tjin EPM, Vermaelen K, Clark RA, et al. First histopathological and immunophenotypic analysis of early dynamic events in a patient with segmental vitiligo associated with halo nevi. *Pigment Cell Melanoma Res* (2010) 23(3):375–84. doi: 10.1111/j.1755-148X.2010.00703.x
41. Wu CS, Hu SCS, Lan CCE, Chen GS, Chuo WH, Yu HS. Low-energy helium-neon laser therapy induces repigmentation and improves the abnormalities of cutaneous microcirculation in segmental-type vitiligo lesions. *Kaohsiung J Med Sci* (2008) 24(4):180–9. doi: 10.1016/S1607-551X(08)70115-3
42. Puri N, Mojamdar M, Ramaiah A. In Vitro Growth Characteristics of Melanocytes Obtained From Adult Normal and Vitiligo Subjects. *J Invest Dermatol* (1987) 88:434–8. doi: 10.1111/1523-1747.ep12469795
43. Maresca V, Roccella M, Roccella F, Camera E, Del Porto G, Passi S, et al. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. *J Invest Dermatol* (1997) 109(3):310–13. doi: 10.1111/1523-1747.ep12335801
44. Schallreuter KU, Moore J, Wood JM, Beazley WD, Gaze DC, Tobin DJ, et al. In vivo and in vitro evidence for hydrogen peroxide (H2O2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase *J Invest Dermatol Symp Proc* 4(1):91–6. doi: 10.1038/sj.jidsp.5640189
45. Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. *J Invest Dermatol* (1991) 97(6):1081–5. doi: 10.1111/1523-1747.ep12492612
46. Gawkrodger DJ. Pseudocatalase and narrowband ultraviolet B for vitiligo: Clearing the picture. *Br J Dermatol* (2009) 161(4):721–22. doi: 10.1111/j.1365-2133.2009.09292.x
47. Schallreuter KU, Wood JM, Lemke KR, Levenig C. Treatment of vitiligo with a topical application of pseudocatalase and calcium in combination with short-term UVB exposure: A case study on 33 patients. *Dermatology* (1995) 190(3):223–9. doi: 10.1159/000246690
48. Gilhar A, Pillar T, Eidelman S, Etzioni A. Vitiligo and Idiopathic Guttate Hypomelanosis: Repigmentation of Skin Following Engraftment Onto Nude Mice. *Arch Dermatol* (1989) 125(10):1363. doi: 10.1001/archderm.1989.01670220059008
49. Gauthier Y, Cario-Andre M, Lepreux S, Pain C, Taïeb A . Melanocyte detachment after skin friction in non lesional skin of patients with generalized vitiligo. *Br J Dermatol* (2003) 148(1):95–101. doi: 10.1046/j.1365-2133.2003.05024.x
50. Le Poole IC, Van Den Wijngaard RMJGJ, Westerhof W, Das PK. Tenascin is overexpressed in vitiligo lesional skin and inhibits melanocyte adhesion. *Br J Dermatol* (1997) 137(2):171–8. doi: 10.1046/j.1365-2133.1997.18011894.x
51. Wagner RY, Luciani F, Cario-André M, Rubod A, Petit V, Benzekri L, et al. Altered E-cadherin levels and distribution in melanocytes precede clinical

manifestations of vitiligo. *J Invest Dermatol* (2015) 135(7):1810–9. doi: 10.1038/jid.2015.25
52. Bastonini E, Bellei B, Filoni A, Kovacs D, Iacovelli P, Picardo M. Involvement of non-melanocytic skin cells in vitiligo. *Exp Dermatol* (2019) 28(6):667–73. doi: 10.1111/exd.13868
53. Becatti M, Prignano F, Fiorillo C, Pescitelli L, Nassi P, Lotti T, et al. The involvement of Smac/DIABLO, p53, NF-kB, and MAPK pathways in apoptosis of keratinocytes from perilesional vitiligo skin: Protective effects of curcumin and capsaicin. *Antioxid Redox Signal* (2010) 13(9):1309–21. doi: 10.1089/ars.2009.2779
54. Passi S, Grandinetti M, Maggio F, Stancato A, De Luca C. Epidermal Oxidative Stress in Vitiligo. *Pigment Cell Res* (1998) 11(2):81–85. doi: 10.1111/j.1600-0749.1998.tb00714.x
55. Richmond JM, Bangari DS, Essien KI, Currimbhoy SD, Groom JR, Pandya AG, et al. Keratinocyte-Derived Chemokines Orchestrate T-Cell Positioning in the Epidermis during Vitiligo and May Serve as Biomarkers of Disease. *J Invest Dermatol* (2017) 137(2):350–8. doi: 10.1016/j.jid.2016.09.016
56. Boukhedouni N, Martins C, Darrigade AS, Drullion C, Rambert J, Barrault C, et al. Type-1 cytokines regulate MMP-9 production and E-cadherin disruption to promote melanocyte loss in vitiligo. *JCI Insight* (2020) 5(11): e133772. doi: 10.1172/jci.insight.133772
57. Hertz KC, Gazze LA, Kirkpatrick CH, Katz SI. Autoimmune Vitiligo: Detection of Antibodies to Melanin-Producing Cells. *N Engl J Med* (1977) 297(12):634–7. doi: 10.1056/NEJM197709222971204
58. Gilhar A, Zelickson B, Ulman Y, Etzioni A. In vivo destruction of melanocytes by the IgG fraction of serum from patients with vitiligo. *J Invest Dermatol* (1995) 105(5):683–6. doi: 10.1111/1523-1747.ep12324456
59. Kroon MW, Kemp EH, Wind BS, Krebbers G, Bos JD, Gawkrodger DJ, et al. Melanocyte antigen-specific antibodies cannot be used as markers for recent disease activity in patients with vitiligo. *J Eur Acad Dermatol Venereol* (2013) 27(9):1172–75. doi: 10.1111/j.1468-3083.2012.04501.x
60. le Poole IC, van den Wijngaard RMJGJ, Westerhof W, Dutrieux RP, Das PK. Presence or Absence of Melanocytes in Vitiligo Lesions: An Immunohistochemical Investigation. *J Invest Dermatol* (1993) 100:816–22. doi: 10.1111/1523-1747.ep12476645
61. Badri AMTA, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG, Goudie RB. An immunohistological study of cutaneous lymphocytes in vitiligo. *J Pathol* (1993) 170(2):149–55. doi: 10.1002/path.1711700209
62. van den Boorn JG, Konijnenberg D, Dellemijn TAM, Wietze van der Veen JP, Bos JD, Melief CJM, et al. Autoimmune Destruction of Skin Melanocytes by Perilesional T Cells from Vitiligo Patients. *J Invest Dermatol* (2009) 129:2220–32. doi: 10.1038/jid.2009.32
63. Shen C, Gao J, Sheng Y, Dou J, Zhou F, Zheng X, et al. Genetic susceptibility to vitiligo: GWAS approaches for identifying vitiligo susceptibility genes and loci. *Front Genet* (2016) 7(3). doi: 10.3389/fgene.2016.00003
64. Chen GY, Nuñez G. Sterile inflammation: Sensing and reacting to damage. *Nat Rev Immunol* (2010) 10(12):826–837. doi: 10.1038/nri2873
65. Toosi S, Orlow SJ, Manga P. Vitiligo-inducing phenols activate the unfolded protein response in melanocytes resulting in upregulation of IL6 and IL8. *J Invest Dermatol* (2012) 132(11):2601–9. doi: 10.1038/jid.2012.181
66. 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(174):174ra28. doi: 10.1126/scitranslmed.3005127
67. Yu R, Broady R, Huang Y, Wang Y, Yu J, Gao M, et al. Transcriptome Analysis Reveals Markers of Aberrantly Activated Innate Immunity in Vitiligo Lesional and Non-Lesional Skin. *PloS One* (2012) 7(12):e51040. doi: 10.1371/journal.pone.0051040
68. Spritz RA, Andersen GHL. Genetics of Vitiligo. *Dermatol Clin* (2017) 35 (2):245–55. doi: 10.1016/j.det.2016.11.013
69. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res* (2003) 16(3):208–14. doi: 10.1034/j.1600-0749.2003.00032.x
70. Nath SK, Majumder PP, Nordlund JJ. Genetic epidemiology of vitiligo: Multilocus recessivity cross-validated. *Am J Hum Genet* (1994) 55(5):981–90.
71. Kim HJ, Ahn HS, Kazmi SZ, Kang T, Kim HS, Kang MJ, et al. Familial risk of vitiligo among first degree relatives and spouses: a population-based cohort study in Korea. *J Invest Dermatol* (2020). doi: 10.1016/j.jid.2020.07.029
72. Zhang Y, Cai Y, Shi M, Jiang S, Cui S, Wu Y, et al. The Prevalence of Vitiligo: A Meta-Analysis. *PloS One* (2016) 11:e0163806–e0163806. doi: 10.1371/journal.pone.0163806
73. Liu L, Li C, Gao J, Li K, Gao L, Gao T. Genetic polymorphisms of glutathione s-transferase and risk of vitiligo in the chinese population. *J Invest Dermatol* (2009) 129(11):2646–2652. doi: 10.1038/jid.2009.143
74. Laddha NC, Dwivedi M, Gani AR, Shajil EM, Begum R. Involvement of superoxide dismutase isoenzymes and their genetic variants in progression of and higher susceptibility to vitiligo. *Free Radic Biol Med* (2013) 65:1110–25. doi: 10.1016/j.freeradbiomed.2013.08.189
75. Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gómez-Marín C, et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature* (2014) 507(7492):371–5. doi: 10.1038/nature13138
76. Claussnitzer M, Dankel SN, Kim KH, Quon G, Meuleman W, Haugen C, et al. FTO obesity variant circuitry and adipocyte browning in humans. *N Engl J Med* (2015) 373(10):895–907. doi: 10.1056/NEJMoa1502214
77. Rusk N. SPRITE maps the 3D genome. *Nat Methods* (2018) 15(8):572. doi: 10.1038/s41592-018-0092-1
78. Harris JE. Chemical-Induced Vitiligo. *Dermatol Clin* (2017) 35(2):151–61. doi: 10.1016/j.det.2016.11.006
79. Okamura K, Abe Y, Naka I, Ohashi J, Yagami A, Matsunaga K, et al. Genome-wide association study identifies CDH13 as a susceptibility gene for rhododendrol-induced leukoderma. *Pigment Cell Melanoma Res* (2020) 33 (6):826–33. doi: 10.1111/pcmr.12904
80. Wu S, Li WQ, Cho E, Harris JE, Speizer F, Qureshi AA. Use of permanent hair dyes and risk of vitiligo in women. *Pigment Cell Melanoma Res* (2015) 28(6):744–6. doi: 10.1111/pcmr.12402
81. Theveneau E, Mayor R. Neural crest delamination and migration: From epithelium-to-mesenchyme transition to collective cell migration. *Dev Biol* (2012) 366(1):34–54. doi: 10.1016/j.ydbio.2011.12.041
82. Rodrigues M, Ezzedine K, Hamzavi I, Pandya AG, Harris JE. New discoveries in the pathogenesis and classification of vitiligo. *J Am Acad Dermatol* (2017) 77(1):1–13. doi: 10.1016/j.jaad.2016.10.048
83. Oiso N, Tanemura A, Kotobuki Y, Kimura M, Katayama I, Kawada A. Role of macrophage infiltration in successful repigmentation in a new periphery-spreading vitiligo lesion in a male Japanese patient. *J Dermatol* (2013) 40 (11):915–8. doi: 10.1111/1346-8138.12263
84. Dunbar PR, Chen JL, Chao D, Rust N, Teisserenc H, Ogg GS, et al. Cutting edge: rapid cloning of tumor-specific CTL suitable for adoptive immunotherapy of melanoma. *J Immunol* (1999) 162(12):6959–62.
85. Ogg GS, Dunbar PR, Romero P, Chen JL, Cerundolo V. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J Exp Med* (1998) 188(6):1203–8. doi: 10.1084/jem.188.6.1203
86. Shalbaf M, Gibbons NCJ, Wood JM, Maitland DJ, Rokos H, Elwary SM, et al. Presence of epidermal allantoin further supports oxidative stress in vitiligo. *Exp Dermatol* (2008) 17(9):761–70. doi: 10.1111/j.1600-0625.2008.00697.x
87. Boissy RE, Liu YY, Medrano EE, Nordlund JJ. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. *J Invest Dermatol* (1991) 97(3):395–404. doi: 10.1111/1523-1747.ep12480976
88. Koca R, Armutcu F, Altinyazar HC, Gürel A. Oxidant-antioxidant enzymes and lipid peroxidation in generalized vitiligo. *Clin Exp Dermatol* (2004) 29 (4):406–9. doi: 10.1111/j.1365-2230.2004.01524.x
89. Mosenson JA, Zloza A, Klarquist J, Barfuss AJ, Guevara-Patino JA, Le Poole IC. HSP70i is a critical component of the immune response leading to vitiligo. *Pigment Cell Melanoma Res* (2012) 25(1):88–89. doi: 10.1111/j.1755-148X.2011.00916.x
90. Shoab Mansuri M, Singh M. Could ER Stress Be A Major Link Between Oxidative Stress And Autoimmunity In Vitiligo? *J Pigment Disord* (2014) 1 (3). doi: 10.4172/2376-0427.1000123
91. Todd DJ, Lee AH, Glimcher LH. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* (2008) 8(9):663–74. doi: 10.1038/nri2359
92. Harris JE. Cellular stress and innate inflammation in organ-specific autoimmunity: Lessons learned from vitiligo. *Immunol Rev* (2016) 269 (1):11–25. doi: 10.1111/imr.12369

93. Ren Y, Yang S, Xu S, Gao M, Huang W, Gao T, et al. Genetic variation of promoter sequence modulates XBP1 expression and genetic risk for vitiligo. *PloS Genet* (2009) 5(6):e1000523. doi: 10.1371/journal.pgen.1000523
94. Rubio DMG, Schoenbaum EE, Lee LS, Schteingart DE, Marantz PR, Anderson KE, et al. Defining translational research: Implications for training. *Acad Med* (2010) 85(3):470–5. doi: 10.1097/ACM.0b013e3181ccd618
95. Kiistala U, Mustakallio KK. In-Vivo Separation of Epidermis by production of Suction Blisters. *Lancet* (1964) 283(7348):1444–5. doi: 10.1016/S0140-6736(64)92011-2
96. Mustakallio KK, Salo OP, Kiistala R, Kiistala U. Counting the number of Aerobic Bacteria in Full-Thickness Human Epidermis Separated by Suction. *Acta Pathol Microbiol Scand* (1967) 69(3):477–8. doi: 10.1111/j.1699-0463.1967.tb03754.x
97. Ingemansson-Nordqvist B, Kiistala U, Rorsman H. Culture of adult human epidermal cells obtained from roofs of suction blisters. *Acta Derm Venereol* (1967) 47(4):237–40.
98. Mustakallio KK, Kiistala U, Piha HJ, Nieminen E. Epidermal lipids in Besnier's prurigo (atopic eczema). *Ann Med Exp Biol Fenn* (1967) 45:323–5.
99. Kiistala U. Suction blister device for separation of viable epidermis from dermis. *J Invest Dermatol* (1968) 50(2):129–37. doi: 10.1038/jid.1968.15
100. Kiistala U, Mustakallio KK, Rorsman H. Suction blisters in the study of cellular dynamics of inflammation. *Acta Derm Venereol* (1967) 47(3):150–3.
101. Dellatorre G, Bertolini W, de Castro CCS. Optimizing suction blister epidermal graft technique in the surgical treatment of vitiligo. *Bras Dermatol* (2017) 92(6):888–90. doi: 10.1590/abd1806-4841.20176332
102. Caixia T, Hongwen F, Xiran L. Levels of soluble interleukin-2 receptor in the sera and skin tissue fluids of patients with vitiligo. *J Dermatol Sci* (1999) 21(1):59–62. doi: 10.1016/S0923-1811(99)00029-8
103. Özdemir M, Yillar G, Wolf R, Yillar O, Ünal G, Tüzün B, et al. Increased basic fibroblast growth factor levels in serum and blister fluid from patients with vitiligo. *Acta Derm Venereol* (2000) 80(6):438–9. doi: 10.1080/000155500300012918
104. Strassner JP, Rashighi M, Ahmed Refat M, Richmond JM, Harris JE. Suction blistering the lesional skin of vitiligo patients reveals useful biomarkers of disease activity. *J Am Acad Dermatol* (2017) 79(6):847–55.e5. doi: 10.1016/j.jaad.2016.12.021
105. Svoboda M, Hlobilová M, Marešová M, Sochorová M, Kováčik A, Vávrová K, et al. Comparison of suction blistering and tape stripping for analysis of epidermal genes, proteins and lipids. *Arch Dermatol Res* (2017) 309(9):757–65. doi: 10.1007/s00403-017-1776-6
106. Kendall AC, Pilkington SM, Massey KA, Sassano G, Rhodes LE, Nicolaou A. Distribution of bioactive lipid mediators in human skin. *J Invest Dermatol* (2015) 135(6):1510–20. doi: 10.1038/jid.2015.41
107. Wu X, Yang B, Udo-Inyang I, Ji S, Ozog D, Zhou L, et al. Research Techniques Made Simple: Single-Cell RNA Sequencing and its Applications in Dermatology. *J Invest Dermatol* (2018) 138(5):1004–9. doi: 10.1016/j.jid.2018.01.026
108. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: A method for assaying chromatin accessibility genome-wide. *Curr Protoc Mol Biol* (2015) 109(1):21.29.1–21.29.9. doi: 10.1002/0471142727.mb2129s109
109. Buenrostro JD, Wu B, Litzenburger UM, Ruff D, Gonzales ML, Snyder MP, et al. Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature* (2015) 523(7561):486–90. doi: 10.1038/nature14590
110. Li Z, Schulz MH, Look T, Begemann M, Zenke M, Costa IG. Identification of transcription factor binding sites using ATAC-seq. *Genome Biol* (2019) 20(1):45. doi: 10.1186/s13059-019-1642-2
111. Scharer CD, Blalock EL, Barwick BG, Haines RR, Wei C, Sanz I, et al. ATAC-seq on biobanked specimens defines a unique chromatin accessibility structure in naïve SLE B cells. *Sci Rep* (2016) 6(1):27930. doi: 10.1038/srep27030
112. Niedzwiecki MM, Samant P, Walker DI, Tran V, Jones DP, Prausnitz MR, et al. Human Suction Blister Fluid Composition Determined Using High-Resolution Metabolomics. *Anal Chem* (2018) 90(6):3786–3792. doi: 10.1021/acs.analchem.7b04073
113. Burgess DJ. Spatial transcriptomics coming of age. *Nat Rev Genet* (2019) 20(6):317. doi: 10.1038/s41576-019-0129-z
114. Buchberger AR, DeLaney K, Johnson J, Li L. Mass Spectrometry Imaging: A Review of Emerging Advancements and Future Insights. *Anal Chem* (2018) 90(1):240–65. doi: 10.1021/acs.analchem.7b04733
115. Puri N, Mojamdar M, Ramaiah A. Growth defects of melanocytes in culture from vitiligo subjects are spontaneously corrected in vivo in repigmenting subjects and can be partially corrected by the addition of fibroblast-derived growth factors in vitro. *Arch Dermatol Res* (1989) 281(3):178–84. doi: 10.1007/BF00456389
116. Medrano EE, Nordlund JJ. Successful culture of adult human melanocytes obtained from normal and vitiligo donors. *J Invest Dermatol* (1990) 95(4):441–5. doi: 10.5555/uri:pii:0022202X9090223S
117. Barker CL, McHale MT, Gillies AK, Waller J, Pearce DM, Osborne J, et al. The development and characterization of an in vitro model of psoriasis. *J Invest Dermatol* (2004) 123(5):892–901. doi: 10.1111/j.0022-202X.2004.23435.x
118. Jean J, Lapointe M, Soucy J, Pouliot R. Development of an in vitro psoriatic skin model by tissue engineering. *J Dermatol Sci* (2009) 53(1):19–25. doi: 10.1016/j.jdermsci.2008.07.009
119. Duval C, Chagnoleau C, Pouradier F, Sextius P, Condom E, Bernerd F. Human skin model containing melanocytes: essential role of keratinocyte growth factor for constitutive pigmentation-functional response to α-melanocyte stimulating hormone and forskolin. *Tissue Eng Part C Methods* (2012) 18(12):947–57. doi: 10.1089/ten.tec.2011.0676
120. Essien KI, Harris JE. Animal models of vitiligo: Matching the model to the question. *Dermatologica Sin* (2014) 32(4):240–7. doi: 10.1016/j.dsi.2014.09.008
121. Lerner AB, Shiohara T, Boissy RE, Jacobson KA, Lamoreux ML, Moellmann GE. A mouse model for vitiligo. *J Invest Dermatol* (1986) 87(3):299–304. doi: 10.1111/1523-1747.ep12524353
122. Boissy RE, Smyth JR, Fite KV. Progressive cytologic changes during the development of delayed feather amelanosis and associated choroidal defects in the DAM chicken line. A vitiligo model. *Am J Pathol* (1983) 111(2):197–212.
123. Naughton GK, Bystryn JC, Mahaffey M. Antibodies to Surface Antigens of Pigmented Cells in Animals with Vitiligo. *Proc Soc Exp Biol Med* (1986) 181(3):423–6. doi: 10.3181/00379727-181-42275
124. Curik I, Druml T, Seltenhammer M, Sundström E, Pielberg GR, Andersson L, et al. Complex Inheritance of Melanoma and Pigmentation of Coat and Skin in Grey Horses. *PloS Genet* (2013) 9(2):e1003248. doi: 10.1371/journal.pgen.1003248
125. Lentz KJ, Burns RP, Loeffler K, Feeney-Burns L, Berkelhammer J, Hook RR. Uveitis caused by cytotoxic immune response to cutaneous malignant melanoma in swine: Destruction of uveal melanocytes during tumor regression. *Investig Ophthalmol Vis Sci* (1983) 24(8):1063–9.
126. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive CD8+ T-cell accumulation in the skin. *J Invest Dermatol* (2012) 132(7):1869–76. doi: 10.1038/jid.2011.463
127. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci Transl Med* (2014) 6(223):223ra23. doi: 10.1126/scitranslmed.3007811
128. Riding RL, Richmond JM, Harris JE. Mouse Model for Human Vitiligo. *Curr Protoc Immunol* (2019) 124:e63. doi: 10.1002/cpim.63
129. Grimes PE, Ghoneum M, Stockton T, Payne C, Kelly AP, Alfred L. T cell profiles in vitiligo. *J Am Acad Dermatol* (1986) 14(2):196–201. doi: 10.1016/S0190-9622(86)70021-2
130. Bertolotti A, Boniface K, Vergier B, Mossalayi D, Taieb A, Ezzedine K, et al. Type I interferon signature in the initiation of the immune response in vitiligo. *Pigment Cell Melanoma Res* (2014) 27(3):298–407. doi: 10.1111/pcmr.12219
131. Jacquemin C, Martins C, Lucchese F, Thiolat D, Taieb A, Seneschal J, et al. NKG2D Defines a Subset of Skin Effector Memory CD8 T Cells with Proinflammatory Functions in Vitiligo. *J Invest Dermatol* (2020) 140(6):1143–53.e5. doi: 10.1016/j.jid.2019.11.013
132. Riding RL, Richmond JM, Fukuda K, Harris JE. Type I interferon signaling limits viral vector priming of CD8 + T cells during initiation of vitiligo and

melanoma immunotherapy. *Pigment Cell Melanoma Res* (2020) pcmr.12935. doi: 10.1111/pcmr.12935
133. Boniface K, Jacquemin C, Darrigade AS, Dessarthe B, Martins C, Boukhedouni N, et al. Vitiligo Skin Is Imprinted with Resident Memory CD8 T Cells Expressing CXCR3. *J Invest Dermatol* (2018) 138(2):355–64. doi: 10.1016/j.jid.2017.08.038
134. Wang XX, Wang QQ, Wu JQ, Jiang M, Chen L, Zhang CF, et al. Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo. *Br J Dermatol* (2016) 174(6):1318–26. doi: 10.1111/bjd.14416
135. Richmond JM, Masterjohn E, Chu R, Tedstone J, Youd ME, Harris JE. CXCR3 Depleting Antibodies Prevent and Reverse Vitiligo in Mice. *J Invest Dermatol* (2017) 137(4):982–5. doi: 10.1016/j.jid.2016.10.048
136. Gregg RK, Nichols L, Chen Y, Lu B, Engelhard VH. Mechanisms of Spatial and Temporal Development of Autoimmune Vitiligo in Tyrosinase-Specific TCR Transgenic Mice. *J Immunol* (2010) 184(4):1909–17. doi: 10.4049/jimmunol.0902778
137. Liu LY, Strassner JP, Refat MA, Harris JE, King BA. Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. *J Am Acad Dermatol* (2017) 77(4):675–82.e1. doi: 10.1016/j.jaad.2017.05.043
138. Rothstein B, Joshipura D, Saraiya A, Abdat R, Ashkar H, Turkowski Y, et al. Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib. *J Am Acad Dermatol* (2017) 76(6):1054–60.e1. doi: 10.1016/j.jaad.2017.02.049
139. Harris JE, Rashighi M, Nguyen N, Jabbari A, Ulerio G, Clynes R, et al. Rapid skin repigmentation on oral ruxolitinib in a patient with coexistent vitiligo and alopecia areata (AA). *J Am Acad Dermatol* (2016) 74(2):370–1. doi: 10.1016/j.jaad.2015.09.073
140. Craiglow BG, King BA. Tofacitinib citrate for the treatment of Vitiligo a pathogenesis-directed therapy. *JAMA Dermatol* (2015) 151(10):1110. doi: 10.1001/jamadermatol.2015.1520
141. Kim SR, Heaton H, Liu LY, King BA. Rapid repigmentation of vitiligo using tofacitinib plus low-dose, narrowband UV-B phototherapy. *JAMA Dermatol* (2018) 154(3):370. doi: 10.1001/jamadermatol.2017.5778
142. Komnitski M, Komnitski A, Komnitski Junior A, Silva de Castro CC. Partial repigmentation of vitiligo with tofacitinib, without exposure to ultraviolet radiation. *Bras Dermatol* (2020) 95(4):473–6. doi: 10.1016/j.abd.2019.08.032
143. Rosmarin D, Pandya AG, Lebwohl M, Grimes P, Hamzavi I, Gottlieb AB, et al. Ruxolitinib cream for treatment of vitiligo: a randomised, controlled, phase 2 trial. *Lancet* (2020) 396(10244):110–20. doi: 10.1016/S0140-6736(20)30609-7
144. Khemis A, Fontas E, Moulin S, Montaudié H, Lacour JP, Passeron T. Apremilast in Combination with Narrowband UVB in the Treatment of Vitiligo: A 52-Week Monocentric Prospective Randomized Placebo-Controlled Study. *J Invest Dermatol* (2020) 140(8):1533–7.e2. doi: 10.1016/j.jid.2019.11.031
145. Cavalié M, Ezzedine K, Fontas E, Montaudié H, Castela E, Bahadoran P, et al. Maintenance Therapy of Adult Vitiligo with 0.1% Tacrolimus Ointment: A Randomized, Double Blind, Placebo-Controlled Study. *J Invest Dermatol* (2015) 135(4):970–4. doi: 10.1038/jid.2014.527
146. Richmond JM, Strassner JP, Zapata LZ, Garg M, Riding RL, Refat MA, et al. Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo. *Sci Transl Med* (2018) 10(450):eaam7710. doi: 10.1126/scitranslmed.aam7710
147. Cheuk S, Schlums H, Gallais Sérézal I, Martini E, Chiang SC, Marquardt N, et al. CD49a Expression Defines Tissue-Resident CD8+ T Cells Poised for Cytotoxic Function in Human Skin. *Immunity* (2017) 46(2):287–300. doi: 10.1016/j.immuni.2017.01.009
148. Richmond JM, Strassner JP, Rashighi M, Agarwal P, Garg M, Essien KI, et al. Resident Memory and Recirculating Memory T Cells Cooperate to Maintain Disease in a Mouse Model of Vitiligo. *J Invest Dermatol* (2019) 139(4):769–78. doi: 10.1016/j.jid.2018.10.032
149. Azzolino V, Zapata L, Garg M, Gjoni M, Riding RL, Strassner JP, et al. Jak Inhibitors Reverse Vitiligo in Mice but Do Not Deplete Skin Resident Memory T Cells. *J Invest Dermatol* (2020) 141(1):182–4.e1. doi: 10.1016/j.jid.2020.04.027
150. Malik BT, Byrne KT, Vella JL, Zhang P, Shabaneh TB, Steinberg SM, et al. Resident memory T cells in the skin mediate durable immunity to melanoma. *Sci Immunol* (2017) 2(10):eaam6346. doi: 10.1126/sciimmunol.aam6346
151. Chen X, Guo W, Chang Y, Chen J, Kang P, Yi X, et al. Oxidative stress-induced IL-15 trans-presentation in keratinocytes contributes to CD8+ T cells activation via JAK-STAT pathway in vitiligo. *Free Radic Biol Med* (2019) 139:80–91. doi: 10.1016/j.freeradbiomed.2019.05.011
152. Frisoli ML, Harris JE. Vitiligo: Mechanistic insights lead to novel treatments. *J Allergy Clin Immunol* (2017) 140(3):654–62. doi: 10.1016/j.jaci.2017.07.011
153. Richmond JM, Frisoli ML, Harris JE. Innate immune mechanisms in vitiligo: Danger from within. *Curr Opin Immunol* (2013) 25(6):676–82. doi: 10.1016/j.coi.2013.10.010
154. Regazzetti C, Joly F, Marty C, Rivier M, Mehul B, Reiniche P, et al. Transcriptional analysis of vitiligo skin reveals the alteration of WNT pathway: A promising target for repigmenting vitiligo patients. *J Invest Dermatol* (2015) 135(12):3105–14. doi: 10.1038/jid.2015.335
155. Yamada T, Hasegawa S, Inoue Y, Date Y, Yamamoto N, Mizutani H, et al. Wnt/β-Catenin and kit signaling sequentially regulate melanocyte stem cell differentiation in uvb-induced epidermal pigmentation. *J Invest Dermatol* (2013) 133(12):2753–62. doi: 10.1038/jid.2013.235
156. Nelson WJ, Nusse R. Convergence of Wnt, β-Catenin, and Cadherin pathways. *Science (80-)* (2004) 303(5663):1483–7. doi: 10.1126/science.1094291
157. Speeckaert R, van Geel N. Targeting CTLA-4, PD-L1 and IDO to modulate immune responses in vitiligo. *Exp Dermatol* (2017) 26(7):630–4. doi: 10.1111/exd.13069
158. Willemsen M, Melief CJM, Bekkenk MW, Luiten RM. Targeting the PD-1/PD-L1 Axis in Human Vitiligo. *Front Immunol* (2020) 11:579022. doi: 10.3389/fimmu.2020.579022
159. Belum VR, Benhuri B, Postow MA, Hellmann MD, Lesokhin AM, Segal NH, et al. Characterisation and management of dermatologic adverse events to agents targeting the PD-1 receptor. *Eur J Cancer* (2016) 60:12–25. doi: 10.1016/j.ejca.2016.02.010
160. Bertrand A, Kostine M, Barnetche T, Truchetet ME, Schaeverbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: Systematic review and meta-analysis. *BMC Med* (2015) 13(1):211. doi: 10.1186/s12916-015-0455-8
161. Lacouture ME, Wolchok JD, Yosipovitch G, Kähler KC, Busam KJ, Hauschild A. Ipilimumab in patients with cancer and the management of dermatologic adverse events. *J Am Acad Dermatol* (2014) 71(1):161–9. doi: 10.1016/j.jaad.2014.02.035
162. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. *N Engl J Med* (2012) 366(26):2443–54. doi: 10.1056/nejmoa1200690
163. 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. doi: 10.1111/j.1755-148X.2011.00892.x
164. Rahimi A, Hossein-Nataj H, Hajheydari Z, Aryanian Z, Shayannia A, Ajami A, et al. Expression analysis of PD-1 and Tim-3 immune checkpoint receptors in patients with vitiligo; positive association with disease activity. *Exp Dermatol* (2019) 28(6):674–81. doi: 10.1111/exd.13952
165. Gianchecchi E, Fierabracci A. Inhibitory receptors and pathways of lymphocytes: The role of PD-1 in Treg development and their involvement in autoimmunity onset and cancer progression. *Front Immunol* (2018) 9. doi: 10.3389/fimmu.2018.02374
166. Mazanet MM, Hughes CCW. B7-H1 Is Expressed by Human Endothelial Cells and Suppresses T Cell Cytokine Synthesis. *J Immunol* (2002) 169(7):3581–8. doi: 10.4049/jimmunol.169.7.3581
167. Miao X, Xu R, Fan B, Chen J, Li X, Mao W, et al. PD-L1 reverses depigmentation in Pmel-1 vitiligo mice by increasing the abundance of Tregs in the skin. *Sci Rep* (2018) 8(1):1605. doi: 10.1038/s41598-018-19407-w
168. Tembhre MK, Parihar AS, Sharma VK, Sharma A, Chattopadhyay P, Gupta S. Alteration in regulatory T cells and programmed cell death 1-expressing regulatory T cells in active generalized vitiligo and their clinical correlation. *Br J Dermatol* (2015) 172(4):940–50. doi: 10.1111/bjd.13511
169. Franceschini D, Paroli M, Francavilla V, Videtta M, Morrone S, Labbadia G, et al. PD-L1 negatively regulates CD4+CD25+Foxp3+ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. *J Clin Invest* (2009) 119(3):551–64. doi: 10.1172/JCI36604

170. Maeda Y, Nishikawa H, Sugiyama D, Ha D, Hamaguchi M, Saito T, et al. Detection of self-reactive CD8+ T cells with an anergic phenotype in healthy individuals. *Science (80-)* (2014) 346(6216):1536–40. doi: 10.1126/science.aaa1292
171. Klarquist J, Denman CJ, Hernandez C, Wainwright DJ, Strickland FM, Overbeck A, et al. Reduced skin homing by functional Treg in vitiligo. *Pigment Cell Melanoma Res* (2010) 23(2):276–86. doi: 10.1111/j.1755-148X.2010.00688.x
172. Hegab DS, Attia MAS. Decreased circulating T regulatory cells in egyptian patients with nonsegmental vitiligo: Correlation with disease activity. *Dermatol Res Pract* (2015) 2015:1–7. doi: 10.1155/2015/145409
173. 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(4):586–91. doi: 10.1111/pcmr.12105
174. 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(5):e37513. doi: 10.1371/journal.pone.0037513
175. Giri PS, Dwivedi M, Begum R. Decreased suppression of CD8+ and CD4+ T cells by peripheral regulatory T cells in generalized vitiligo due to reduced NFATC1 and FOXP3 proteins. *Exp Dermatol* (2020) 29(8):759–75. doi: 10.1111/exd.14157
176. Giri PS, Dwivedi M, Laddha NC, Begum R, Bharti AH. Altered expression of nuclear factor of activated T cells, forkhead box P3, and immune-suppressive genes in regulatory T cells of generalized vitiligo patients. *Pigment Cell Melanoma Res* (2020) 33(4):566–78. doi: 10.1111/pcmr.12862
177. Li LZ, Zhang Z, Bhoj VG. Conventional T cell therapies pave the way for novel Treg therapeutics. *Cell Immunol* (2020) 359:104234. doi: 10.1016/j.cellimm.2020.104234
178. Dwivedi M, Helen Kemp E, Laddha NC, Mansuri MS, Weetman AP, Begum R. Regulatory T cells in vitiligo: Implications for pathogenesis and therapeutics. *Autoimmun Rev* (2015) 14(1):49–56. doi: 10.1016/j.autrev.2014.10.002
179. Stephens LA, Malpass KH, Anderton SM. Curing CNS autoimmune disease with myelin-reactive Foxp3+ Treg. *Eur J Immunol* (2009) 39(4):1108–17. doi: 10.1002/eji.200839073
180. Golshayan D, Jiang S, Tsang J, Garin MI, Mottet C, Lechler RI. In vitro-expanded donor alloantigen-specific CD4+CD25 + regulatory T cells promote experimental transplantation tolerance. *Blood* (2007) 109(2):827–35. doi: 10.1182/blood-2006-05-025460
181. Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J Exp Med* (2004) 199(11):1467–77. doi: 10.1084/jem.20040180
182. Zhang Q, Lu W, Liang CL, Chen Y, Liu H, Qiu F, et al. Chimeric antigen receptor (CAR) Treg: A promising approach to inducing immunological tolerance. *Front Immunol* (2018) 9. doi: 10.3389/fimmu.2018.02359
183. Mohseni YR, Tung SL, Dudreuilh C, Lechler RI, Fruhwirth GO, Lombardi G. The Future of Regulatory T Cell Therapy: Promises and Challenges of Implementing CAR Technology. *Front Immunol* (2020) 11:1608. doi: 10.3389/fimmu.2020.01608
184. 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(5):1285–94. doi: 10.1038/jid.2013.540
185. Beecham AH, Patsopoulos NA, Xifara DK, Davis MF, Kemppinen A, Cotsapas C, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat Genet* (2013) 45(11):1353–60. doi: 10.1038/ng.2770
186. Falorni A, Brozzetti A, La Torre D, Tortoioli C, Gambelunghe G. Association of genetic polymorphisms and autoimmune Addison's disease. *Expert Rev Clin Immunol* (2008) 4(4):441–56. doi: 10.1586/1744666X.4.4.441
187. Morran MP, Vonberg A, Khadra A, Pietropaolo M. Immunogenetics of type 1 diabetes mellitus. *Mol Aspects Med* (2015) 42:42–60. doi: 10.1016/j.mam.2014.12.004
188. Zaletel K, Gaberscek S. Hashimotos Thyroiditis: From Genes to the Disease. *Curr Genomics* (2011) 12(8):576–88. doi: 10.2174/138920211798120763
189. Luty J, Ruckemann-Dziurdzińska K, Witkowski JM, Bryl E. Immunological aspects of autoimmune thyroid disease – Complex interplay between cells and cytokines. *Cytokine* (2019) 116:128–33. doi: 10.1016/j.cyto.2019.01.003
190. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol* (2015) 14(2):183–93. doi: 10.1016/S1474-4422(14)70256-X
191. Dawoodji A, Chen J-L, Shepherd D, Dalin F, Tarlton A, Alimohammadi M, et al. High Frequency of Cytolytic 21-Hydroxylase–Specific CD8 + T Cells in Autoimmune Addison's Disease Patients. *J Immunol* (2014) 193(5):2118–26. doi: 10.4049/jimmunol.1400056
192. Salou M, Nicol B, Garcia A, Laplaud DA. Involvement of CD8+ T cells in multiple sclerosis. *Front Immunol* (2015) 6. doi: 10.3389/fimmu.2015.00604
193. Weetman AP. Thyroid disease. In: *The Autoimmune Diseases*. Elsevier Academic Press (2020). doi: 10.1016/B978-0-12-812102-3.00040-3
194. Frigerio S, Junt T, Lu B, Gerard C, Zumsteg U, Holländer GA, et al. β cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nat Med* (2002) 8(12):1414–20. doi: 10.1038/nm792
195. Bratland E, Husebye ES. Cellular immunity and immunopathology in autoimmune Addison's disease. *Mol Cell Endocrinol* (2011) 336(1-2):180–90. doi: 10.1016/j.mce.2010.12.015
196. Bossowski A, Harasymczuk J, Moniuszko A, Bossowska A, Hilczer M, Ratomski K. Cytometric evaluation of intracellular IFN-γ and IL-4 levels in thyroid follicular cells from patients with autoimmune thyroid diseases. *Thyroid Res* (2011) 2011(1):13. doi: 10.1186/1756-6614-4-13
197. Arellano G, Ottum PA, Reyes LI, Burgos PI, Naves R. Stage-specific role of interferon-gamma in experimental autoimmune encephalomyelitis and multiple sclerosis. *Front Immunol* (2015) 6. doi: 10.3389/fimmu.2015.00492
198. Bobbala D, Chen XL, Leblanc C, Mayhue M, Stankova J, Tanaka T, et al. Interleukin-15 plays an essential role in the pathogenesis of autoimmune diabetes in the NOD mouse. *Diabetologia* (2012) 55(11):3010–20. doi: 10.1007/s00125-012-2675-1

Conflict of Interest: JEH is a consultant for Pfizer, Genzyme/Sanofi, Aclaris Therapeutics Inc, Incyte, Rheos Medicines, Sun Pharmaceuticals, LEO Pharma, Villaris Therapeutics Inc, Dermavant, Temprian, AbbVie Inc, Janssen, TeVido BioDevices, EMD Serono, Almirall, Boston Pharma, Sonoma Biotherapeutics Inc, Methuselah Health, Twi Biotech, Pandion, Cogen Therapeutics Inc, Admirx, BridgeBio, AnaptysBio, Avita, and Frazier Management. JEH is an investigator for Pfizer, Genzyme/Sanofi, Aclaris Therapeutics Inc, Incyte, Rheos Medicines, Sun Pharmaceuticals, LEO Pharma, Villaris Therapeutics Inc, Dermavant, AbbVie, TeVido BioDevices, EMD Serono, and Pandion. JEH is scientific founder of Villaris Therapeutics, Inc, which develops therapeutic treatments for vitiligo, and NIRA Biosciences. JEH is an inventor on patent #62489191 "Diagnosis and Treatment of Vitiligo" that includes targeting IL-15 and Trm for treatment of vitiligo, as well as #067988 "Anti-Human CXCR3 Antibodies for Treatment of Vitiligo" and #029531 "Compositions and Methods for Treating Vitiligo".

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 05 March 2021
doi: 10.3389/fimmu.2021.613031

Vitiligo Skin Biomarkers Associated With Favorable Therapeutic Response

Qianli Yang[1,2†], Guohong Zhang[3†], Mingwan Su[1], Gigi Leung[1], Harvey Lui[1], Pingyu Zhou[4], Yan Wu[5], Joshua Zhou[6], Jinhua Xu[2], Xuejun Zhang[2,7*] and Youwen Zhou[1*]*

[1] Department of Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada, [2] Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China, [3] Department of Pathology, Shantou University Medical College, Shantou, China, [4] Shanghai Skin Hospital, Tongji University, Shanghai, China, [5] Department of Dermatology, First Hospital, China Medical University, Shenyang, China, [6] Faculty of Dentistry, University of British Columbia, Vancouver, BC, Canada, [7] Institute of Dermatology, Anhui Medical University, Hefei, China

OPEN ACCESS

Edited by:
John E. Harris,
University of Massachusetts Medical School, United States

Reviewed by:
Mehdi Rashighi,
University of Massachusetts Medical School, United States
Stanca Birlea,
University of Colorado, United States

***Correspondence:**
Youwen Zhou
youwen.zhou@ubc.ca
Jinhua Xu
xjhhsyy@163.com
Xuejun Zhang
ayzxj@vip.sina.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: 01 October 2020
Accepted: 08 January 2021
Published: 05 March 2021

Citation:
Yang Q, Zhang G, Su M, Leung G, Lui H, Zhou P, Wu Y, Zhou J, Xu J, Zhang X and Zhou Y (2021) Vitiligo Skin Biomarkers Associated With Favorable Therapeutic Response. Front. Immunol. 12:613031. doi: 10.3389/fimmu.2021.613031

Vitiligo is an acquired depigmentation skin disease caused by immune-mediated death of melanocytes. The most common treatment for vitiligo is narrow band ultraviolet B phototherapy, which often is combined with topical therapies such as tacrolimus. However, patients' responses to these treatments show large variations. To date, the mechanism for this heterogeneity is unknown, and there are no molecular indicators that can predict an individual patient's response to therapy. The goal of this study is to identify clinical parameters and gene expression biomarkers associated with vitiligo response to therapy. Six patients with segmental vitiligo and 30 patients with non-segmental vitiligo underwent transcriptome sequencing of lesional and nonlesional skin at baseline before receiving combined UBUVB and tacrolimus therapy for 6 month, and were separated into good response and bad response groups based on target lesion achieving > 10% repigmentation or not. Our study revealed that treatment-responsive vitiligo lesions had significantly shorter disease duration compared with non-responsive vitiligo lesions (2.5 years vs 11.5 years, p=0.046, t-Test), while showing no significant differences in the age, gender, ethnicity, vitiligo subtype, or disease severity. Transcriptomic analyses identified a panel of 68 genes separating the good response from bad response lesions including upregulation of immune active genes, such as CXCL10, FCRL3, and TCR, Further, compared with vitiligo lesions with long disease duration, the lesions with short duration also have much higher level of expression of immune-active genes, including some (such as FCRL3 and TCR genes) that are associated with favorable therapeutic response. In conclusion, our study has identified clinical parameters such as short disease duration and a panel of immune active and other gene expression biomarkers that are associated with favorable response to immune suppressive NBUVB + tacrolimus therapy. These markers may be useful clinically for individualized therapeutic management of vitiligo patients in the future.

Keywords: vitiligo, RNA sequencing, biomarkers, response to therapy, phototherapy, tacrolimus, therapeutic markers

INTRODUCTION

Vitiligo is an acquired depigmentation skin disorder that affects 0.5%–2% of world's population (1). Patients with vitiligo develop white patches on their skin, including in visible areas such as the face, neck, hands, and forearms. As a result, the quality of life of vitiligo patients can be severely reduced (2–5). Clinically two major types of vitiligo are recognized: segmental vitiligo (SV), which affects a localized asymmetrical area of the body, and non-segmental vitiligo (NSV), the more common subtype, which often involves multiple body sites in a symmetrical fashion.

In addition to loss of melanocytes, vitiligo patients have increased risk of developing other autoimmune diseases, such as diabetes, thyroiditis, rheumatoid arthritis, alopecia areata, lupus erythematosus, and adrenal insufficiency (6, 7). The pathogenesis of vitiligo is unknown, although previous studies identified multiple factors that are potentially involved in the development of vitiligo, including genetic predisposition (such as polymorphisms in genes involved in the immune response and in melanogenesis) (8–14), inducible heat shock protein 70 (15, 16), activation in vitiligo lesions of adaptive immunity [such as CXCL10 (17)] and innate immunity [such as NK cells (18)], environmental factors (such as exposure to certain chemicals (19–21), abnormalities in metabolic and oxidative stress responses (22–24), abnormalities in melanocyte cell adhesion (25), and neurogenic inflammation (9, 10, 26–28) It is unknown if segmental and non-segmental vitiligo involve the same pathogenic mechanisms.

Treatment of vitiligo is challenging. Repigmentation is often incomplete, and requires prolonged therapeutic exposure and careful optimization of treatment protocols (29–31). At present, the most widely available therapy is narrow band ultraviolet B phototherapy (NBUVB) (30, 31), and topical calcineurin inhibitors such as tacrolimus (32, 33), often used in combination (34–41). These therapies are limited in that they are modestly effective (31), require prolonged maintenance, and can be associated with side effects, such as accelerated photo-aging, and the development of photo-lichenoid papules (42). Numerous novel therapies are under investigation, mostly by targeting the immune response regulators, such as JAK inhibitors (43–46), are under investigation.

At present, there are no reliable methods for predicting a given individual patient's treatment outcomes to vitiligo therapies, apart from the well-established clinical observations that facial vitiligo and pediatric patients are more responsive to therapy. Adult vitiligo patients show a high degree of heterogeneity in response to therapy that is not explained by these factors. To date there are no biomarkers shown to predict treatment response to vitiligo therapy.

In the current study, we performed a clinical-transcriptomic correlational analysis to systemically examine the molecular landscape and specific biological pathways of vitiligo skin microenvironment prior to the initiation of NBUVB phototherapy, and evaluate if any clinical features, such as disease subtype and lesional duration, or gene expression features are correlated with a favorable response to combined photo-topical therapy.

Our results revealed that adult vitiligo lesions with recent onset and the vitiligo lesions with biomarkers of active innate and adaptive immune responses respond more favorably to the immune suppressive combined photo-topical therapy. Therefore, vitiligo therapy should be started as early as possible to suppress active innate and adaptive immune activities.

MATERIALS AND METHODS

Vitiligo Patient Clinical Information and Skin Biopsies

With approval from the Clinical Ethics Board of University of British Columbia, 36 vitiligo patients (30 non-segmental vitiligo and 6 segmental vitiligo) attending the outpatient dermatology clinics at the Skin Care Center of Vancouver General Hospital and nine healthy volunteers participated in this study.

The baseline clinical information was collected from each individual, including demographics, general medical, and medication history, vitiligo specific information such as onset, duration, extent of skin involvement, anatomical distribution, previous therapies and associated responses. A target lesion at least 2 cm in diameter was selected from a non-cosmetically sensitive area of the skin such as the torso or the proximal extremities and photographed. With informed consent, two 4 mm punch full-thickness biopsies were performed, one from the lesional skin 1 cm inside border of the target lesion (lesional skin, or LS), the other, nonlesional skin 1 cm outside of the lesional border (nonlesional skin, or NLS). The biopsies were bisected, with one portion placed immediately in RNA Later solution (Life Labs) and stored at -20° until further use. The other portion was placed in formalin for histological assessment. One subject's lesional skin biopsy did not contain significant reduction of melanocytes, thus the diagnosis of vitiligo was not confirmed, and was excluded from the rest of the analyses. For the nine healthy volunteers, a single 4 mm punch biopsy was performed from the torso or the proximal extremities.

After the biopsies were obtained, the vitiligo patients received NBUVB-tacrolimus combination therapy that included NBUVB phototherapy three times per week according to the protocol previous reported (31, 42) and topical application of tacrolimus 0.1% ointment twice daily on non-phototherapy days. The target lesions were assessed every three months for the percentage of re-pigmentation in comparison to the baseline photographs until the end of 6th month. Ten percent (10%) re-pigmentation was used as the criteria separating the responsive from non-responsive groups. This was chosen to maximizing the statistical power of the study as it roughly separated the vitiligo patients undergoing treatment to two groups with similar sizes.

RNA Extraction and Transcriptome Sequencing

Bulk RNA was extracted from each skin biopsy using the RNeasy® Fibrous Tissue Mini Kit as we had described previously (18), and used for transcription sequencing using the Novo Gene Ilumina platform (HiSeq PE150, Tianjin China),

TABLE 1 | Clinical Information of vitiligo patients.

ID #	Sub-type	Sex	Ethnic Origin	Age at Onset (yr)	Age at Biopsy (yr)	Vitiligo Duration (yr)	Vitiligo Severity (BSA %)	Location of Biopsied Lesion	Responsive to therapy?
Vit 01	NSV	F	SA	30	31.5	1.5	5	Arm	~
Vit 02	NSV	F	SA	30	31.5	1.5	5	Arm	~
Vit 05	NSV	F	SA	26	30	4	2	Arm	no
Vit 12	NSV	M	Cau	70	72	2	2	Hand	no
Vit 13	NSV	M	SA	54	54.5	0.5	5	Abdo	yes
Vit 14	NSV	M	Chi	10	17	7	11	Leg	no
Vit 20	NSV	F	SA	35	39	4	10	Abdo	no
Vit 21	NSV	M	Chi	28	38	10	30	Arm	no
Vit 22	NSV	M	Chi	19	69	50	20	Back	no
Vit 27	NSV	M	Cau	35	55	20	10	Torso	~
Vit 28	NSV	M	Cau	43	43.75	0.75	5	Torso	~
Vit 29	NSV	F	Cau	14	14.2	0.2	10	Arm	yes
Vit 31	SV	F	SA	57	58	1	3	Neck	~
Vit 33	NSV	F	Cau	23	33	10	10	Knee	no
Vit 34	NSV	M	SA	8	8.5	0.5	22	Abdo	yes
Vit 35	NSV	M	SA	30	70	40	10	Back	no
Vit 36	SV	M	Chi	51	51.6	0.6	2	Neck	yes
Vit 37	NSV	F	SA	55	56	1	15	Torso	no
Vit 39	NSV	M	Cau	64	65	1	7	Neck	yes
Vit 40	NSV	F	Chi	71	71.4	0.4	2	Abdo	yes
Vit 42	NSV	M	Chi	36	59	23	30	Hand	yes
Vit 43	SV	F	Cau	36	36.4	0.4	2.5	Face	yes
Vit 44	SV	F	SA	47	48	1	3	Neck	no
Vit 45	SV	M	Chi	26	26.5	0.5	5	Buttock	yes
Vit 47	NSV	F	Cau	17	32	15	5	Axilla	~
Vit 48	NSV	M	Cau	12	27	15	10	Back	~
Vit 49	NSV	F	Cau	63	75	12	3	Arm	no
Vit 50	NSV	F	Cau	58	59	1	6	Back	~
Vit 51	NSV	F	Chi	36	46	10	5	Back	no
Vit 54	NSV	M	Cau	43	53	10	5	Back	no

(Continued)

TABLE 1 | Continued

ID #	Sub-type	Sex	Ethnic Origin	Age at Onset (yr)	Age at Biopsy (yr)	Vitiligo Duration (yr)	Vitiligo Severity (BSA %)	Location of Biopsied Lesion	Responsive to therapy?
Vit 55	NSV	F	Kor	28	28.5	0.5	20	Flank	yes
Vit 56	NSV	F	SA	40	40.2	0.2	6	Shoulder	yes
Vit 57	NSV	M	Cau	50	60	10	8	Arm	~
Vit 58	SV	F	Cau	63	63.3	0.3	3	Neck	~
Vit 59	NSV	M	Chi	15	25	10	3	Torso	no
Vit 61	SV	F	Chi	15	15.75	0.75	1	Neck	~

SV, segmental vitiligo; NSV, nonsegmental vitiligo; Cau, Caucasians; Chi, Chinese, Kor, Korean; SA, South Asian; BSA, Body surface area; ~ not available.

generating at least 30 million clean reads for each sample. The abundance of each transcript is normalized to the total number of transcripts and the length of the transcripts and expressed in FPKM. The averages of the depigmented biopsies (LS), nonlesional biopsies (NLS), and control healthy normal skin (HNS) were calculated for all of the coding genes of the human genome. All of the genes showing more than 2 fold differences (up or down regulated) between the LS and NHS ($p<0.05$ two tailed t test) were identified (723 genes in total) (See **Supplemental Table 1**). This table also shows the details of expression of these genes between NLS and HNS, and between LS and NLS (ratios and p values).

The R, IPA, Reactome PA, and SPSS packages were used for datamining and statistical analyses.

RESULT

Vitiligo Patient Characteristics and Therapeutic Response

The demographics and vitiligo-specific parameters are summarized in **Table 1**. There was a slight female dominance (1.12 F: M ratio), with an average age of 44.5 years (range 14.2 to 72 years) at time of assessment, and 37.2 years at time of vitiligo onset (range 14 to 71 years). The average vitiligo severity is 8.4% BSA (body surface area, range 1% to 30%). Thirty patient had non-segmental vitiligo whereas six patients had segmental vitiligo. There were 14 Caucasians and 22 Asians (including 11 South Asians and 11 East Asians).

Of the 36 vitiligo patients, 11 patients did not undergo treatment, and were not included in the therapeutic response analysis. The remaining 25 patients underwent NBUVB-tacrolimus combination therapy. The NBUVB phototherapy was administered according to protocols previously reported by Hemzavi et al. (31). On the days when not on NBUVB phototherapy, the patients applied topical tacrolimus 0.1% ointment BID. The repigmentation of the target lesions was assessed at three-month intervals, with 11 achieving more than 10% repigmentation at 6 months compared with photographs taken at the baseline.

The 11 responders and the 14 non-responders (achieving less than 10% repigmentation at 6 months) did not show any significant difference in sex (female 54.5% vs 53.3%), ethnicity (Caucasian/Asian ratio 0.38 vs 0.36), age at onset (38.9 yrs vs 35 yrs), age at biopsy (41.4 vs 46.4), vitiligo severity at baseline (10.1% BSA vs 8.6% BSA), vitiligo subtype composition (SV/NSG ratio 3/8 vs 1/13) (Chi-square test, $p>0.05$), or anatomical location of the target lesions (acral in 1/10 patients vs 1/13 patients)(Chi-square test, $p>0.05$). However, there was a significant difference in the lesional duration between these two groups. The responding lesions had an average duration of 2.5 years (range 0.2 to 23 years) whereas the average duration of non-responding target lesions was 8.6 years (range: 1 to 40 years) (two tailed t test, $p=0.047$) (**Table 2**).

Transcriptomic Features of Lesional and Non-Lesional Vitiligo Compared with Healthy Normal Skin

Complete transcriptome analysis revealed 19,041 coding transcripts with detectable expression in at least one of the

TABLE 2 | Correlational Analysis of Response to NBUVB-Tacrolimus Therapy.

Parameters	Favorable Response to Therapy	Non-Favorable Response to Therapy	Response Not assessed	p
Sex (% males)	54.5%	53.30%	36.4%	ns
Ethnicity (Caucasians vs Asians)	3 vs 8	4 vs 10	7 vs 4	ns
Age at biopsy	41.4	46.4	43.3	ns
Age at onset	38.9	35.0	37.3	ns
Duration of lesion	2.5	11.4	6.1	0.047
Vitiligo severity (%BSA)	10.1	8.6	5.5	ns
Segmental vs Non-segmental ratio	3 vs 8	1 vs 13	3 vs 8	ns
Acral vs non-acral	1 vs 10	1 vs 13	0 vs 11	ns

BSA, body surface area; ns, not significant.

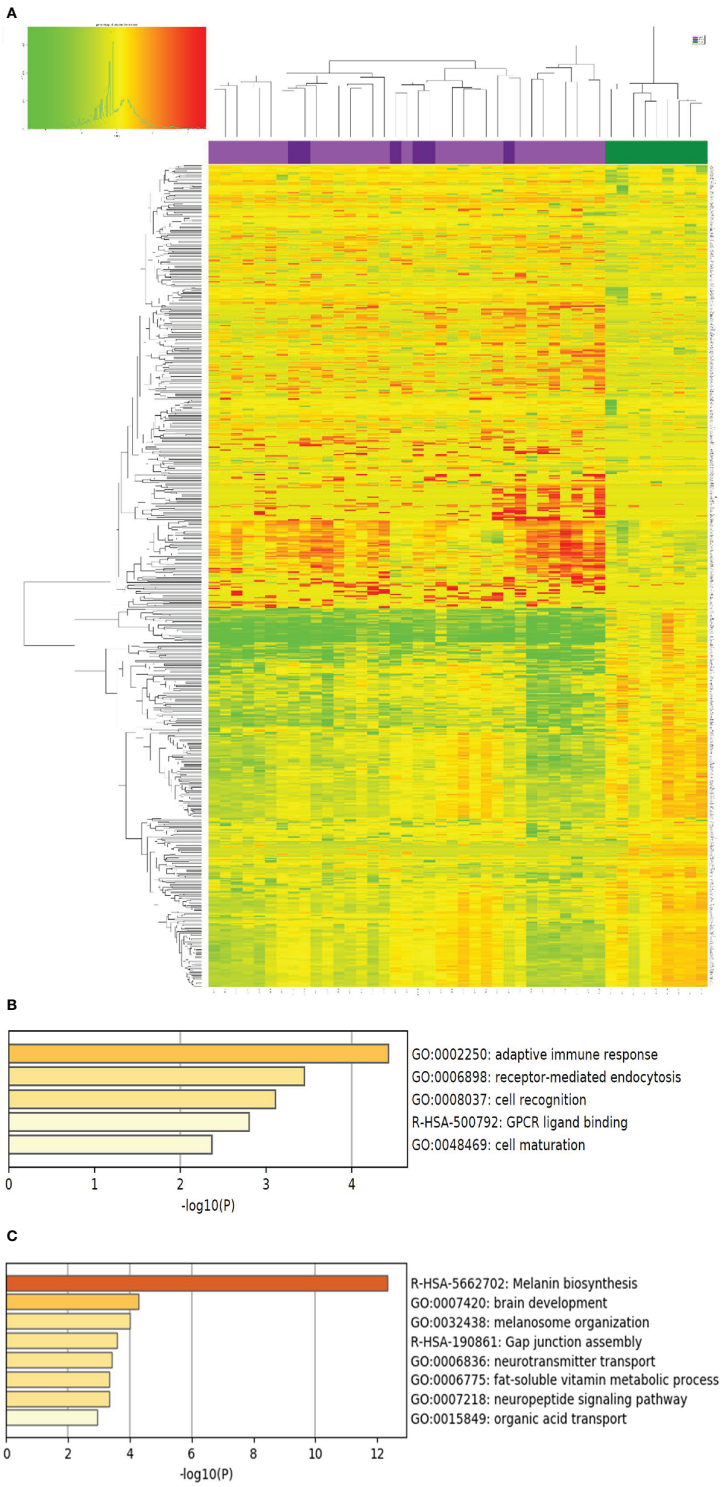


FIGURE 1 | Genes with differential expression between vitiligo lesional skin and normal health skin. **(A)** The expression of the 723 genes with significant differential expression (> 2 fold change, p<0.05) compared with normal healthy skin (NHS, shown in green on the top panel, N=9), lesional skin of segmental vitiligo (shown in dark purple, N=6), and non-segmental vitiligo (bright purple, N=30) is presented as a heat map with non-supervised clustering analysis. Red and green color designate up or down regulation, respectively, of the gene in that particular sample compared to the average of healthy normal skin. The complete list of these genes are included in **Supplemental Table 1**. **(B, C)** Biological pathways represented by genes up and down regulated in LS over healthy normal skin, respectively. The p values were obtained by Metascape analysis.

skin biopsies. Compared with normal healthy skin, there were 392 and 331 genes with increased and decreased expression in vitiligo lesional skin, respectively (>2 fold change, average level of each group, p<0.05, two tailed t test) (See **Supplemental Table 1** for details). The upregulated genes in vitiligo lesional skin include markers of inflammation (such as MARCO, NLRP10, PTGS2, and IL36G), oxidative response genes (DUOXA2), and NK cell receptors (NCR3LG1), revealing presence of activated immune response in vitiligo lesions.

The down regulated genes include those involved in melanin synthesis and melanocyte development (TYRP1, PMEL, DCT, TYR, MLANA, and MC5R), neural crest cell development (PLP1, SYN2) and lipid and surfactant metabolism (ACOT1, ACOT2, ACOT4, ACOX2, ACSBG1, ACSM3, ACSM6, PCSK2, and SFPTC), consistent with death of melanocytes, and disturbance of lipid metabolism in vitiligo lesions.

Some of the differentially expressed genes in the lesional skin were also differentially expressed in the nonlesional skin albeit to a lesser degree (**Supplemental Table 1**).

SV and NSV Skin Lesions Are Indistinguishable at the Transcriptome Level

As shown in **Figure 1**, the lesional skin of SV and NSV were not separable at the transcriptome level, suggesting that despite the stark contrast in the lesional distribution, SV and NSG are similar at the molecular level based on this small sample size comparison involving 6 SV patients and 30 NSV patients.

Melanocyte-Signature Genes Show No Significant Association With Therapeutic Response or Vitiligo Duration

Given that recent research showed that preservation of perifollicular pigmentation under dermatoscope (47), we evaluated if there were more residual melanocytic biomarkers in lesions of good therapeutic response or short duration. As shown in **Figure 2**, all of the melanocyte marker genes showed a trend of higher residual level of expression in good response and short duration lesions, the degree of higher expression did not reach the level of statistical significance (p>0.05, two tailed t test), suggesting the level of residual melanocytic marker genes cannot be reliably used to predict therapeutic response.

Biomarkers of Vitiligo Therapeutic Response to NBUVB-Tacrolimus Combination Therapy

We then compared the transcriptomes of the responsive and non-response vitiligo patients, and identified 68 genes with >2 fold differential expression (average level of each group, p<0.05, two tailed t test) between these two groups (**Figure 3**). Sixteen of

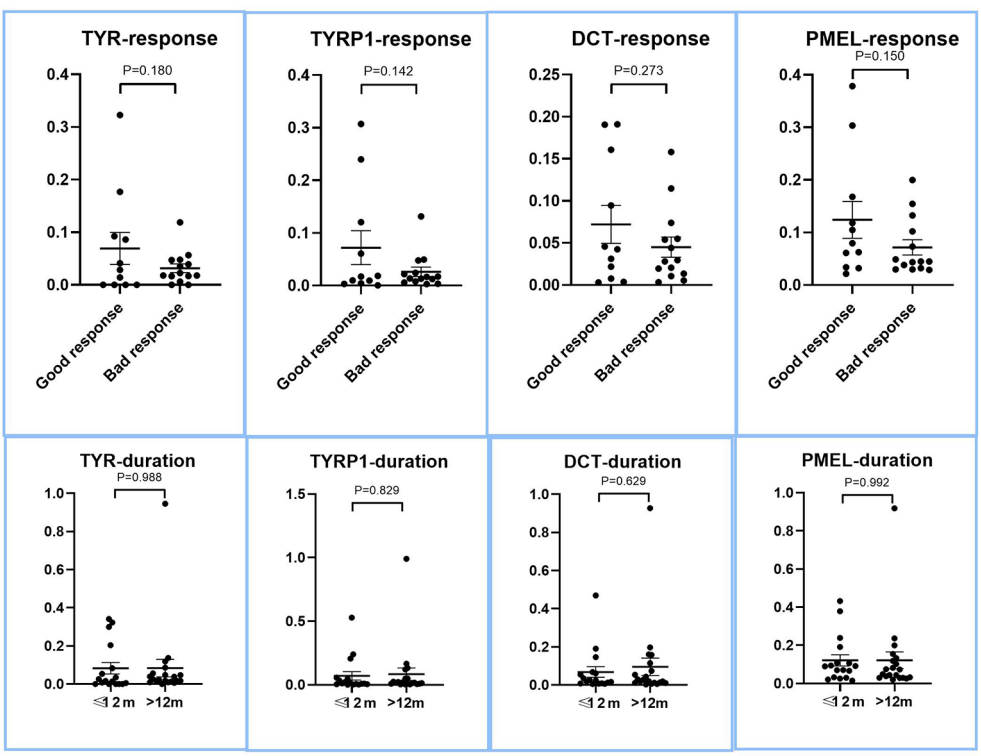


FIGURE 2 | Expression of melanocyte marker genes in lesions with good and bad response to therapy, and between long and short duration lesions. The LS/NLS expression ratios of major melanocyte marker genes, DCT, TYR, TYRP1, and PMEL of good and bad response groups are presented as dot plots. There was no significant relationship between the expression levels of these genes and therapeutic response (p > 0.05).

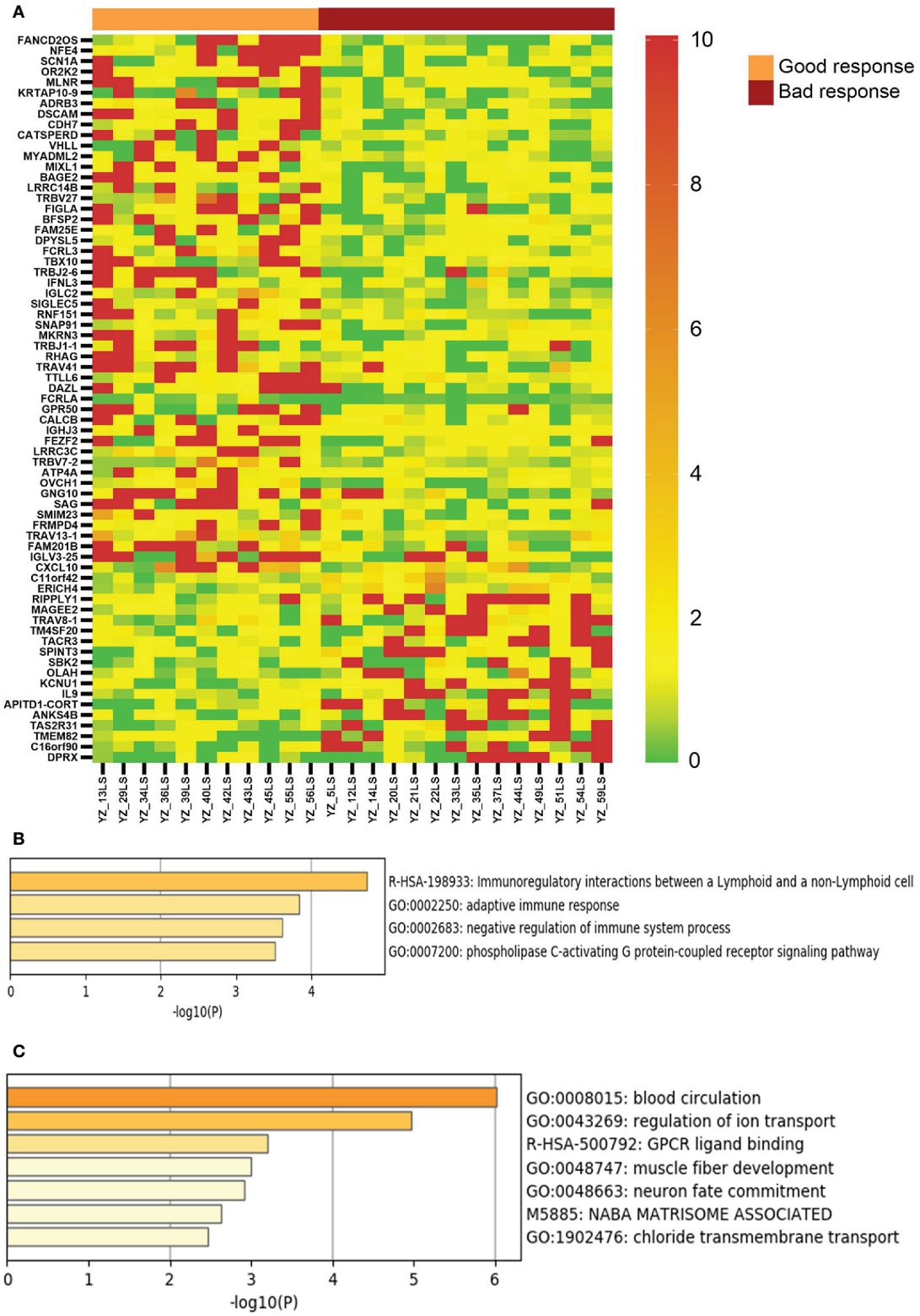


FIGURE 3 | Genes with differential expression between vitiligo lesions with favorable response and lesions with non-favorable response to therapy. **(A)** LS/NLS ratios of 69 genes showing >2 fold difference between good response and bac response lesions ($p < 0.05$); **(B, C)** Pathways represented by markers of good and bad response, respectively.

the 50 up-regulated genes in good response lesions are involved in regulation of immune response (such as CXCL10, FCRL3, and T cell receptor genes). These 68 genes were able to completely separate the good response lesions from the bad response lesions, demonstrating the potential of them being used for therapeutic response prediction in the future.

Biomarkers of Vitiligo Disease Duration

Given that vitiligo lesions with shorter duration have better response to NBUVB-tacrolimus therapy, we examined the genes showing significant differential expression between duration less than 12 months and duration longer that 12 months. As can be seen in **Figure 4**, vitiligo lesions with shorter duration express significantly higher levels of genes in the regulation of adaptive and cellular immunity, including some of the genes associated with good therapeutic response such as CXCL10, FCRL3, and T cell receptor genes.

DISCUSSION

At present, several clinical parameters have been associated with more favorable response to vitiligo repigmentation therapies, including pediatric age, facial location, short disease duration, and absence of leukotrichia (poliosis) (48, 49). Since our study focused on patients with target lesions without leukotrichia that are mostly from torso and proximal extremities, our study was not designed to evaluate the impact of location on facial or acral sites, or leukotrichia on therapeutic responses. Only three of our patients were of pediatric age (two from the good response group and one from bad response group), therefore, our study was not designed to yield information on the impact of pediatric age on therapeutic response. We performed additional analyses excluding these three patients (data not shown), the results and the conclusions did not change.

Further, since we did not evaluate glabrous vitiligo lesions, this study could not generate information on the impact of hair follicles on therapeutic response. Our study did confirm that shorter duration is associated with better therapeutic response to NBUVB-tacrolimus combinations therapy. However, since we did not collect information on lesional stability art baseline, we could not comment on if the short lesional disease duration was correlated with disease activities such as lesional progression or regression.

The relationship between lesional skin pigmentation and the patient's therapeutic response to vitiligo therapy has been unclear as the existing literature provided conflicting conclusions. On the one hand, vitiligo lesions with peri-follicular pigmentation under dermatoscope is associated with more favorable response (47). On the other, the degree of depigmentation in general did not show correlation to NBUVB therapy (50). We systematically evaluated the level of residual melanocyte-signature markers such as DCT, TYR, TYRP1, and MLANA in lesions of good and bad therapeutic response. Although there was a strong trend that good response lesions tend to have higher levels of melanocyte-signature gene expression, there was a wide range of variation, making the difference non-significant statistically. This suggests that melanocyte-signature genes are not robust markers of therapeutic response.

Our study attempted to take a novel approach to identify parameters associated with therapeutic response by performing transcriptional sequencing of vitiligo patients' lesional and non-lesional skin, and identified a panel of 68 genes that could completely differentiate the vitiligo lesions with good response to therapy from those with bad response. It is of interest that these response biomarker genes include many with functions in immune activation, including CXCL10, which had been shown to play critical roles in the development of vitiligo (17). This discovery showed that there are still residual subclinical immune activity present in established skin lesions and that these lesions are more responsive to NBUVB and tacrolimus therapy, which are known to be immune suppressive in nature.

Previous studies showed that short disease duration is associated with better response to vitiligo therapy, which was confirmed in our study (**Table 2**). Further, our results demonstrated an inverse relationship between vitiligo lesional duration and the level of persistent immune response, including some of the immune biomarkers associated with good therapeutic response such as CXCL10, FCRL3, and T cell receptor genes.

Taken together, our study showed that in order to maximize the chances of therapeutic success for patients with vitiligo, one needs to initiate immune suppressive therapies, such as NBUVB and/or tacrolimus therapy as early as possible.

The current study has several limitations. First, the sample size is relatively small, with transcriptomic analysis involving 36 patients (only six with segmental vitiligo) and therapeutic response evaluable in 25 patients, limiting the statistical power of our study. It is possible that a larger sample sized study may uncover additional biomarkers that have not been detected in the current study. Another limitation is that we did not examine the biomarkers of the lesional borders, where inflammatory and immune activities are the strongest. Future studies will be needed to address this issue.

It should be pointed out that our study evaluated only a form of immune-suppressive therapy, NBUVB combined with tacrolimus, and did not directly address the therapeutic response prediction of surgical treatments of vitiligo lesions, which have been reported to have higher chances of success for lesions that are stable over time with no signs of recent lesional progression. We speculate that the favorable response biomarkers of immune suppressive therapy discovered in this study (high expression of immune response genes) would be biomarkers of non-favorable response to surgical therapies of vitiligo.

In addition, since this study was designed to evaluate skin microenvironment of well-established vitiligo lesions, the lesional and non-lesional skin biopsies were obtained at approximately 1 cm inside or outside the lesional borders, this selection criteria excluded vitiligo lesions smaller than 2 cm in sizes from being included in this study.

Further, our study was focused on the assessment of target vitiligo lesions' response to therapy, we could not answer the

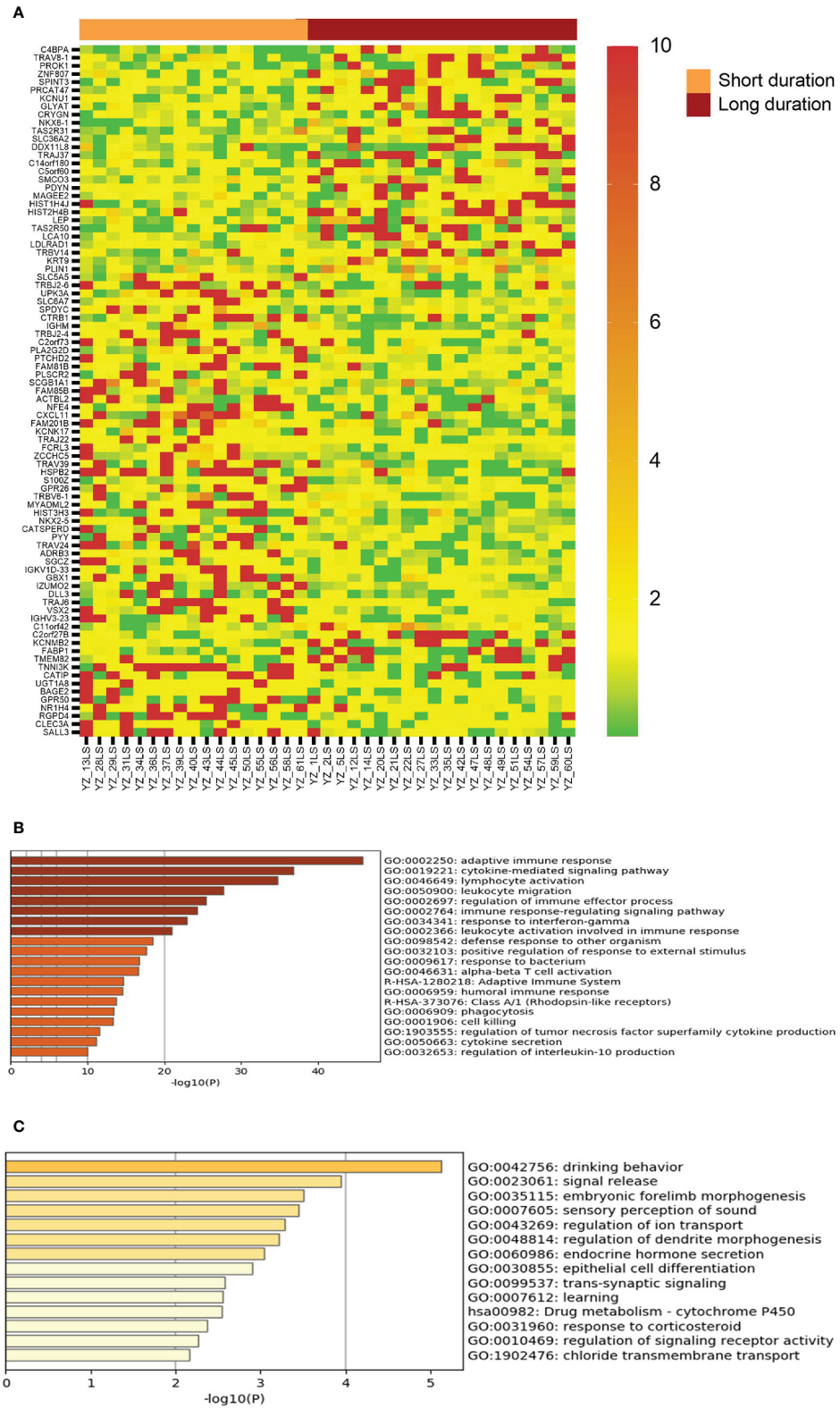


FIGURE 4 | Genes with differential expression between lesions with short duration and lesions with long duration. **(A)** The LS/NLS ratios of 85 genes with >2 fold up or down regulated between lesions of <12 month duration and lesions >12 month duration. **(B, C)** Top biological genes enriched or decreased in short and long duration lesions, respectively.

question if biomarkers obtained by sampling one lesion would have predictive power for the un-sampled lesions of the vitiligo patients, which often have multiple lesions. Further studies are needed to address this question.

Our study involves obtaining full-thickness biopsies of the skin lesions, which makes it impractical for routine testing in the clinic. However, this shortcoming could be easily overcome in the future by modification of the sampling technique, capitalizing on the technical advance of using non-invasive techniques such as tape-stripping to quantify biomarkers of the skin (51–53).

Finally, this study also uncovered previously unknown gene expression changes in the skin of patients with vitiligo, including dysregulation of genes involved in lipid metabolism (such as SFPC and PCSK2). These indicate that the pathophysiology of vitiligo is more complex than what have been reported at present, such as loss of melanocytes. The functional and clinical significance remains to be further elucidated in the future.

In summary, this study showed that short disease duration is associated with better therapeutic response, and that higher expression of genes regulating innate and adaptive immune response are factors predictive of more favorable response to vitiligo therapies that are immune suppressive in nature. Therefore, early therapeutic initiation and suppressing persistent immune activities of vitiligo skin lesions are required to restore melanocytes in vitiligo.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Ethics Board of University of British Columbia. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

QY: Statistical analyses, statistical mapping, and drafting of the manuscript. GZ and JZ: Bioinformatics and statistical analyses. MS and GL: Biopsy collecting and RNA extracting. HL: Conceptual development of the project and therapeutic response data. PZ and JX: Bioinformatics and statistical analyses and critical revision of the manuscript. YW: Statistical analyses. XZ: RNA sequencing and funding. YZ: Patients enrolling, demographical information and clinical parameters, development of the project and therapeutic response data, drafting and revision of the manuscript, and funding. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

This study was funded in part by grants from Canadian Institutes of Health Research, Canadian Dermatology Foundation, National Natural Sciences Foundation of China, and Canadian Melanoma Foundation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.613031/full#supplementary-material

REFERENCES

1. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. *J Am Acad Dermatol* (2011) 65(3):473–91. doi: 10.1016/j.jaad.2010.11.061
2. Linthorst Homan MW, Sprangers MA, de Korte J, Bos JD, van der Veen JP. Characteristics of patients with universal vitiligo and health-related quality of life. *Arch Dermatol* (2008) 144(8):1062–4. doi: 10.1001/archderm.144.8.1062
3. Linthorst Homan MW, Spuls PI, de Korte J, Bos JD, Sprangers MA, van der Veen JP. The burden of vitiligo: patient characteristics associated with quality of life. *J Am Acad Dermatol* (2009) 61(3):411–20. doi: 10.1016/j.jaad.2009.03.022
4. Mattoo SK, Handa S, Kaur I, Gupta N, Malhotra R. Psychiatric morbidity in vitiligo and psoriasis: a comparative study from India. *J Dermatol* (2001) 28 (8):424–32. doi: 10.1111/j.1346-8138.2001.tb00004.x
5. Ongenae K, Beelaert L, van Geel N, Naeyaert JM. Psychosocial effects of vitiligo. *J Eur Acad Dermatol Venereol* (2006) 20(1):1–8. doi: 10.1111/j.1468-3083.2005.01369.x
6. Gill L, Zarbo A, Isedeh P, Jacobsen G, Lim HW, Hamzavi I. Comorbid autoimmune diseases in patients with vitiligo: A cross-sectional study. *J Am Acad Dermatol* (2016) 74(2):295–302. doi: 10.1016/j.jaad.2015.08.063
7. Sheth VM, Guo Y, Qureshi AA. Comorbidities associated with vitiligo: a ten-year retrospective study. *Dermatology* (2013) 227(4):311–5. doi: 10.1159/000354607
8. Yang C, Wu J, Zhang X, Wen L, Sun J, Cheng Y, et al. Fine-mapping analysis of the MHC region for vitiligo based on a new Han-MHC reference panel. *Gene* (2018) 648:76–81. doi: 10.1016/j.gene.2018.01.053
9. Tang XF, Zhang Z, Hu DY, Xu AE, Zhou HS, Sun LD, et al. Association analyses identify three susceptibility Loci for vitiligo in the Chinese Han population. *J Invest Dermatol* (2013) 133(2):403–10. doi: 10.1038/jid.2012.320
10. Quan C, Ren YQ, Xiang LH, Sun LD, Xu AE, Gao XH, et al. Genome-wide association study for vitiligo identifies susceptibility loci at 6q27 and the MHC. *Nat Genet* (2010) 42(7):614–8. doi: 10.1038/ng.603
11. Xu S, Zhou Y, Yang S, Ren Y, Zhang C, Quan C, et al. Platelet-derived growth factor receptor alpha gene mutations in vitiligo vulgaris. *Acta Derm Venereol* (2010) 90(2):131–5. doi: 10.2340/00015555-0820
12. Spritz RA, Santorico SA. The Genetic Basis of Vitiligo. *J Invest Dermatol* (2020) 141(2):265–73. doi: 10.1016/j.jid.2020.06.004

13. Jin Y, Birlea SA, Fain PR, Gowan K, Riccardi SL, Holland PJ, et al. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. *N Engl J Med* (2010) 362(18):1686–97. doi: 10.1056/NEJMoa0908547
14. Jin Y, Mailloux CM, Gowan K, Riccardi SL, LaBerge G, Bennett DC, et al. NALP1 in vitiligo-associated multiple autoimmune disease. *N Engl J Med* (2007) 356(12):1216–25. doi: 10.1056/NEJMoa061592
15. Henning SW, Jaishankar D, Barse LW, Dellacecca ER, Lancki N, Webb K, et al. The relationship between stress and vitiligo: Evaluating perceived stress and electronic medical record data. *PloS One* (2020) 15(1):e0227909. doi: 10.1371/journal.pone.0227909
16. 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 (9):566–9. doi: 10.1111/exd.12183
17. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci Transl Med* (2014) 6(223):223ra23. doi: 10.1126/scitranslmed.3007811
18. Yu R, Broady R, Huang Y, Wang Y, Yu J, Gao M, et al. Transcriptome analysis reveals markers of aberrantly activated innate immunity in vitiligo lesional and non-lesional skin. *PloS One* (2012) 7(12):e51040. doi: 10.1371/journal.pone.0051040
19. Harris JE. Chemical-Induced Vitiligo. *Dermatol Clin* (2017) 35(2):151–61. doi: 10.1016/j.det.2016.11.006
20. Vine K, Meulener M, Shieh S, Silverberg NB. Vitiliginous lesions induced by amyl nitrite exposure. *Cutis* (2013) 91(3):129–36.
21. Vrijman C, Hosseinpour D, Bakker JG, Wolkerstorfer A, Bos JD, van der Veen JP, et al. Provoking factors, including chemicals, in Dutch patients with vitiligo. *Br J Dermatol* (2013) 168(5):1003–11. doi: 10.1111/bjd.12162
22. Chen S, Wang X, Nisar MF, Lin M, Zhong JL. Heme Oxygenases: Cellular Multifunctional and Protective Molecules against UV-Induced Oxidative Stress. *Oxid Med Cell Longev* (2019) 2019:5416728. doi: 10.1155/2019/5416728
23. Sastry KS, Naeem H, Mokrab Y, Chouchane AI. RNA-seq Reveals Dysregulation of Novel Melanocyte Genes upon Oxidative Stress: Implications in Vitiligo Pathogenesis. *Oxid Med Cell Longev* (2019) 2019:2841814. doi: 10.1155/2019/2841814
24. Nandi A, Yan LJ, Jana CK, Das N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. *Oxid Med Cell Longev* (2019) 2019:9613090. doi: 10.1155/2019/9613090
25. Tarle RG, Silva de Castro CC, do Nascimento LM, Mira MT. Polymorphism of the E-cadherin gene CDH1 is associated with susceptibility to vitiligo. *Exp Dermatol* (2015) 24(4):300–2. doi: 10.1111/exd.12641
26. Picardo M, Dell'Anna ML, Ezzedine K, Hamzavi I, Harris JE, Parsad D, et al. Vitiligo. *Nat Rev Dis Primers* (2015) 1:15011. doi: 10.1038/nrdp.2015.11
27. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, et al. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res* (2012) 25(3):E1–13. doi: 10.1111/j.1755-148X.2012.00997.x
28. Ren Y, Yang S, Xu S, Gao M, Huang W, Gao T, et al. Genetic variation of promoter sequence modulates XBP1 expression and genetic risk for vitiligo. *PloS Genet* (2009) 5(6):e1000523. doi: 10.1371/journal.pgen.1000523
29. Zubair R, Hamzavi IH. Phototherapy for Vitiligo. *Dermatol Clin* (2020) 38 (1):55–62. doi: 10.1016/j.det.2019.08.005
30. Gan EY, Eleftheriadou V, Esmat S, Hamzavi I, Passeron T, Bohm M, et al. Repigmentation in vitiligo: position paper of the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res* (2017) 30(1):28–40. doi: 10.1111/pcmr.12561
31. Hamzavi I, Jain H, McLean D, Shapiro J, Zeng H, Lui H. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. *Arch Dermatol* (2004) 140(6):677–83. doi: 10.1001/archderm.140.6.677
32. 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(3):654–9. doi: 10.1111/j.1365-2133.2009.09217.x
33. Grimes PE, Soriano T, Dytoc MT. Topical tacrolimus for repigmentation of vitiligo. *J Am Acad Dermatol* (2002) 47(5):789–91. doi: 10.1067/mjd.2002.126250
34. Chang HC, Sung CW. Efficacy of combination therapy of narrowband-ultraviolet B phototherapy or excimer laser with topical tacrolimus for vitiligo: An updated systematic review and meta-analysis. *Photodermatol Photoimmunol Photomed* (2021) 37(1):74–7. doi: 10.1111/phpp.12593
35. Gauthier Y, Almasi-Nasrabadi M, Cario-Andre M, Pain C, Rakhshan A, Ghalamkarpour F. Tacrolimus (FK506) ointment combined with Nb-UVB could activate both hair follicle (HF) and dermal melanocyte precursors in vitiligo: the first histopathological and clinical study. *Arch Dermatol Res* (2020). doi: 10.1007/s00403-020-02068-z
36. Arora CJ, Rafiq M, Shumack S, Gupta M. The efficacy and safety of tacrolimus as mono- and adjunctive therapy for vitiligo: A systematic review of randomised clinical trials. *Australas J Dermatol* (2020) 61(1):e1–9. doi: 10.1111/ajd.13096
37. Satyanarayan HS, Kanwar AJ, Parsad D, Vinay K. Efficacy and tolerability of combined treatment with NB-UVB and topical tacrolimus versus NB-UVB alone in patients with vitiligo vulgaris: a randomized intra-individual open comparative trial. *Indian J Dermatol Venereol Leprol* (2013) 79(4):525–7. doi: 10.4103/0378-6323.113091
38. Nordal EJ, Guleng GE, Ronnevig JR. Treatment of vitiligo with narrowband-UVB (TL01) combined with tacrolimus ointment (0.1%) vs. placebo ointment, a randomized right/left double-blind comparative study. *J Eur Acad Dermatol Venereol* (2011) 25(12):1440–3. doi: 10.1111/j.1468-3083.2011.04002.x
39. Hossani-Madani AR, Halder RM. Topical treatment and combination approaches for vitiligo: new insights, new developments. *G Ital Dermatol Venereol* (2010) 145(1):57–78.
40. Esfandiarpour I, Ekhlasi A, Farajzadeh S, Shamsadini S. The efficacy of pimecrolimus 1% cream plus narrow-band ultraviolet B in the treatment of vitiligo: a double-blind, placebo-controlled clinical trial. *J Dermatol Treat* (2009) 20(1):14–8. doi: 10.1080/09546630802155057
41. Fai D, Cassano N, Vena GA. Narrow-band UVB phototherapy combined with tacrolimus ointment in vitiligo: a review of 110 patients. *J Eur Acad Dermatol Venereol* (2007) 21(7):916–20. doi: 10.1111/j.1468-3083.2006.02101.x
42. AlJasser M, Richer V, Ball N, Lui H, Zhou Y. Photolichenoid papules within vitiligo induced by narrowband UVB phototherapy. *J Eur Acad Dermatol Venereol* (2016) 30(8):1428–9. doi: 10.1111/jdv.13271
43. Craiglow BG, King BA. Tofacitinib Citrate for the Treatment of Vitiligo: A Pathogenesis-Directed Therapy. *JAMA Dermatol* (2015) 151(10):1110–2. doi: 10.1001/jamadermatol.2015.1520
44. Ezzedine K, Vance TM, Hamzavi IH, Qureshi AA. Vitiligo: Targeted Therapies Add Color to Disease Pathophysiology. *J Invest Dermatol* (2020) 140(8):1498–500. doi: 10.1016/j.jid.2020.01.033
45. Rosmarin D, Pandya AG, Lebwohl M, Grimes P, Hamzavi I, Gottlieb AB, et al. Ruxolitinib cream for treatment of vitiligo: a randomised, controlled, phase 2 trial. *Lancet* (2020) 396(10244):110–20. doi: 10.1016/S0140-6736(20)30609-7
46. Joshipura D, Alomran A, Zancanaro P, Rosmarin D. Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib: A 32-week open-label extension study with optional narrow-band ultraviolet B. *J Am Acad Dermatol* (2018) 78(6):1205–7. doi: 10.1016/j.jaad.2018.02.023
47. Errichetti E, Zelin E, Pinzani C, Kyrgidis A, Lallas A, Stinco G. Dermoscopic and Clinical Response Predictor Factors in Nonsegmental Vitiligo Treated with Narrowband Ultraviolet B Phototherapy: A Prospective Observational Study. *Dermatol Ther (Heidelb)* (2020) 10(5):1089–98. doi: 10.1007/s13555-020-00431-6
48. Anbar TS, Westerhof W, Abdel-Rahman AT, El-Khayyat MA. Evaluation of the effects of NB-UVB in both segmental and non-segmental vitiligo affecting different body sites. *Photodermatol Photoimmunol Photomed* (2006) 22 (3):157–63. doi: 10.1111/j.1600-0781.2006.00222.x
49. Taieb A, Picardo M. Clinical practice. Vitiligo. *N Engl J Med* (2009) 360 (2):160–9. doi: 10.1056/NEJMcp0804388
50. Westerhof W, Nieuweboer-Krobotova L. Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A. *Arch Dermatol* (1997) 133(12):1525–8. doi: 10.1001/archderm.133.12.1525
51. Fried L, Tan A, Bajaj S, Liebman TN, Polsky D, Stein JA. Technological advances for the detection of melanoma: Advances in molecular techniques. *J Am Acad Dermatol* (2020) 83(4):996–1004. doi: 10.1016/j.jaad.2020.03.122
52. Kim BE, Goleva E, Kim PS, Norquest K, Bronchick C, Taylor P, et al. Side-by-Side Comparison of Skin Biopsies and Skin Tape Stripping Highlights Abnormal Stratum Corneum in Atopic Dermatitis. *J Invest Dermatol* (2019) 139(11):2387–9.e1. doi: 10.1016/j.jid.2019.03.1160

53. Wong R, Tran V, Talwalker S, Benson NR. Analysis of RNA recovery and gene expression in the epidermis using non-invasive tape stripping. *J Dermatol Sci* (2006) 44(2):81–92. doi: 10.1016/j.jdermsci.2006.08.007

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 10 March 2021
doi: 10.3389/fimmu.2021.587440

Transcriptome and Differential Methylation Integration Analysis Identified Important Differential Methylation Annotation Genes and Functional Epigenetic Modules Related to Vitiligo

Yihuan Pu[1†], Xuenuo Chen[2†], Yangmei Chen[1], Lingzhao Zhang[1], Jiayi Chen[1], Yujie Zhang[1], Xinyi Shao[1] and Jin Chen[1]*

[1] Department of Dermatology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China, [2] Department of Gastroenterology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

OPEN ACCESS

Edited by:
Reinhart Speeckaert,
Ghent University Hospital, Belgium

Reviewed by:
Mitesh Dwivedi,
Uka Tarsadia University, India
Stanca Birlea,
University of Colorado, United States

***Correspondence:**
Jin Chen
chenjin771010@163.com

[†] These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: 26 July 2020
Accepted: 19 February 2021
Published: 10 March 2021

Citation:
Pu Y, Chen X, Chen Y, Zhang L, Chen J, Zhang Y, Shao X and Chen J (2021) Transcriptome and Differential Methylation Integration Analysis Identified Important Differential Methylation Annotation Genes and Functional Epigenetic Modules Related to Vitiligo. Front. Immunol. 12:587440. doi: 10.3389/fimmu.2021.587440

Vitiligo is an pigmentation disorder caused by a variety of pathogenic factors; its main pathophysiological conditions include oxidative stress, immune activation, and genetic background. Additionally, DNA methylation is often associated with the pathogenesis of vitiligo; however, the underlying mechanism remains unknown. In the present study, we used the Human Methylation 850K BeadChip platform to detect DNA methylation changes in the vitiligo melanocytes. We then integrated the results with the transcriptome data of vitiligo melanocytes and lesions to analyse the correlation between differentially methylated levels and differentially expressed genes. The results showed that there was a significant negative correlation between methylation levels and differentially expressed genes. Subsequently, we enriched GO and KEGG based on methylated differentially expressed genes (MDEGs) using R package ClusterProfiler, and the results were closely related to the pathogenesis of vitiligo. In addition, we also constructed a PPI network of MDEGs and excavated three important functional epigenetic modules, involving a total of 12 (BCL2L1, CDK1, ECT2, HELLS, HSP90AA1, KIF23, MC1R, MLANA, PBK, PTGS2, SOX10, and TYRP1) genes. These genes affect melanocyte melanogenesis, cellular oxidative stress and other important biological processes. Our comprehensive analysis results support the significant contribution of the status of DNA methylation modification to vitiligo, which will help us to better understand the molecular mechanism of vitiligo and explore new therapeutic strategies.

Keywords: vitiligo melanocyte, 850K, MDEGs, PPI, functional epigenetic modules

INTRODUCTION

Vitiligo is an acquired chronic skin pigmentation disease that affects 0.5–2% of the world's population (1). The reported epidemiological data varies according to region, and the disease impact may be related to the respective social and cultural stigmas (2). The pathogenesis of vitiligo manifests as oxidative stress, infiltration of inflammatory mediators, melanocyte loss, and

autoimmune responses (3). It is caused due to the complex interaction among environmental and genetic factors that eventually leads to melanocyte dysfunction (4). However, the inherent defect in melanocytes is an early event in vitiligo; the gradual disappearance of melanocytes may involve multiple processes, including immune system attack, cell degeneration, and exfoliation among others (1).

Epigenetic factors such as DNA methylation, histone modification, and gene silencing through microRNAs play an important role in the pathogenesis and progression of autoimmune dermatoses, such as psoriasis and atopic dermatitis (5). Increasing number of studies have shown that failure to maintain the level and pattern of DNA methylation can lead to abnormal cell function and proliferative activity (6). Although epigenetics plays a key role in the pathogenesis of skin tumors, researchers have recently begun to pay more attention to the epigenetics in the pathogenesis of inflammatory skin diseases, such as psoriasis, atopic dermatitis, and other inflammatory skin diseases (7–9). Abnormal DNA methylation has been identified as a major epigenetic change leading to the development of various skin cancers (10). However, DNA methylation is reversible and is therefore considered an attractive therapeutic intervention (11, 12) to provide the possibility treatment methods for various skin diseases.

Evidences have shown that abnormal DNA methylation is involved in the development of vitiligo. For example, the DNA methylation level of peripheral blood mononuclear cells (PBMC) in vitiligo have been investigated and the emerging evidence suggests an epigenetic regulation of CD8+ T cells. Zhao M et al. reported that in vitiligo patients, DNA methylation of peripheral blood mononuclear cells affects the mRNA expression level of DNMT, MBD, IL-10, and other genes (13). Another study suggests that in autoimmune diseases such as type 1 diabetes (T1D), systemic lupus erythematosus (SLE), and vitiligo genes associated with the proliferation or activation of CD8+ T cells are affected by epigenetic modification (14). However, the important biological pathway regulated by abnormal methylation has not been elucidated yet, and the role of epigenetic factors in the vitiligo pathogenesis needs further research.

In the present study, we integrated and analyzed the differential transcriptomic data (from vitiligo melanocyte cell lines and vitiligo lesions) and differential methylation data (from vitiligo melanocyte cell lines). We used Infinium Methylation EPIC BeadChip for the methylation sequencing of vitiligo melanocyte PIG3V and normal melanocyte PIG1. The 850K microarray covered the gene promoter region, gene coding region, CpG island, and enhancer regions present in ENCODE and FANTOM5. The data of the transcriptional group were derived from vitiligo lesion transcriptional sequencing datasets GSE75819 (15 non-segmental vitiligo patients with lesion skin and peri-lesion skin biopsies, the peri-lesion skin biopsies as controls) in the GEO database and our cell line transcriptional sequencing data (PIG3V and PIG1). Subsequently, we conducted GO/KEGG pathway analysis, PPI, and functional epigenetic module analysis of MDEGs to further explore the potential mechanism driven by abnormal methylation of vitiligo. Flowchart of bioinformatics analysis is shown in **Figure 1**.

MATERIALS AND METHODS

Study Subjects

The human vitiligo melanocyte cell line PIG3V and the human normal melanocyte cell line PIG1 (gifted by Dr. Li Chunying, Xijing Hospital, Fourth Military Medical University, Xi'an, Shanxi Province) were cultured in human melanocyte growth medium 254 supplemented with 5% fetal bovine serum and penicillin- streptomycin at 37°C in a 5% CO2 incubator. To integrate and study the correlation between the methylation status and transcriptome dataset, we sequenced the whole genome of two cases of PIG1 cell line and two cases of PIG3V cell line, and used it as cell line methylation dataset and expression dataset. Vitiligo lesion expression GSE75819 was downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). Fifteen vitiligo focal skin samples and fifteen vitiligo peri-focal skin samples were included in the GSE75819 to verify the expression dataset.

Infinium Human Methylation EPIC Array Using 850K BeadChip and Data Analysis

DNA was isolated from cell samples, using DNeasy Blood & Tissue Kit (Qiagen, Germany). The purity and concentration of DNA was estimated using Nanodrop 2000 (Thermo Fisher Scientific, China). Approximately 500 ng of genomic DNA from each sample was used for sodium bisulfite treatment using the EZ DNA methylation Gold Kit (Zymo Research, USA) following the manufacturer's protocol. Genome-wide DNA methylation was assessed using the Infinium Human Methylation 850K BeadChip (Illumina Inc, USA) according to the manufacturer's instructions. The array data (IDAT files) were analyzed using the ChAMP package in R for deriving the level of methylation. The methylation status of all the probes was denoted as β value, which is the ratio of the methylated probe intensity to the overall probe intensity (sum of methylated and unmethylated probe intensities plus constant α, where α = 100). A CpG site with $|\Delta\beta| \geq 0.20$ (in test vs. control) and adjusted *P*-value ≤ 0.05 was considered as a differentially methylated site. A CpG was considered hypermethylated if $\Delta\beta \geq 0.20$ or hypomethylated if $\Delta\beta \leq -0.20$. The average β value of promoters and CpG islands was compared between diseased and normal conditions. Promoters and CGIs with $|\Delta\beta| \geq 0.20$ and adjusted *P*-value ≤ 0.05 were considered for further analysis.

Copy Number Variation Analysis

We used the consumer package to analyse the human measurement epic data to identify copy number variation (CNV). CNV analysis was carried out in two steps. First, the combined signal values of "methylated" and "unmethylated" CPGs were normalized using the control group (PIG1 as control). This step required correcting the probe and sample deviation. Second, the adjacent probes were combined to generate a probe bin with the minimum size and quantity to reduce the remaining technical changes and achieve meaningful segmentation results. For CNV results and differential methylation sites, we used the Circos map for joint analysis.

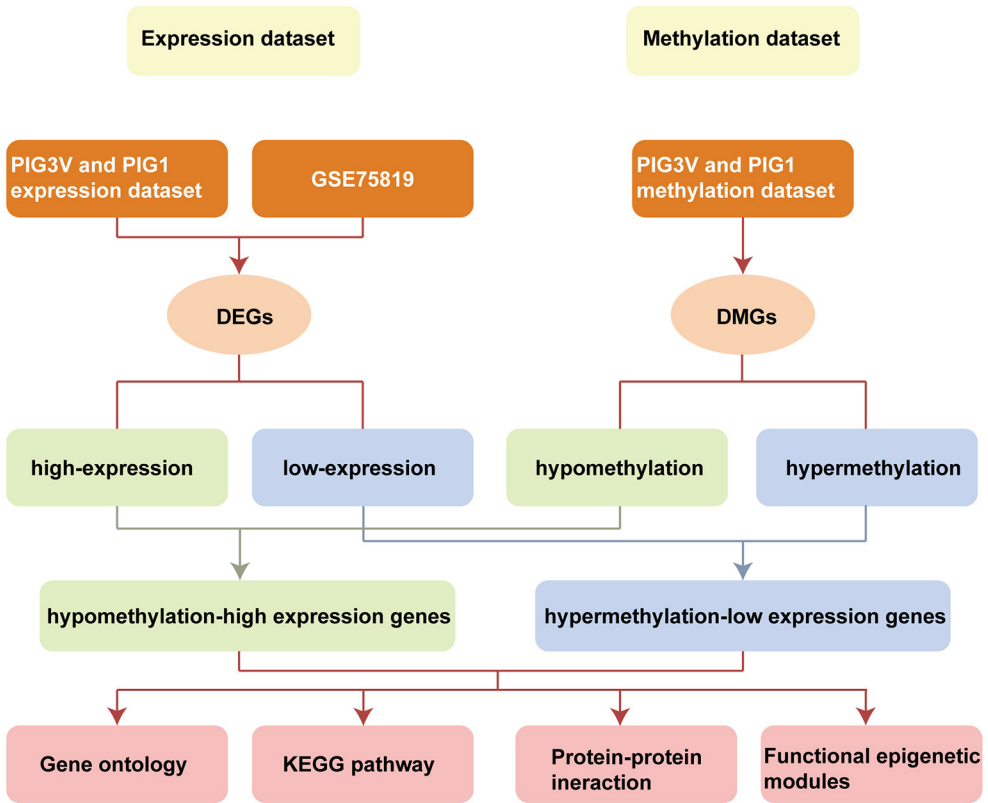


FIGURE 1 | Flowchart of bioinformatics analysis. DMGs, Differentially methylated genes; DEGs, Differentially expressed genes; PIG1, human normal melanocyte; PIG3V, human vitiligo melanocyte.

Acquisition and Processing of Expression Spectrum Data

RNA-Seq strand-specific libraries were constructed using a VAHTS Total RNA-Seq (H/M/R) Library Prep Kit (Vazyme, China). The original gene expression data set GSE75819 was downloaded from the GEO public database. We used R 4.0.0 to analyse the expression matrix. The robust multiarray average (RMA) method was used to pre-process data, including background adjustment, normalization, and log value conversion. The Limma package was used to search for differential genes. The threshold of up and down genes was set as $|\log FC| \geq 1$ and $P \leq 0.05$.

FunRich

FunRich 3.1.3 (http://www.funrich.org) is an independent software tool, used mainly for the functional enrichment and interaction network analysis of genes and proteins (15). The software was used to identify hypomethylation and hypermethylation genes for further analysis.

GO and KEGG Enrichment

GO and KEGG enrichment were carried out for hypomethylation and hypermethylation genes using R package clusterProfiler. Results with $P < 0.05$ were considered statistically significant. We used R package ggplot2 to visualize the important projects of each group function and pathway enrichment analysis.

Construction of PPI Network and Related Analysis

To further explain the interactions between the two sets of genes in the pathogenesis of vitiligo and the specific molecular mechanisms, PPI analysis was performed. Since the number of hypomethylation and hypermethylation genes was small, we integrated them to construct PPI networks. STRING 11.0 was used to generate a PPI network (https://string-db.org/). The cutoff value was set to 0.4 of the interaction score. The results were imported into Cytoscape 3.8.0 for subsequent analysis. CytoHubba app (http://apps.cytoscape.org/apps/cytohubba) was used to screen the hub genes. The first 3 modules were applied in Cytoscape by the MCODE app software package. We used FunRich 3.1.3 to identify and visualize the potential genes related to each module.

Statistical Analysis

The correlation between differentially expressed genes and methylation levels was analyzed by Pearson's correlation coefficient. Results with $P < 0.05$ were considered statistically significant. The statistical analyses were performed using R 4.0.0.

GraphPad Prism 8 (GraphPad Prism Software Inc., San Diego, California) to correlate analysis and graphic display.

RESULTS

Global Methylation Pattern of Vitiligo Melanocytes and Normal Melanocytes

The characteristics of DNA methylation were significantly different between vitiligo melanocyte cell line and normal melanocyte cell line. The analysis of differential methylation position (DMP) showed 83 451 differential methylation positions in PIG3V compared with PIG1. Among them, 53 895 were hypermethylated and 29 556 were hypomethylated. CpGs showed bimodal methylation distribution in PIG3V, and most of them showed hyper/hypo methylation levels. The differential methylation positions are shown in the cluster heat map (**Figure 2A**) and the volcano map (**Figure 2B**). The genes corresponding to the top 20 differences in methylation positions are listed at the two tips of the volcanic map. In addition, to examine the methylation changes throughout the genome, we used Circos maps for joint analysis (**Figure 2C**).

Integration of Epigenomic and the Transcriptomic Data

The intersection of transcriptomic data on vitiligo cell lines and lesions was visualized in the veen map (**Figure 3A**); there were 117 down-regulated and 149 up-regulated genes. The intersection of transcriptomic methylation data of vitiligo melanocyte cell line was also visualized by veen map (**Figure 3B**) and 126 overlapping genes were obtained. We analyzed the correlation between intersectional genes and their methylation levels, and the results showed that DNA methylation in vitiligo melanocyte cell line is negatively correlated with gene expression (**Figure 3C**), but there was no significant positive correlation (**Figure 3D**). Therefore, we selected the genes with a high expression of low methylation and low expression of high methylation for further study. There were 69 genes that met these requirements, including 14 down-regulated genes with hypermethylation and 55 up-regulated genes with low methylation. It is important to note that GEO currently does not have DNA methylation data for vitiligo, so there is no verification set for vitiligo methylation.

Enrichment Analysis of GO and KEGG Pathways

In PIG3V, the biological processes of MDEGs, such as oxidation–reduction oxidoreductase activity, are mainly related to oxidative stress, immune responses, and melanogenesis (**Figures 4A,B**). The response to oxidative stress is related to the positive regulation of T cell proliferation, whereas regulation of acute inflammatory responses is involved in inflammation, and melanocyte differentiation and melanosome transport affect melanogenesis. The specific genes involved in each GO term are shown in the **Supplementary Table 1**. Apart from these, some other processes such as the regulation of cell aging, response to vitamin D, autophagy, and the positive regulation of fatty acid metabolic processes are related to the pathogenesis of vitiligo.

KEGG analysis identified a variety of pathways in enrichment analysis, and we visualized the top 10 (**Figures 4C,D**). According to KEGG classification, the pathways rich in MDEGs are commonly involved in IL-17 signaling, Th17 cell differentiation, TNF signaling, and NOD-like receptor signaling pathway. Further, different pathways may overlap due to common elements. For the transcripts of differential regulation, some pathways are concentrated in the glucagon signaling such as insulin resistance, insulin signaling, and thyroid hormone signaling. This may be related to the crosstalk among vitiligo, diabetes, thyroid, and other autoimmune diseases, and the MDEGs involved in these pathways may be important biomarkers for the mutual crosstalk in immune diseases.

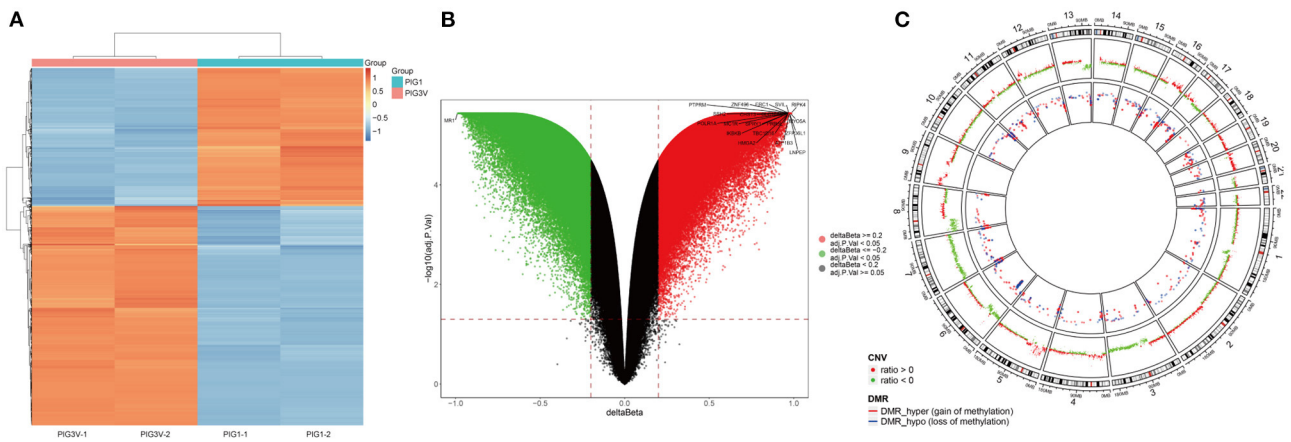


FIGURE 2 | (A) The heat map, **(B)** the Volcano map analysis of genes with differential methylation between vitiligo melanocyte and normal melanocyte. **(C)** A joint analysis of CNV results and differential methylation sites performde by the Circos diagram. CNV and the outermost circle represent chromosome regions, and the second inward circle represents the CNV copy number (red represents ratio > 0, green represents ratio < 0), The inner circle represents the DMR area.

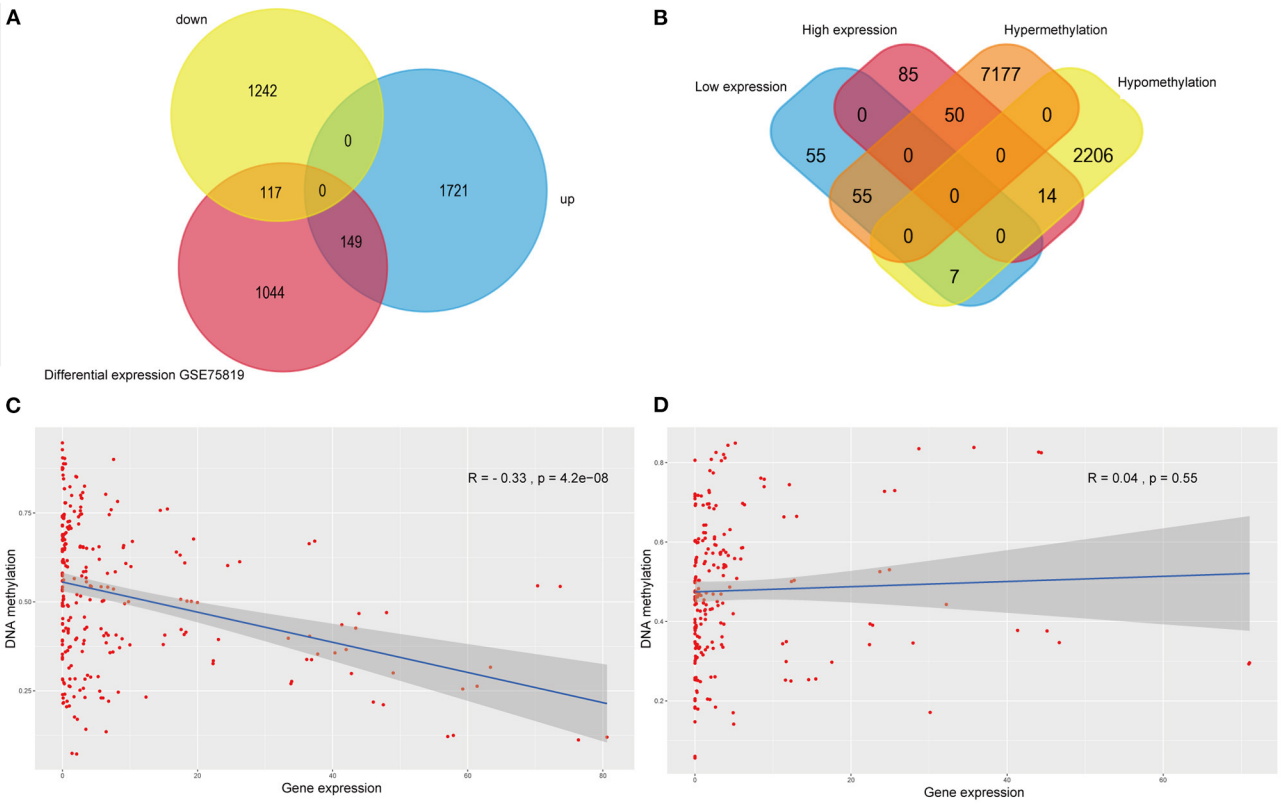


FIGURE 3 | Integrative analysis of methylome and transcriptome of vitiligo. **(A)** Veen diagram of differentially expressed genes between vitiligo skin lesion microarray and vitiligo melanocyte microarray. **(B)** Venn diagram between differentially methylated genes and differentially expressed genes of vitiligo. Correlation analysis between differentially methylated genes and differentially expressed genes: **(C)** negative correlation analysis, **(D)** positive correlation analysis.

PPI Network and Functional Epigenetic Modules

The PPI network analyzed by STRING showed 69 nodes and 66 edges. Cytoscape 3.8.0 was used to visualize the PPI network (**Figure 5A**), CytoHubba app in Cytoscape software is used to select the hub nodes genes of the PPI network. In the results, a total of 7 genes of the top 10 hub genes detected by six ranked methods in cytoHubba were overlap hub genes (**Figure 5B**), including CDK1 (cyclin dependent kinase 1), HSP90AA1 (heat shock protein 90 alpha family class A member 1), AKT1 (AKT serine/threonine kinase 1), BCL2L1 (BCL2 like 1), HDAC2 (histone deacetylase 2), HELLS (helicase, lymphoid specific), and KIF23 (kinesin family member 23). Among them, BCL2L1 was hypermethylated and its expression was downregulated, whereas the other six genes are hypomethylated and their expression was upregulated. Finally, based on the PPI network, three important epigenetic function modules were identified and were established as statistically significant. The results of enrichment analysis of functional epigenetic modules showed that the MDEGs of module 1 (**Figure 5C**) played an important role in the regulation of cell cycle and cell division, and the MDEGs of module 2 (**Figure 5D**) affected melanogenesis and pigmentation. Module 3 (**Figure 5E**) involved the regulation of cellular response to stress, negative regulation of intrinsic apoptotic signaling pathway, and complex pathway regulation. The enrichment results are presented in detail in **Table 1**.

DISCUSSION

Vitiligo is considered to be an autoimmune disease because cytokines such as (IFN)-γ, IL-1, IL-6, IL-8, and IL-10 are overexpressed in lesions, and activated CD8 + T lymphocytes, TH17, and other immune cells are significantly aggregated in the lesion area (16–19). However, some researchers have suggested that autoimmunity may be secondary to high oxidative stress in vitiligo skin, leading to inherent defects of melanocytes and changes in its microenvironment (4). At present, the unified view is that the inherent defect of melanocytes is an early event in vitiligo, and the gradual disappearance of epidermal melanocytes leads to skin depigmentation of vitiligo lesions.

GO enrichment analyses of the oxidation-reduction process, response to oxidative stress, and oxidoreductase activity have been partially studied in vitiligo (20–23). Inflammation-related processes such as positive regulation of T cell proliferation, regulation of acute inflammatory response, and melanogenesis-related processes such as cellular pigmentation, melanocyte differentiation, melanosome transport were also widely studied in vitiligo (24–26). However, previous studied did not consider

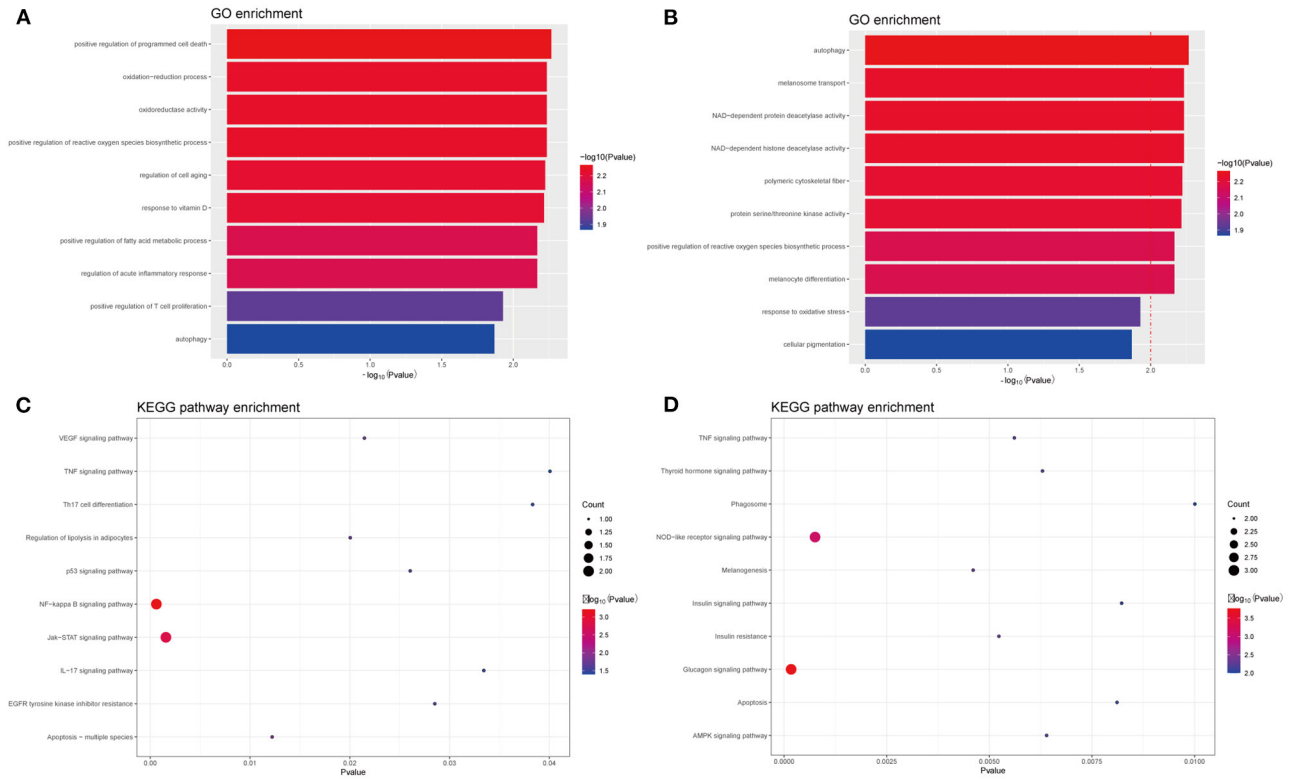
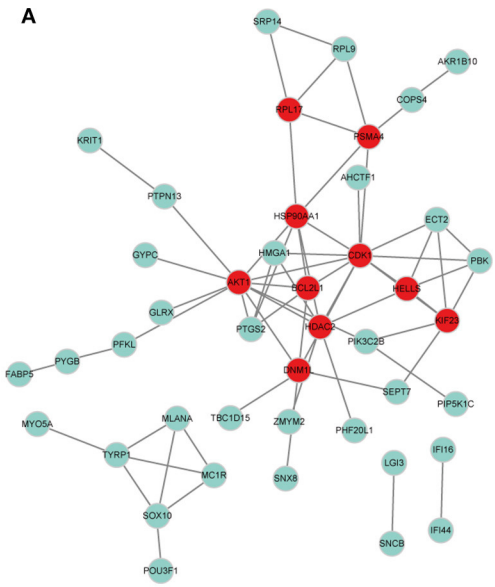


FIGURE 4 | GO and KEGG enrichment analysis of methylated-differentially expressed genes related with vitiligo melanocyte. Top 10 GO terms in **(A)** GO enrichment analysis of Hyper-MDGEs, **(B)** GO enrichment analysis of Hypo-MDGEs. **(C)** KEGG pathway analysis of Hyper-MDGEs, **(D)** KEGG pathway analysis of Hypo-MDGEs. The horizontal axes shows -log10 transformed *P*-value and $p < 0.05$ is considered significant.

methylation modification, and our study shows that the methylation levels of key genes involved in these important regulatory processes, such as TYP1, IL17, and MC1R, are altered in vitiligo melanocyte PIG3V. Therefore, based on our findings, we propose that the change in methylation level may regulate the differential expression of functional genes in vitiligo. To the best of our knowledge, this is the first study to analyze the methylation profile of vitiligo melanocytes.

The infiltration of immune cells and the release of pro-and anti-inflammatory cytokines is key to the pathogenesis of vitiligo. KEGG results showed that MDEGs were significantly enriched in IL-17 signaling pathway, Th17 cell differentiation, TNF signaling pathway, and NOD-like receptor signaling pathways, and these signaling pathways have been proved to be involved in mediating the immune regulation of vitiligo (18, 27, 28). In addition, differential methylation of AKT1, PYGB, HDAC2 were frequently observed in the insulin signaling and thyroid hormone signaling pathways. These pathways play an important role in abnormal glucose metabolism in diabetes and thyroid diseases (29–31), while vitiligo is frequently associated with other autoimmune diseases, particularly autoimmune thyroid diseases (Hashimoto's thyroiditis and Graves' disease), adult-onset type 1 diabetes mellitus, psoriasis, and so on (32). Thus, genes in these signaling pathways are regulated by methylation modification may play an important role in crosstalk between immune diseases. Previous studies have shown that the effective treatment of psoriasis, an immune inflammatory skin disease, can reverse DNA methylation in the epidermis (33, 34), suggesting that further studies on vitiligo methylation may be potentially identify new treatment strategies for vitiligo.

Futhermore, the PPI network showed that CDK1, HSP90AA1, AKT1, BCL2L1, HDAC2, HELLS, and KIF23 are 7 central genes identified by the top 10 high hub nodes after gene overlap. Among these central genes, CDK1 is a set of Ser/Thr kinase system corresponding to cell cycle progression (35), and its differential expression was found in another vitiligo study (36); HSP90AA1 plays a key role in signal transduction, protein folding, protein degradation, and morphological evolution (37); AKT1 and BCL2L1 are involved in apoptosis (38, 39), and AKT methylation has been shown to promote skin tumors (40); HDAC2 interferes with histone deacetylase function (41); KIF23 plays an important role in the changes of cellular motor cytoskeleton (42); HELLS is considered to be a regulator of cell senescence and is necessary for DNMT1-mediated methylation maintenance and DNMT3A/DNMT3B-mediated remethylation (43). Therefore, we speculate that these hub genes may be involved in the pathological process in the early stage of melanocyte loss in vitiligo, but further functional experiments are needed to further explore.



B

The hub genes for hypo/hyper methylated differential expressed genes ranked in cytoHubba

Catelogy	Rank methods in cytoHubba					
	MNC	MCC	EPC	Degree	Closeness	Radiality
Gene symbol top 10	CDK1	CDK1	CDK1	CDK1	CDK1	AKT1
	HSP90AA1	AKT1	AKT1	AKT1	AKT1	CDK1
	AKT1	KIF23	HSP90AA1	HSP90AA1	HSP90AA1	HSP90AA1
	BCL2L1	HELLS	BCL2L1	HDAC2	HDAC2	HDAC2
	HDAC2	ECT2	HDAC2	KIF23	DNM1L	BCL2L1
	HELLS	PBK	HELLS	DNM1L	BCL2L1	DNM1L
	RPL17	HSP90AA1	KIF23	BCL2L1	PSMA4	PSMA4
	ECT2	BCL2L1	PTGS2	PSMA4	KIF23	PTGS2
	KIF23	HDAC2	DNM1L	HELLS	HELLS	KIF23
	PBK	DNM1L	ECT2	RPL17	PTGS2	HELLS

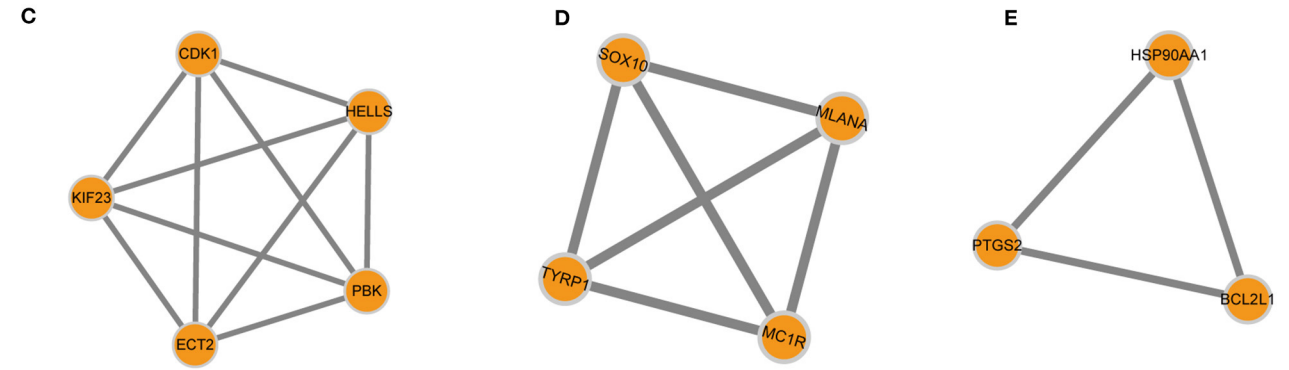


FIGURE 5 | (A) Protein-protein interaction network of MDEGs, Disconnected nodes were hid in the network. **(B)** Hub genes for MDEGs ranked in cytoHubba, functional epigenetic modules of the protein-protein interaction network: **(C)** module1, **(D)** module2, **(E)** module3.

The analysis of the epigenetic module shows that the MDEGs in module 1 is highly enriched in the processes of cell cycle regulation, whereas the MDEGs in module 2 is involved in the process of pigmentation and the pathway of regulating melanin production. Interestingly, the functions of MDEGs in these modules are relatively concentrated, which suggests that these biological processes may be collectively regulated by methylation. Whether demethylation can reverse these processes needs to be verified by further studies. Module 3 involves cellular stress, apoptosis, and regulation of complex biological processes and pathways (44, 45) key to the pathogenesis of vitiligo. These findings suggest that MDEGs in vitiligo melanocytes may have regulatory functions in these biological processes and molecular functions. However, some genes and pathways identified in this study have not been formally studied as targets of the vitiligo process, and need to be evaluated further.

Although our research attempts to bridge an important gap, it does have several limitations that can be addressed in the future studies. Firstly, the sample size was relatively small as only four microarray profiles were analyzed and there was no vitiligo methylation data available on the GEO platform. Hence, a replication with larger samples will be required to validate the findings. Secondly, the study lacked experimental verification of the effects of aberrant methylation on gene expression and functions in vitiligo melanocytes. Therefore, supplementary molecular experiments should be conducted to verify the results of our investigation. Furthermore, due to the limited sample size, the GEO verification set of differentially expressed genes selected only differential datasets rather than general high and low expression sets due to the differences between cell lines and human samples. This strict intersection may mask genes that play significant roles.

In conclusion, using a series of bioinformatics databases and tools, we found that the interactions among MDEGs with different functions and signaling pathways is related to the pathogenesis of vitiligo melanocytes. The hub genes of vitiligo melanocytes include CDK1, HSP90AA1, AKT1, BCL2L1, HDAC2, HELLS, and KIF23. The genes involved in three important epigenetic modules include BCL2L1, CDK1, ECT2, HELLS, HSP90AA1, KIF23, MC1R, MLANA, PBK, PTGS2, SOX10, and TYRP1. This study provides hypothetical and biological characteristic insight into the pathogenesis of vitiligo. However, additional molecular-level studies are needed to

TABLE 1 | GO and KEGG enrichment analysis of functional epigenetic modules related with vitiligo melanocyte.

Category	Term	Count	FDR	Gene
Module1	GO:0072686→ mitotic spindle	3	4.33E-05	CDK1, ECT2, KIF23
	GO:0097149→ centralspindlin complex	2	4.33E-05	ECT2, KIF23
	GO:0030496→ midbody	3	0.00017	CDK1, ECT2, KIF23
	GO:0005524→ ATP binding	4	0.0097	CDK1, HELLS, KIF23, PBK
	GO:0004674→ protein serine/threonine kinase activity	2	0.0233	CDK1, PBK
	GO:0007049→ cell cycle	5	0.00072	CDK1, ECT2, HELLS, KIF23, PBK
	GO:0051301→ cell division	4	0.00072	CDK1, ECT2, HELLS, KIF23
	GO:0000278→ mitotic cell cycle	4	0.0011	CDK1, ECT2, KIF23, PBK
	GO:0090068→ positive regulation of cell cycle process	3	0.0043	CDK1, ECT2, KIF23
	GO:0032467→ positive regulation of cytokinesis	2	0.0052	ECT2, KIF23
	GO:0042307→ positive regulation of protein import into nucleus	2	0.0052	CDK1, ECT2
	GO:0000281→ mitotic cytokinesis	2	0.0055	ECT2, KIF23
	GO:0070301→ cellular response to hydrogen peroxide	2	0.0071	CDK1, ECT2
	GO:1903047→ mitotic cell cycle process	3	0.0082	CDK1, ECT2, KIF23
	GO:0071478→ cellular response to radiation	2	0.0144	ECT2, PBK
	GO:0006323→ DNA packaging	2	0.019	CDK1, HELLS
	GO:0022607→ cellular component assembly	4	0.019	CDK1, ECT2, HELLS, KIF23
	GO:0051276→ chromosome organization	3	0.0218	CDK1, HELLS, KIF23
	GO:0010038→ response to metal ion	2	0.0395	CDK1, ECT2
	GO:0001932→ regulation of protein phosphorylation	3	0.0405	CDK1, ECT2, PBK
	GO:0032147→ activation of protein kinase activity	2	0.0405	CDK1, ECT2
	GO:0016569→ covalent chromatin modification	2	0.0411	CDK1, HELLS
	GO:0000226→ microtubule cytoskeleton organization	2	0.0459	CDK1, KIF23
	GO:0065003→ protein-containing complex assembly	3	0.0468	CDK1, ECT2, HELLS
Module2	GO:0042470→ melanosome	2	0.0141	MLANA, TYRP1
	GO:0043473→ pigmentation	2	0.0332	MC1R, TYRP1
	hsa04916 → Melanogenesis	2	0.00062	MC1R, TYRP1
Module3	GO:0042803→ protein homodimerization activity	3	0.0057	BCL2L1, HSP90AA1, PTGS2
	GO:0019899→ enzyme binding	3	0.0263	BCL2L1, HSP90AA1, PTGS2
	GO:0019901→ protein kinase binding	2	0.041	BCL2L1, HSP90AA1
	GO:0019904→ protein domain specific binding	2	0.0436	BCL2L1, HSP90AA1
	GO:0007006→ mitochondrial membrane organization	2	0.0114	BCL2L1, HSP90AA1
	GO:0009408→ response to heat	2	0.0114	HSP90AA1, PTGS2
	GO:0009628→ response to abiotic stimulus	3	0.0114	BCL2L1, HSP90AA1, PTGS2
	GO:0019221→ cytokine-mediated signaling pathway	3	0.0114	BCL2L1, HSP90AA1, PTGS2
	GO:0033138→ positive regulation of peptidyl-serine phosphorylation	2	0.0114	HSP90AA1, PTGS2
	GO:0045429→ positive regulation of nitric oxide biosynthetic process	2	0.0114	HSP90AA1, PTGS2
	GO:0051726→ regulation of cell cycle	3	0.0114	BCL2L1, HSP90AA1, PTGS2
	GO:0071478→ cellular response to radiation	2	0.0114	BCL2L1, PTGS2
	GO:0080135→ regulation of cellular response to stress	3	0.0114	BCL2L1, HSP90AA1, PTGS2
	GO:1903827→ regulation of cellular protein localization	3	0.0114	BCL2L1, HSP90AA1, PTGS2
	GO:1904407→ positive regulation of nitric oxide metabolic process	2	0.0114	HSP90AA1, PTGS2
	GO:2001243→ negative regulation of intrinsic apoptotic signaling pathway	2	0.0114	BCL2L1, PTGS2
	GO:0006839→ mitochondrial transport	2	0.0122	BCL2L1, HSP90AA1
	GO:0010647→ positive regulation of cell communication	3	0.0174	BCL2L1, HSP90AA1, PTGS2
	GO:0023056→ positive regulation of signaling	3	0.0174	BCL2L1, HSP90AA1, PTGS2
	GO:0046677→ response to antibiotic	2	0.0185	BCL2L1, HSP90AA1
	GO:1902531→ regulation of intracellular signal transduction	3	0.0185	BCL2L1, HSP90AA1, PTGS2
	GO:0001101→ response to acid chemical	2	0.0196	BCL2L1, PTGS2
	GO:0009653→ anatomical structure morphogenesis	3	0.0247	BCL2L1, HSP90AA1, PTGS2

(Continued)

TABLE 1 | Continued

Category	Term	Count	FDR	Gene
	GO:0048584→ positive regulation of response to stimulus	3	0.0263	BCL2L1, HSP90AA1, PTGS2
	GO:0048608→ reproductive structure development	2	0.0275	BCL2L1, PTGS2
	GO:0009636→ response to toxic substance	2	0.0316	BCL2L1, PTGS2
	GO:0032990→ cell part morphogenesis	2	0.0316	BCL2L1, HSP90AA1
	GO:0051186→ cofactor metabolic process	2	0.0316	HSP90AA1, PTGS2
	GO:0071417→ cellular response to organonitrogen compound	2	0.0319	BCL2L1, PTGS2
	GO:0006897→ endocytosis	2	0.0345	BCL2L1, HSP90AA1
	GO:0045786→ negative regulation of cell cycle	2	0.0345	BCL2L1, PTGS2
	GO:1903047→ mitotic cell cycle process	2	0.0389	BCL2L1, HSP90AA1
	GO:0007276→ gamete generation	2	0.0421	BCL2L1, PTGS2
	GO:0007346→ regulation of mitotic cell cycle	2	0.0421	BCL2L1, HSP90AA1
	GO:0043065→ positive regulation of apoptotic process	2	0.0421	BCL2L1, PTGS2
	GO:0010564→ regulation of cell cycle process	2	0.0464	BCL2L1, HSP90AA1
	hsa05200→ Pathways in cancer	3	0.00079	BCL2L1, HSP90AA1, PTGS2
	hsa04064→ NF-kappa B signaling pathway	2	0.0015	BCL2L1, PTGS2
	hsa04657→ IL-17 signaling pathway	2	0.0015	HSP90AA1, PTGS2
	hsa05222→ Small cell lung cancer	2	0.0015	BCL2L1, PTGS2
	hsa04621→ NOD-like receptor signaling pathway	2	0.0019	BCL2L1, HSP90AA1
	hsa04151→ PI3K-Akt signaling pathway	2	0.0068	BCL2L1, HSP90AA1

confirm the identified genes and pathways in vitiligo to elucidate potential mechanisms, and *in vitro* and *in vivo* functional studies are also required to find the crucial role of the identified genes in vitiligo pathogenesis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

YP and XC prepared the figures and writing. YC and LZ collected and organized the data. JiaC cultured cells. YZ and XS prepared the tables. JinC critically revised the data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was financially supported by the National Natural Science Foundation of China (No. 81773307) and Chongqing Science and Technology Commission (No. cstc2018jcyjAX0195), which offered the financial support.

ACKNOWLEDGMENTS

We would like to thank the Sinotech Genomics Biotechnology Company for technical support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.587440/full#supplementary-material

REFERENCES

1. Picardo M, Dell'Anna ML, Ezzedine K, Hamzavi I, Harris JE, Parsad D, et al. Vitiligo. *Nat Rev Dis Primers.* (2020) 312:461–6.
2. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. *J Am Acad Dermatol.* (2011) 65:473–91. doi: 10.1016/j.jaad.2010.11.061
3. Schallreuter KU, Bahadoran P, Picardo M, Slominski A, Elassiuty YE, Kemp EH, et al. Vitiligo pathogenesis: autoimmune disease, genetic defect, excessive reactive oxygen species, calcium imbalance, or what else? *Exp Dermatol.* (2008) 17:139–60. doi: 10.1111/j.1600-0625.2007.00666.x
4. Abdel-Malek ZA, Jordan C, Ho T, Upadhyay PR, Fleischer A, Hamzavi I. The enigma and challenges of vitiligo pathophysiology and treatment. *Pigment Cell Melanoma Res.* (2020) 33:778–87. doi: 10.1111/pcmr.12878
5. Mervis JS, McGee JS. DNA methylation and inflammatory skin diseases. *Arch Dermatol Res.* (2020) 312:461–6. doi: 10.1007/s00403-019-02005-9
6. Gopisetty G, Ramachandran K, Singal R. DNA methylation and apoptosis. *Mol Immunol.* (2006) 43:1729–40. doi: 10.1016/j.molimm.2005.11.010
7. Millington GW. Epigenetics and dermatological disease. *Pharmacogenomics.* (2008) 9:1835–50. doi: 10.2217/14622416.9.12.1835
8. Liang Y, Chang C, Lu Q. The genetics and epigenetics of atopic dermatitis-filaggrin and other polymorphisms. *Clin Rev Allergy Immunol.* (2016) 51:315–28. doi: 10.1007/s12016-015-8508-5

9. Chandra A, Senapati S, Roy S, Chatterjee G, Chatterjee R. Epigenome-wide DNA methylation regulates cardinal pathological features of psoriasis. *Clin Epigenetics.* (2018) 10:108. doi: 10.1186/s13148-018-0541-9
10. Sang Y, Deng Y. Current insights into the epigenetic mechanisms of skin cancer. *Dermatol Ther.* (2019) 32:e12964. doi: 10.1111/dth.12964
11. Zhang X, Hu M, Lyu X, Li C, Thannickal VJ, Sanders YY. DNA methylation regulated gene expression in organ fibrosis. *Biochim Biophys Acta Mol Basis Dis.* (2017) 1863:2389–2397. doi: 10.1016/j.bbadis.2017.05.010
12. Yung PYK, Elsasser SJ. Evolution of epigenetic chromatin states. *Curr Opin Chem Biol.* (2017) 41:36–42. doi: 10.1016/j.cbpa.2017.10.001
13. Zhao M, Gao F, Wu X, Tang J, Lu Q. Abnormal DNA methylation in peripheral blood mononuclear cells from patients with vitiligo. *Br J Dermatol.* (2010) 163:736–42. doi: 10.1111/j.1365-2133.2010.09919.x
14. Deng Q, Luo Y, Chang C, Wu H, Ding Y, Xiao R. The emerging epigenetic role of CD8+T cells in autoimmune diseases: a systematic review. *Front Immunol.* (2019) 10:856. doi: 10.3389/fimmu.2019.00856
15. Pathan M, Keerthikumar S, Ang CS, Gangoda L, Quek CYJ, Williamson NA, et al. FunRich: an open access standalone functional enrichment and interaction network analysis tool. *Proteomics.* (2015) 15:2597–601. doi: 10.1002/pmic.201400515
16. Martins LT, Frigeri HR, de Castro CCS, Mira MT. Association study between vitiligo and autoimmune-related genes CYP27B1, REL, TNFAIP3, IL2 and IL21. *Exp Dermatol.* (2020) 29:535–8. doi: 10.1111/exd.14100
17. Fraczek A, Owczarczyk-Saczonek A, Placek W. The role of TRM cells in the pathogenesis of vitiligo-a review of the current state-of-the-art. *Int J Mol Sci.* (2020) 21:3552. doi: 10.3390/ijms21103552
18. Gholijani N, Yazdani MR, Dastgheib L. Predominant role of innate pro-inflammatory cytokines in vitiligo disease. *Arch Dermatol Res.* (2020) 312:123–294. doi: 10.1007/s00403-019-01996-9
19. Frisoli ML, Essien K, Harris JE. Vitiligo: mechanisms of pathogenesis and treatment. *Annu Rev Immunol.* (2020) 38:621–48. doi: 10.1146/annurev-immunol-100919-023531
20. Speeckaert R, Dugardin J, Lambert J, Lapeere H, Verhaeghe E, Speeckaert MM, et al. Critical appraisal of the oxidative stress pathway in vitiligo: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol.* (2018) 32:1089–98. doi: 10.1111/jdv.14792
21. Qiao Z, Wang X, Xiang L, Zhang C. Dysfunction of autophagy: a possible mechanism involved in the pathogenesis of vitiligo by breaking the redox balance of melanocytes. *Oxid Med Cell Longev.* (2016) 2016:3401570. doi: 10.1155/2016/3401570
22. Yang L, Yang F, Teng L, Katayama I. 6-Shogaol protects human melanocytes against oxidative stress through activation of the Nrf2-antioxidant response element signaling pathway. *Int J Mol Sci.* (2020) 21:3537. doi: 10.3390/ijms21103537
23. Passeron T, Ortonne JP. Physiopathology and genetics of vitiligo. *J Autoimmun.* (2005) 25:63–68. doi: 10.1016/j.jaut.2005.10.001
24. Liu XM, Zhou Q, Xu SZ, Wakamatsu K, Lei TC. Maintenance of immune hyporesponsiveness to melanosomal proteins by DHICA-mediated antioxidation: possible implications for autoimmune vitiligo. *Free Radic Biol Med.* (2011) 50:1177–85. doi: 10.1016/j.freeradbiomed.2011.01.017
25. Tang L, Li J, Lin X, Wu W, Kang K, Fu W. Oxidation levels differentially impact melanocytes: low versus high concentration of hydrogen peroxide promotes melanin synthesis and melanosome transfer. *Dermatology.* (2012) 224:145–53. doi: 10.1159/000336777
26. Tofani LB, Depieri LV, Campos PM, Riul TB, Antonietto KS, de Abreu Fantini MC, et al. *In vitro* TyRP-1 knockdown based on siRNA carried by liquid crystalline nanodispersions: an alternative approach for topical treatment of vitiligo. *Pharm Res.* (2018) 35:104. doi: 10.1007/s11095-017-2330-0
27. Pyo JJ, Ahn S, Jin SH, An S, Lee E, Choi J, et al. Keratinocyte-derived IL-36gamma plays a role in hydroquinone-induced chemical leukoderma through inhibition of melanogenesis in human epidermal melanocytes. *Arch Toxicol.* (2019) 93:2307–20. doi: 10.1007/s00204-019-02506-6
28. Yang CA, Chiang B-L. Inflammasomes and human autoimmunity: a comprehensive review. *J Autoimmun.* (2015) 61:1–8. doi: 10.1016/j.jaut.2015.05.001
29. Xuehan Wu, Weilu Huang, Minxue Quan, Yongqi Chen, Jiaxin Tu, Jialu Zhou, et al. Inhibition of brain-type glycogen phosphorylase ameliorates high glucose-induced cardiomyocyte apoptosis via Akt-HIF-1α activation. *Biochem Cell Biol.* (2020) 6:458–65. doi: 10.1139/bcb-2019-0247
30. Sasaki S, Lesoon-Wood LA, Dey A, Kuwata T, Weintraub BD, Humphrey G, et al. Ligand-induced recruitment of a histone deacetylase in the negative-feedback regulation of the thyrotropin beta gene. *EMBO J.* (1999) 18:5389–98. doi: 10.1093/emboj/18.19.5389
31. Bergman RN, Iyer MS. Indirect regulation of endogenous glucose production by insulin: the single gateway hypothesis revisited. *Diabetes.* (2017) 66:1742–7. doi: 10.2337/db16-1320
32. Narita T, Oiso N, Fukai K, Kabashima K, Kawada A, Suzuki T. Generalized vitiligo and associated autoimmune diseases in Japanese patients and their families. *Allergol Int.* (2011) 60:505–8. doi: 10.2332/allergolint.11-OA-0303
33. Ovejero-Benito MC, Reolid A, Sanchez-Jimenez P, Saiz-Rodriguez M, Munoz-Aceituno E, Llamas-Velasco M, et al. Histone modifications associated with biological drug response in moderate-to-severe psoriasis. *Exp Dermatol.* (2018) 27:1361–71. doi: 10.1111/exd.13790
34. Gu X, Nylander E, Coates PJ, Fahraeus R, Nylander K. Correlation between reversal of DNA methylation and clinical symptoms in psoriatic epidermis following narrow-band UVB phototherapy. *J Invest Dermatol.* (2015) 135:2077–83. doi: 10.1038/jid.2015.128
35. Prevo R, Pirovano G, Puliyadi R, Herbert KJ, Rodriguez-Berriguete G, O'Docherty A, et al. CDK1 inhibition sensitizes normal cells to DNA damage in a cell cycle dependent manner. *Cell Cycle.* (2018) 17:1513–23. doi: 10.1080/15384101.2018.1491236
36. Li S, Zeng H, Huang J, Lu J, Chen J, Zhou Y, et al. Identification the competing endogenous RNA networks in oxidative stress injury of melanocytes. *DNA Cell Biol.* (2021) 40:192–208. doi: 10.1089/dna.2020.5455
37. Zuehlke AD, Beebe K, Neckers L, Prince T. Regulation and function of the human HSP90AA1 gene. *Gene.* (2015) 570:8–16. doi: 10.1016/j.gene.2015.06.018
38. Duggal S, Jailkhani N, Midha MK, Agrawal N, Rao KVS, Kumar A. Defining the Akt1 interactome and its role in regulating the cell cycle. *Sci Rep.* (2018) 8:1303. doi: 10.1038/s41598-018-19689-0
39. Warren CFA, Wong-Brown MW, Bowden NA. BCL-2 family isoforms in apoptosis and cancer. *Cell Death Dis.* (2019) 10:177. doi: 10.1038/s41419-019-1407-6
40. Guo J, Dai X, Laurent B, Zheng N, Gan W, Zhang J, et al. AKT methylation by SETDB1 promotes AKT kinase activity and oncogenic functions. *Nat Cell 323 Biol.* (2019) 21:226–37. doi: 10.1038/s41556-018-0261-6
41. Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, et al. HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature.* (2009) 459:55–60. doi: 10.1038/nature07925
42. Capalbo L, Bassi ZI, Geymonat M, Todesca S, Copoiu L, Enright AJ, et al. The midbody interactome reveals unexpected roles for PP1 phosphatases in cytokinesis. *Nat Commun.* (2019) 10:4513. doi: 10.1038/s41467-019-12507-9
43. Jung HJ, Byun HO, Jee BA, Min S, Jeoun UW, Lee YK, et al. The ubiquitin-like with PHD and ring finger domains 1 (UHRF1)/DNA methyltransferase 1 (DNMT1) axis is a primary regulator of cell senescence. *J Biol Chem.* (2017) 292:3729–39. doi: 10.1074/jbc.M116.750539
44. Harris JE. Cellular stress and innate inflammation in organ-specific autoimmunity: lessons learned from vitiligo. *Immunol Rev.* (2016) 269:11–25. doi: 10.1111/imr.12369
45. Bellei B, Pitisci A, Ottaviani M, Ludovici M, Cota C, Luzi F, et al. Vitiligo: a possible model of degenerative diseases. *PLoS ONE.* (2013) 8:e59782. doi: 10.1371/journal.pone.0059782

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 14 April 2021
doi: 10.3389/fimmu.2021.613056

Targeting Innate Immunity to Combat Cutaneous Stress: The Vitiligo Perspective

Katia Boniface[1]*, *Thierry Passeron*[2,3], *Julien Seneschal*[1,4] and *Meri K. Tulic*[2]

[1] Univ. Bordeaux, INSERM, BMGIC, U1035, Immuno-dermatology Team, Bordeaux, France, [2] INSERM U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Côte d'Azur University, Nice, France, [3] Côte d'Azur University, Department of Dermatology, CHU Nice, Nice, France, [4] Department of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Disorders, Hôpital Saint-André, Bordeaux, France

Multiple factors are involved in the process leading to melanocyte loss in vitiligo including environmental triggers, genetic polymorphisms, metabolic alterations, and autoimmunity. This review aims to highlight current knowledge on how danger signals released by stressed epidermal cells in a predisposed patient can trigger the innate immune system and initiate a cascade of events leading to an autoreactive immune response, ultimately contributing to melanocyte disappearance in vitiligo. We will explore the genetic data available, the specific role of damage-associated-molecular patterns, and pattern-recognition receptors, as well as the cellular players involved in the innate immune response. Finally, the relevance of therapeutic strategies targeting this pathway to improve this inflammatory and autoimmune condition is also discussed.

Keywords: innate immunity, vitiligo, PAMPs, DAMPs, ILC, DC, melanocytes

OPEN ACCESS

Edited by:
J. Michelle Kahlenberg, University of Michigan, United States

Reviewed by:
Jillian M. Richmond, University of Massachusetts Medical School, United States
Rasheedunnisa Begum, Maharaja Sayajirao, University of Baroda, India

***Correspondence:**
Katia Boniface
katia.boniface@u-bordeaux.fr

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *01 October 2020*
Accepted: *22 March 2021*
Published: *14 April 2021*

Citation:
Boniface K, Passeron T, Seneschal J and Tulic MK (2021) Targeting Innate Immunity to Combat Cutaneous Stress: The Vitiligo Perspective. Front. Immunol. 12:613056. doi: 10.3389/fimmu.2021.613056

INTRODUCTION

Clinical, translational, and fundamental research studies performed over the last decade have tremendously improved our understanding of vitiligo physiopathology and new therapeutic perspectives are emerging for this disease which suffers from the lack of effective treatments. Vitiligo is a puzzling disease combining multiple intertwined components including environmental triggers, genetic predisposition, increased oxidative stress, and abnormal immune and inflammatory response (1, 2). Vitiligo is defined by the loss of epidermal melanocytes, nonetheless several cell subsets of immune and non-immune cells are involved to induce and/or contribute to their disappearance. Vitiligo skin is consistently associated with infiltration of T cells with a Th1/Tc1 skewed immune profile which target melanocytes (3, 4). Besides the role of the adaptive immune response, increasing data highlight a major role of innate immune cell subsets and their immune-related pathways that could spark the induction of the disease in the "normal-appearing" skin. Therefore, this short review is focusing on the innate side of the disease, discussing how genetic and transcriptomic data revealed the importance of innate immunity in vitiligo, as well as the interplay between epidermal cells (keratinocytes and melanocytes) and innate immune cells to contribute to the initiation and/or progression of the disease through the release of danger signals, cytokines, and chemokines, leading to activation of the adaptive immune response and ultimately the loss of

melanocyte. This better understanding now offers novel insight into the development of targeted therapies that could prevent the induction as well as the recurrence of the disease.

GENETIC AND TRANSCRIPTOME DATA

Genome wide association studies (GWAS) have identified over 50 susceptibility loci involved in melanogenesis and immunity in vitiligo patients (5). On the other hand, a delay in vitiligo age-of-onset over the past 30 years emphasizes the key role of environmental factors in triggering vitiligo in genetically predisposed individuals (6, 7). These GWAS studies not only demonstrated the implication of genes involved in melanogenesis and adaptive immunity but also revealed allelic variations in key genes involved in the innate immune responses, such as IFIH1, NLRP1, or TICAM1 (7–9).

Transcriptional analysis comparing gene expression profiles of skin from vitiligo patients with normal skin of healthy volunteers also emphasized the role of innate immunity (10, 11). Thus, natural killer (NK) cell activation markers, such as NKG2D, KLRC2, and KLRC4, ligands for NK receptor (CLEC2B), as well as markers of oxidative stress (CANP and POSTN) and innate immunity (DEFB103A) were shown to be increased in vitiligo skin (10). In our study, we also found a significant increase in NK receptors, including NKTR and KLRC1, as well as trends for increased EOMES (master regulator of NK cells), CCL20, and NK-related cytokines (TNFα and IL-15) (11). Interestingly, activation of these innate immunity markers was found in the non-lesional skin of vitiligo patients, suggesting that the activation of the innate immunity may be present throughout the entire skin surface of patients (10, 11).

Taken together, these data illustrate that vitiligo patients have genetic predisposition affecting their innate immune response in their apparent non-affected skin. Such findings may be indicative of a subclinical activation of innate immunity, loss of protective mechanisms to stress (such as defective unfolded protein response in target cells following endoplasmic reticulum stress), and/or increased sensitivity to endogenous or external stress, such as several damage-associated-molecular patterns (DAMPs) or pathogen-associated-molecular patterns (PAMPs) (12).

ACTIVATION OF INNATE IMMUNE CELLS BY DANGER SIGNALS

DAMPs

Several DAMPs have been detected in perilesional skin of vitiligo patients. Previous studies have shown that the chromatin-associated nuclear protein High-mobility group-box-1 (HMGB1) could be released by melanocytes under oxidative stress and could directly impact melanocyte survival (13–15). Additionally, HMGB1 could bind free DNA and HMGB1-DNA complexes and induce maturation of vitiligo patients' dendritic cells (DC), as well as the production of cytokines and chemokines by keratinocytes (16). Another candidate for sensing the immune system in vitiligo is calreticulin (CRT). In response to stress, CRT can localize at the surface of immune cells, affecting their antigen presentation, complement activation, and clearance of apoptotic cells. Moreover, CRT can translocate to the melanocyte surface when these cells undergo H_2O_2-mediated oxidative stress, increasing melanocyte immunogenicity. CRT may also enhance the immunogenic potential of melanocytes through their induction of pro-inflammatory cytokine production, such as IL-6 and TNFα (17).

Heat shock proteins (HSP) are likely important candidates bridging stress to the skin with the innate immune response. Indeed, inducible HSP70 (HSP70i) released in the context of cellular stress, notably by epidermal cells (including keratinocytes and melanocytes) has been shown to accelerate the progression of the disease in a preclinical model (18–20). Likewise, modified HSP70i prevented or reversed vitiligo in a mouse and Sinclair Swine models of the disease (21, 22). In vitiligo patients, the expression of HSP70 in the skin correlated with disease activity and was lower in patients with stable disease (23). As discussed below, HSP70i could interact with several cell subsets, leading to their activation.

Pattern Recognition Receptors

PAMPs are critical in initiation of the innate immune response through activation of pattern recognition receptors (PRRs). Implication of PRRs in vitiligo has been demonstrated in several GWAS, in particular genes encoding TLRs and their signaling pathway (24, 25). In addition, polymorphisms in NLRs have been described in patients with non-segmental vitiligo. Upregulated NLRP3 expression has been detected in perilesional keratinocytes in vitiligo skin and associated with higher cutaneous IL-1β expression and increased severity of the disease (26, 27).

Viral components are likely involved in vitiligo pathogenesis, as they can trigger activation of the immune system, however whether viruses can activate the innate immune response in the context of vitiligo is poorly described. Viruses possess several structurally diverse PAMPs, including surface glycoproteins, DNA, and RNA species (28). Virus infection could thus activate the innate response and potentially trigger a vitiligo flare. There is some evidence that viral infections in a genetically predisposed host may induce excessive ROS production by recruited lymphocytes leading to destruction of epidermal melanocytes (29). Furthermore, IFIH1, encoding intracellular virus sensor MDA5, has been identified as a vitiligo susceptibility gene capable of inducing secretion of CXCL10 and CXCL16 from keratinocytes and inducing infiltration of $CD8^+$ T cells in vitiligo (30).

Bacteria are among the top producers of PAMPs and could directly trigger PRRs activation and therefore participate in activation of the innate immune response in vitiligo, however their direct role in triggering vitiligo has yet to be proven. While gut dysbiosis has been reported in several auto-immune disorders, there exists only one study suggesting skin dysbiosis in lesional zone of vitiligo patients compared to their non-

lesional skin; however in that study there was no comparison to skin microbiota from healthy skin (31). The second study in mouse model of vitiligo treated with antibiotics has shown that depletion of certain bacterial strains in the gut induces skin depigmentation, suggesting possible gut-skin axis in the disease (32). We have recently demonstrated gut and skin dysbiosis in vitiligo compared to healthy controls; the most striking differences were seen in the deeper regions of vitiligo skin (33). Importantly, these changes were associated with mitochondrial damage and loss of protective bacteria at the same site with elevated systemic innate immunity in vitiligo patients.

ROLE OF INNATE IMMUNE CELLS IN VITILIGO

As suggested above, a large number of innate immunity genes that confer risk for vitiligo have been identified in genetic studies. Collectively, these papers undeniably support innate immunity pathways as critical in the development of the disease, which was further confirmed at the transcriptional level, with increased expression of innate immune related genes both in non-lesional and lesional skin of vitiligo patients (10, 11). If there is activation of innate immune pathways, naturally we would expect to see influx or activation of resident innate immune cells in the skin of vitiligo patients; however, the data in this area of research is only now starting to emerge. Although it has been known for a while that there is infiltration of macrophages, inflammatory DCs, dermal DCs, Langerhans cells, and NK cells to the leading edge or the lesional sites (34–37), their roles in vitiligo have not been thoroughly explored. The contribution of inflammatory DCs ($CD11c^+$ $CD11b^+$) has been demonstrated in DAMP-induced animal model of vitiligo driven by HSP70 (21), however their role in human disease remains to be proven. Studies dating over 10 years ago have demonstrated a positive correlation between levels of macrophage migration inhibitory factor (MIF) in the blood of vitiligo patients and their disease duration suggesting MIF may be a useful serum biomarker of vitiligo activity (38–40), however direct contribution of macrophages to the disease in the skin is unknown.

Plasmacytoid Dendritic Cells

Plasmacytoid dendritic cells (pDCs) certainly represent an important player in the initiation of the inflammatory response and the type I/II IFN signature in vitiligo skin. pDCs have been involved in various chronic inflammatory dermatoses, including cutaneous lupus erythematosus and psoriasis, mainly through their propensity to release high levels of IFNα (41). We showed that perilesional skin of vitiligo patients in the active phase of the disease harbors infiltrates of pDCs, associated with a local IFN response (36). Activation of this cell subset is likely mediated by the release of DAMPs from epidermal cells, as shown with HSP70i, potentiating IFNα secretion by pDCs and subsequent production CXCL9 and CXCL10 chemokines by epidermal cells, leading to the Th1 adaptive immune response establishment characteristic of vitiligo skin (42).

Furthermore, the other question which has been puzzling researchers was the initial source of the signature vitiligo cytokine IFNγ. We know that IFNγ is critical for the progression of vitiligo through 1) its induction of CXCL9 and CXCL10 chemokines and thereby recruitment of $CD8^+$ T cells expressing CXCR3, which are without doubt responsible for the loss of melanocytes and 2) its direct effect together with TNFα on melanocyte, through induction of melanocyte detachment from the basal layer of the epidermis (43). We recently highlighted that type 1 innate lymphoid cells (ILC1) are also poised to release IFNγ (37).

NK Cells

NK cells are described as a bridge between innate and adaptive immune system. They are characterized by their early and potent production of IFNγ. As discussed above, the transcriptional data supporting role of innate immunity in vitiligo is primarily based on differential gene expression associated with NK cell function, activity, and cytotoxicity (10, 11). It has been known for almost 30 years that there is an increase in circulating NK cells in the blood of vitiligo patients with abnormalities in their expression of inhibitory receptor CD158a and their activity (44–47), yet their role in vitiligo skin remained unexplored until recently. We have now confirmed the increased number of cytotoxic NK cells in not only the blood but also in the skin of vitiligo patients compared to healthy controls, predominantly in non-lesional skin (37). Furthermore, vitiligo NK cells are much more sensitive to stress, produce much larger amounts of IFNγ following stress, and are directly implicated in initiation of long-term adaptive immunity against melanocytes (37).

Innate Lymphoid Cells

Innate lymphoid cells (ILCs) are the innate counterparts of T cells. In response to IL-12, IL-15, and IL-18, they secrete IFNγ; a signature vitiligo cytokine. We have recently demonstrated increased presence of ILC1 (but not ILC2 or ILC3) in vitiligo blood and skin and these cells to be the initial source of IFNγ, which is involved in early melanocyte apoptosis and subsequent T-cell mediated destruction of melanocytes (37).

Melanocytes

It has been known for a long time that melanocytes from vitiligo patients are intrinsically abnormal and are more sensitive to external stress (48, 49), however this defect alone doesn't explain the disease pathology as stressed melanocytes remain viable. As shown in a chicken model of spontaneous vitiligo, innate immunity is an important link between melanocyte stress and long-term adaptive immunity (50). Melanocytes have also been proposed as immunocompetent cells being able to process and present antigen, upregulate their own co-stimulatory markers and directly stimulate cytotoxic T lymphocytes following IFNγ stimulation (51, 52). We have recently shown that human melanocytes express chemokine receptor of the B-isoform (CXCR3B), whose expression is upregulated in vitiligo melanocytes compared to healthy melanocytes and this receptor to play a critical role in anti-melanocyte immunity in vitiligo (37).

Together, recent literature highlighted that innate immune pDCs, NK cells and ILC1 are capable of directly responding to stressed melanocytes and are critical in initiation of the disease, making these cells ideal primary target for therapeutic intervention.

THERAPEUTIC PERSPECTIVES

Vitiligo is a chronic inflammatory skin disorder and future therapeutic strategies might consider targeting the innate immunity side of the disease to halt initiation and/or progression of the disease, but such approach could also be envisioned as a maintenance therapy to prevent relapse.

Topical or systemic immunosuppressive drugs that are actually used for treating vitiligo such as corticosteroids, methotrexate or calcineurin inhibitors, have some potential impact on the innate immune response (53, 54). However, these agents have a broad impact on innate and adaptive immunity. Future approaches targeting more specifically the pathways involved in vitiligo could provide better responses with safer profile.

The elicitation of DAMPs depends on endoplasmic reticulum stress and oxidative stress. Many studies have described the role of the oxidative stress in vitiligo and how it can trigger the immune response (55–58). However, the efficacy of antioxidants in treating vitiligo is still a matter of debate as it relies on inconclusive studies or studies with contradictory results (59). This discrepancy between robust fundamental evidences and questionable clinical data could be explained by the differences in the type of antioxidant therapies used. More effective antioxidants with better bioavailability could effectively reduce the oxidative stress in the skin and provide a useful approach in treating vitiligo. There is increasing evidence showing mitochondrial alterations with increased production of ROS in vitiligo skin (60, 61). Compounds protecting specifically against this kind of mitochondrial damages could be of great interest in treating or preventing vitiligo relapses. Inhibition of DAMPs released by epidermal cells could also represent an interesting approach to prevent activation of innate cells. Indeed, mutant HSP70i have been shown to prevent auto-immune depigmentation or induce repigmentation both in mouse and Sinclair swine models (21, 22).

As detailed above, bacteria are major producers of PAMPs and alteration of skin and gut microbiome could participate in activation of the innate immune response in vitiligo (31–33). Modulating the skin or gut microbiome appears as an appealing approach. Recent data conducted in atopic dermatitis skin, demonstrated that topical formulation containing specific strains of probiotics could improve skin lesions (62). Additional studies are urgently needed, especially those in vitiligo, but modulation of microbiome, using prebiotics, probiotics, postbiotics, or fecal microbiota transplantation, could be an alternative approach for secondary prevention in vitiligo.

The development of antibodies targeting specifically the B isoform of CXCR3, could prevent the initial apoptosis of melanocytes and thus could be an effective preventive approach (37). Another strategy could rely on direct action on innate cells themselves. BDCA2 is a C-type lectin specifically expressed on pDCs, whose engagement inhibits the release of IFNα. Of interest, the use of a monoclonal antibody targeting BDCA2 showed improvement of skin lesions in systemic lupus erythematosus (63), however no preclinical studies have been performed so far to evaluate the efficacy of such strategy in vitiligo. In line with the important role of IFNα in disease pathogenesis, hydroxychoroquine, a TLR7 and TLR9 inhibitor downregulating IFNα production by pDCs, was shown to induce repigmentation of vitiligo lesions in a clinical case reports (64, 65). Whether inhibition of IFNα or its receptor could be an alternative strategy to block the type I IFN pathway in vitiligo has not yet been assessed.

NKG2D is one of the most frequent allelic variation found in vitiligo population. It also regulates both NK and T cell responses and thus, targets both innate and adaptive immune responses (66), and has been involved in vitiligo pathogenesis (67, 68). The use of anti-NKG2D antibodies could be a very promising approach for treating vitiligo.

In line with a therapy that would dampen both innate and adaptive immune response, IL-15 could represent another attractive strategy. Indeed, this cytokine is important for both T cells and NK cells maintenance and function. Recent studies highlighted the role of IL-15 on resident memory T cells in vitiligo pathogenesis and the interest to inhibit this cytokine or its receptor in vitiligo (68–70). Clinical phase II study is about to start, evaluating the efficacy of AMG714 for treatment of vitiligo (NCT04338581). Whether such targeting would also impact the innate response remains to be evaluated.

Targeting multiple cytokine pathways with JAK inhibitors is showing promising clinical outcome in vitiligo patients, as shown with the use of tofacitinib (blocking JAK1/3) or ruxolitinib (blocking JAK1/2) (71–73). Besides targeting IFNγ signaling, such therapies will also likely block IFNα impact on epidermal cells, as this cytokine signals through JAK1/TYK2. Interestingly, a phase 2 clinical trial evaluating the efficacy of systemic administration of a JAK1/TYK2 inhibitor is ongoing (NCT03715829) and will provide new insights into the physiopathology of vitiligo.

CONCLUSION

Innate immunity has long time been overlooked in autoimmune disorders, including in vitiligo. However, from genetic and transcriptome data to modulation of key innate cells in vitiligo skin and blood, there are now accumulating and strong evidences supporting the key role of the innate immunity in pathogenesis of vitiligo (**Figure 1**). Activated by the PAMPs and DAMPs, the innate immunity appears as the bridge between potential triggering factors of vitiligo flares such as stress, Koebner phenomenon or infections, and the secondary activation of the adaptative immune response. These data foster new therapeutic

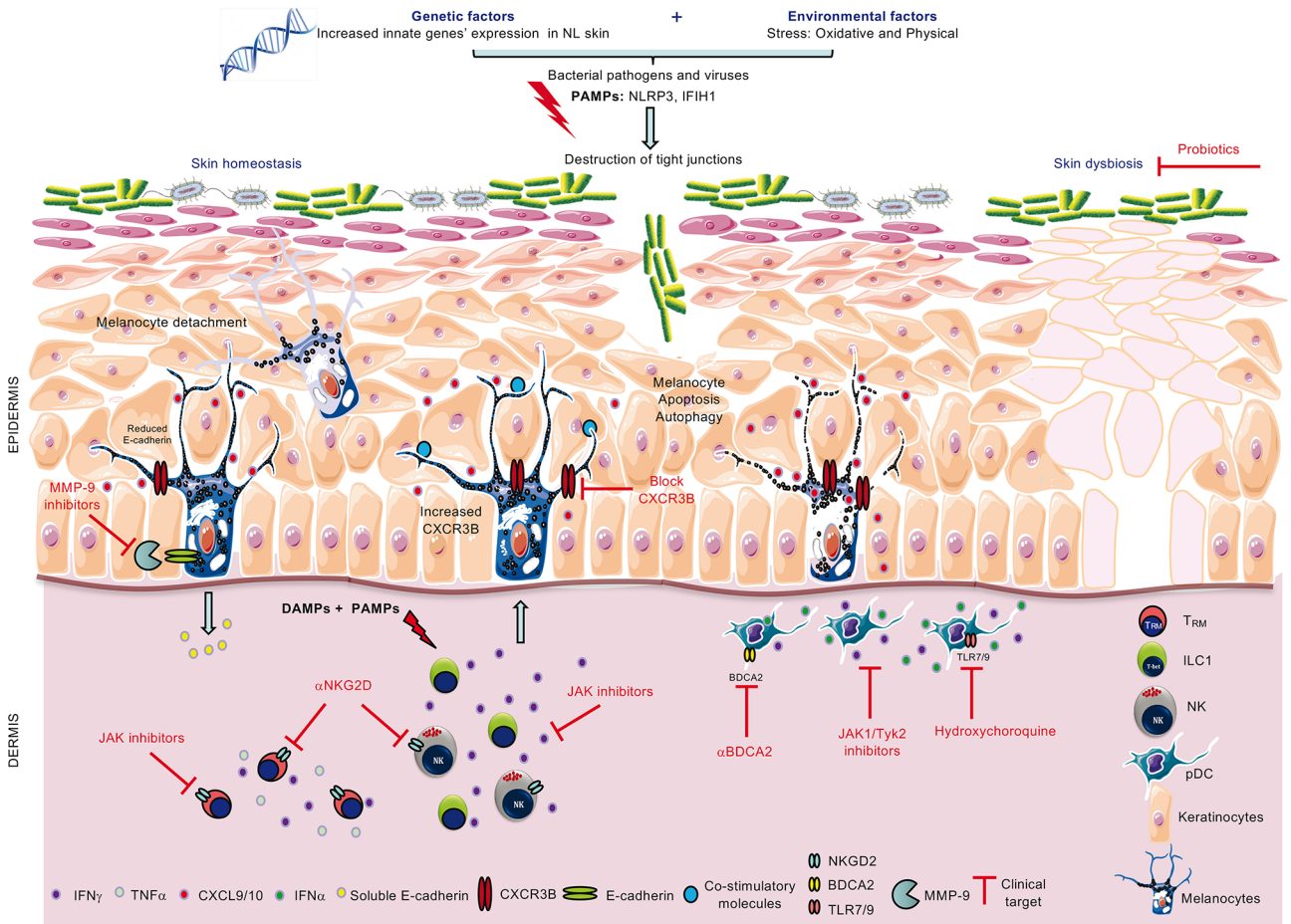


FIGURE 1 | Role of innate immunity in loss of melanocytes and therapeutic strategies to combat the disease. Combined genetic and environmental (PAMPs) or endogenous (DAMPs) stress activates innate immune cells ILC1/NK and pDCs to increase their production of IFNγ and IFNα, respectively. In turn, IFNγ is involved in melanocyte detachment from the basal layer of the epidermis and upregulates melanocytic CXCR3B expression. In some melanocytes, CXCR3B activation induces melanocyte apoptosis which triggers increased expression of co-stimulatory molecules in other melanocytes and subsequent recruitment of cytotoxic T cells. Possible clinical therapeutic targets include the use of anti-CXCR3B (to block initial apoptosis), JAK inhibitors (to inhibit cytokine release from ILC1, pDCs and memory T cells, T_{RM}), anti-NKG2D (to block activating NKG2D receptor on NK and T_{RM}), anti-BDCA2 and hydroxychloroquine (to inhibit IFNα production from pDCs) and probiotics to restore skin homeostasis.

opportunities for vitiligo treatment but also for primary and secondary prevention. It will also be important to further characterize the role of the innate immune response in preventing repigmentation in patients with a stable disease.

AUTHOR CONTRIBUTIONS

KB, TP, JS, and MKT wrote the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Boniface K, Seneschal J, Picardo M, Taïeb A. Vitiligo: Focus on Clinical Aspects, Immunopathogenesis, and Therapy. *Clin Rev Allergy Immunol* (2018) 54:52–67. doi: 10.1007/s12016-017-8622-7
2. Picardo M, Dell'Anna ML, Ezzedine K, Hamzavi I, Harris JE, Parsad D, et al. Vitiligo. *Nat Rev Dis Primers* (2015) 1:15011. doi: 10.1038/nrdp.2015.11
3. Willemsen M, Linkutė R, Luiten RM, Matos TR. Skin-resident memory T cells as a potential new therapeutic target in vitiligo and melanoma. *Pigment Cell Melanoma Res* (2019) 32:612–22. doi: 10.1111/pcmr.12803
4. Riding RL, Harris JE. The Role of Memory CD8+ T Cells in Vitiligo. *J Immunol* (2019) 203:11–9. doi: 10.4049/jimmunol.1900027
5. Roberts GHL, Santorico SA, Spritz RA. The genetic architecture of vitiligo. *Pigment Cell Melanoma Res* (2020) 33:8–15. doi: 10.1111/pcmr.12848
6. Jin Y, Santorico SA, Spritz RA. Pediatric to Adult Shift in Vitiligo Onset Suggests Altered Environmental Triggering. *J Invest Dermatol* (2020) 140:241–243.e4. doi: 10.1016/j.jid.2019.06.131
7. Spritz RA, Santorico SA. The Genetic Basis of Vitiligo. *J Invest Dermatol* (2020) 41(2):265–73. doi: 10.1016/j.jid.2020.06.004
8. Jin Y, Andersen G, Yorgov D, Ferrara TM, Ben S, Brownson KM, et al. Genome-wide association studies of autoimmune vitiligo identify 23 new risk

loci and highlight key pathways and regulatory variants. *Nat Genet* (2016) 48:1418–24. doi: 10.1038/ng.3680
9. Jin Y, Birlea SA, Fain PR, Ferrara TM, Ben S, Riccardi SL, et al. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. *Nat Genet* (2012) 44:676–80. doi: 10.1038/ng.2272
10. Yu R, Broady R, Huang Y, Wang Y, Yu J, Gao M, et al. Transcriptome analysis reveals markers of aberrantly activated innate immunity in vitiligo lesional and non-lesional skin. *PloS One* (2012) 7:e51040. doi: 10.1371/journal.pone.0051040
11. Regazzetti C, Joly F, Marty C, Rivier M, Mehul B, Reiniche P, et al. Transcriptional Analysis of Vitiligo Skin Reveals the Alteration of WNT Pathway: A Promising Target for Repigmenting Vitiligo Patients. *J Invest Dermatol* (2015) 135:3105–14. doi: 10.1038/jid.2015.335
12. Jadeja SD, Mayatra JM, Vaishnav J, Shukla N. Begum R. A Concise Review on the Role of Endoplasmic Reticulum Stress in the Development of Autoimmunity in Vitiligo Pathogenesis. *Front Immunol* (2020) 11:624566. doi: 10.3389/fimmu.2020.624566
13. Kim JY, Lee EJ, Seo J, Oh SH. Impact of high-mobility group box 1 on melanocytic survival and its involvement in the pathogenesis of vitiligo. *Br J Dermatol* (2017) 176:1558–68. doi: 10.1111/bjd.15151
14. Becatti M. Oxidative stress and high-mobility group box 1 (HMGB1) protein release in vitiligo. *Br J Dermatol* (2017) 176:1436–7. doi: 10.1111/bjd.15538
15. Mou K, Liu W, Miao Y, Cao F, Li P. HMGB1 deficiency reduces H2 O2 -induced oxidative damage in human melanocytes *via the* Nrf2 pathway. *J Cell Mol Med* (2018) 22:6148–56. doi: 10.1111/jcmm.13895
16. Cui T, Zhang W, Li S, Chen X, Chang Y, Yi X, et al. Oxidative Stress-Induced HMGB1 Release from Melanocytes: A Paracrine Mechanism Underlying the Cutaneous Inflammation in Vitiligo. *J Invest Dermatol* (2019) 139:2174–2184.e4. doi: 10.1016/j.jid.2019.03.1148
17. Zhang Y, Liu L, Jin L, Yi X, Dang E, Yang Y, et al. Oxidative stress-induced calreticulin expression and translocation: new insights into the destruction of melanocytes. *J Invest Dermatol* (2014) 134:183–91. doi: 10.1038/jid.2013.268
18. Mosenson JA, Flood K, Klarquist J, Eby JM, Koshoffer A, Boissy RE, et al. Preferential secretion of inducible HSP70 by vitiligo melanocytes under stress. *Pigment Cell Melanoma Res* (2014) 27:209–20. doi: 10.1111/pcmr.12208
19. 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. doi: 10.1111/exd.12183
20. Denman CJ, McCracken J, Hariharan V, Klarquist J, Oyarbide-Valencia K, Guevara-Patiño JA, et al. HSP70i accelerates depigmentation in a mouse model of autoimmune vitiligo. *J Invest Dermatol* (2008) 128:2041–8. doi: 10.1038/jid.2008.45
21. 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. doi: 10.1126/scitranslmed.3005127
22. Henning SW, Fernandez MF, Mahon JP, Duff R, Azarafrooz F, Guevara-Patiño JA, et al. HSP70iQ435A-Encoding DNA Repigments Vitiligo Lesions in Sinclair Swine. *J Invest Dermatol* (2018) 138:2531–9. doi: 10.1016/j.jid.2018.06.186
23. Doss RW, El-Rifaie A-AA, Abdel-Wahab AM, Gohary YM, Rashed LA. Heat Shock Protein-70 Expression in Vitiligo and its Relation to the Disease Activity. *Indian J Dermatol* (2016) 61:408–12. doi: 10.4103/0019-5154.185704
24. Traks T, Keermann M, Karelson M, Rätsep R, Reimann E, Silm H, et al. Polymorphisms in Toll-like receptor genes are associated with vitiligo. *Front Genet* (2015) 6:278. doi: 10.3389/fgene.2015.00278
25. Karaca N, Ozturk G, Gerceker BT, Turkmen M, Berdeli A. TLR2 and TLR4 gene polymorphisms in Turkish vitiligo patients. *J Eur Acad Dermatol Venereol* (2013) 27:e85–90. doi: 10.1111/j.1468-3083.2012.04514.x
26. Li S, Kang P, Zhang W, Jian Z, Zhang Q, Yi X, et al. Activated NLR family pyrin domain containing 3 (NLRP3) inflammasome in keratinocytes promotes cutaneous T-cell response in patients with vitiligo. *J Allergy Clin Immunol* (2020) 145:632–45. doi: 10.1016/j.jaci.2019.10.036
27. Levandowski CB, Mailloux CM, Ferrara TM, Gowan K, Ben S, Jin Y, et al. NLRP1 haplotypes associated with vitiligo and autoimmunity increase interleukin-1β processing *via the* NLRP1 inflammasome. *Proc Natl Acad Sci USA* (2013) 110:2952–6. doi: 10.1073/pnas.1222808110
28. Mogensen TH, Paludan SR. Reading the viral signature by Toll-like receptors and other pattern recognition receptors. *J Mol Med (Berl)* (2005) 83:180–92. doi: 10.1007/s00109-004-0620-6
29. Dwivedi M, Laddha NC, Begum R. Viral causes of Vitiligo: A New Perspective for Vitiligo Pathogenesis. *Virol Immunol J* (2018) 2:000181. doi: 10.23880/VIJ-16000181
30. Zhuang T, Yi X, Chen J, Kang P, Chen X, Chen J, et al. Intracellular virus sensor MDA5 exacerbates vitiligo by inducing the secretion of chemokines in keratinocytes under virus invasion. *Cell Death Dis* (2020) 11:453. doi: 10.1038/s41419-020-2665-z
31. Ganju P, Nagpal S, Mohammed MH, Nishal Kumar P, Pandey R, Natarajan VT, et al. Microbial community profiling shows dysbiosis in the lesional skin of Vitiligo subjects. *Sci Rep* (2016) 6:18761. doi: 10.1038/srep18761
32. Dellacecca ER, Cosgrove C, Mukhatayev Z, Akhtar S, Engelhard VH, Rademaker AW, et al. Antibiotics Drive Microbial Imbalance and Vitiligo Development in Mice. *J Invest Dermatol* (2020) 140:676–687.e6. doi: 10.1016/j.jid.2019.08.435
33. Bzioueche H, Simonyte Sjodin K, West C, Khemis A, Passeron T, Tulik MK. Analysis of Matched Skin and Gut Microbiome of Vitiligo Patients Reveals Deep Skin Dysbiosis: Link With Mitochondrial and Immune Changes. *J Invest Dermatol* (2021) S0022-202X(21)01006-X. doi: 10.1016/j.jid.2021.01.036
34. Wang CQF, Cruz-Inigo AE, Fuentes-Duculan J, Moussai D, Gulati N, Sullivan-Whalen M, et al. Th17 cells and activated dendritic cells are increased in vitiligo lesions. *PloS One* (2011) 6:e18907. doi: 10.1371/journal.pone.0018907
35. Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J Pathol* (1996) 148:1219–28.
36. Bertolotti A, Boniface K, Vergier B, Mossalayi D, Taieb A, Ezzedine K, et al. Type I interferon signature in the initiation of the immune response in vitiligo. *Pigment Cell Melanoma Res* (2014) 27:398–407. doi: 10.1111/pcmr.12219
37. Tulic MK, Cavazza E, Cheli Y, Jacquel A, Luci C, Cardot-Leccia N, et al. Innate lymphocyte-induced CXCR3B-mediated melanocyte apoptosis is a potential initiator of T-cell autoreactivity in vitiligo. *Nat Commun* (2019) 10:2178. doi: 10.1038/s41467-019-09963-8
38. Serarslan G, Yönden Z, Söğüt S, Savaş N, Celik E, Arpaci A. Macrophage migration inhibitory factor in patients with vitiligo and relationship between duration and clinical type of disease. *Clin Exp Dermatol* (2010) 35:487–90. doi: 10.1111/j.1365-2230.2009.03617.x
39. Ma L, Xue H-B, Guan X-H, Shu C-M, Zhang Y-J, Zhang J-H, et al. Relationship of macrophage migration inhibitory factor levels in PBMCs, lesional skin and serum with disease severity and activity in vitiligo vulgaris. *Braz J Med Biol Res* (2013) 46:460–4. doi: 10.1590/S0100-879X2012007500152
40. Farag AGA, Hammam MA, Habib MS, Elnaidany NF, Kamh ME. Macrophage migration inhibitory factor as an incriminating agent in vitiligo. *Bras Dermatol* (2018) 93:191–6. doi: 10.1590/abd1806-4841.20186068
41. Conrad C, Gilliet M. Type I IFNs at the interface between cutaneous immunity and epidermal remodeling. *J Invest Dermatol* (2012) 132:1759–62. doi: 10.1038/jid.2012.149
42. Jacquemin C, Rambert J, Guillet S, Thiolat D, Boukhedouni N, Doutre M-S, et al. Heat shock protein 70 potentiates interferon alpha production by plasmacytoid dendritic cells: relevance for cutaneous lupus and vitiligo pathogenesis. *Br J Dermatol* (2017) 177:1367–75. doi: 10.1111/bjd.15550
43. Boukhedouni N, Martins C, Darrigade A-S, Drullion C, Rambert J, Barrault C, et al. Type-1 cytokines regulate MMP-9 production and E-cadherin disruption to promote melanocyte loss in vitiligo. *JCI Insight* (2020) 5(11): e133772. doi: 10.1172/jci.insight.133772
44. Halder RM, Walters CS, Johnson BA, Chakrabarti SG, Kenney JA. Aberrations in T lymphocytes and natural killer cells in vitiligo: a flow cytometric study. *J Am Acad Dermatol* (1986) 14:733–7. doi: 10.1016/s0190-9622(86)70085-6
45. Basak PY, Adiloglu AK, Koc IG, Tas T, Akkaya VB. Evaluation of activatory and inhibitory natural killer cell receptors in non-segmental vitiligo: a flow cytometric study. *J Eur Acad Dermatol Venereol* (2008) 22:970–6. doi: 10.1111/j.1468-3083.2008.02681.x
46. Mozzanica N, Frigerio U, Negri M, Tadini G, Villa ML, Mantovani M, et al. Circadian rhythm of natural killer cell activity in vitiligo. *J Am Acad Dermatol* (1989) 20:591–6. doi: 10.1016/s0190-9622(89)70069-4
47. Mozzanica N, Villa ML, Foppa S, Vignati G, Cattaneo A, Diotti R, et al. Plasma alpha-melanocyte-stimulating hormone, beta-endorphin, met-

enkephalin, and natural killer cell activity in vitiligo. *J Am Acad Dermatol* (1992) 26:693–700. doi: 10.1016/0190-9622(92)70094-v
48. Jimbow K, Chen H, Park JS, Thomas PD. Increased sensitivity of melanocytes to oxidative stress and abnormal expression of tyrosinase-related protein in vitiligo. *Br J Dermatol* (2001) 144:55–65. doi: 10.1046/j.1365-2133.2001.03952.x
49. Maresca V, Roccella M, Roccella F, Camera E, Del Porto G, Passi S, et al. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. *J Invest Dermatol* (1997) 109:310–3. doi: 10.1111/1523-1747.ep12335801
50. Shi F, Kong B-W, Song JJ, Lee JY, Dienglewicz RL, Erf GF. Understanding mechanisms of vitiligo development in Smyth line of chickens by transcriptomic microarray analysis of evolving autoimmune lesions. *BMC Immunol* (2012) 13:18. doi: 10.1186/1471-2172-13-18
51. Lu Y, Zhu W-Y, Tan C, Yu G-H, Gu J-X. Melanocytes are potential immunocompetent cells: evidence from recognition of immunological characteristics of cultured human melanocytes. *Pigment Cell Res* (2002) 15:454–60. doi: 10.1034/j.1600-0749.2002.02065.x
52. Le Poole IC, Mutis T, van den Wijngaard RM, Westerhof W, Ottenhoff T, de Vries RR, et al. A novel, antigen-presenting function of melanocytes and its possible relationship to hypopigmentary disorders. *J Immunol* (1993) 151:7284–92.
53. Passeron T. Medical and Maintenance Treatments for Vitiligo. *Dermatol Clin* (2017) 35:163–70. doi: 10.1016/j.det.2016.11.007
54. Chang H-C, Hsu Y-P, Huang Y-C. The effectiveness of topical calcineurin inhibitors compared with topical corticosteroids in the treatment of vitiligo: A systematic review and meta-analysis. *J Am Acad Dermatol* (2020) 82:243–5. doi: 10.1016/j.jaad.2019.07.108
55. Dell'Anna ML, Ottaviani M, Bellei B, Albanesi V, Cossarizza A, Rossi L, et al. Membrane lipid defects are responsible for the generation of reactive oxygen species in peripheral blood mononuclear cells from vitiligo patients. *J Cell Physiol* (2010) 223:187–93. doi: 10.1002/jcp.22027
56. Passeron T, Ortonne J-P. Activation of the unfolded protein response in vitiligo: the missing link? *J Invest Dermatol* (2012) 132:2502–4. doi: 10.1038/jid.2012.328
57. Schallreuter KU, Salem MAEL, Holtz S, Panske A. Basic evidence for epidermal H2O2/ONOO(-)-mediated oxidation/nitration in segmental vitiligo is supported by repigmentation of skin and eyelashes after reduction of epidermal H2O2 with topical NB-UVB-activated pseudocatalase PC-KUS. *FASEB J* (2013) 27:3113–22. doi: 10.1096/fj.12-226779
58. Schallreuter KU, Moore J, Wood JM, Beazley WD, Gaze DC, Tobin DJ, et al. In vivo and *in vitro* evidence for hydrogen peroxide (H2O2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase. *J Investig Dermatol Symp Proc* (1999) 4:91–6. doi: 10.1038/sj.jidsp.5640189
59. Speeckaert R, Dugardin J, Lambert J, Lapeere H, Verhaeghe E, Speeckaert MM, et al. Critical appraisal of the oxidative stress pathway in vitiligo: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol* (2018) 32:1089–98. doi: 10.1111/jdv.14792
60. Dell'Anna ML, Ottaviani M, Kovacs D, Mirabilii S, Brown DA, Cota C, et al. Energetic mitochondrial failing in vitiligo and possible rescue by cardiolipin. *Sci Rep* (2017) 7:13663. doi: 10.1038/s41598-017-13961-5
61. Sahoo A, Lee B, Boniface K, Seneschal J, Sahoo SK, Seki T, et al. MicroRNA-211 Regulates Oxidative Phosphorylation and Energy Metabolism in Human Vitiligo. *J Invest Dermatol* (2017) 137:1965–74. doi: 10.1016/j.jid.2017.04.025
62. Myles IA, Castillo CR, Barbian KD, Kanakabandi K, Virtaneva K, Fitzmeyer E, et al. Therapeutic responses to Roseomonas mucosa in atopic dermatitis may involve lipid-mediated TNF-related epithelial repair. *Sci Transl Med* (2020) 12 (560):eaaz8631. doi: 10.1126/scitranslmed.aaz8631
63. Furie R, Werth VP, Merola JF, Stevenson L, Reynolds TL, Naik H, et al. Monoclonal antibody targeting BDCA2 ameliorates skin lesions in systemic lupus erythematosus. *J Clin Invest* (2019) 129:1359–71. doi: 10.1172/JCI124466
64. Li D-G, Hu W-Z, Ma H-J, Liu W, Yang Q-Q, Zhao G. Hydroxychloroquine protects melanocytes from autoantibody-induced injury by reducing the binding of antigen-antibody complexes. *Mol Med Rep* (2016) 14:1275–82. doi: 10.3892/mmr.2016.5354
65. Joo K, Park W, Kwon SR, Lim MJ, Jung K-H. Improvement of vitiligo in a patient with rheumatoid arthritis after hydroxychloroquine treatment. *Int J Rheum Dis* (2015) 18:679–80. doi: 10.1111/1756-185X.12442
66. Wensveen FM, Jelenčić V, Polić B. NKG2D: A Master Regulator of Immune Cell Responsiveness. *Front Immunol* (2018) 9:441. doi: 10.3389/fimmu.2018.00441
67. Zloza A, Lyons GE, Chlewicki LK, Kohlhapp FJ, O'Sullivan JA, Lacek AT, et al. Engagement of NK receptor NKG2D, but not 2B4, results in self-reactive CD8+ T cells and autoimmune vitiligo. *Autoimmunity* (2011) 44:599–606. doi: 10.3109/08916934.2011.593599
68. Jacquemin C, Martins C, Lucchese F, Thiolat D, Taieb A, Seneschal J, et al. NKG2D Defines a Subset of Skin Effector Memory CD8 T Cells with Proinflammatory Functions in Vitiligo. *J Invest Dermatol* (2020) 140:1143–1153.e5. doi: 10.1016/j.jid.2019.11.013
69. Cheuk S, Schlums H, Gallais Sérézal I, Martini E, Chiang SC, Marquardt N, et al. CD49a Expression Defines Tissue-Resident CD8+ T Cells Poised for Cytotoxic Function in Human Skin. *Immunity* (2017) 46:287–300. doi: 10.1016/j.immuni.2017.01.009
70. Richmond JM, Strassner JP, Zapata L, Garg M, Riding RL, Refat MA, et al. Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo. *Sci Transl Med* (2018) 10(450):eaam7710. doi: 10.1126/scitranslmed.aam7710
71. Phan K, Phan S, Shumack S, Gupta M. Repigmentation in vitiligo using janus kinase (JAK) inhibitors with phototherapy: systematic review and Meta-analysis. *J Dermatolog Treat* (2020) 1–5. doi: 10.1080/09546634.2020.1735615
72. Relke N, Gooderham M. The Use of Janus Kinase Inhibitors in Vitiligo: A Review of the Literature. *J Cutan Med Surg* (2019) 23:298–306. doi: 10.1177/1203475419833609
73. Rosmarin D, Pandya AG, Lebwohl M, Grimes P, Hamzavi I, Gottlieb AB, et al. Ruxolitinib cream for treatment of vitiligo: a randomised, controlled, phase 2 trial. *Lancet* (2020) 396:110–20. doi: 10.1016/S0140-6736(20)30609-7

Conflict of Interest: JS has been an advisor, speaker or investigator for Abbvie, Calypso Biotech, Lilly, Novartis, Pierre Fabre, Sanofi-Genzyme. KB has been an advisor, investigator for Calypso Biotech.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership