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Brief Reviews

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Vitiligo is an autoimmune skin disease mediated by autoreactive CD8$^+$ T cells that destroy the pigment-producing cells of the epidermis, melanocytes, leading to areas of depigmentation. Patients with vitiligo require lifelong treatment to regain and maintain their pigment. Clinical observations uncovered the importance of autoimmune memory in vitiligo because cessation of treatment frequently led to relapse of disease at the site of previous lesions. A subset of memory T cells known as CD8$^+$ resident memory T cells (T_{RM}) are long-lived, nonmigratory memory cells that persist in most nonlymphoid tissues, including the skin. Recent reports describe the presence of CD8$^+$ T_{RM} in lesional vitiligo patient skin and suggest their role as active players in disease maintenance. In this review, we will discuss the role of skin CD8$^+$ T_{RM} in maintaining disease in vitiligo and the opportunity to target this population to induce a long-lasting reversal of disease. *The Journal of Immunology*, **2019, 203: 11–19.**

Vitiligo is an autoimmune skin disease in which melanocytes, the pigment-producing cells of the skin, are targeted for destruction by autoreactive CD8$^+$ T cells. As a result, patients develop patchy white spots on their skin. Vitiligo affects roughly 1% of the population, has no sex bias, and most patients are diagnosed before the age of 30.

Vitiligo: an introduction

Vitiligo has a significant impact on the patients' quality of life and self-esteem (1–7). Similar to many other autoimmune diseases, complex interactions among genetic, environmental, and stochastic factors contribute to vitiligo susceptibility (8, 9). Although it is still unclear how disease is initiated, intrinsic or extrinsic cellular stress may play a role (10). Melanocytes in healthy human skin are present in both the epidermis and the hair follicle to which they provide pigment (11). In the majority of vitiligo patients, only the epidermal melanocytes are targeted for destruction, and melanocytes present in the hair follicles remain unaffected, likely resulting from the mechanisms of immune privilege within the hair follicle (12). Because of this, vitiligo can be reversed by both suppressing the immune attack and by stimulating melanocyte precursors that live in the hair follicle to proliferate, migrate, and replenish lost epidermal melanocytes through a process known as perifollicular repigmentation (13, 14).

Conventional treatment uses a combination of topical corticosteroids or calcineurin inhibitors that broadly suppress the local immune response in the skin, together with narrow band UV light B therapy (nbUVB), which contributes to immunosuppression but also stimulates melanocyte regeneration from the hair follicles. Current therapy can be effective with up to 100% repigmentation possible but is often unpredictable, time consuming, and insufficient for many patients (15, 16). Studies reveal that not all vitiligo patients respond to nbUVB treatment, which highlights the need for better targeted therapies. Because hair follicles harbor the melanocyte precursors required for repigmentation, anatomical sites devoid of hair follicles, such as the fingertips, knuckles, ventral wrists, and elbows, often have poor treatment responses. Also, lesions in which the follicular melanocytes have been destroyed, resulting in white hair, often do not regain pigment following treatment.

Vitiligo is a chronic disease that requires lifelong therapy, and ∼40% of vitiligo patients relapse within 1 y after stopping treatment (16, 17). Clinical observations revealed that depigmented lesions return to the exact same location of a previously depigmented spot (17). These insights suggest that the formation of autoimmune memory plays an important role in the recurrence of vitiligo lesions.

CD8$^+$ T cells are sufficient to mediate melanocyte destruction

It is well established that CD8$^+$ T cells are both necessary and sufficient to mediate human vitiligo. Early studies showed that the number of HLA-A2 melanocyte-specific CD8$^+$ T cells in the blood of vitiligo patients correlated with disease severity and expressed high levels of the skin homing receptor, cutaneous lymphocyte-associated Ag (18). Furthermore, isolated melanocyte-specific CD8$^+$ T cells from vitiligo patients were able to lyse HLA-A2–matched peptide pulsed cells and melanoma cells ex vivo, whereas nonspecific CD8$^+$ T cells had no cytolytic ability. Examination of vitiligo patient skin cells using suction blistering found that the number of CD8$^+$ T cells is significantly increased in active disease compared with stable, nonlesional, and healthy control skin (19). Elegant studies showed that perilesional CD8$^+$ T cells isolated

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Abbreviations used in this article: nbUVB, narrow band UV light B therapy; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; Treg, regulatory T cell; T_{RM}, resident memory T cell; VACV, vaccinia virus.

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from vitiligo skin could kill melanocytes from normal pigmented skin isolated from the same patient when cultured ex vivo, demonstrating that melanocyte-specific $CD8^+$ T cells are both necessary and sufficient for the destruction of melanocytes (20). A better understanding of the development, formation, and survival of memory $CD8^+$ T cells in vitiligo is important to understand their role in the recurrence of vitiligo lesions.

Memory $CD8^+$ T cell subsets

Much of what we know about the generation of skin memory $CD8^+$ T cells comes from studies in mouse models of viral infections. Naive T cells circulate between the blood and secondary lymphoid organs because of their expression of CD62L, also known as L-selectin, and the chemokine receptor CCR7, which allow their entry into the lymph nodes through high endothelial venules (21, 22). Naive T cells are generally not found in peripheral tissues and require recognition of Ag, costimulation, and activation by cytokines to enter nonlymphoid tissues (23, 24). Activation of naive T cells stimulates reprogramming into effector T cells, which includes upregulation of various adhesion molecules and tissue-specific chemokine receptors required to position effector cells at the site of infection (25, 26). Following the clearance of a pathogen or resolution of inflammation, a population of Ag-experienced T cells remain in the host as memory T cells to protect against reinfection (27, 28). Peripheral tissues such as the skin are considered restrictive, meaning that effector and memory $CD8^+$ T cells do not enter the tissue in the steady-state (29, 30). Thus, the skin becomes accessible to effector $CD8^+$ T cells only after local inflammation and induction of inflammatory chemokines that trigger T cell recruitment.

Memory T cells are divided into different subsets based on their patterns of migration. Circulating memory T cells expressing high levels of CD62L and CCR7 are known as central memory T cells (T_{CM}), trafficking between the blood and secondary lymph organs. Memory T cells that lack expression of these lymphoid homing molecules and also express effector molecules upon stimulation are called effector memory T cells (T_{EM}) (31). T_{EM} cells are recruited to peripheral tissues to fight infection but can return back to the circulation upon upregulation of CCR7, which permits their exit from the tissue (32, 33). Studies suggest that T_{EM} are seemingly in constant transit between the circulation and peripheral tissues and are responsible for continued immunosurveillance (34, 35). Elegant work originally identified a subset of memory T cells that persist in the peripheral tissue without any replenishment of T cells from the circulation (36). These cells were labeled resident memory T cells (T_{RM}), defined as nonmigratory, tissue-resident cells. T_{RM} have been found in most peripheral tissues, including the gut, lung, reproductive tract, and skin, where they are positioned to provide robust protection against reinfection with pathogens (36–38).

Skin T_{RM}

Healthy human skin contains a surprisingly large number of T cells, roughly 20 billion, with 80% of T cells identified as $CD45RO^+$ memory T cells (39). The majority of memory T cells in healthy skin tissue are $CD62L^-CCR7^-$ T_{EM}, and T_{CM} make up less than 20% of T cells in resting skin. Of the memory T cells present in healthy skin tissue, between 20 and 60% are T_{RM} (40, 41), revealing that the percentage of T_{RM} is highly variable among healthy individuals. Healthy human skin is populated by both $CD4^+$ and $CD8^+$ T_{RM} populations, which are enriched in the epidermis, compared with the dermal skin compartment (40, 41).

$CD8^+$ T_{RM} are positioned at the basement membrane between basal keratinocytes (41) and are characterized by the expression of CD103, a subunit of the $\alpha_E\beta_7$ integrin receptor that binds to E-cadherin expressed on cells of the epidermis (36), as well as the activation marker CD69. CD69 associates with the sphingosine 1-phosphate receptor 1 required for lymphocyte egress from peripheral tissues and promotes its internalization and degradation, thereby maintaining T cell residence in the skin (42). Upregulation of CD103 and CD69 on T_{RM} is important for their development and survival in the skin because deletion of CD103 or CD69 on virus-specific T cells led to a significant reduction in T_{RM} numbers in the skin following virus infection (43). Additional local signals including TGF-β, which promotes CD103 expression, and IL-15 signaling are also important for their development and survival (44, 45).

Directly targeting T_{RM} in vitiligo is of interest after multiple reports reveal the importance of T_{RM} in promoting allergic responses, inflammation, and autoimmune disease (46–50). Early clues that skin T_{RM} may induce pathogenic immune responses come from clinical observations. For example, fixed drug eruption is a $CD8^+$ T cell–mediated allergic reaction of the skin following exposure to a drug, which resolves when the drug is discontinued. However, if the drug is taken again years or even decades later, the inflammation rapidly reappears in the same location. It is thought that the recurrent skin inflammation is a result of a persisting population of drug-reactive $CD8^+$ T cells located at the site of the first encounter with the drug (51). Disease relapse is also seen in patients with the inflammatory skin disease psoriasis (47, 52) and in vitiligo (17). Because vitiligo is a chronic disease, continuous treatment is necessary to regain and maintain pigment. Disease relapse in vitiligo suggests that autoimmune memory forms within lesions and is involved in their recurrence. A better understanding of the signals driving immune memory formation, maintenance, and activation in vitiligo will provide insights into more durable treatments.

IFN-γ–CXCR3–CXCL9/10 axis drives vitiligo

Our laboratory and others have focused on understanding the pathways driving vitiligo pathogenesis as well as the signals responsible for the recruitment, positioning, and survival of melanocyte-specific $CD8^+$ T cells in the skin. Early clinical studies showed that production of the proinflammatory cytokines IFN-γ and TNF-α by $CD8^+$ T cells isolated from perilesional vitiligo skin positively correlated with disease severity and could predict the success of nbUVB therapy (20). Likewise, gene expression analysis of vitiligo lesional skin revealed an IFN-γ–specific gene signature and no upregulation of IL-17 transcripts (53–57). These human studies point to IFN-γ as the central cytokine in disease, and mechanistic studies in mice support this hypothesis (53, 58). The IFN-γ signature in mice parallels that seen in human patient skin. Affected skin in mice induces a significant increase in IFN-γ,

and melanocyte-specific CD8$^+$ T cells from vitiligo mice produce IFN-γ after stimulation with melanocyte Ag. Most importantly, vitiligo progression is dependent on IFN-γ as a therapeutic blockade of IFN-γ significantly reduced the severity of disease (53).

IFN-γ stimulates transcription of the chemokine ligands CXCL9, CXCL10, and CXCL11, which drive the migration of immune cells into tissues in many type 1 inflammatory diseases and infections. All three chemokine ligands bind to the shared receptor, CXCR3 (59, 60), which is expressed on activated immune cells, including effector CD4$^+$ T cells, CD8$^+$ T cells, and NK cells (59). Expression of CXCR3 on melanocyte-specific CD8$^+$ T cells in vitiligo is required for skin tissue homing because CXCR3-deficient CD8$^+$ T cells are unable to mediate vitiligo in mice (54). This CXCR3-dependent migration is a result of the upregulation of CXCL9 and CXCL10 in the skin of vitiligo mice (61). Treatment of mice with CXCR3-depleting Abs both prevented and reversed disease (62). In vitiligo patients, the majority of CD8$^+$ T cells in lesional skin express CXCR3 (40, 63, 64), and the chemokine ligands CXCL9 and CXCL10 are enriched within lesional skin compared with nonlesional and healthy control skin (19, 54). These studies establish the IFN-γ–CXCR3–CXCL9/10 axis as the central pathway in mediating the recruitment of CD8$^+$ T cells in mouse and human vitiligo.

The functional roles of CXCL9 and CXCL10 in vitiligo

Additionally, mice that report the expression of CXCL9 and CXCL10 were used to determine the kinetics and source of CXCR3 ligands in epidermal vitiligo skin (61). Global epidermal expression of CXCL9 followed a bimodal pattern; CXCL9 was maximally upregulated early after disease induction and then again at the peak of disease (61). Epidermal CXCL10 expression gradually increased over the duration of disease with the highest expression at the peak of the immune response in vitiligo (61). Induction of vitiligo in CXCL9-deficient mice led to a significant reduction of melanocyte-specific CD8$^+$ T cells in both the epidermal and dermal skin compartments, suggesting the importance of CXCL9 for early recruitment of CD8$^+$ T_{EM} cells to the skin and the development of new lesions (54). However, CXCL9-deficient mice still developed vitiligo as the few melanocyte-specific CD8$^+$ T cells that did make it into the skin were evidently sufficient to induce disease (54). Interestingly, CXCL10-deficient mice and those treated with CXCL10 Ab did not show significant defects in bulk recruitment of melanocyte-specific CD8$^+$ T_{EM} cells into the dermis, but CD8$^+$ T_{EM} did not efficiently reach the epidermis, which may be partly due to low CD44 expression (54). CD44 is important for memory T cell survival, activation, and directed migration through the basement membrane (65). As a result, CXCL10-deficient mice were protected from vitiligo progression. In addition, vitiligo could be reversed in mice by treatment with a CXCL10-neutralizing Ab (54). These results suggest that CXCL10 is critical in both disease progression and maintenance, playing an active role in the directed migration and tethering of CD8$^+$ memory T cells in the epidermis as well as possibly modulating their effector function.

A recent study by our group used suction blistering of human vitiligo skin to measure IFN-γ–induced chemokine expression in situ (19). We found that active vitiligo patient skin contains significantly higher levels of CXCL9 protein than nonlesional and stable skin from vitiligo patients as well as healthy control skin. In fact, the presence of CXCL9 protein in the skin is sensitive and specific for disease activity and may serve as a biomarker of early treatment responses.

CD8$^+$ T_{RM} in mouse models of vitiligo

In a mouse model of vitiligo (53, 58), transferred naive melanocyte-specific CD8$^+$ T cells are activated and recruited to the skin through expression of CXCR3 ligands. T_{EM} traffic to the skin and kill epidermal melanocytes, which leads to patchy white depigmentation on the tail, ears, nose, and footpads (53). We found that T_{RM} seed peripheral tissues during the effector phase of the immune response, which peaks at 7 wk in mice. At this point, ∼60–90% of the melanocyte-specific CD8$^+$ T cells express the canonical T_{RM} markers CD69 and CD103 (63). T_{RM} in vitiligo mice persist in the epidermis and dermis for over a year and are enriched within the epidermis (63). It remains unknown exactly how T_{RM} are generated during vitiligo, but multiple reports in infection models suggest that KLRG1$^-$ precursors give rise to T_{RM} and that dendritic cell signals during cross-priming, including IL-2 and IL-15, direct their differentiation into T_{RM} (43, 66, 67). The tissue microenvironment also helps shape T_{RM} development because hair follicle and keratinocyte derived IL-7 and IL-15 are critical for the maintenance of T_{RM} (68) as is TGF-β (44).

Epidermal skin T_{RM} adopt a dendritic shape and have limited mobility, allowing for their surveillance of the tissue (69). In contrast to virus-specific CD8$^+$ T_{RM}, which do not encounter viral Ag unless reinfected, self-reactive CD8$^+$ T_{RM} have the potential for frequent exposure to autoantigens. To determine whether CD8$^+$ T_{RM} detect Ag in the skin, vitiligo was induced using melanocyte-specific CD8$^+$ T cells expressing the reporter Nur77-GFP (70, 71). In this system, GFP positivity indicates Ag recognition through the activation of the TCR. About 10% of epidermal CD8$^+$ T_{RM} were positive for Nur77-GFP (70). Similar to human disease, melanocytes in our mouse model of vitiligo repigment the epidermis through migration from the hair follicle (58), and we suspect that epidermal CD8$^+$ T_{RM} sense Ag as hair follicle melanocytes migrate to repopulate the epidermis. In the dermis, 30% of melanocyte-specific CD8$^+$ T_{RM} expressed GFP from the Nur77 reporter (70). As melanocytes predominantly reside in the epidermis, this data suggests that either CD8$^+$ T_{RM} sense Ag from an unknown dermal resident melanocyte population or from the cross-presentation of melanocyte Ag by resident dendritic cells. To determine whether T_{RM} produce effector cytokines in response to Ag recognition, we used the IFN-γ reporter with internal ribosome entry site poly A tail (GREAT) mouse, to visualize IFN-γ in situ (72). Interestingly, during stable disease in mice, 60–80% of melanocyte-specific CD8$^+$ T_{RM} in the epidermis appeared to express the effector cytokine IFN-γ (70). As the percentage of IFN-γ$^+$CD8$^+$ T_{RM} exceeds the percentage of CD8$^+$ T_{RM} sensing Ag, it suggests that IFN-γ production by CD8$^+$ T_{RM} may be induced independent of Ag sensing or that brief Ag exposure leads to extended production of IFN-γ. The expression of IFN-γ by CD8$^+$ T_{RM} also suggests that they have the potential to recruit additional T cells via the production of effector cytokine and downstream

chemokines (70). Together, these studies reveal that IFN-γ signaling is not only important for disease progression, but expression is chronically maintained in stable disease from $CD8^+$ T_{RM}.

Another recent paper identified $CD8^+$ T_{RM} in the skin of vitiligo mice using a mouse model in which dermal inoculation with B16 melanoma cells, depletion of regulatory T cells (Tregs), and excision of the melanoma tumor led to autoimmune vitiligo, visible by hair depigmentation (73). In this model, melanoma/melanocyte-specific $CD8^+$ T cells were highly enriched in lesional skin and expressed the T_{RM} markers CD69 and CD103. These cells were nonmigratory and, upon ex vivo stimulation, produced IFN-γ. Likewise, melanoma/melanocyte $CD8^+$ T_{RM} were found located at the epidermal/dermal junction near melanocyte-depleted hair follicles (73).

The authors questioned whether the formation of $CD8^+$ T_{RM} in this model is dependent on autoimmune vitiligo. The authors found significant enrichment of skin $CD8^+$ T_{RM} in mice that developed vitiligo compared with mice without vitiligo (70–12%, respectively) (73), suggesting that the development of vitiligo drives the formation and retention of melanoma/melanocyte T_{RM} in the skin. Melanoma/melanocyte $CD8^+$ T_{RM} formation was dependent on the expression of CD103, and CD103 expression was required for protection against melanoma rechallenge. $CD103^+$ T_{RM} were both necessary and sufficient for tumor protection because treatment of mice with fingolimod (FTY720) did not alter the tumor response. Although development of $CD8^+$ T_{RM} was enhanced by vitiligo, tumor protection after rechallenge was independent of vitiligo and suggests that small pools of seeded melanoma/melanocyte $CD8^+$ T_{RM} are sufficient to mediate protection (73).

An additional report also describes the role of T_{RM} in mediating protection against melanoma (74). Using a mouse model of epicutaneous melanoma, the authors reported that resident memory $CD8^+$ T cells restrained the outgrowth of melanoma cells and persist in the epidermis for continued tumor surveillance even in the absence of tumor growth. Melanoma T_{RM} alone were able to mediate protection against melanoma challenge in the majority of mice, but complete protection was established when both T_{RM} and recirculating memory T cells were present.

$CD8^+$ T_{RM} in human vitiligo

Recently, we and others identified T_{RM} in human vitiligo patient skin (40, 41, 63). Similar to healthy skin, vitiligo skin is populated by $T_{CM,}$ T_{EM}, and T_{RM}. The majority of $CD8^+$ T cells are $CD45RO^+CCR7^-$ T_{EM} with only 10% expressing $CCR7^+$, a marker for circulating memory T cells (40). Of the memory $CD8^+$ T cells in vitiligo skin, many express the canonical T_{RM} markers CD69 and CD103. It is worth noting that $CD69^+CD103^+$ T_{RM} are enriched in stable vitiligo skin compared with patients with active disease (42 or 93%, respectively) (40, 63), consistent with their role as memory cells that persist after active inflammation clears. $CD8^+$ T_{RM} are located in both the epidermis and dermis but are enriched in the epidermal compartment (40, 41).

Melanocyte-specific $CD8^+$ T cells were identified using HLA-A2*0201 pentamers for MART1, gp100, and tyrosinase in the blood and skin of healthy controls and vitiligo patients. To identify these cells in the skin, we induce a blister through negative pressure or suction to obtain the fluid, which represents interstitial skin fluid and contains cells and proteins involved in local inflammation. This fluid is distinct from intravascular fluid and thus provides an excellent source for sampling immune components involved in peripheral tissue inflammation. In vitiligo patients, melanocyte-specific $CD8^+$ T cells were highly enriched in the skin fluid in both lesional and nonlesional skin compared with blood (63). The presence of melanocyte-specific $CD8^+$ T cells in nonlesional skin suggests that peripheral tolerance mechanisms might prevent the formation of lesions at these sites. As previously shown by other groups, melanocyte-specific $CD8^+$ T cells were detected in the blood of healthy controls, but no pentamer-positive cells were found in the skin fluid (18, 20, 63, 75, 76). Expression of the chemokine receptor CXCR3 is present on the majority of skin $CD8^+$ T_{RM} in both healthy and vitiligo patients. Interestingly, circulating $CXCR3^+CD8^+$ T_{EM} in vitiligo patients have enhanced proliferative capacity compared with $CXCR3^+CD8^+$ T_{EM} in healthy controls and to their CXCR3-negative counterparts (40). Furthermore, CXCR3 expression is enriched on melanocyte-specific $CD8^+$ T_{RM} compared with nonmelanocyte-reactive T_{RM} (40), highlighting the functional importance of this chemokine receptor in vitiligo.

Further studies investigated the effector and cytolytic functions of $CD8^+$ T_{RM} in vitiligo. Compared with healthy control skin, vitiligo patient skin shows a significant increase in IFN-γ^+ and IFN-γ^+TNF-α^+, producing $CD8^+$ T_{RM} (40, 41, 63). Increases in IFN-γ^+ $CD8^+$ T_{RM} was not seen in psoriasis patient skin, which is mediated by IL-17–producing effector T cells. Polyfunctional IFN-γ^+TNF-α^+CD8^+ T_{RM} are enriched in active disease, a state in which there is new lesion formation, and high numbers of T_{EM} are recruited to kill melanocytes. Cytotoxic ability of $CD8^+$ T_{RM} measured by granzyme B and perforin was not different between healthy control and vitiligo $CD8^+$ T_{RM} in one study (40), whereas another study found that granzyme B– and perforin-expressing $CD8^+$ T_{RM} were increased in vitiligo skin (41). These differences may be a result of a high variability between the healthy and vitiligo patient cohorts tested. Nevertheless, the results suggest that $CD8^+$ T_{RM} in vitiligo have cytolytic capabilities in the skin tissue.

Phenotypic analyses of $CD8^+$ T_{RM} in healthy human skin revealed heterogeneity within the population (41). Researchers identified a specialized subset of $CD8^+$ T_{RM} that express the integrin α subunit, CD49a (41). In healthy human skin, $CD49a^-$-expressing T_{RM} are enriched within epidermal $CD8^+$ T_{RM} compared with dermal $CD8^+$ T_{RM}. To determine whether $CD49a^+$ T_{RM} were functionally distinct, RNA-sequencing studies were performed. Interestingly, $CD49a^+$ $CD8^+$ T_{RM} were enriched in transcripts encoding cytotoxic components, including granzymes and perforin. Of interest, stimulation of epidermal cell suspensions with IL-2 and IL-15, but not other proinflammatory cytokines, induced protein expression of granzyme B and perforin. In vitro assays revealed that the cytotoxic ability of $CD49a^+CD8^+$ T_{RM} was dependent on stimulation with IL-15 and that IL-15 augments IFN-γ production by $CD49a^+$ T_{RM}. The authors looked for $CD49a^+$ T_{RM} in vitiligo and identified a significant population of $CD49a^+$ T_{RM} in lesional vitiligo skin limited to $CD8^+$ but not $CD4^+$ $T_{RM.}$ Furthermore, $CD49a^+CD8^+$ T_{RM} are significantly increased in the epidermis and dermis of lesional vitiligo skin compared with healthy control skin (41).

Collectively, these studies reveal the presence of CD8$^+$ T_{RM} in vitiligo lesions and identify a subset of T_{RM} with enhanced cytolytic and effector cytokine potential.

Sentinel and alarm function of T_{RM} in vitiligo

It is clear from recent reports that CD8$^+$ T_{RM} possess cytotoxic ability, including those in vitiligo skin, but whether they are actively involved in the killing of target cells is still under debate. Elegant studies in viral models report that skin CD8$^+$ T_{RM} alone are sufficient to mediate viral clearance (44, 77, 78), whereas other studies suggest that CD8$^+$ T_{RM} act as sentinel cells that recruit recirculating memory cells to mediate viral clearance (79–81). In the former studies, mice with vaccinia virus (VACV)–specific CD8$^+$ T_{RM} were able to clear skin rechallenge more efficiently than mice without VACV CD8$^+$ T_{RM}, demonstrating that skin CD8$^+$ T_{RM} enhance viral clearance during reinfection (77, 78). Virus was efficiently controlled by VACV T_{RM} even in mice treated with FTY720, an $S1P_1$ inhibitor, which blocks T cell egress from the lymph nodes, suggesting that the recruitment of T_{CM} was not required for clearance. However, additional studies reported that after sensing viral Ag, local T_{RM} cells produce IFN-γ and CXCL9 to promote the migration of recirculating memory CD8$^+$ T cells (79). Two additional reports revealed that reactivation of T_{RM} by viral Ag was critical to the amplification of both innate and adaptive antiviral responses, including the induction of dendritic cell maturation, and NK cell activity. T_{RM} reactivation abruptly reprogrammed the tissue and induced the activation of many inflammatory genes within 3 h of sensing Ag (80, 81). Thus, some studies report that T_{RM} are effective killers that can control viral infection alone, whereas others suggest that T_{RM} are not efficient killers but instead serve to promote the recruitment of appropriate effector cells to the site of infection.

In vitiligo, skin T_{RM} appear to be responsible for maintaining disease and specifically for relapse of disease after discontinuing treatment (40, 41, 63, 70). During stable disease, melanocyte-specific CD8$^+$ T_{RM} likely sense Ag as melanocytes migrate out of the hair follicles to replenish the skin (70). However, the blockade of CXCL10 reversed established disease, suggesting that continued recruitment of T cells was required even for the maintenance of disease (54). Therefore, we asked whether vitiligo CD8$^+$ T_{RM} are sufficient to kill the repopulating melanocytes and maintain disease or whether recruitment of additional circulating memory CD8$^+$ T cells are required to kill repigmenting melanocytes (70). We found that FTY720 treatment of mice with vitiligo led to a rapid reversal of disease despite the persistence of T_{RM}. In addition, the depletion of only recirculating autoimmune T cells reversed disease as well, supporting the concept that CD8$^+$ T_{RM} alone are not sufficient for the maintenance of vitiligo but instead cooperate with recirculating memory CD8$^+$ T cells to maintain disease (70). Because T_{CM} downregulate CD62L and CCR7 to enter the skin tissue and thus resemble T_{EM}, skin migrating melanocyte-specific CD8$^+$ T cells may be newly recruited T_{CM} or recirculating T_{EM}. Active recruitment of recirculating memory CD8$^+$ T cells by T_{RM} may occur through the production of IFN-γ and downstream chemokines CXCL9 and CXCL10, which CD8$^+$ T_{RM} produce during disease (63).

Collectively, this data supports the model by which vitiligo skin-resident CD8$^+$ T_{RM} do not kill melanocytes directly but instead actively recruit melanocyte-specific recirculating memory T cells to the skin to kill repigmenting melanocytes. In this feed-forward loop, the cooperation between resident and recirculating memory leads to the progression of disease. The local signals inducing CD8$^+$ T_{RM} production of IFN-γ and downstream chemokines needs further investigation, but IL-15, which augments IFN-γ production by CD8$^+$ T cells (41), may play a role. Because there is significant heterogeneity within mucosal CD8$^+$ T_{RM}, a distinct subgroup of T_{RM} may function in direct cytotoxicity, whereas another may function to recruit circulating cytotoxic cells. Nevertheless, in vitiligo, recirculating memory T cells play an important role in maintaining disease, presumably through the destruction of repopulating melanocytes (41, 82).

Checks and balances on T_{RM}

Seeding of CD8$^+$ T_{RM} occurs during the effector phase of the immune response (83), and Ag-specific CD8$^+$ T_{RM} are found not only at the site of infection but throughout the tissue. Vitiligo is a focal disease in which islands of skin are affected and border unaffected areas. Because melanocyte-specific CD8$^+$ T_{RM} are found in both lesional and nonlesional skin, it is likely that peripheral tolerance mechanisms prevent the formation of lesions at these sites, and their presence could be a sign of subclinical disease held in check by those mechanisms. Tregs, which are required to maintain peripheral tolerance to self, have been implicated in suppressing melanocyte-specific CD8$^+$ T cells in vitiligo. Multiple studies report that the ratio of CD4$^+$ to CD8$^+$ T cells in the blood are reduced in vitiligo patients compared with healthy controls (84, 85) and that there is a significant decrease in the ability of Tregs to suppress CD8$^+$ T cell proliferation and cytolytic function in vitiligo patients (84). Interestingly, the overexpression of CCL22, a chemokine attractant for Tregs, in the skin of mice led to enhanced Treg numbers in the skin and significantly reduced depigmentation in a mouse model of vitiligo (86). Another study, which adoptively transferred Tregs into host mice and treated mice with rapamycin, led to lasting remission of vitiligo (87). These studies suggest that an imbalance of Treg number or Treg dysfunction may lead to vitiligo development and that Tregs play a critical role in suppressing melanocyte-specific CD8$^+$ T cells.

In healthy control subjects, although melanocyte-specific CD8$^+$ T cells are present in the blood, no pentamer-positive cells are found in the skin tissue (63). It is reported that Tregs control potentially dangerous circulating melanocyte-specific CD8$^+$ T cells by suppressing their proliferation and cytokine production and by inducing anergy (88). Specifically, natural occurring Tregs induced the expression of CTLA-4 and CCR7 on circulating melanocyte-specific CD8$^+$ T cells. Melanocyte-specific CTLA-4$^+$CCR7$^+$CD8$^+$ T cells were detected in larger numbers in healthy individuals compared with vitiligo patients, suggesting that Tregs actively keep autoreactive cells in check within the circulation and that this tolerance is disrupted in vitiligo patients (88).

A better understanding of the potential functional differences between lesional and nonlesional CD8$^+$ T_{RM}, as well as the identification of the local suppressive mechanisms in

place, will provide insight into how CD8+ T_{RM} are controlled. Further studies will help to identify the breaks in peripheral tolerance in vitiligo patient skin and will determine what signals prompt changes in the reactivation state of CD8+ T_{RM}.

Targeting T_{RM} in vitiligo

Studies in mice show that the cooperation of CD8+ T_{RM} with recirculating memory CD8+ T cells leads to the persistence of disease (70). Although inhibiting the function of T_{RM} can be an effective approach to treating vitiligo, this alone would not lead to long-lasting treatment responses because these cells can become active again once the treatment is discontinued. This is presumably why vitiligo patients relapse after stopping conventional treatments (17) as well as newer treatments that inhibit Janus kinase signaling (89). However, a treatment approach that results in the depletion of T_{RM} from the skin could have long-lasting effects because the autoimmune memory within the skin would be eliminated.

Multiple tissue-derived CD8+ T_{RM} survival signals have been identified, including TGF-β, IL-7, and IL-15 (43, 44, 68). Local IL-15 signaling was reported to be important for skin T_{RM} development, survival, and effector function (41, 44). A major source of IL-15 production in the skin is from epidermal basal keratinocytes that constitutively express the cytokine as well as the IL-15R α-chain, also known as CD215, which is required for *trans*-presentation of IL-15 to promote signaling (90). We found that expression of CD215 by keratinocytes in lesional vitiligo patient skin is increased compared with nonlesional skin, suggesting that local IL-15 signaling is dysregulated (63). Interestingly, the majority of melanocyte-specific CD8+ T_{RM} in vitiligo skin express the IL-2/IL-15R β-chain CD122, and CD122 expression was significantly higher on melanocyte-specific CD8+ T_{RM} compared with nonmelanocyte-reactive CD8+ T_{RM}. These observations suggest that local IL-15 signaling is important for the maintenance of melanocyte-specific CD8+ T_{RM} in vitiligo lesional skin (63).

To determine whether targeting IL-15 signaling would lead to changes in skin CD8+ T_{RM}, we treated mice exhibiting stable vitiligo with an anti-CD122 Ab. Anti-CD122 treatment resulted in significant skin repigmentation and a reduction of melanocyte-specific CD8+ T_{RM} in the epidermis and dermis (63). Not only did long-term anti-CD122 treatment deplete CD8+ T_{RM} in the skin, but even short-term treatment that was insufficient to deplete these cells reduced IFN-γ production by T_{RM} (63). This effect on CD8+ T_{RM} was limited to melanocyte-specific cells, and treatment did not reduce host T_{RM} or T_{CM}, presumably because autoreactive CD8+ T_{RM} in both humans and mice express higher amounts of CD122 and may be more dependent on IL-15 signaling (63). Importantly, short-term treatment with anti-CD122 Ab had long-lasting effects on repigmentation (63). This study supports the hypothesis that skin CD8+ T_{RM} are responsible for the maintenance of vitiligo and that targeting this population in vitiligo patients would have durable effects.

Recent studies have identified CD8+ T_{RM} in the female reproductive mucosa that are not dependent on IL-15 for survival or homeostatic proliferation. These studies provide further evidence that CD8+ T_{RM} are heterogeneous and that subpopulations may rely on different signals for survival and maintenance at each tissue site (82). This may explain why a small number of CD8+ T_{RM} remained in the skin after anti-CD122 treatment. Whether IL-15–independent CD8+ T_{RM} populations are also present in vitiligo patient skin and whether these heterogeneous populations cluster in different microanatomical locations is not known. For example, CD8+ T_{RM} located at the hair follicle may rely more on IL-7 than IL-15 (68). Identification of these additional local signals will be important for targeting melanocyte-specific CD8+ T_{RM}, although sparing other skin resident T cells, and would improve the specificity of the therapy.

Conclusions

In vitiligo, clinical observations reveal that white spots of depigmentation frequently return in the same exact location after cessation of treatment (16), suggesting that autoimmune memory drives the pathogenesis and maintenance of the disease. In this review, we highlight recent studies that identify the signals driving CD8+ T cell memory formation and retention in vitiligo as well as the potential to develop new therapies that target the memory cells responsible for maintaining disease (Fig. 1).

Early events in vitiligo pathogenesis are dependent on IFN-γ signaling on keratinocytes, which induces expression of the chemokine ligands CXCL9 and CXCL10 by skin-resident cells (Fig. 1A). This chemokine gradient triggers the migration of CXCR3+ melanocyte-specific CD8+ T cells to enter the skin and directs their migration through the dermis to the epidermal/dermal junction where they can identify and destroy melanocytes (Fig. 1A). Once T cells enter the epidermis, a subset of melanocyte-specific CD8+ T cells differentiate into T_{RM} because of the expression of local retention signals, and these cells persist long term within the lesional skin (Fig. 1B). Melanocyte-specific CD8+ T_{RM} in mice and human vitiligo skin are enriched in the epidermis and located both at the dermal–epidermal junction in humans and at the interfollicular epidermis close to hair follicles in mice.

CD8+ T_{RM} retention within the epidermis results from the local production of the survival signal IL-15, which is produced by hair follicle and basal keratinocytes (Fig. 1B) (91). The hair follicles are also a source of Ag because melanocytes migrate from their niche at that location to repigment the skin. Detection of Ag and/or local inflammatory cues, including IFN-γ signaling and IL-15 (41), may induce the reactivation of quiescent CD8+ T_{RM} to recruit recirculating memory CD8+ T cells to the skin where they can kill repopulating melanocytes. This positive feedback circuit leads to the persistence of disease and recalcitrance to treatment.

Targeting CD8+ T_{RM} by blocking IL-15 signaling using an anti-CD122 Ab led to a significant reversal of vitiligo by both reducing CD8+ T_{RM} numbers in the skin and by suppressing their effector function (63). Importantly, short-term anti-CD122 treatment led to a durable response in mice. These recent studies provide an important mechanistic insight into the role of T_{RM} in the maintenance of vitiligo and support targeting this population as a new, durable treatment strategy (Fig. 1B). We are hopeful that targeting

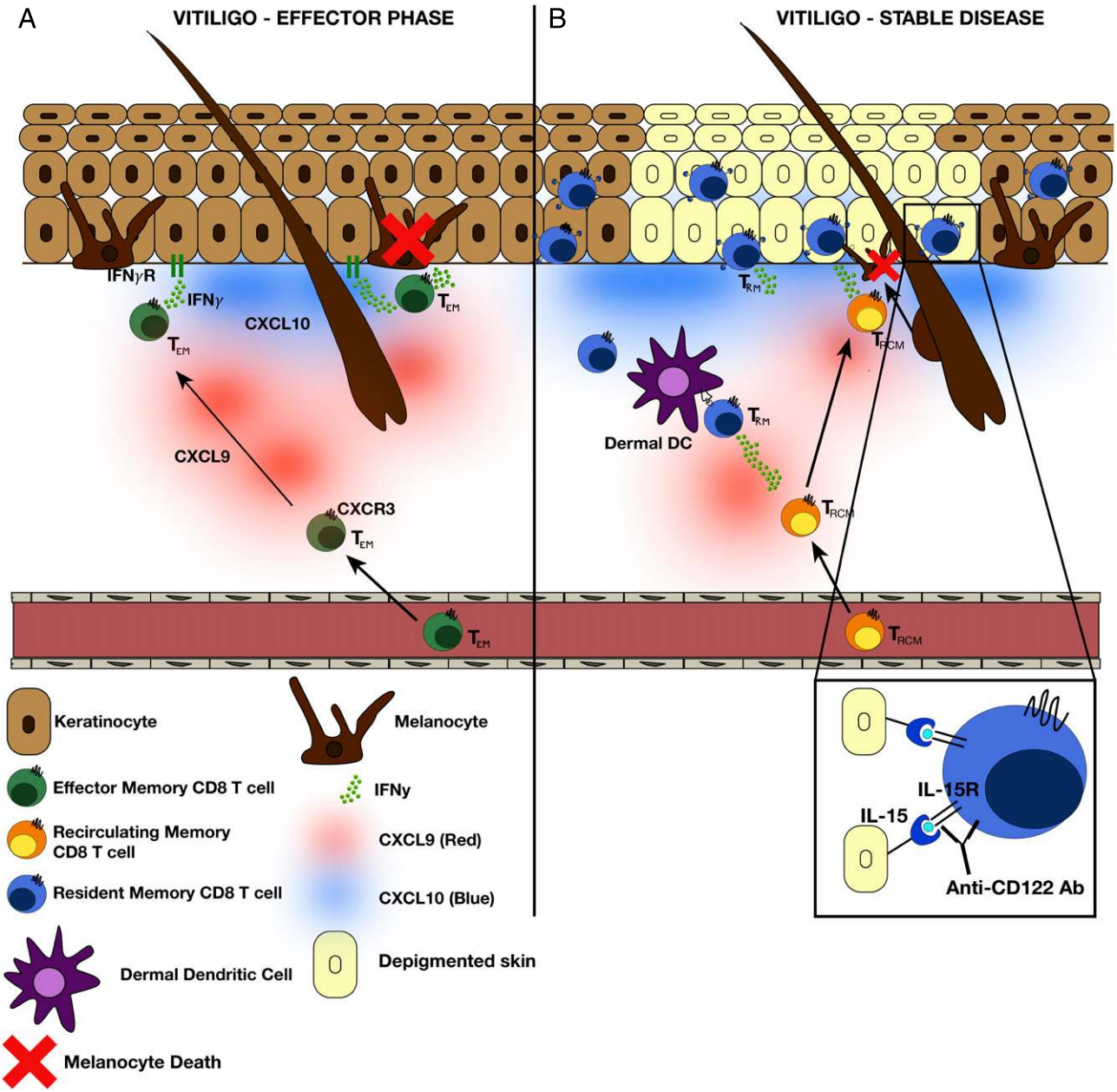

FIGURE 1. Summary of vitiligo pathogenesis during both the effector phase and stable vitiligo. (**A**) In active vitiligo or early lesion development, IFN-γ signaling on keratinocytes stimulates the production of the IFN-γ–dependent chemokines, CXCL9 and CXCL10. Many epidermal cell types produce CXCL9 and CXCL10, but keratinocytes, which are numerous, produce the greatest total amounts. This chemokine gradient supports continued migration of melanocyte-specific $CD8^+$ T_{EM} through the dermis to the dermal–epidermal junction (DEJ) where melanocytes reside. CXCL9 and CXCL10 both play a role in T_{EM} trafficking; CXCL9 is responsible for bulk recruitment of $CD8^+$ T_{EM} into the skin, whereas CXCL10 is required for T_{EM} tethering and microanatomical positioning at the DEJ as well as $CD8^+$ T_{EM} function. Chemokine-dependent recruitment and positioning of $CD8^+$ T_{EM} leads to melanocyte death. Activated $CD8^+$ T_{EM} express IFN-γ, which induces recruitment of additional $CD8^+$ T_{EM} to the skin and further melanocyte destruction. (**B**) Over the course of the disease, a subset of $CD8^+$ T_{EM} differentiate into T_{RM}, resulting from expression of local retention signals, and seed the skin tissue, including the depigmented lesion. Melanocyte-specific T_{RM} are retained in the tissue because of *trans*-presentation of IL-15 by keratinocytes. Within a depigmented lesion, T_{RM} likely sense melanocytes migrating from hair follicles, and the activation of T_{RM} induces the production of the inflammatory cytokines IFN-γ and CXCL9 that help to recruit recirculating melanocyte-specific memory T cells (T_{RCM}) to the skin, where they can kill the repopulating melanocytes. This feed-forward loop and cooperation between T_{RM} and T_{RCM} leads to the persistence of disease. In vitiligo, IL-15R expression is enriched on melanocyte-specific $CD8^+$ T_{RM}, and the treatment of mice with an anti-CD122 Ab (IL-15Rβ) led to a significant reversal of disease characterized by a reduction of T_{RM} numbers and effector function in the skin. Importantly, short-term treatment with anti-CD122 Ab proved to be durable in mice.

T_{RM} will provide a long-lasting treatment option for vitiligo patients.

Disclosures

J.E.H. is scientific founder of Villaris Therapeutics Inc., a company focused on developing targeted treatments for vitiligo, and he holds equity in the company.

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