AUTOIMMUNITY

Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo

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Vitiligo is an autoimmune disease of the skin mediated by CD8$^+$ T cells that kill melanocytes and create white spots. Skin lesions in vitiligo frequently return after discontinuing conventional treatments, supporting the hypothesis that autoimmune memory is formed at these locations. We found that lesional T cells in mice and humans with vitiligo display a resident memory (T_{RM}) phenotype, similar to those that provide rapid, localized protection against reinfection from skin and mucosal-tropic viruses. Interleukin-15 (IL-15)–deficient mice reportedly have impaired T_{RM} formation, and IL-15 promotes T_{RM} function ex vivo. We found that both human and mouse T_{RM} express the CD122 subunit of the IL-15 receptor and that keratinocytes up-regulate CD215, the subunit required to display the cytokine on their surface to promote activation of T cells. Targeting IL-15 signaling with an anti-CD122 antibody reverses disease in mice with established vitiligo. Short-term treatment with anti-CD122 inhibits T_{RM} production of interferon-γ (IFNγ), and long-term treatment depletes T_{RM} from skin lesions. Short-term treatment with anti-CD122 can provide durable repigmentation when administered either systemically or locally in the skin. On the basis of these data, we propose that targeting CD122 may be a highly effective and even durable treatment strategy for vitiligo and other tissue-specific autoimmune diseases involving T_{RM}.

INTRODUCTION

Vitiligo is caused by CD8$^+$ T cells that target melanocytes for destruction (*1*), resulting in patchy depigmentation that is disfiguring and distressing to patients (*2*). It affects about 1% of the population worldwide, yet there are currently no U.S. Food and Drug Administration–approved medical treatments to reverse the disease (*2*, *3*). Depigmentation typically recurs rapidly at the same location after therapy is stopped (*4*), indicating that autoimmune memory persists in the skin and permits disease reactivation after cessation of treatment. The presence of resident memory T cells (T_{RM}) in vitiligo has now been reported by several laboratories, strongly supporting their role in disease (*5*–*7*). However, it has been difficult to implicate them directly as necessary and/or sufficient for the disease because of the lack of tools available to remove them or inhibit their function in tissues without disrupting other memory T cell populations. These previous studies have been limited to identifying their presence and their immunophenotype as well as describing their ability to make cytokines, granzymes, and other effector molecules.

Previous studies in mice have shown that interleukin-15 (IL-15) is important for the generation of T_{RM} in viral infections and in cutaneous lymphomas (*8*, *9*). Further, it has been proposed that targeting IL-15 might be useful for the treatment of autoimmune diseases (*10*, *11*). We therefore sought to target IL-15 signaling to determine whether (i) it was important for function and/or maintenance of T_{RM} in skin and (ii) targeting this pathway could provide a durable treatment strategy for vitiligo. The biology of the IL-15 receptor is complicated in that it can exist in several forms: as a heterodimer, a heterotrimer, or a monomer to present IL-15 in trans to other cells. The heterotrimeric IL-15 receptor is composed of CD122 (which can be shared by the IL-2 receptor when paired with CD25), CD215, and CD132 (the common γ chain). This trimeric receptor is found on natural killer (NK) cells and some T cell subsets. The heterodimeric IL-15 receptor is composed of CD122 and CD132 only and is expressed on memory T cell populations. IL-15 can bind to CD215 alone to be trans-presented to cells bearing the heterodimeric receptor [reviewed in (*12*)]. Here, we first confirmed that human and mouse T_{RM} are present in lesional skin and that they express components of the IL-15 receptor. Specifically, melanocyte-specific T cells express the CD122 subunit, whereas keratinocytes express CD215, consistent with an ability to present IL-15 to T cells in trans (*13*).

We therefore decided to test whether blockade of CD122 signaling with a monoclonal antibody (*14*) could serve as a treatment for vitiligo. To do this, we used our mouse model of vitiligo, which uses the adoptive transfer of T cell receptor (TCR) transgenic T cells recognizing the human melanocyte antigen premelanosome protein (*15*). These T cells, called PMEL, target mouse melanocytes and induce patchy epidermal depigmentation that mirrors human disease (*16*). Long-term CD122 blockade depleted PMEL T_{RM} from the skin, whereas short-term CD122 blockade reduced their effector function, as measured by interferon-γ (IFNγ) production and provided durable repigmentation. Our data suggest that targeted treatment of IL-15 signaling may provide a novel, durable treatment strategy for vitiligo and other organ-specific autoimmune diseases.

RESULTS

Autoreactive T cells within lesions of vitiligo patients have a T_{RM} phenotype and express CD122

To phenotype skin-resident T cells, we performed blister biopsies (*17*) on histocompatibility leukocyte antigen (HLA)–A2$^+$ vitiligo patients with active and stable disease. Melanocyte antigen–specific T cells were identified using HLA-A2*0201 pentamers against MART-1,

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gp100, and tyrosinase in both blood and skin fluid from these patients (Fig. 1A and table S1). Initially, we performed screens on blood using each pentamer separately before choosing one to stain skin fluid due to the limited number of cells. However, we found that the highest staining pentamer in blood did not always correlate to skin enrichment; therefore, we pooled all three pentamers to more reliably identify melanocyte-specific cells in the skin (fig. S1) and colabeled these cells with the T_{RM} markers CD69 and CD103.

In vitiligo patients, melanocyte-specific $CD8^+$ T cells can be detected in their blood and skin fluid. These antigen-specific T cells were highly enriched in lesional and nonlesional skin compared to blood. In patients with active disease, there were large populations of CD69 single-positive and CD69/CD103 double-positive antigen-specific T cells in the lesional skin, whereas in patients with stable disease, most were double-positive (Fig. 1B). We hypothesize that the presence of autoreactive T cells in nonlesional skin indicates that tolerance mechanisms at those locations in the skin prevent the formation of lesions. Melanocyte-specific $CD8^+$ T cells with a T_{RM} phenotype were enriched in lesional skin as compared to blood in all patients, regardless of whether their disease status was active or stable (Fig. 1C). We have found that antigen specificities vary from site to site, which may explain the variance in MART-1 versus the pooled pentamer reactivity in Fig. 1C. In accordance with previously published studies (*1*, *18*–*20*), we found that healthy control patients also have melanocyte-specific T cells in their blood, although healthy controls had far fewer T cells in blister fluid, and no pentamer$^+$

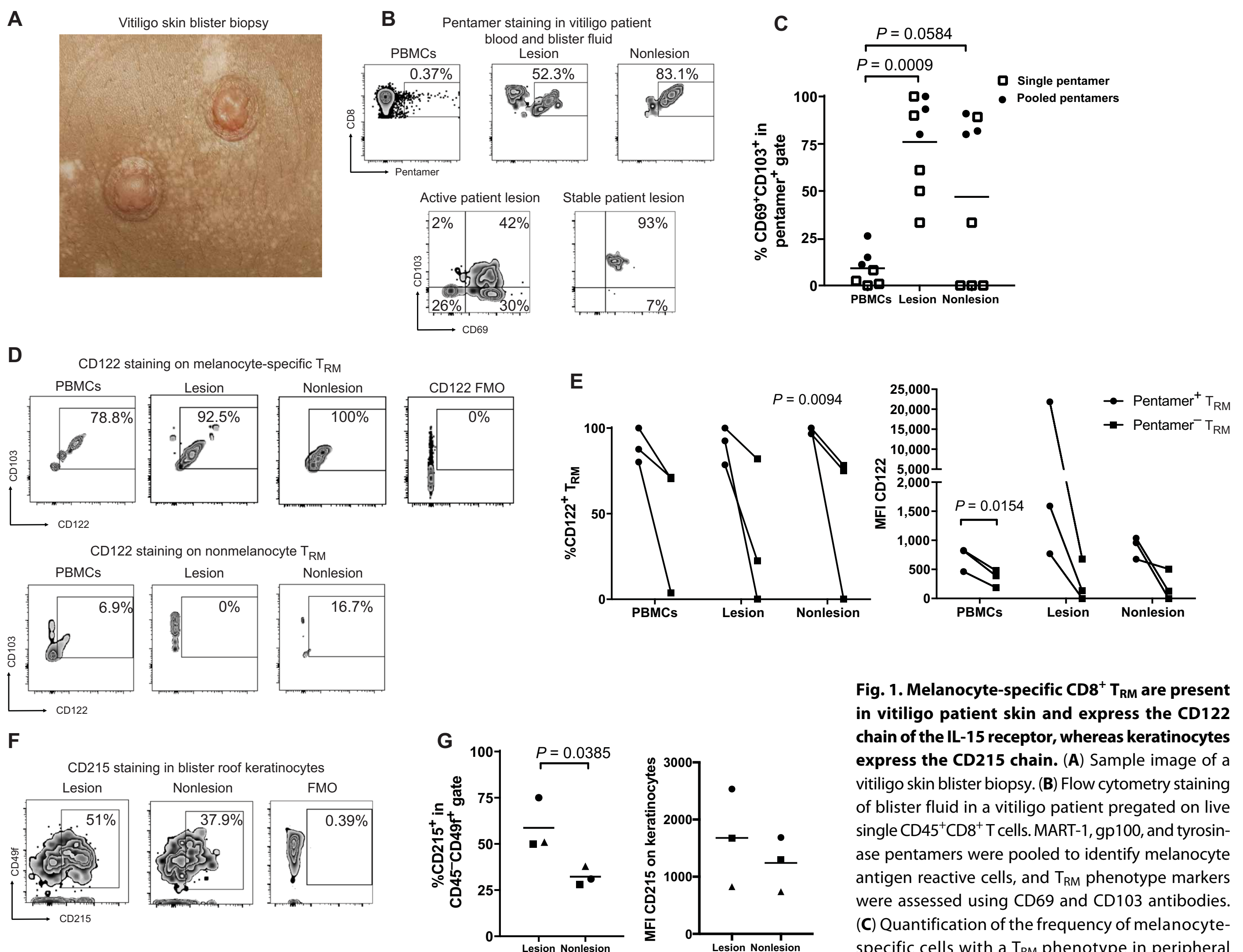

Fig. 1. Melanocyte-specific $CD8^+$ T_{RM} are present in vitiligo patient skin and express the CD122 chain of the IL-15 receptor, whereas keratinocytes express the CD215 chain. (**A**) Sample image of a vitiligo skin blister biopsy. (**B**) Flow cytometry staining of blister fluid in a vitiligo patient pregated on live single $CD45^+CD8^+$ T cells. MART-1, gp100, and tyrosinase pentamers were pooled to identify melanocyte antigen reactive cells, and T_{RM} phenotype markers were assessed using CD69 and CD103 antibodies. (**C**) Quantification of the frequency of melanocyte-specific cells with a T_{RM} phenotype in peripheral blood mononuclear cells (PBMCs) and lesional and nonlesional skin. Melanocyte-specific T_{RM} were significantly enriched in lesional skin. [Each symbol represents one patient; open squares were stained with MART-1 pentamer alone, and closed circles were stained with all three pentamers MART-1, gp100, and tyrosinase; one-way analysis of variance (ANOVA) with Tukey's post tests significant as indicated.] (**D**) Flow cytometry staining of CD122 on pentamer$^+$ and pentamer$^-$ T_{RM} and (**E**) quantification in PBMCs and lesional and nonlesional skin. Cells were pregated on live single $CD45^+CD8^+CD69^+CD103^+pentamer^{+/-}$ T cells [two-way ANOVA for $\%CD122^+$, $P = 0.0094$ significant for differences between pentamer$^+$ and pentamer$^-$ cells; post tests, not significant (ns); two-way ANOVA for median fluorescence intensity (MFI) CD122, ns; paired t test, $P = 0.0154$ for PBMCs]. (**F**) Flow cytometry staining of CD215 on keratinocytes from blister roofs and (**G**) quantification in lesional and nonlesional skin. Cells were pregated on live single $CD45^-$ cells. CD215 expression was more frequent in lesional than nonlesional skin (Student's t tests significant as indicated, $P = 0.0385$). FMO, Flourescence Minus One control.

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T cells in blister fluid (fig. S2). In healthy donors or nonlesional skin, these melanocyte-specific T cells may either provide tumor surveillance or represent prelesions.

To determine whether melanocyte-specific T_{RM} expressed the receptor for IL-15 (IL-15R), we stained for the CD215 and CD122 subunits of the receptor in our samples. We found that T_{RM} primarily express the CD122 subunit, a shared component of the receptors for IL-2 and IL-15, but rarely expressed the IL-15–specific subunit CD215 (Fig. 1D and fig. S3). Further, pentamer$^+$ T_{RM} had higher CD122 by both frequency and MFI than pentamer$^-$ T_{RM} (Fig. 1E). In contrast, keratinocytes and dendritic cells constitutively expressed CD215. However, only keratinocytes expressed more CD215 in lesional skin compared to nonlesional skin (Fig. 1, F and G, and fig. S4). Further analysis of skin from vitiligo patients using immunohistochemistry revealed that the microanatomical locations with mononuclear infiltrates characteristic of interface dermatitis had more CD215 expression on keratinocytes than areas without mononuclear infiltrates (fig. S5). Together, these data indicate that human vitiligo patients have antigen-specific T_{RM} in lesional skin that can respond to IL-15 displayed on CD215 by keratinocytes through CD122.

Autoreactive T cells in the epidermis of a mouse model of vitiligo have a resident memory phenotype and express the IL-15R

To address the functional roles of T_{RM} in vitiligo, we used our mouse model, which uses the adoptive transfer of CD8$^+$ T cells that recognize the autoantigen premelanosome protein (PMEL) physiologically expressed in melanocytes. PMEL-reactive cells are activated in vivo using intraperitoneal injection of attenuated recombinant vaccinia virus expressing their cognate antigen (human gp100), while their recruitment to the skin and identification of melanocytes are undirected or spontaneous. Previous studies using this model revealed that adoptively transferred, autoreactive T cells accumulate within the epidermis of the skin during the progression of vitiligo in an IFNγ/CXCR3-mediated fashion (*15*, *16*, *21*, *22*). We found that these cells accumulate in the epidermis where melanocytes reside and that a large fraction of these cells increasingly expressed both CD69 and CD103, markers of T_{RM} formation, over time (Fig. 2, A and B). Like human pentamer$^+$ T_{RM}, mouse PMEL T_{RM} expressed more CD122 by both frequency and MFI than host mouse CD8$^+$ T_{RM} ($P = 0.009$ and 0.0018, respectively; Fig. 2, C and D). PMEL T_{RM} expressed not only CD122 but also CD215 (fig. S3), a distinction between the mouse model and human disease. Together, these data indicate that our mouse model recapitulates autoimmune memory and could be used to study T_{RM} biology in the context of vitiligo.

CD122 blockade reverses disease in mice with established vitiligo

Previous studies reported that IL-15 signaling was important for the generation of skin T_{RM} that reside in hair follicles (*9*), and we hypothesized that targeting this cytokine might also deplete T_{RM} in the skin and result in long-lasting repigmentation. To test the impact of IL-15 on the survival and maintenance of the T_{RM} population in the epidermis, we treated mice with long-standing, stable vitiligo (>12 weeks after disease initiation) intraperitoneally three times weekly with 100 μg of anti-mouse CD122 antibody, which blocks IL-15 signaling (fig. S6) (*14*, *23*, *24*). The antibody used, ChMBC7, is a chimeric rat/mouse antibody with diminished Fc-mediated effector functions to eliminate Fc-mediated depletion of CD122$^+$ cells (*14*). After 8 weeks of systemic treatment, we observed significant repigmentation in treated mice compared to controls ($P = 0.0001$; Fig. 3, A to C). We found that PMEL were depleted from the epidermis in long-standing lesions, and within the PMEL population, T_{RM} (CD69$^+$CD103$^+$) were largely affected. There were also fewer PMEL in the dermis, lymph nodes, spleen, and blood of treated mice compared to controls (Fig. 3D). However, anti-CD122 treatment had only small effects on host CD8$^+$ T cell populations, indicating that targeting this pathway preferentially had an effect on the transferred cells (Fig. 3E). Together, these data indicate that CD122 blockade may be used to treat vitiligo and that chronic systemic administration results in depletion of autoreactive T_{RM} and other memory T cell pools.

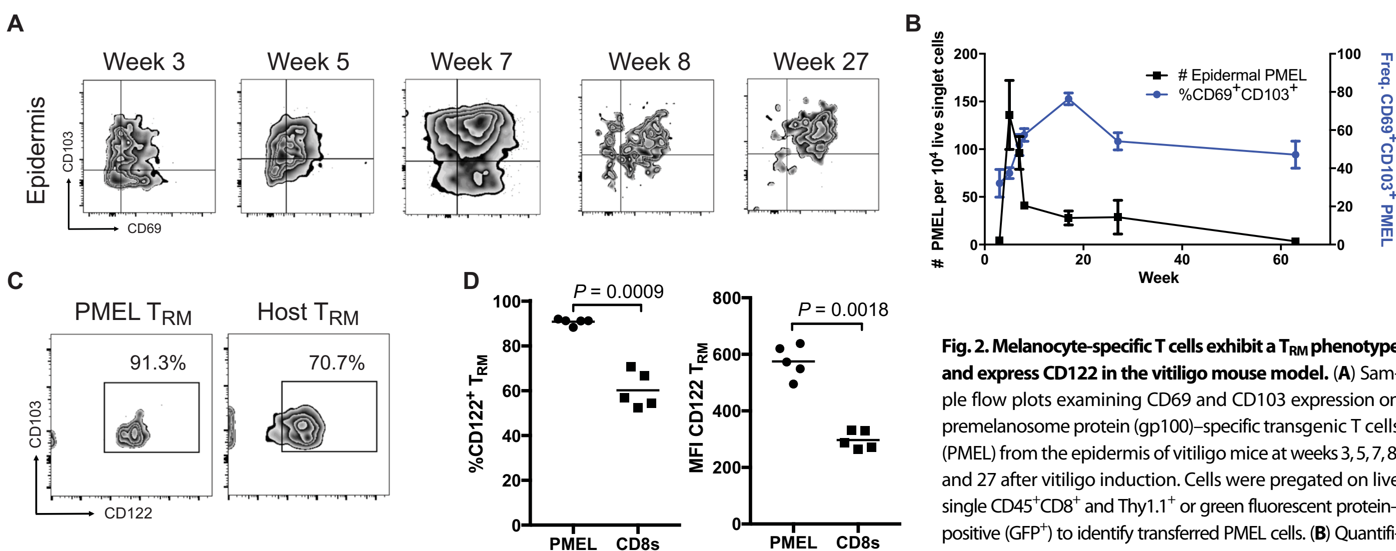

Fig. 2. Melanocyte-specific T cells exhibit a T_{RM} phenotype and express CD122 in the vitiligo mouse model. (**A**) Sample flow plots examining CD69 and CD103 expression on premelanosome protein (gp100)–specific transgenic T cells (PMEL) from the epidermis of vitiligo mice at weeks 3, 5, 7, 8, and 27 after vitiligo induction. Cells were pregated on live single CD45$^+$CD8$^+$ and Thy1.1$^+$ or green fluorescent protein–positive (GFP$^+$) to identify transferred PMEL cells. (**B**) Quantification of the total number of PMEL in the epidermis (left axis, black line) and the frequency of CD69$^+$CD103$^+$ PMEL T_{RM} in the epidermis (right axis, blue line) over time after vitiligo induction ($n = 4$ mice at week 3, 5 mice at week 5, 5 mice at week 7, 2 mice at week 8, 3 mice at week 17, 4 mice at week 27, and 2 mice at week 63 pooled from four separate experiments). (**C**) Representative flow cytometry staining of CD122 on PMEL and host CD8$^+$ T_{RM} and (**D**) quantification in mouse epidermis at week 8 (Student's *t* test significant as indicated, $P = 0.0009$ and $P = 0.0018$; $n = 5$ mice pooled from two representative experiments).

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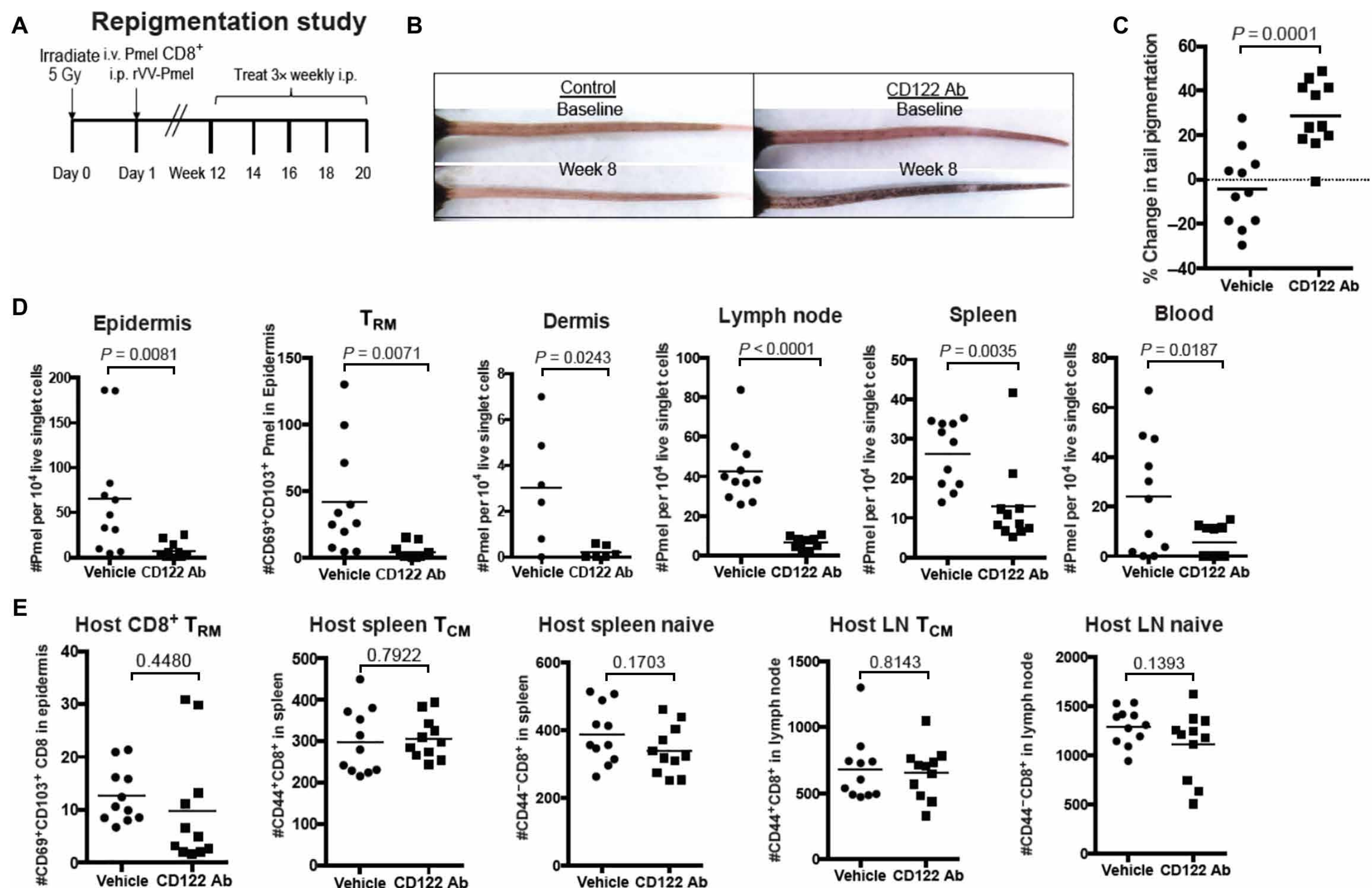

Fig. 3. Treatment with anti-CD122 antibody reverses disease in mice with established vitiligo. (**A**) Timing of treatments in the efficacy/repigmentation model. i.v., intravenous; i.p., intraperitoneal. (**B**) Sample photos of vehicle control [phosphate-buffered saline (PBS) or isotype] and anti-CD122 antibody (Ab)–treated animals at treatment baseline and week 8. (**C**) Comparison of the final percent change in pigmentation in vehicle and anti-CD122 antibody–treated animals. (**D**) Quantification of PMEL numbers in treated animals within the indicated tissues. (**E**) Quantification of host $CD8^+$ T cell numbers in treated animals within the indicated tissues (each dot represents one animal; pooled from two separate experiments, $n = 11$ vehicle mice and $n = 11$ anti-CD122 antibody–treated mice; t tests significant as indicated). LN, lymph node.

CD122 serves as a durable treatment in mice with established vitiligo

Existing treatments for vitiligo are not durable, because the disease frequently returns after discontinuation. To determine whether CD122 blockade could provide a durable treatment option, we treated mice with established vitiligo with anti-CD122 for only 2 weeks, then stopped the treatment, and observed repigmentation over a full 8-week period. Treatment with this short-term approach resulted in significant repigmentation over the total 10-week period ($P = 0.0448$; Fig. 4, A to D). Analysis of PMEL depletion after short-term anti-CD122 treatment revealed variable effects in the lymph node, spleen, blood, and skin (table S2). Therefore, we assessed PMEL function after short-term anti-CD122 treatment and found that PMEL cells in the skin produced significantly less IFNγ when stimulated ex vivo than cells from vehicle-treated animals, whereas lymph node cells were not significantly affected (dermis, $P = 0.0066$; epidermis, $P = 0.1583$; lymph node, $P = 0.2692$; Fig. 4, E to G).

Because short-term systemic administration of anti-CD122 antibody durably reversed disease, we tested a short course of local, intradermal treatment to determine whether we could achieve similar clinical efficacy with less drug and less impact on circulating T cell populations. We treated mice with established vitiligo with anti-CD122 intradermally for 4 weeks (2 weeks at 5 μg for loading dose and 2 weeks at 1 μg for maintenance dose), then stopped the treatment, and observed repigmentation over a 10-week period. Experimental mice treated with intradermal anti-CD122 had significant repigmentation compared to control mice ($P = 0.0058$; Fig. 5) without depleting PMEL (fig. S7). Therefore, we believe that T cells are recruited to the skin via the CXCR3 chemokine axis (*16*, *21*, *22*, *25*), encounter IL-15 presented in trans on keratinocytes, up-regulate IFNγ at the dermal-epidermal junction to bolster their function, and become dependent on IL-15 for their survival as they establish residence in the epidermis. Our proposed model for the biology of CD122 and IL-15/CD215 in vitiligo is summarized in fig. S8. These data suggest that targeting IL-15 in vitiligo, unlike existing therapies, could provide a durable treatment option for patients, with longstanding effects after a limited treatment course.

DISCUSSION

The clinical characteristics of vitiligo, such as its recalcitrant nature and well-defined lesions that return after stopping treatment,

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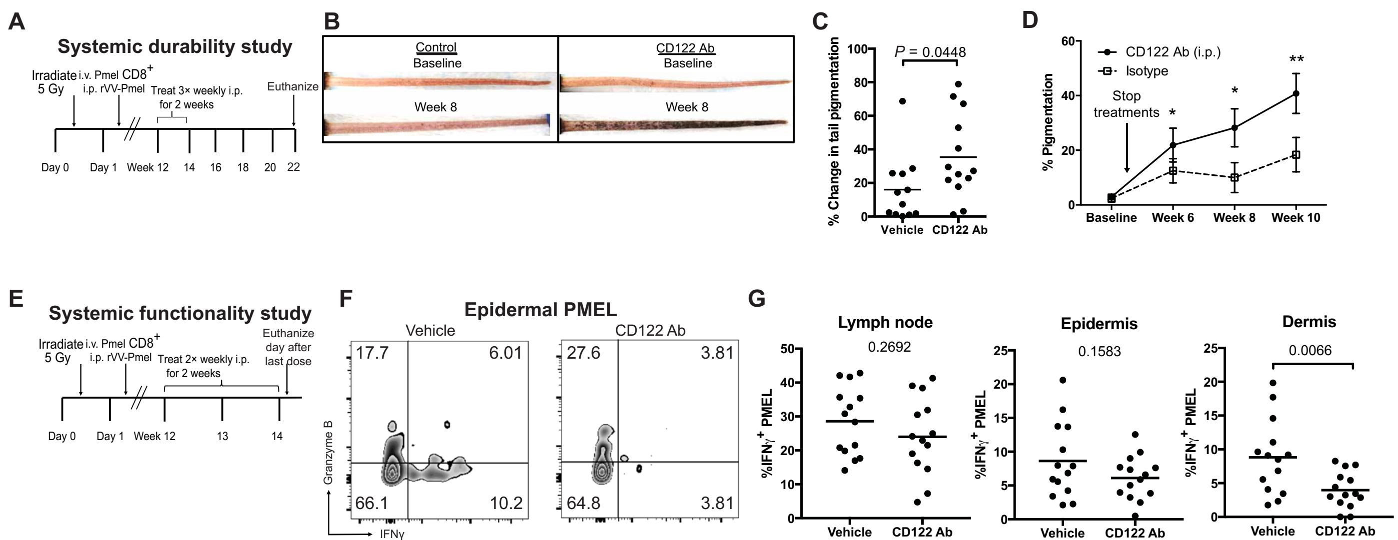

Fig. 4. Systemic anti-CD122 antibody treatment durably reverses disease in mice via inhibition of antigen-specific T cell IFNγ production. (**A**) Timing of treatments in the systemic durability study. (**B**) Sample photos of vehicle control (PBS or isotype) and anti-CD122 antibody–treated animals at treatment baseline and week 8. (**C**) Comparison of the final percent change in pigmentation in vehicle and CD122 antibody–treated animals (each dot represents one animal; pooled from three separate experiments, $n = 12$ control mice and $n = 13$ anti-CD122 antibody–treated mice; *t* test significant as indicated). (**D**) Analysis of the percent of tail with pigmentation over time (two-way ANOVA, $P = 0.0015$ for treatment, $P < 0.0001$ for time, and ns for interaction with Dunnett's comparisons to baseline pigmentation with main time effect significant as indicated by asterisks; baseline versus week 6, $P = 0.0143$; baseline versus week 8, $P = 0.0105$; baseline versus week 10, $P = 0.0001$). (**E**) Timing of the treatments in the functionality study. (**F**) Sample flow plots of epidermal PMEL production of granzyme B and IFNγ. (**G**) Quantification of PMEL producing IFNγ in the indicated tissues (Student's *t* tests significant as indicated; $n = 14$ vehicle mice and $n = 14$ anti-CD122 antibody–treated mice; pooled from three separate experiments).

implicate T_{RM} as functionally responsible for its persistence. Previous reports studying viral T_{RM} have used parabiotic mice to create a system where recirculating and central memory T cells (T_{CM}) exist without T_{RM} and then tested their ability to respond to reinfection (*26*). These studies demonstrated that T_{RM} were required to fight local viral reinfection effectively and efficiently. A few recent studies have characterized cells with T_{RM} phenotypes in mouse and human vitiligo (*5*–*7*). However, depleting T_{RM} without affecting T_{CM} to definitively confirm this hypothesis has proven difficult within existing models of autoimmunity, including our own. Thus, because it seems plausible and even likely that autoreactive T_{RM} are required for maintenance of disease within skin lesions, future strategies to selectively deplete T_{RM} while leaving T_{CM} and other populations intact could be very useful.

The role of IL-15 in T_{RM} formation and function prompted us to target the cytokine as a potential "Achilles' heel" of autoreactive T_{RM}. Although IL-15 is constitutively expressed by keratinocytes (*27*), we found that those in lesional skin express more CD215 than in nonlesional skin, which may help to better promote the formation and/or function of T_{RM} during active inflammation, as well as their microanatomical localization to areas of active melanocyte destruction in the epidermis. In contrast, dendritic cells also expressed CD215 but in similar amounts in lesional and nonlesional skin. Whereas CD215 on accessory cells is known to play an important role to trans-present IL-15 to memory T cells to support their activation and survival, its function on memory T cells and NK cells themselves is less well understood. We found that human T_{RM} did not express CD215, consistent with studies reporting that CD215 is dispensable on memory T cells (*28*) but is required on parenchymal cells for memory CD8 T cell development (*29*). CD215 knockout $CD8^+$ memory T cells and NK cells both developed normally when complemented with accessory cells expressing wild-type CD215 and IL-15, indicating that its presence is dispensable for in vivo IL-15–mediated function (*30*).

Therefore, blocking the interaction of the trans-presented IL-15 to autoreactive T_{RM} in the skin is crucial to achieving a durable treatment effect, and an anti-CD122 antibody enabled this approach. The lower CD122 expression on non–melanocyte-specific T_{RM} in mice and humans implies that targeting this molecule may preferentially deplete autoreactive memory T cell populations. We found that chronic systemic administration of anti-CD122 depleted memory PMEL T cells in multiple tissues. Similarly, another recent study of ChMBC7 CD122 antibody treatment in diabetic animals found depletion of pathogenic $CD8^+$ memory T cells and NK cells from islets, as well as diminished IFNγ production by islet T cells (*14*). Others found a similar depletion of $CD8^+$ T cells using TMβ-1 antibody against CD122 in a mouse model of experimental autoimmune encephalomyelitis, although they did not distinguish memory subtypes of these cells (*31*). Systemic administration of anti-CD122 had minimal effects on host $CD8^+$ T cell populations. This may indicate differences in the biology of autoreactive clones from endogenous clones, or it may be a result of the high-affinity PMEL TCR. Nevertheless, we are excited by these results, and future studies could be conducted to examine differences in CD122 biology in different T cell clones. Short-term systemic administration had variable effects on PMEL numbers in tissues but did reduce IFNγ secretion by these cells. A recent study identified T cells with a T_{RM} phenotype in human vitiligo skin and reported that IL-15 promotes their effector function in vitro (*6*), therefore supporting our data that short-term administration of anti-CD122 reduced PMEL effector functions in the skin. Intradermal administration, which would be attractive from a clinical/safety standpoint, did not

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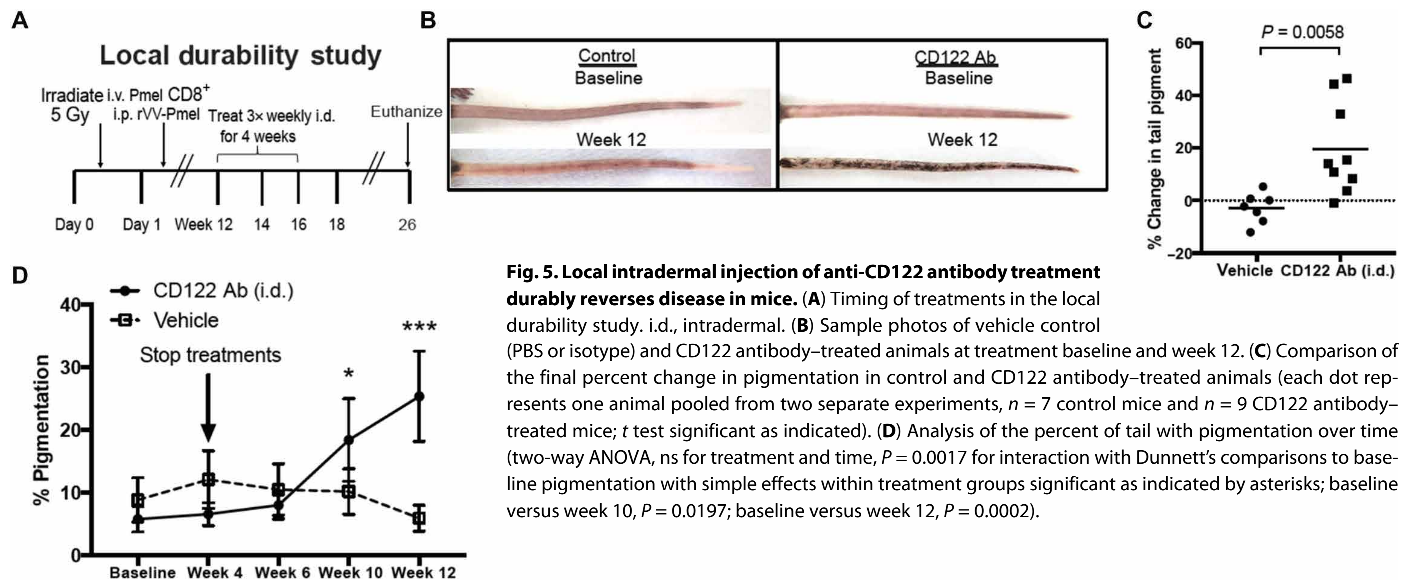

Fig. 5. Local intradermal injection of anti-CD122 antibody treatment durably reverses disease in mice. (**A**) Timing of treatments in the local durability study. i.d., intradermal. (**B**) Sample photos of vehicle control (PBS or isotype) and CD122 antibody–treated animals at treatment baseline and week 12. (**C**) Comparison of the final percent change in pigmentation in control and CD122 antibody–treated animals (each dot represents one animal pooled from two separate experiments, $n = 7$ control mice and $n = 9$ CD122 antibody–treated mice; t test significant as indicated). (**D**) Analysis of the percent of tail with pigmentation over time (two-way ANOVA, ns for treatment and time, $P = 0.0017$ for interaction with Dunnett's comparisons to baseline pigmentation with simple effects within treatment groups significant as indicated by asterisks; baseline versus week 10, $P = 0.0197$; baseline versus week 12, $P = 0.0002$).

deplete PMEL T_{RM} or other populations but still achieved clinical efficacy.

A caveat to our study is that CD122 is a shared receptor for both IL-2 and IL-15. Our in vitro studies indicated that anti-CD122 was able to block IL-15–mediated T cell survival but not IL-2–mediated proliferation and survival. We did not observe IL-15–mediated proliferation, which may be due to the requirement of IL-15 complexed with CD215 for maximal bioactivity (*32*, *33*). Although we cannot completely exclude a role for IL-2 in the maintenance of T_{RM} in vivo, others have shown that IL-15 is much more potent at generating $CD8^+$ T_{RM} pools than residual IL-2 signaling in skin tissue (*9*), and IL-15 is required for the generation of $CD8^+$ T_{RM} in viral models in mice (*8*). Further, $CD8^+$ T cells often express T_{RM} without CD25 (*34*), and mice lacking IL-2 or CD25 develop autoimmunity due to regulatory T cell (T_{reg}) impairments, whereas mice lacking IL-15 are protected from autoimmunity [reviewed in (*35*)]. In support of these studies and our data, two recent studies found that anti-CD122 treatment in rhesus macaques greatly reduced tissue effector memory T cell populations, whereas recirculating populations rebounded (*36*). In addition, anti-CD122 administration in diabetic mice only mildly affected T_{regs} but depleted memory $CD8^+$ T cells and reduced the ability of remaining T cells to produce IFNγ (*14*). We found that targeting IL-15 signaling through antibody treatment even after the generation of T_{RM} in the tissue was effective at clearing these cells from the epidermis, indicating that IL-15 signaling is required for maintenance, and not just formation, of these cells. Another limitation to our study is that we have not addressed the mechanism of action of anti-CD122 in the skin in human patients. Elucidating this will be important for future targeting of IL-15 in vitiligo and other T_{RM}-driven autoimmune diseases.

Our data support targeting IL-15 as a strategy to clear autoreactive memory cells from the tissue, resulting in a long-lasting, durable response to treatment. This is in contrast to existing therapies for inflammation in skin and other tissues, which result in rapid relapse after they are discontinued. Further, targeting IL-15 in our mouse model preferentially affects autoreactive T cells while leaving most endogenous T cell populations intact. Whether short-term IL-15 antibody treatment in humans would result in only localized effects or systemic depletion of other subsets needs to be determined in clinical studies. On the basis of our results in mice, if widespread depletion is observed with systemic treatment in humans, then localized injection of antibody within skin lesions may be a viable alternative with similar efficacy but an improved safety profile. Further, the option of treating intradermally is clinically attractive because other skin diseases are often treated by dermatologists using local injections in the skin. Thus, future trials in patients will provide important insights into targeting T_{RM} via IL-15 signaling, and vitiligo may be used as a model autoimmune disease to understand this process in other tissues.

MATERIALS AND METHODS

Study design

The objectives of this study were to determine whether T_{RM} exist in human vitiligo, how they contribute to disease in a mouse model of vitiligo, and to determine whether these cells could be targeted therapeutically. These objectives were proposed to test the hypothesis that vitiligo is resistant to treatment because T_{RM} persist in the skin and reactivate disease upon cessation of treatment. This hypothesis was formed on the basis of clinical observations and others reported in the literature (*8*, *26*, *37*–*41*). Sample size was determined using the approach described by Dell *et al.* (*42*). Briefly, each experiment was powered to detect a difference between group means of twice the observed SD, with a power of 0.8 and a significance level of 0.05. Replicate experiments were performed two or three times, as indicated in the figure legends.

Mice used for these studies were on the C57BL/6J (B6) background. Mice with greater than 75% depigmentation were selected and randomly assigned to treatment groups, as described below. Repigmentation in mice was quantified objectively using ImageJ without blinding. Mouse epidermis, dermis, and lymph node samples were run on the flow cytometer on a high flow rate with a time cutoff of 60 s per sample, and all cell numbers were normalized to live single cells, as previously described (*16*, *21*, *22*, *43*).

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Human blood and skin samples were collected from patients examined by a dermatologist (J.E.H. or A.G.P.) and diagnosed with vitiligo, as well as from healthy volunteers with no known autoimmune diseases. Inclusion/exclusion criteria for human patient samples were as follows: Vitiligo lesional skin was obtained from clinical biopsies from patients with confirmed vitiligo regardless of race, gender, or age, and nonlesional skin was obtained from these same patients at sites at least 15 cm away from lesions. Patients who were actively treating their vitiligo within the past month were excluded from the study due to potential effects on their T cell populations. For blister biopsies, the entire sample of cells from the blister fluid was analyzed on a flow cytometer. For histology, vitiligo shave biopsy samples with interface dermatitis indicative of an active T cell infiltrate were selected for CD215 staining. Primary data are located in table S3.

Mice

All mice were housed in pathogen-free facilities at the University of Massachusetts Medical School (UMMS), and procedures were approved under protocol #2266 by the UMMS Institutional Animal Care and Use Committee and in accordance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals*. Mice used for these studies were on the C57BL/6J (B6) background or a mixed 129 × C57BL/6 background that had been backcrossed to B6 for more than 10 generations. Age- and sex-matched mice were used, and both male and female mice of all strains were tested to avoid gender bias. Replicate experiments were performed two to five times.

KRT14-Kitl*4XTG2Bjl (Krt14-Kitl*) mice, which express membrane-bound stem cell factor under the keratin 14 promoter to retain epidermal melanocytes and therefore have pigmented skin, were a gift from B. J. Longley (University of Wisconsin, Madison, WI; now available at The Jackson Laboratory, stock no. 009687). The Krt14-Kitl* allele was heterozygous on all mice used in vitiligo experiments. Thy1.1^{+} PMEL TCR transgenic mice were obtained from The Jackson Laboratory (stock no. 005023) and were used as T cell donors in the vitiligo model. GFP-PMEL TCR transgenic mice were produced by crossing PMEL transgenic mice with DPE^{GFP} mice, which express GFP in T cells (provided by U. von Andrian, Harvard Medical School, Boston, MA).

Vitiligo induction

Vitiligo was induced as previously described (*15*). Briefly, PMEL CD8^{+} T cells were isolated from the spleens of PMEL TCR transgenic mice through negative selection on microbeads (Miltenyi Biotec) according to the manufacturer's instructions. Purified CD8^{+} T cells (1×10^{6}) were injected intravenously into sublethally irradiated (5 Gy 1 day before transfer) Krt14-Kitl* hosts (8 to 16 weeks of age). On the same day of transfer, recipient mice received intraperitoneal injection of 1×10^{6} plaque-forming units of recombinant vaccinia virus expressing PMEL (N. Restifo, National Cancer Institute, NIH) (*44*).

Vitiligo score was objectively quantified by an observer blinded to the experimental groups, using a point scale based on the extent of depigmentation at four easily visible locations, including the ears, nose, rear footpads, and tails, as described previously (*15*). The extent of depigmentation was estimated as a percentage of the anatomic site; both left and right ears and left and right rear footpads were averaged and therefore evaluated as single sites. Points were awarded as follows: No evidence of depigmentation (0%) received a score of 0, >0 to 10% = 1 point, >10 to 25% = 2 points, >25 to 75% = 3 points, >75 to <100% = 4 points, and 100% = 5 points. The "vitiligo score" was the sum of the scores at all four sites, with a maximum score of 20 points.

Repigmentation and durability experiments

Vitiligo mice with >75% depigmentation and stable disease (between week 10 and 20 after vitiligo induction; about 25% of all mice) were used for repigmentation studies. CD122 antibody treatment was performed by intraperitoneal injection of 100 μg of (i) anti-CD122 antibody ChMBC7 (JN Biosciences LLC) or (ii) isotype control (JN Biosciences LLC) or vehicle (PBS, Corning Cellgro) three times weekly. For long-term repigmentation studies, mice were treated for the duration of the observation period (8 weeks). For systemic durability studies, mice were treated intraperitoneally for 2 weeks with 100 μg of antibody three times each week and monitored for an additional 8 weeks after cessation of treatment. For systemic functionality studies, mice were treated intraperitoneally twice weekly for 2 weeks with 100 μg of antibody and were sacrificed 24 hours after the final injection. For local durability studies, mice were treated intradermally three times weekly for 2 weeks with 5 μg of antibody for loading dose and then tapered to 1 μg of antibody for maintenance dose for an additional 2 weeks for a total of 4 weeks of treatment. Mice were monitored for an additional 10 weeks after cessation of treatment. Repigmentation analysis was performed with ImageJ. Photos were taken of each individual mouse before treatment and again after treatment was completed. The images were converted into black and white, and the change in pigment was quantified with ImageJ software, as previously described (*43*).

Study subjects

Patient suction blister biopsies were collected under institutional review board (IRB)–approved protocols at UMMS by board-certified dermatologists, and all samples were deidentified before use in experiments. For suction blister skin biopsies, lesional sites were chosen on the basis of the presence of depigmentation. Active patients were defined as having changes in their lesions over the last 6 months, as well as the presence of confetti depigmentation, a recently described clinical sign of active vitiligo (*45*), or inflammatory vitiligo (*46*). Stable patients were defined as lacking confetti depigmentation and having no changes in their lesions over the previous 6 months. Nonlesional sites were selected as normal-appearing, nondepigmented skin when examined by Wood's lamp, at least 15 cm from the nearest depigmented macule.

Ellipse biopsies about 4 mm × 8 mm were collected from vitiligo patients under IRB-approved protocols at the University of Texas Southwestern Medical Center by board-certified dermatologists. Samples were fixed and paraffin-embedded for use in immunohistochemistry experiments. The depigmented and pigmented edges of the ellipse biopsy were marked with red and blue ink, respectively, for orientation during sectioning.

Immunohistochemistry

Immunohistochemical studies for CD215 were performed on 5-μm sections from formalin-fixed, paraffin-embedded ellipse biopsy specimens from vitiligo patients using a Cell & Tissue staining kit according to the manufacturer's instructions (R&D Systems) with a slight modification. Briefly, after deparaffinization and rehydration,

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the antigen retrieval was performed with citrate buffer (pH 6.0). The sections were permeabilized with PBST (0.2% Triton X-100 in PBS) and then blocked with blocking reagents of peroxidase, serum, avidin, and biotin sequentially. The sections were blocked with Human TruStain (BioLegend) and incubated with goat anti-human CD215 (6 to 10 μg/ml; sc-1524 or AF-247) or goat immunoglobulin G isotype control (R&D Systems) overnight at 4°C, followed by incubation with biotinylated secondary antibody at room temperature for 1 hour. The staining was visualized using the R&D Systems HRP-DAB detection reagent. All the sections were counterstained with hematoxylin, and images were taken using an Olympus BX51 microscope with Nikon NIS Elements software version 3.10.

Blister induction and processing

Suction blisters were induced on the skin using the Negative Pressure Instrument Model NP-4 (Electronic Diversities), as previously described (*17*). Briefly, the suction chambers were applied to the patient skin with 10 to 12 mmHg of negative pressure and a constant temperature of 40°C; blisters formed between 30 min and 1 hour after initiation of the procedure. After blister formation, the blister fluid was aspirated using 1-ml insulin syringes. Blister roofs were removed with scissors and forceps using aseptic technique and were processed by crushing through 100-μm nylon mesh in PBS. Cells were pelleted at 330*g* for 10 min for cell staining.

Flow cytometry and cell sorting

Mouse tissues were harvested and processed as previously described (*21*). Briefly, tail skin and draining lymph nodes were harvested at the indicated times. Lymph nodes were disrupted, and tail skin was incubated with Dispase II (5 U/ml; Roche) for 1 hour at 37°C. Epidermis was removed and mechanically dissociated using 100-μm filters. Dermis was incubated with collagenase IV (1 mg/ml) and deoxyribonuclease I (2 mg/ml; Sigma-Aldrich) for 1 hour at 37°C before mechanical dissociation. Samples were filtered before staining and analysis, and UltraComp eBeads (eBioscience) were used for compensation controls. All mouse flow cytometry samples were blocked with Fc block 2.4G2 (Bio X Cell) and stained with LIVE/DEAD Blue (1:1000; Invitrogen). The following antibodies were used at a 1:200 dilution: CD45, Thy1.1, CD3, CD8β, CD69, CD44, CD103, CD122/IL-15Rβ, and CD62L (BioLegend). CD215/IL-15Rα (R&D Systems) was used at a 1:10 dilution (10 μl per 100 μl).

All human flow cytometry samples were blocked with Human TruStain FcX (BioLegend) and LIVE/DEAD Blue (1:1000; Invitrogen). The following antibodies were used at a 1:20 dilution: CD45 and CD4 (Tonbo Biosciences) and CD8, HLA-A2, HLA-ABC, CD215, CD122, CD49f, CD69, and CD103 (BioLegend). CD3 (BioLegend) was used at a 1:200 dilution. Human blood was screened for HLA-A2 (clone BB7.2, BioLegend)–expressing cells by flow cytometry, and HLA-A2–positive patient samples were treated with 50 nM dasatinib (Axon Medchem BV) for 30 min at 37°C before Fc blockade and labeling with MART-1–, tyrosinase-, and gp100-loaded class I HLA-A2*0201 pentamers (ProImmune) per the manufacturer's protocol. Additional surface staining was performed to identify phenotypes of antigen-specific cells. Peripheral blood was used to make Flouresence Minus One controls to assist in gating, and samples were stained and then fixed/lysed using RBC Fixation/Lysis Buffer (BioLegend) per the manufacturer's instructions. Data were collected with an LSR II and were analyzed with FlowJo software.

Statistical analysis

All statistical analyses were performed with GraphPad Prism software. Dual comparisons were made with unpaired Student's *t* test, and groups of three or more were analyzed by ANOVA with Tukey's or Dunnett's post tests. $P < 0.05$ was considered significant.

SUPPLEMENTARY MATERIALS

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Fig. S1. Analysis of single versus pooled pentamer stains.
Fig. S2. Analysis of melanocyte-specific cells in blister biopsies from healthy controls.
Fig. S3. CD215 staining on human and mouse melanocyte-specific T cells.
Fig. S4. Examining CD215 expression on dendritic cells in vitiligo patient blister fluid.
Fig. S5. Immunohistochemistry of CD215 in vitiligo skin biopsies.
Fig. S6. CD122 antibody blocks IL-15 activity, but not IL-2 activity, in mouse T cell bioassays.
Fig. S7. Analysis of PMEL T cell populations in mice treated with intradermal CD122 antibody.
Fig. S8. Proposed model for CD122 and IL-15/CD215 biology in vitiligo.
Table S1. Characteristics of patients used in this study.
Table S2. PMEL analysis in CD122 antibody systemic durability studies.
Table S3. Primary data.

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Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo

Jillian M. Richmond, James P. Strassner, Lucio Zapata, Jr., Madhuri Garg, Rebecca L. Riding, Maggi A. Refat, Xueli Fan, Vincent Azzolino, Andrea Tovar-Garza, Naoya Tsurushita, Amit G. Pandya, J. Yun Tso and John E. Harris

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Forcing memory T cells to forget

In vitiligo, autoreactive T cells attack melanocytes, leading to white spots on the skin. Depigmentation typically recurs upon cessation of treatment, so new therapies are needed for permanent patient relief. Richmond and colleagues reasoned that targeting tissue-resident memory T cells may allow for durable therapy. They observed that T cells from patient lesional samples expressed the receptor for IL-15, an important survival cytokine. T cells in a mouse model of vitiligo also expressed the IL-15 receptor, and blocking IL-15 signaling with an antibody was able to reverse disease symptoms. A clinical trial to test this therapy is now in the works.

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