CD4 T Cell-Dependent Autoimmunity against a Melanocyte Neoantigen Induces Spontaneous Vitiligo and Depends upon Fas-Fas Ligand Interactions

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Better understanding of tolerance and autoimmunity toward melanocyte-specific Ags is needed to develop effective treatment for vitiligo and malignant melanoma; yet, a systematic assessment of these mechanisms has been hampered by the difficulty in tracking autoreactive T cells. To address this issue, we have generated transgenic mice that express hen egg lysozyme as a melanocyte-specific neoantigen. By crossing these animals to a hen egg lysozyme-specific CD4 TCR transgenic line we have been able to track autoreactive CD4+ T cells from their development in the thymus to their involvement in spontaneous autoimmune disease with striking similarity to human vitiligo vulgaris and Vogt-Koyanagi-Harada syndrome. Our findings show that CD4-dependent destruction of melanocytes is partially inhibited by blocking Fas-Fas ligand interactions and also highlights the importance of local control of autoimmunity, as vitiligo remains patchy and never proceeds to confluence even when Ag and autoreactive CD4+ T cells are abundant. Immune therapy to enhance or suppress melanocyte-specific T cells can be directed at a series of semiredundant pathways involving tolerance and cell death. The Journal of Immunology, 2006, 177: 3055–3062.

Vitiligo is an acquired and highly stigmatizing disease that affects between 1 and 2% of the worldwide population (1–3). It is characterized by variable numbers of depigmented macules, which are often symmetrically placed and frequently affect exposed body areas and the skin around orifices (1, 2). Although the precise etiology is unknown, an autoimmune component is strongly suggested by immune infiltrates and skin-homing autoreactive T cells (4–6) that target melanocyte-specific Ags, such as melan-A/MART1, Gp100, tyrosinase, and tyrosinase-related protein (TRP)-1α and TRP-2 (7, 8).

The role of central and peripheral tolerance directed toward melanocyte-specific Ags has been difficult to dissect due to the difficulty in tracking rare autoreactive T cells. Vitiligo is observed in patients suffering defects in central tolerance including autoimmune polyglandular syndrome 1 (or APECED) and is common in patients with other identified causes of polyglandular autoimmune disease (9, 10). However, recent studies have shown that the level of thymic expression of melanocyte-specific Ags is highly variable between individuals (11). The patchy nature of vitiligo and variation in disease severity suggest that the autoreactive precursor frequency is not the only determinant of disease and that peripheral and local mechanisms are involved (5, 8, 12). The induction of melanocyte-specific peripheral CD8+ T cell tolerance has been demonstrated in the neonate, when the epidermis is accessible to migrating T cells (13), but the situation in the adult is less clear. There is some evidence for peripheral tolerance to melanocyte-specific Ags (14, 15) and roles for ignorance and anergy have been suggested (16, 17). However, the mechanism of tolerance induction and level of persistence are unclear.

The same melanocyte-specific Ags targeted in vitiligo are expressed on melanoma cells and are frequently targeted during cancer progression (8, 18). A natural corollary of melanocyte-specific autoimmunity is the induction of autoreactive T cells capable of mediating tumor rejection, a supposition supported by the development of vitiligo in melanoma patients successfully treated with IL-2 (19). However, reliable immunization strategies to enhance autoimmunity against malignant melanoma have been difficult to achieve and trials with a wide range of vectors and adjuvant therapies have typically produced clinical response rates of <3% (20).

A more effective therapeutic strategy might involve inhibiting melanocyte-specific tolerance and augmenting subsequent autoimmune reactions by blocking signaling moieties such as CTLA-4 (21–23). A rational approach requires a more detailed understanding of the basic mechanisms underlying tolerance to melanocyte Ags.

To track autoreactive CD4+ T cells specific for melanocyte Ags, we have generated transgenic mice that express an intracellular melanocyte-restricted neo self-Ag, hen egg lysozyme (HEL). By crossing these animals to a HEL-specific CD4 TCR transgenic, we have been able to track autoreactive CD4+ T cells from their development in the thymus to their involvement in melanocyte-specific autoimmunity leading to vitiligo and uveoretinitis. The distribution of lesions in this disease is strikingly similar to human vitiligo vulgaris and the Vogt-Koyanagi-Harada (VKH) syndrome. We show that CD4+ T cells are sufficient for vitiligo and that the...
destruction of melanocytes depends on signaling via Fas-Fas ligand (Fas-Fasl). The chronicity of disease and the progressive development of autoimmune foci allowed us to monitor and linearly score vitiligo development. Our findings highlight the complexity and redundancy in melanocyte-specific tolerance with implications for the design of therapy to treat melanoma and vitiligo.

Materials and Methods

Generation of TrpHEL mice

The TrpHEL transgene was prepared by replacing the promoter of the previously described intracellular membrane-bound HEL construct (mHEL-KK) (24) with the TRP-2 promoter, which was a gift from P. Overbeek (Baylor College of Medicine, Houston, TX) (25). The mHEL-KK construct was linearized with ClaI; the MHC class I promoter was removed by BamHI partial digest to yield a 10.4-kb fragment, containing the lysozyme gene linked to MHC class I transmembrane region along with the endoplasmic reticulum retention motif; and Nofl and ClaI restriction sites were inserted using an oligonucleotide linker. The TRP-2 promoter was subcloned via Sall and XbaI into pBluescript together with a linker introducing new Nofl and a ClaI site. The final construct (TrpHEL) was obtained by ligating the 1.5-kb TRP-2 promoter and 10.4-kb mHEL-KK fragment; the TrpHEL construct was then excised from the vector by digestion with Sall and Nofl and prepared for microinjection into (C57BL/6 × CBA/Cas)F1 oocytes as previously described (24). Transgenic founders were identified by PCR, and animals were kept in specific pathogen-free conditions. Mice were backcrossed to B10.BR mice or to the 3A9 TCR transgenic mouse on the B10.BR background (a gift from M. Davis, Stanford University School of Medicine, Stanford, CA) for five to seven generations and then to gld/gld, rag-1−/−, and myd88−/− (a gift from S. Akira, Osaka University, Osaka, Japan) mice (26, 27). All of the animal experiments were approved by local ethical review committees and performed under Home Office license.

RT-PCR

Total RNA was isolated from thymus using TRIzol reagent (Invitrogen Life Technologies), and 1–2 μg of DNase I-treated RNA was reverse transcribed using a Superscript First Strand Synthesis system (Invitrogen Life Technologies). One to 2 μl of cDNA was amplified for HEL (forward) CGA TGG GAG TAC CGA CTA CG and (reverse) TTC ACG CTC GCT GTT ATG TC at 95°C for 2 min, 95°C for 30 s, 57°C for 30 s, 72°C for 1 min and for actin (forward) TAC AGC TTC ACC ACA GC and (reverse) CCT CTG CAT CCT GTC AGC CA at 95°C for 2 min, 95°C for 30 s, 59°C for 30 s, 72°C for 1 min. Samples were analyzed after 25, 30, 35, and 40 cycles for HEL and after 15, 20, 25, and 30 cycles for β-actin on a 2% agarose gel.

Histology

Eyes for histology were fixed in 2.5% buffered glutaraldehyde and embedded in resin for standard H&E staining. OCT-embedded skin was sectioned and stained, as described previously (24, 28). Additional Abs used were rabbit anti-mouse CD4 + (BD Biosciences), polyclonal goat anti-mouse TRP-2 (The Jackson Laboratory), and anti-goat FITC (BD Biosciences). Images were captured with a Zeiss Axioskop microscope or a Leica TCS SP confocal microscope.

Vitiligo scoring

Vitiligo was scored as follows: 0, wild-type; 1, spotting in the ears; 2, ears severely affected; 3, ears severely affected and body affected; and 4, ears and body severely affected. Weak or background vitiligo was defined as 0–1 and was characterized by affected animals having less pigment in the ears than wild-type control.

Eye scoring

Disease severity was scored in a masked fashion after examination of four sections of each globe cut at different levels using a novel semiquantitative system (J. V. Forrester, manuscript in preparation): 0, no disease; 1, occasional inflammatory cell, and large round pigment cell on iris and in choroid; rare patches of depigmentation; 2, more of above plus cells in vitreous (vitritis); 3, as above plus evidence of iritis, choroiditis, significant depigmentation, beginning retinal infiltration with large pigment cells; and 4, moderate inflammatory cell infiltrate, extensive pigment cell migration and areas of depigmentation, widespread retinal destruction, cataract.

Flow cytometry

Whole cell suspensions were stained, as previously described (29) with the following mAbs: 1G12 anti-clonotype culture supernatant (a gift from E. Unanue and D. Peterson, Washington University, St. Louis, MO) (30) followed by rat anti-mouse IgGl-allophycocyanin (BD Biosciences), anti-CD44 FITC, anti-CD4 FITC, anti-CD8 PE, anti-Vj8.2/8.3 PE, anti-CD25 PE, anti-CD4 TriColor, anti-CD45RB TriColor (Caltag Laboratories), anti-CD25 TriColor, anti-CD4 allophycocyanin Cy7, anti-CD3 PE, and anti-CD5 FITC (BD Biosciences). Foxp3 staining was performed with the PE anti-mouse/rat Foxp3 staining set from eBioscience as per the manufacturer’s instruction. Data were collected on a FACSCalibur or a FACSCanto flow cytometer and analyzed with CellQuest or FACSDiva software (BD Biosciences).

In vivo proliferation assays for CD4+ T cells

Splenocytes from 3A9 TCR mice were isolated and labeled with 1 μM CFSE as described (31). A total of 1 × 10 5 cells was adoptively transferred by i.v. injection into TrpHEL, B10.BR, and ML5 (H-2k; express soluble HEL at 10–20 ng/ml) mice. Recipient mice were sacrificed 72 h later and assessed for lymphocyte proliferation by flow cytometry.

In vivo anergy assays for CD4+ T cells

The Th assay was performed essentially as described elsewhere (32). The percentage of purified CD4+ cells was typically 90% or above.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 4.0. Statistical analysis was performed using unpaired, two-tailed, t test, with a 95% confidence index. Values for p < 0.05 and p < 0.01 are indicated.

Results

The generation of mice expressing a melanocyte neoantigen

To explore tolerance to melanocyte-specific Ags, we generated a series of transgenic mice expressing HEL under the TRP-2 promoter (Fig. 1a). To sequester the Ag in melanocytes we used an endoplasmic reticulum-restricted membrane-bound transmembrane form of HEL (mHEL-KK) (24). Transgenic mice were produced from (CBA/Cas × C57BL/6)F1 mice, and two lines with similar levels of HEL expression, designated TrpHEL1 and TrpHEL2, were both backcrossed five to seven generations to B10.BR. The comparison between two lines and careful use of age-matched littermates controlled for backcrossing. Both lines appeared healthy with normal coat color, bred well and no significant differences were found between mice of different generations or genders. Microscopy and semiquantitative real-time PCR was used

FIGURE 1. Characterization of TrpHEL transgenic mouse. A, Schematic diagram of the transmembrane (TM) TrpHEL construct. B, Representative semiquantitative limited cycle RT-PCR of HEL (top) and β-actin (bottom), from thymi after 25, 30, 35, and 40 cycles for HEL and 15, 20, 25, and 30 cycles for β-actin. C, OCT-embedded skin sections stained for HEL (dark red). No positive staining was seen in nontransgenic controls (NON).
to assess the expression of HEL in the skin and thymus (Fig. 1, B and C). Confocal microscopy of the skin showed colocalization of intracellular HEL and TRP-2 in melanocytes (data not shown). Soluble HEL (>0.5 ng/ml) was detected in the serum of 1 of 10 TrpHEL1 and 1 of 12 TrpHEL2 mice (data not shown), suggesting that HEL is relatively well sequestered in the skin. Immunization with melanocyte-specific Ags and adjuvant has been shown to induce depigmentation in a number of mouse models (33–36). However, immunizing adult TrpHEL1 mice with HEL in CFA resulted in only one mouse of eight tested developing depigmentation, and the diffuse pattern of graying was unlike spontaneous vitiligo (data not shown). This result implies that immunological tolerance to HEL is robust.

Spontaneous vitiligo and uveitis develop in TCR-TrpHEL mice

To track melanocyte-specific T cells, we crossed the TrpHEL mice with the 3A9 CD4 TCR transgenic line, which recognizes the immunodominant HEL peptide 46–61 in the context of I-A^k (26, 32, 37). To our surprise, TCR-TrpHEL-1 and -2 double transgenic mice developed spontaneous vitiligo (Fig. 2) that had a distribution strikingly similar to human vitiligo vulgaris. The disease had a penetrance of 87% in animals scored (22 of 26 TCR-TrpHEL1 and 58 of 66 TCR-TrpHEL2). It was first evident in the ears at 21 days, and as animals matured, it principally affected the eyes, genitalia, back, and abdomen (Fig. 2, B–D), but although disease was generally progressive, it never developed to confluence, even after substantial aging (Fig. 2E). To track vitiligo over time, we developed a semiquantitative grading system (Fig. 3A). In general, mice without vitiligo at weaning did not go on to develop florid disease, whereas mice with more obvious lesions were later associated with grade 3–4 disease (Fig. 3, B and C, and data not shown). Again, this variation was not due to backcrossing because unaffected TCR-TrpHEL2 mice were able to transmit vitiligo to offspring when bred to B10.BR.

Uveitis, poliosis, and vitiligo are associated with low-grade meningitis/meningism in VKH disease, which is a major cause of blindness in East Asia and Turkey. Recent studies suggest that the autoimmune target in VKH is TRP-1 or TRP-2 (38). Accordingly, we scored the TCR-TrpHEL lines for disease affecting the melanocytes of the uveal tract of the eye (comprising the iris, the choroid, and the ciliary body). Inflammatory changes were observed in the uveal tract and, in severe cases, in the retina (Fig. 3, E and F). The eye disease (Fig. 3F) was unlike previously described uveitis and included several unusual histological features, including large rounded melanin-containing cells, patches of depigmentation in the iris and choroid, and low-grade inflammatory cell infiltration in the form of macrophages and lymphocytes. Interestingly, the retinal pigment epithelium was unaffected except in severe disease.

FIGURE 2. Spontaneous autoimmune vitiligo. A, Vitiligo affecting the torso, ears, and eyes of a double transgenic (top); shown with an unaffected littermate control (bottom). B, Depigmentation spots in the ears and around the eyes of a double transgenic (top); shown with an unaffected littermate control (bottom). C, A complete lack of pigment in the ears of a 21-day-old double transgenic mouse (top); shown with an unaffected littermate control (bottom). D, Severe vitiligo in matured mice with the susceptible genotype.

FIGURE 3. Spontaneous vitiligo and uveitis in TCR-TrpHEL mice. A, Scale depicting vitiligo severity in TCR-TrpHEL mice. B, Weekly vitiligo scores, from weaning, of double transgenic TCR-TrpHEL mice. C, Weekly vitiligo scores of double transgenic TCR-TrpHEL line. D, Vitiligo scores of adult TCR-TrpHEL transgenic lines. E, Eye disease scores in TCR-TrpHEL mice. F, Histology of eye disease in TCR-TrpHEL double transgenic mice. Panels show (top to bottom): pristine retina; mild retinal vasculitis (black arrow); choroid pathology (rounded presumed migratory melanocytes, black arrow), normally pigmented retinal pigment epithelium (gray arrow), and patch of depigmented choroid without melanocytes (white arrow); retinal degeneration with migrated (presumed choroidal/iris) melanocytes in the retinal parenchyma; and rounded migratory iris melanocytes with low-grade iritis. All images are magnified to ×20.
The histology from diseased TCR-TrpHEL mice may be representative of undocumented early stage human VKH. However, our animals remained healthy and there was no histological evidence of meningitis in mice with established vitiligo (data not shown).

Central, peripheral, and local checkpoints control melanocyte-specific tolerance

To analyze tolerance to melanocyte-specific Ags through development, we enumerated HEL-binding clonotype-specific (1G12+CD4+) CD4+ T cells in the thymus and secondary lymphoid organs. Clonotype-specific CD4+ T cells were reduced 3-fold in the thymus and 4- to 9-fold in the periphery of the TCR-TrpHEL1 line (Fig. 4). In contrast, there was no loss of clonotype-specific cells in the thymus of TrpHEL2 mice but cells were depleted in a majority of secondary organs (Fig. 4). The level of central tolerance is likely to reflect the absolute and local expression of HEL in the thymus. Significant receptor down-modulation or editing, evidenced by lower binding to the 1G12+ or V8.2/8.3 Ab, was not seen (data not shown). Thymic control of T cell responses also involves the release of Ag-specific regulatory T (Treg) cells (39).

Peripheral clonotype-specific CD4+CD25+ cells were enriched as a fraction of total 1G12+ CD4+ T cells in TCR-TrpHEL mice (Fig. 5A). However, the absolute number of 1G12+ CD4+CD25+ or Foxp3+ CD4+CD25+ cells was not significantly increased in the thymus or periphery of TCR-TrpHEL double transgenic mice (Fig. 5, B and C), suggesting that Treg populations are preserved relative to conventional T cells and are not subject to the same stringent central and peripheral deletion regimens.

The induction of peripheral tolerance requires self-Ag presentation in the periphery (40, 41) and to measure this we transferred naive CFSE-labeled 3A9 cells into each of the TrpHEL lines. After 3 days there was marked proliferation of the transferred cells in the skin draining lymph nodes but lower proliferation in the mesenteric lymph nodes (Fig. 6A). In agreement with efficient HEL presentation, we found evidence of previously activated clonotype-specific CD4+CD44high T cells (42) in the inguinal lymph node of TCR-TrpHEL mice (Fig. 6B). To measure the activation of CD4+ T cells in each line we used a sensitive in vivo B cell helper assay (32). Briefly, CD4+ T cells were isolated from TCR or TCR-TrpHEL mice, mixed with spleen cells from anti-HEL Ig-transgenic mice (MD4, H-2d; or reconstituted chimera) and transferred into partially irradiated soluble HEL expressing recipients (MLS, H-2b). Naïve CD4+ T cells from TCR mice initiated a potent B cell response with >10⁶ Ab-forming cells per spleen being produced within 5 days. In contrast, the response was up to 1000-fold lower when CD4+ T cells from double transgenic mice provided help (Fig. 6C). This response may reflect both anergy and Treg cell functions. Anergic 3A9 T cells have previously been shown to up-regulate CD5 and down-regulate CD3 (32, 43), and TCR-TrpHEL1 and TCR-TrpHEL2 show moderate modulation of these receptors (Fig. 6D). Therefore, although both central and peripheral tolerogenic mechanisms are evident in our model, sufficient numbers of immunocompetent anti-melanocyte CD4+ T cells persist and can mediate autoimmunity in both TCR-TrpHEL lines. Nevertheless, the patchiness and limited progression of vitiligo foci suggest that, in addition to tolerogenic pathways, local mechanisms may prevent florid disease.

Activation of CD4+ T cells is not limited by MyD88-dependent signaling

Previous studies have shown that localized trauma and inflammation can initiate vitiligo (12, 36, 44) and the predilection for degeneration at body orifices suggests that a localized innate immune response may augment the activation of autoreactive CD4+ T cells in the vicinity (45). The innate immune response has been shown to be critical for inducing dendritic cell (DC) maturation.
Nontransgenic controls (NON) are also shown. Values from one recipient mouse. Results are shown as the mean with SD.

CD4-dependent vitiligo requires Fas-FasL interactions. A, Score of grade 4 vitiligo in a pfp−/− TCR-TrpHEL2 line. B, Vitiligo scores of wt/wt, wt/gld, and gld/gld TCR-TrpHEL1 mice. C, Absolute number of CD4+ single positive cells and 1G12+ CD4+ T cells in thymus, spleen, pooled submandibular and superficial cervical lymph nodes (SLN), and inguinal lymph nodes (IAL) in wt/wt, wt/gld, and gld/gld TCR-TrpHEL1 mice. Results are shown as the mean with SD. Statistical analysis was performed using unpaired t test to wild-type TCR-TrpHEL1 mice. *p < 0.05; **p < 0.01.

Fas-dependent destruction of melanocytes by CD4+ T cells

To assess the importance of CD4+ T cells in the autoimmune vitiligo we generated Rag-deficient double transgenic TCR-TrpHEL1 and TCR-TrpHEL2 mice. The fact that these mice still developed vitiligo and uveitis with a severity similar to Rag-deficient animals suggests that CD4+ T cells can induce autoimmunity and destroy melanocytes without the need for accessory Ag-specific cells (data not shown). The two principal mechanisms through which CTLs mediate targeted cell death involve perforin and Fas-mediated signaling (47, 48). When we crossed TCR-TrpHEL2 onto the perforin knockout background the pfp−/− TCR-TrpHEL2 mice continued to develop severe vitiligo (Fig. 7A). However, the incidence and severity of vitiligo in TCR-TrpHEL1 carrying a mutation in FasL (gld/gld) was considerably reduced (Fig. 7, B and C). Disease penetrance was reduced to 43% in gld/gld TCR-TrpHEL1 mice (10 of 23 gld/gld TCR-TrpHEL1 mice) (Fig. 7B) and disease severity was also reduced in heterozygote mutants (wt/gld, TCR-TrpHEL1 mice) (Fig. 7C).

There is conflicting data on the role of Fas-FasL in thymic selection (30, 49) and so we were keen to exclude this interaction as an indirect cause of peripheral immune modulation. We found no difference in the number of thymic CD4+ or 1G12+ CD4+ T cells between wild-type and gld/gld TCR-TrpHEL1 mice (Fig. 7D). There was also no difference in peripheral numbers of 1G12+ CD4+ T cells (Fig. 7D). Only the spleen showed elevated total CD4+ T cell number in gld/gld TCR-TrpHEL1 mice (Fig. 7D). These results demonstrate the destruction of melanocytes and the severity of disease are both partially dependent on Fas.

Discussion

In this study, we describe a mouse model of spontaneous autoimmune vitiligo that closely mimics human disease in appearance and progress. In our model we have been able to track melanocyte-specific CD4+ T cells and highlight the importance of these cells in disease onset. Our ability to follow and quantify this autoimmune disease over time helps to highlight the importance of local through TLR signaling thus differentiating between immunogenic and tolerogenic Ag presentation (45, 46). We sought to address the importance of TLR signaling by crossing our double transgenic mice with mice deficient in the key TLR adaptor protein MyD88 (45, 46). However, vitiligo still developed in the myd88−/− TCR-TrpHEL2 line (data not shown), indicating that this pathway is dispensable for T cell activation and subsequent autoimmunity in this model.
as well as central and peripheral mechanisms of tolerance. Indeed, the same patchiness that we observe in vitiligo is likely to occur in less visible and subclinical forms of autoimmune disease in internal organs, such as type 1 diabetes and multiple sclerosis (32, 50), which also begin with distinct foci of CD4-dependent autoimmunity. Local tolerance is poorly understood but may hold the key to treatments that can prevent the transition from subclinical to overt disease in subclinical autoimmunity and cancer.

Thymic expression of Ag is associated with the deletion of autoreactive cells, which was significantly induced in the TCR-TrpHEL1 line (32, 43, 51, 52). Ag expression in the thymus also determines the release of natural Treg cells (39) and while self-Ag expression had no effect on the absolute number of clonotype-specific CD4⁺CD25⁺ cells or Foxp3⁺ CD4⁺CD25⁺ cells, the preservation of this cell population in TCR-TrpHEL mice led to an increase in their number relative to autoreactive T cells. Broadly similar findings have been reported in TCR-insHEL mice (43, 53) and in mice expressing HEL in the retina (TCR-rHEL) (T. Lambe, unpublished data). Treg cells may play a role in controlling vitiligo and in mice expressing HEL in the retina (TCR-rHEL) (T. Lambe, unpublished data). We have also crossed TrpHEL1 mice to VDJx animals that have the VDJ gene segment of the HEL-specific H HEL10 Ab knocked in to replace segments J1-J4 of the H chain (L. Tang and J. Cyster, unpublished data) and the transgene for the anti-HELκ L chain (68). Comparison of VDJx and VDJx-TrpHEL1 double transgenic mice showed no evidence of B cell tolerance in the latter, with normal numbers of peripheral anti-HHEL-specific B cells and titers of switched and unswitched anti-melanocyte specific Abs similar to controls (K. Silver, unpublished data). Therefore, B cells are immunologically competent to respond to the melanocyte specific self-Ag but even high titers of IgG autoantibodies are insufficient to cause disease.

With respect to the mechanisms of CD4-mediated killing, there is ample evidence that CD4⁺ T cells are able to destroy MHC class II-expressing cells via Fas-FasL interactions (69, 70) and melanocytes are able to express both MHC class II and Fas (71, 72). The importance of Fas-FasL signaling in mediating melanocyte cell death is emphasized by the high incidence of melanoma cells, which have evaded the immune system through down-regulation of Fas and up-regulation of FasL (59, 73). Fas-FasL signaling has also been implicated in other forms of cell-specific autoimmunity including experimental autoimmune encephalomyelitis and autoimmune diabetes (74). Fas-FasL signaling certainly plays a role in our model, as demonstrated by the lower frequency and severity of disease in gld/gld double transgenic mice. The absolute numbers of clonotype-specific cells was not affected by the gld mutation; thus, defective clonal deletion is not responsible for the lower frequency of autoimmunity.

It can be argued that effective immunotherapy against malignant melanoma is only possible when we have a better understanding of the basic mechanisms of normal tolerance to melanocyte-specific self-Ags. Indeed, many of the current models of tumor immunity are likely to be flawed by the induction of immunity against non-self-Ags, including retroviruses, in the transplanted tumors (75). There are a number of vaccination models for melanoma rejection, which have demonstrated successful therapeutic activity with concurrent depigmentation (22, 33). It has also been demonstrated that the activation of CD4⁺ T cells is critical during the immunization phase of the immune response, though other cells may be required in the effector phase (35, 76). As a direct result of experimental findings and the isolation of Ag-specific cytotoxic CD8⁺ T cells from melanoma patients, a concerted effort has focused on the development of therapeutics directed at augmenting cytotoxic CD8⁺ T cell activity (5, 8, 20, 77). However, our results suggest that CD4⁺ T cells are sufficient to initiate the destruction of melanocytes, in addition to being adept at providing help to other effector lineages (78, 79). Consequently, CD4⁺ T cells should be considered proficient therapeutic targets for vaccination and treatment of spontaneous melanoma and vitiligo. Better understanding of the complex interplay between different tolerogenic pathways, particularly local control, will lead to new therapies in a variety of clinical and subclinical autoimmune diseases, such as vitiligo and melanoma.
VKH disease, and improved treatment for eradicating metastatic malignant melanoma.

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Disclosures

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