DERMATOLOGICA SINICA 32 (2014) 240–247

Contents lists available at ScienceDirect

Dermatologica Sinica

journal homepage: http://www.derm-sinica.com

REVIEW ARTICLE

Animal models of vitiligo: Matching the model to the question☆

Kingsley I. Essien, John E. Harris*

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

ARTICLE INFO

Article history:
Received: Aug 4, 2014
Revised: Sep 27, 2014
Accepted: Sep 30, 2014

Keywords:
adaptive immunity
animal model
autoimmunity
cellular stress
innate immunity
vitiligo

ABSTRACT

Vitiligo is an autoimmune disease of the skin that is characterized by patchy depigmentation (i.e., white spots) and results from the loss of melanocytes, which are pigment-producing cells. The pathogenesis of human vitiligo consists of an interaction between intrinsic melanocyte defects, environmental factors, and autoimmune mechanisms that target these cells for destruction. Human clinical and translational studies have outlined pathways that are important in human disease; however, combining human correlative studies with mechanistic studies in representative preclinical animal models is a powerful approach to study disease pathogenesis and develop new treatments. Because of the complex pathogenesis of vitiligo, it is unlikely that any one single animal model will adequately reflect all factors implicated in the initiation, progression, and maintenance of the disease. Therefore, vitiligo is best modeled by multiple systems—each with its strengths and weaknesses—that allow insight into specific components of vitiligo pathogenesis. In this paper, we describe some of the available animal models that have been developed to study vitiligo.

Introduction

Human vitiligo pathogenesis

Vitiligo affects 0.5–1% of the population and is characterized by skin depigmentation resulting from the loss of melanocytes that produce epidermal pigment. Men and women are equally affected, and one-half of patients develop vitiligo before the age of 20 years. Patients and their family members are at higher risk for other autoimmune diseases such as thyroiditis, type 1 diabetes, pernicious anemia, Addison's disease, and alopecia areata. Depigmentation frequently affects the face, hands, feet, and genitals, although any part of the skin may be involved. The affected skin lacks epidermal melanocytes, whereas hair follicles within the lesional skin are often spared, likely because of the immune privilege of the hair follicle. Successful treatment results in repigmentation of the skin that begins around the hair follicles, and presumably originates from melanocyte stem cell reservoirs that are protected within the follicle.[1–3] Patients with vitiligo have a three-fold decreased risk of developing melanoma, and patients with melanoma who spontaneously develop vitiligo have a better prognosis because they sometimes clear their tumor.[4] New melanoma treatments that activate antitumor immunity have had impressive success in the clinic, but the treatments also induce vitiligo.[5,6] These observations strongly suggest that melanocyte-specific immune responses are shared between vitiligo and melanoma immunity. The pathogenesis of vitiligo involves multiple components such as intrinsic melanocyte defects, environmental factors, autoimmunity, and genetic factors that influence each of these components.[3,7–9]

Melanocytes from uninvolved vitiligo skin grow less efficiently than melanocytes from healthy patients and they exhibit structural defects, which indicate intrinsic abnormalities.[10,11] They also die more readily in response to exogenous oxidative stressors, compared to normal melanocytes.[12,13] They contain elevated levels of reactive oxygen species[14,15] and greater endoplasmic reticulum stress.[10] These abnormalities may also be induced after exposing the skin to chemical phenols such as 4-tert-butylphenol and monobenzyl ether of hydroquinone (i.e., monobenzone), which

Conflicts of interest: The authors declare no conflicts of interest.

☆ *Scientific significance:* Because of the complex pathogenesis of vitiligo, it is unlikely that any one single animal model will be sufficient to identify all factors implicated in the initiation, progression, and maintenance of disease. Vitiligo is best modeled by multiple systems, each with its strengths and weaknesses, which allow insight into specific components of vitiligo pathogenesis. This article is a discussion of the strengths and weaknesses of the available animal models that have been developed to study vitiligo.

* Corresponding author. Division of Dermatology, Department of Medicine, University of Massachusetts Medical School, Lazare Research Building 325, 364 Plantation Street, Worcester, MA 01605, USA.
E-mail address: John.Harris@umassmed.edu (J.E. Harris).

http://dx.doi.org/10.1016/j.dsi.2014.09.008

worsen clinical depigmentation in patients.[16,17] This chemical-induced stress results in proinflammatory signals that activate autoimmunity and are most likely responsible for inducing vitiligo.[16] Thus intrinsic melanocyte stress has an important role in vitiligo pathogenesis, and chemical tyrosine analogs such as monobenzone appear to be environmental factors that promote the disease.

Innate immunity is apparently activated in the affected and unaffected skin of patients with vitiligo, and innate responses may be initiated by the proinflammatory signals produced by stressed melanocytes.[7] For example, natural killer (NK) cell infiltration is elevated in the affected and unaffected skin in vitiligo patients, compared to controls.[18] Furthermore, inflammatory dendritic cells have been implicated in lesions and in the peripheral blood of vitiligo patients. These cells may be induced by the release of HSP70i from melanocytes, which occurs during chemical-induced stress.[19,20] These observations support a role for innate immunity in vitiligo and suggest that innate immune cells may respond to melanocyte stress.

Elevated levels of melanocyte-specific antibodies have been reported in the serum of vitiligo patients, including those that target VIT40, tyrosinase-related protein 1 (TRP1), tyrosinase-related protein 2 (TRP2), and gp100.[21] These antibodies kill cultured human melanocytes *in vitro*.[22] Purified immunoglobulin G from the sera of patients with vitiligo destroys melanocytes in human skin grafted onto nude mice.[23] However, autoantibodies in vitiligo patients are not correlated with disease severity.[24] Autoantibodies exist systemically, but distinct patches of skin are affected. Therefore, the contribution of autoantibodies to vitiligo pathogenesis is unclear.

T cell-driven immune responses are strongly implicated in disease pathogenesis. $CD8^+$ T cells infiltrate the epidermis of vitiligo patients. They express high levels of perforin and granzyme, which reflects their cytotoxic phenotype.[25,26] $CD8^+$ T cells that recognize melanocyte antigens such as MART1, gp100, and tyrosinase exist in higher numbers in the blood and perilesional skin from vitiligo patients, compared to healthy donors.[27–29] The frequency of autoreactive $CD8^+$ T cells is correlated with disease severity.[27] In fact, $CD8^+$ T cells (but not other T cell populations) isolated directly from lesional vitiligo skin are capable of migrating into the epidermis of normal skin from the same donor and inducing melanocyte apoptosis,[30] which suggests that $CD8^+$ T cells are necessary and sufficient for melanocyte destruction in human vitiligo. Interferon (IFN)-γ and IFN-γ-dependent chemokines are expressed in lesional skin in vitiligo.[29–31] This finding is consistent with a $CD8^+$ T cell-driven response.

It is likely that an interplay between melanocyte stress and autoimmunity initiates vitiligo and sustains melanocyte destruction, and studies to identify genes associated with vitiligo support this concept. Associations include genes that are important in melanocyte function (e.g., tyrosinase, *OCA2*, and *MC1R*), in the cellular stress response (e.g., *XBP1*), in innate immunity (e.g., *NLRP1*, *IFIH1*, and *TICAM1*), and in adaptive immunity (e.g., *GZMB*, *HLA-A*, *FoxP3*, and CD80). Some risk alleles for vitiligo are also protective alleles for melanoma, which supports the clinical observation that vitiligo immunity effectively targets melanoma.[32] Functional studies to determine a role for these genes in vitiligo pathogenesis are now needed, and studies in humans and preclinical animal models should be useful. The connections between intrinsic melanocyte abnormalities, innate immune activation, and adaptive immunity are unclear. The connections may also be difficult to define using only human individuals and their tissues. Therefore, animal models provide an excellent opportunity to further dissect the pathogenesis of vitiligo.

However, no model reflects every aspect of the disease. For example, it is important to consider the differences between melanocytes in humans and those of other species, and to consider whether these differences impact their utility as models for human disease. One key difference is the distribution of melanocytes in the skin. Human melanocytes reside in the hair follicles and in the epidermis of hair-bearing skin. However, mouse melanocytes primarily reside in the follicles and are absent from the hair-bearing epidermis. They are present in low numbers in the epidermis at sites lacking fur such as the ear, nose, tail, and paw.[33,34] Even within human skin, there are differences between melanocytes in the epidermis and melanocytes in the hair follicle.[34] Follicular melanocytes only perform melanogenesis during the anagen phase of hair growth, whereas epidermal melanocytes continuously produce melanin. Compared to epidermal melanocytes, active follicular melanocytes are larger than epidermal melanocytes, have a more extensive Golgi apparatus and rough endoplasmic reticulum, and have an increased sensitivity to aging.[34] Because of these distinctions between human melanocytes at different anatomical locations, melanocytes are likely to vary in form and function between species. Unlike humans, mice are generally nocturnal, and therefore mouse melanocytes may have evolved to fulfill different tasks. Functional differences between murine melanocytes and human melanocytes include the fact that tyrosine related protein 1 (TRP1) acts on a different substrate in mouse melanocytes than in human melanocytes during the biochemical conversion of tyrosine to melanin,[35] and murine melanocytes transfer melanin into the medulla of growing hairs. By contrast, human melanin is transferred to the cortex.[34] Melanocytes in murine follicles and human epidermal melanocytes could have relevant biological differences that may be overlooked in an animal model of vitiligo. This caveat should always be considered when interpreting results.

Spontaneous models and induced models of vitiligo have been identified and developed, and each has distinct advantages and disadvantages. Induced models of vitiligo enable investigation of immune events during disease progression and offer a consistent, synchronized presentation of disease. However, depigmentation results from an intentional breaking of immune tolerance through the adoptive transfer of melanocyte-specific T cells, or the administration of antigen and immune adjuvants, or both. Therefore induced models may not exhibit early initiating events of vitiligo. Spontaneous models develop in a more physiologic way, which provides an opportunity to study the initiating events in the disease and how these events translate to autoimmunity. However, the time and cost required to follow a colony of animals that develop a low incidence of disease can be time-consuming and costly.

Animal models of vitiligo that develop spontaneously

Several animal species reportedly spontaneously develop vitiligo. Sinclair swine depigmentation and cellular and humoral immunity against melanocytes have been reported in association with disease. They were bred to exhibit a high incidence of melanoma—54% of these animals are born with melanoma and 85% of them develop melanomas within the 1st year of life—but in 90% of animals tumors spontaneously resolve and are accompanied by local depigmentation of the skin and hair. Between the 4th week and 16th week of life, depigmentation may then spread from the local site and generally involve the skin, hair, and melanocytes in the iris of the eye[36,37] (Figure 1). Peripheral blood leukocytes from affected individuals kill melanocytes *in vitro*, and antibodies to vitiligo antigens are present in their serum.[38] These events are consistent with melanoma regression, followed by depigmentation in humans, and therefore Sinclair swine may provide an opportunity to study crosstalk between melanoma and vitiligo.

Breeds of horses with the Gray allele (which encodes *STX17* and is involved in vesicle transport) such as Arabians, Andalusians, and

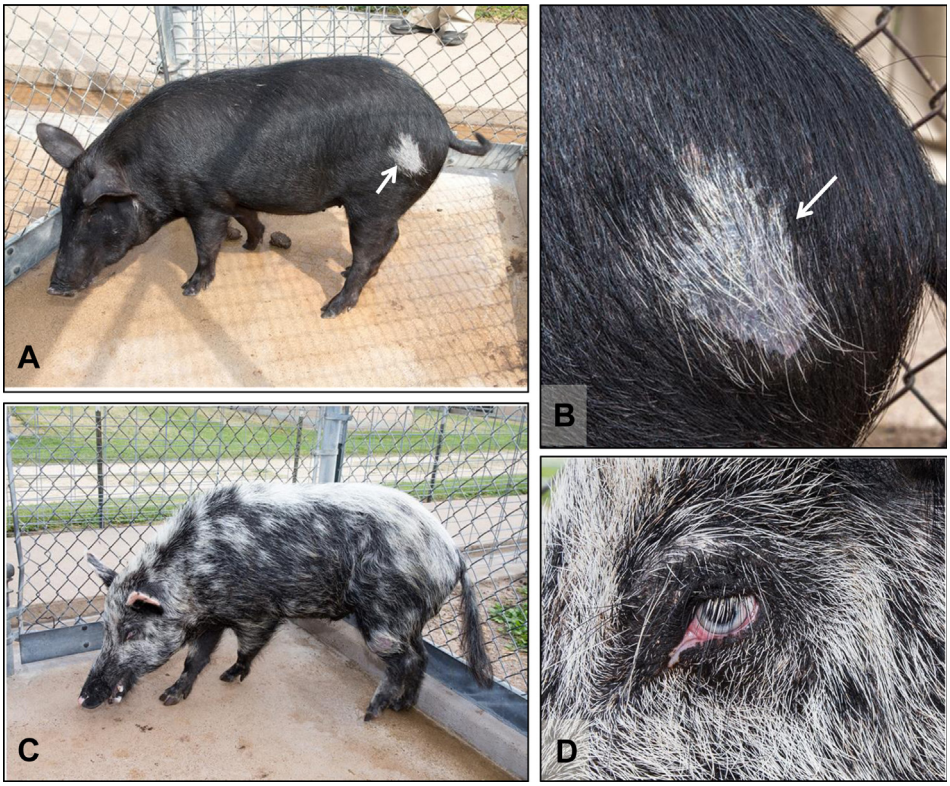

Figure 1 Vitiligo in Sinclair swine. (A, B) Sinclair swine exhibit a high incidence of melanoma. In 90% of animals, tumors spontaneously resolve and are accompanied by local depigmentation of the skin and hair (arrow). (The pig is a 12-month-old female.) In some animals, depigmentation can become more widespread with age and involve (C) the skin and hair and (D) the eye. Disease severity is somewhat unpredictable, but may be correlated with tumor burden. (The pig is a 23-month-old boar.) *Note*: Ino Curik: University of Zagreb, Svetosimunska Zagreb, Croatia; Thomas Druml: University of Veterinary Medicine Vienna, Vienna, Austria; Monika Seltenhammer: Medical University of Vienna, Vienna, Austria; Johan Solkner: University of Natural Resources and Life Science Vienna, Vienna, Austria.

Lipizzaners spontaneously develop vitiligo that typically manifests on the face, perianal, perioral, and perigenital areas.[39] Vitiligo in these horse breeds is also associated with an increased incidence of melanoma, similar to what is seen in Sinclair swine, and early graying of the hair that may or may not be related to vitiligo (Figure 2).[37,40,41] Little is known about the mechanism of vitiligo in horses, but sera from affected horses contain antibodies against surface melanocyte antigens.[42] Therefore, horses may be helpful models to study antibody formation in vitiligo, particularly because they provide large quantities of serum for antibody purification.

Certain dog breeds are genetically predisposed to develop vitiligo such as Rottweilers, German Shepherds, Old English Sheepdogs, Doberman Pinschers, Dachshunds, and German Shorthaired Pointers. In general, affected animals have serum autoantibodies against melanocyte antigens that are not present in healthy animals.[42,43] Dog breeds are genetically homogeneous, enabling powerful genome-wide association studies that require fewer individuals for statistical power than humans.[44] Human genome-wide association studies are preferred; however, identifying additional genes at some point becomes cost-prohibitive because of the small contributions of individual genes to disease pathogenesis and because of the genetic heterogeneity in humans. Therefore, dogs represent a physiologic onset and time course of the disease, and may provide an excellent model in which to further investigate genetic contributions to the disease.

Vitiligo in the Smyth line (SL) chicken exhibits many of the pathogenic characteristics of human vitiligo, which provides an opportunity to investigate the interplay between genetic components, melanocyte defects, stress, and humoral and cellular immune responses that are potentially involved in the onset and pathogenesis of the disease. Smyth line vitiligo (SLV) is characterized by depigmentation of the feathers (Figure 3). Up to 70–95% of hatchlings develop depigmentation when vaccinated to prevent lymphoma, yet without vaccination the incidence is lower.[45] Viral infections have been proposed as environmental triggers of autoimmunity in vitiligo, but definitive evidence of this is lacking. The SLV model nevertheless exhibits a much higher incidence of vitiligo, compared to humans (with an incidence of 0.5–1%).

Patchy depigmentation in SLV presents during early adulthood at 6–14 weeks of age and varies in severity. Smith line chickens are also susceptible to uveitis and other autoimmune diseases associated with human vitiligo such as hypothyroidism and feather loss that is similar to alopecia areata. Melanocyte abnormalities and cellular stress are both implicated in SLV. Reactive oxygen species are increased in SL feathers and in other organs, and melanocytes have numerous notable morphological abnormalities. As in humans, these defects are insufficient for disease, but require immune responses.[45,46]

Similar to human vitiligo, $CD8^+$ T cells infiltrate the feathers of SL chickens with active disease,[47–49] and IFN-γ, and IFN-γ-induced genes are expressed in the affected feathers.[47] Melanocyte autoantibodies have been isolated from SL chickens with vitiligo.[50] Bursectomy decreases the incidence of disease,[51] which suggests that B cells, and possibly autoantibodies, are involved in melanocyte destruction. Because of the sequencing of the chicken genome[52] and the availability of parental control lines, SLV may be a good model for genetic studies. The Brown line chicken is the parental line for SLV and has a low incidence of spontaneous vitiligo that increases after treatment with 5-azacytidine, an inhibitor of DNA methylation. The Light-brown Leghorn line shares an major histocompatibility complex (MHC) haplotype with the Smith line,

Figure 2 Vitiligo due to the Gray allele in Lipizzaner horses. Horses with the Gray mutation in *STX17* develop premature hair graying, an increased incidence of melanomas, and vitiligo-like depigmentation of the skin. (A) A group of young horses, which were born dark but exhibit variable amounts of graying, depending on whether they are homozygous or heterozygous for the Gray allele. (B) The horse in the foreground was originally born dark, as was the horse in the background, but now exhibits hair speckling and vitiligo-like skin depigmentation around the eye. (C) Gradual patchy graying of the hair. (D) Patchy vitiligo of the skin around the nose, cheek, and eye in an aging horse. (E) Perianal patchy depigmentation of the skin, associated with a nodule of melanoma on the underside of the tail (arrow). *Note*: Photos courtesy of Drs. Ino Curik: University of Zagreb, Svetosimunska Zagreb, Croatia, Thomas Druml: University of Veterinary Medicine Vienna, Vienna, Austria, Monika Seltenhammer: Medical University of Vienna, Vienna, Austria, and Johann Sölkner: University of Natural Resources and Life Science Vienna, Vienna, Austria.

but does not develop vitiligo, even after 5-azacytidine treatment, and is therefore a vitiligo-resistant line.[45]

The Jackson Laboratory (Bar Harbor, ME, USA) discovered the mi^{vit}/mi^{vit} mouse strain. It is the first inbred strain with spontaneous depigmentation that is inherited in a recessive manner.[53] Depigmentation in this strain occurs because of the loss of melanocyte function and occurs in the absence of a functional immune system. A point mutation in the microphthalmia-associated transcription factor (*MITF*) gene is responsible for the phenotype.[54] The *MITF* gene directly influences melanocyte development and regulates genes important for melanin production such as tyrosinase.[55] Because of the monogenic nature and lack of autoimmunity in the mi^{vit}/mi^{vit} mouse,[53,54] this strain does not appear to accurately model human vitiligo pathogenesis. The identical mutation in humans, a single arginine deletion in the basic domain of *MITF* does not result in vitiligo, but results in partial albinism and deafness.[56] To date, *MITF* mutations do not appear to be genetically linked to human vitiligo.[57] Because of these clear differences with human vitiligo, this strain is not widely used as a model to study vitiligo pathogenesis.

The spontaneous development of vitiligo in animal models provides an opportunity to study causative factors that initiate disease such as genetic influences and potential environmental triggers. However, the typically low incidence of the disease, the limited resources available for mechanistic studies, and the cost of care for these animals can be difficult. Induced mouse models of vitiligo are limited in providing the ability to investigate genetic influences and other initiating events in vitiligo; however, they provide an opportunity to investigate disease mechanisms during the progression of vitiligo in a synchronized and predictable system with many tools available for mechanistic studies. The aforementioned Brown line chicken develops vitiligo after the administration of 5-azacytidine, whereas the remaining induced models of vitiligo are conducted in mice.

Induced mouse models of vitiligo

Several methods have been developed to induce depigmentation in mice to model vitiligo. Many of these approaches initially induced therapeutic immune responses against melanoma, and result in depigmentation as an unanticipated side effect. These approaches include chemically inducing melanocyte stress, immunizing mice with melanocyte antigens plus immune adjuvants to activate endogenous immune cells, or genetically altering mice to increase the frequency of melanocyte-reactive T cells. Genetically altered mice express a single T cell receptor (TCR) in their T cells that is specific for a particular melanocyte antigen, and therefore all of their T cells only recognize that one antigen. To more closely model human vitiligo in which T cells specific for a particular melanocyte antigen are limited to a small percentage of the total T cell population, some models use the adoptive transfer of a small number of TCR transgenic T cells to a host with a normal immune system. Each approach has advantages and disadvantages, which we will discuss in the following section.

Induced vitiligo through melanocyte stress

As discussed previously, monobenzone is a clinically relevant chemical analog of tyrosine that induces cellular stress in melanocytes, induces proinflammatory signals, and exacerbates depigmentation in vitiligo patients.[16] Zhu et al[58] applied monobenzone to the shaved abdomens of 4-week-old mice; hair depigmentation

Figure 3 Vitiligo in the Smyth line chicken. (A) Normal pigmentation in Smyth line and Brown line chicks. (B) Smyth line adults before developing vitiligo. (C) The onset of vitiligo is evident by depigmentaion of the feathers, which occurs in varying degrees in each bird. *Note*: Photos courtesy of Dr. Gisela F. Erf, Division of Agriculture, University of Arkansas–Fayetteville, Fayetteville, AR, USA.

initially appeared at the application site, but later spread to distant sites such as the ear and tail. $CD8^+$ T cells infiltrate the affected skin after treatment. The same treatment in RAG-deficient mice resulted in hair depigmentation at the treatment site, but not at distant sites.[58] These findings suggest that direct toxicity of monobenzone may be responsible for localized depigmentation at the site of application, but an adaptive immune response is required for depigmentation to spread to other sites. This model may help identify the mechanisms by which monobenzone-induced stress activates the immune response in vitiligo.

Induced vitiligo through immunization

In an approach to induce melanoma immune responses, one group constructed recombinant vaccinia viruses (rVV) that expressed a variety of melanocyte antigens. They used these viruses to vaccinate C57Bl6 mice and found that infection with rVV that expressed human TRP1 resulted in depigmentation of the hair follicles in 80% of mice, while sparing pigmentation in the eye and brain. Depigmentation in this model is correlated with melanocyte-specific autoantibody production, and is dependent on $CD4^+$ T cells rather than $CD8^+$ T cells.[59] An advantage of immunization-induced vitiligo is the role of endogenous host immune cells in depigmentation, which enables the investigation of host immune factors that contribute to melanocyte destruction.

DNA plasmids encoding human TRP2, a melanocyte antigen, have been delivered directly into the skin of mice using a gene gun.[60,61] The DNA is first coated on gold microparticles, which are then delivered into the skin through a carbon dioxide-powered gene gun. Tyrosinase-related protein 2 is then expressed in skin cells transformed by the plasmid. After treatment, the regrowing hairs are depigmented, which is dependent on $CD8^+$ T cells and their expression of perforin.[60] Antibodies against human TRP2 have also been induced.[61] Studies that used gene gun-delivered plasmids encoding human TRP-2 and human HSP70i increased depigmentation in treated mice, whereas immunizations that lacked HSP70i did not.[20] This observation suggests that HSP70i contributes to depigmentation. This connects HSP70i released by stressed melanocytes to functional melanocyte-specific immune responses in vitiligo. A follow-up study revealed that a mutated form of HSP70i was unable to induce disease in this model, and prevented the induction of the disease.[62] This approach is well suited to study the effects of adjuvants such as HSP70i in disease initiation and exacerbation because plasmids encoding key proteins can be simply added during immunization.

TCR transgenic hosts

The AAD + transgenic mouse expresses an MHC I molecule with the peptide binding region of human HLA-A*021 and presents the

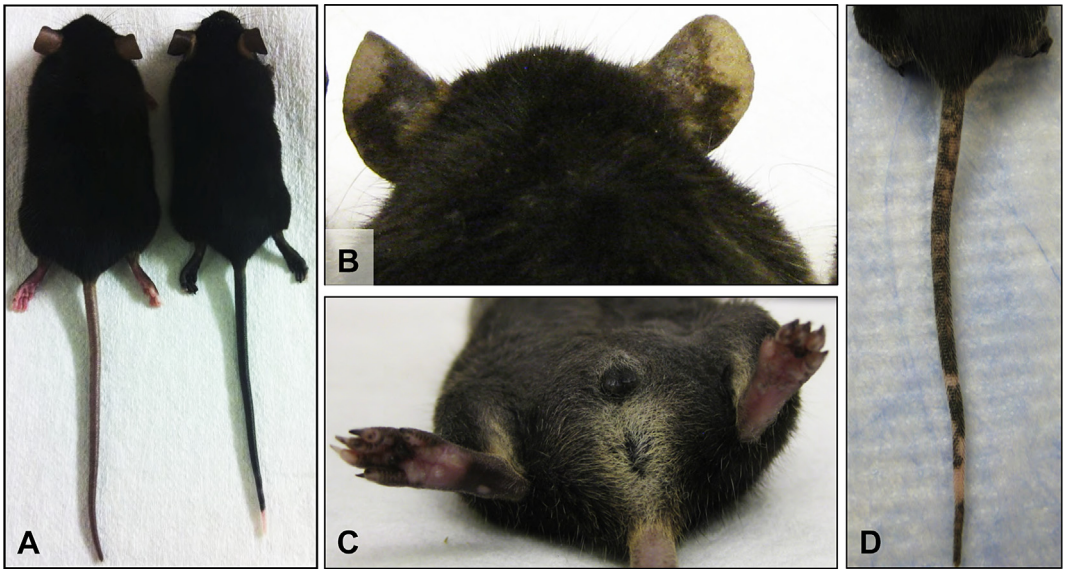

Figure 4 Mouse model of vitiligo with epidermal depigmentation. (A) Hosts for the adoptive transfer of T cells express stem cell factor (SCF) in keratinocytes, which retains melanocytes in the epidermis and result in black skin and hair (a wild-type C57Bl6 mouse is on the left and a K14-SCF mouse is on the right). After the induction of vitiligo, patchy epidermal depigmentation occurs in (B) the ears, (C) the feet, and (D) the tail; however, hair pigmentation is spared.

tyrosinase epitope. Tyr_{369}. AAD + mice were bred with albino mice that lacked the expression of tyrosinase. A T cell clone specific for Tyr_{369} was isolated from these mice. The *TCR* genes from the clone mice were used to generate *TCR* transgenic mice with $CD8^+$ T cells recognizing Tyr_{369} (i.e., FH mice). When FH mice were crossed to AAD + mice, spontaneous depigmentation occurred prominently in the hair and weakly in the tail skin. Immunohistochemistry of the affected skin and hair follicles revealed infiltrating $CD8^+$ and $CD4^+$ T cells. This model is dependent on $CD8^+$ T cells, whereas $CD4^+$ T cells are not required and negatively regulate disease. Depigmentation depended on IFN-γ and the chemokine receptors CXCR3 and CCR5, which bind the IFN-γ-induced chemokines CXCL9, CXCL10, and CCL5.[63]

Mehrotra et al[64] developed a transgenic mouse that develops vitiligo by using mice that express a human melanocyte-specific TCR and human HLA-A2. The human TCR used to create these mice was cloned from a human $CD4^+$ T cell infiltrating a melanoma that had high affinity for the $tyrosinase_{368–376}$ peptide that presented on HLA-A2, which typically interacts with $CD8^+$ T cells. Depigmentation of the hairs in these mice occurs over time, and histological examination reveals a loss of melanocytes in affected hair follicles and antigen specific T cell infiltration of the skin.[64] Because the T cells that are produced in these mice do not express CD4 or CD8, it is unclear whether their effector function is more like $CD4^+$ T cells (from which they are derived), or $CD8^+$ T cells (which normally interact with HLA-A2). Additional studies may reveal how this interesting T cell clone kills melanocytes in this model.

The TrpHEL model results in hair depigmentation that is mediated by $CD4^+$ T cell recognition of a model antigen. Membrane-bound hen egg lysozyme (HEL) was expressed in melanocytes under the control of the TRP-2 promoter. These mice were subsequently crossed to 3A9 $CD4^+$ TCR mice, which have T cells that recognize HEL peptide 46–61. The mice developed depigmentation of their hair beginning at 21 days of age. The mechanism of depigmentation was dependent on Fas-Fas ligand-mediated melanocyte killing.[65] However, human melanocytes appear to be resistant to Fas-mediated killing,[3] and therefore the relevance of this model to human vitiligo is unclear.

Induced through the adoptive transfer of TCR transgenic T cells

Muranski et al[66] generated a transgenic mouse with a MHC II-restricted ($CD4^+$) TCR recognizing TRP1. Adoptive transfer of TRP1-specfiic $CD4^+$ T cells from these mice into a RAG1-deficient or sublethally irradiated C57Bl6 host resulted in rapid depigmentation of the hair. Studies on human vitiligo skin and T cells *ex vivo* have implicated $CD8^+$ T cells in melanocyte destruction, but the role of $CD4^+$ T cells in human disease is unclear. This model provides an opportunity to investigate potential $CD4^+$ T cell contributions to depigmentation in vitiligo. This is an area that requires more insight.

Antony et al[67] injected B16 melanoma cells into RAG-deficient hosts and then adoptively transferred activated $CD8^+$ T cell clones that recognize the melanocyte antigen gp100 (PMEL). The hosts were also infected with fowlpox virus expressing the human gp100 peptide and either IL-2 or $CD4^+$ T cells that had been depleted of T regulatory cells. Tumors regressed in the hosts receiving these cells, and hair depigmentation developed, which represent vitiligo occurring after melanoma treatment. This model is dependent on $CD8^+$ T cell-mediated destruction of melanocytes and IFN-γ,[67] and offers the ability to investigate depigmentation as a consequence of melanoma-targeted immune responses.

Because human vitiligo is characterized by epidermal depigmentation and hairs are often spared within lesions, we developed a mouse model of vitiligo with focused epidermal depigmentation and sparing of the hairs. To do this, we adoptively transferred PMEL $CD8^+$ T cells into hosts that expressed stem cell factor in keratinocytes, as described previously,[68] and therefore retained melanocytes in the epidermis. These mice have black skin and black hair. To activate the T cells *in vivo*, the hosts were then sublethally irradiated and treated with recombinant vaccinia virus expressing the gp100 peptide. From 5 weeks to 7 weeks later, the mice developed patches of depigmentation in the ears, tail, footpads, and nose, with sparing of the hairs[69] (Figure 4). The development of prominent epidermal depigmentation is one strength of this model, and allows investigation of mechanisms that direct T cell migration through the skin to the epidermis. Using this model we discovered that, similar to human disease, IFN-γ induces CXCL9 and

Table 1 Summary of vitiligo models established in chickens and mice.[a]

Model	Animal	Mechanism	Immune	Stress	Genetics	Refs
Smyth	Chicken	Spontaneous	T and B cells are required	Increased ROS in feathers	Inherited depigmentation	45–51
Lerner	Mouse	Spontaneous	N/A	N/A	Point mutation in MITF	53
Zhu	Mouse	Application of MBEH to the shaved abdomens of 4-week-old mice	$CD8^+$ T cells infiltrate	Stress required to induce depigmentation	N/A	58
Overwijk	Mouse	Immunization of B6 mice with recombinant vaccinia virus expressing human TRP1	$CD4^+$ T cells mediate the disease	N/I	N/A	59
Bowne	Mouse	Immunization with plasmids encoding human TRP2 on gold particles *via* gene gun	$CD8^+$ T cells mediate the disease *via* a perforin-dependent mechanism	N/I	N/A	60
Gregg	Mouse	FH TCR transgenic mice expressing human HLA-A*021 with $CD8^+$ T cells recognizing Tyr_{369}	$CD8^+$ T cells mediate the disease and infiltrate the skin; $CD4^+$ infiltration is also present but not required	N/I	N/A	63
Mehrotra	Mouse	The TCR transgene expresses the complete HLA-A2 molecule; TCRs recognize $tyrosinase_{368-376}$	$CD3^+CD8^-CD4^-$ T cells infiltrate the skin	N/I	N/A	64
Lambe	Mouse	$CD4^+$ TCR transgene recognizes HEL, which is expressed under the control of the TRP2 promoter	$CD4^+$ T cells mediate the disease	N/I	N/A	65
Muranski	Mouse	TCR transgenic mouse with $CD4^+$ T cells that recognize TRP1	$CD4^+$ T cells mediate the disease	N/I	N/A	66
Antony	Mouse	Injection of B16 melanoma cells, activated PMEL T cells, virus encoding hgp100 peptide and IL-2	$CD8^+$ T cells mediate the disease; IFN-γ is required	N/I	N/A	67
Harris	Mouse	Adoptive transfer of TCR transgenic $CD8^+$ T cells recognizing melanocyte antigen PMEL into hosts that retain melanocytes in the epidermis	$CD8^+$ T cells mediate depigmentation in an IFN-γ dependent manner	N/I	N/A	69

IFN-γ = interferon-gamma; MBEH = monobenzyl ether of hydroquinone; MITF = microphthalmia-associated transcription factor; N/A = not applicable; N/I = not investigated; ROS = reactive oxygen species; TCR = T cell receptor; TRP1 = tyrosinase-related protein 1; TRP2 = tyrosinase-related protein 2.

[a] The models of vitiligo are listed with identified mechanistic contributions to disease, which include melanocyte stress, autoimmunity, and genetic factors.

CXCL10 in the skin and CXCR3 (their shared receptor) is expressed on melanocyte-specific T cells. In this model, CXCL10 is required for the progression and maintenance of vitiligo, which suggests that targeting CXCL10 or its receptor CXCR3 may be a viable treatment strategy.[29] Because depigmentation in this model is epidermal, it may be the better model to represent epidermal depigmentation in human vitiligo because of the differences between epidermal and follicular melanocytes, as previously described. This model is also well suited to study topical agents that modify disease severity, including immune modulators to treat disease and stress-inducing agents to investigate chemical-induced vitiligo.

A similar model described by Antony et al[67] exhibits hair involvement; however, our model maintains the immune privilege of the hair, thereby providing an opportunity to study the factors that contribute to the immune privilege of the hair follicle. The difference in hair involvement between the models may be because their model uses RAG-deficient host mice, which lack T regulatory cells (Tregs), and Tregs have been reported in other mouse models to limit disease severity.[63,70]

Conclusion

Vitiligo has a complex, multifactorial pathogenesis. Studies conducted directly in humans and their tissues are important, but are primarily limited to correlative observations. Combining mechanistic studies in animal models with observational studies in humans and their tissues is a powerful approach to better understand vitiligo pathogenesis and to develop new treatments for the disease. However, few models reflect the entire spectrum of human disease pathogenesis, and therefore the availability of multiple systems provides complimentary tools for this purpose (Table 1). Spontaneous models of vitiligo provide an opportunity to investigate how vitiligo is initiated, and the genetic contributions to disease. The SL chicken appears to exhibit multiple characteristics of vitiligo that parallel human disease. Future studies in this model may help to clarify interactions between melanocyte stress and autoimmunity that drive depigmentation. Induced models of vitiligo have been primarily conducted in mice, which are less expensive to breed and maintain, and provide a large number of tools to study mechanistic contributions to vitiligo pathogenesis. These models are well suited to study mechanisms that drive disease progression, which are particularly relevant to therapeutic intervention. In summary, animal models provide an opportunity for mechanistic studies to define vitiligo pathogenesis. Each model has its strengths and weaknesses, and should be selected based on the experimental questions being addressed.

References

1. Taïeb A, Picardo M. Clinical practice. Vitiligo. *N Engl J Med* 2009;**360**:160–9.
2. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. *J Am Acad Dermatol* 2011;**65**:473–91.
3. Glassman SJ. Vitiligo, reactive oxygen species and T-cells. *Clin Sci (London)* 2011;**120**:99–120.
4. Teulings HE, Overkamp M, Ceylan E, Nieuweboer-Krobotova L, Bos JD, Nijsten T, et al. Decreased risk of melanoma and nonmelanoma skin cancer in patients with vitiligo: a survey among 1307 patients and their partners. *Br J Dermatol* 2013;**168**:162–71.
5. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;**363**:711–23.
6. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 2013;**369**:134–44.
7. Richmond JM, Frisoli ML, Harris JE. Innate immune mechanisms in vitiligo: danger from within. *Curr Opin Immunol* 2013;**25**:676–82.
8. Laddha NC, Dwivedi M, Mansuri MS, Gani AR, Ansarullah M, Ramachandran AV, et al. Vitiligo: interplay between oxidative stress and immune system. *Exp Dermatol* 2013;**22**:245–50.
9. Passeron T, Ortonne JP. Activation of the unfolded protein response in vitiligo: the missing link? *J Invest Dermatol* 2012;**132**:2502–4.
10. Boissy RE, Liu YY, Medrano EE, Nordlund JJ. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. *J Invest Dermatol* 1991;**97**:395–404.
11. Puri N, Mojamdar M, Ramaiah A. *In vitro* growth characteristics of melanocytes obtained from adult normal and vitiligo subjects. *J Invest Dermatol* 1987;**88**:434–8.
12. Jimbow K, Chen H, Park JS, Thomas PD. Increased sensitivity of melanocytes to oxidative stress and abnormal expression of tyrosinase-related protein in vitiligo. *Br J Dermatol* 2001;**144**:55–65.

13. Maresca V, Roccella M, Roccella F, Camera E, Del Porto G, Passi S, et al. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. *J Invest Dermatol* 1997;**109**:310–3.
14. Schallreuter KU, Moore J, Wood JM, Beazley WD, Gaze DC, Tobin DJ, et al. *In vivo* and *in vitro* evidence for hydrogen peroxide (H2O2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase. *J Invest Dermatol* 1999;**4**:91–6.
15. Shalbaf M, Gibbons NC, Wood JM, Maitland DJ, Rokos H, Elwary SM, et al. Presence of epidermal allantoin further supports oxidative stress in vitiligo. *Exp Dermatol* 2008;**17**:761–70.
16. van den Boorn JG, Picavet DI, van Swieten PF, van Veen HA, Konijnenberg D, van Veelen PA, et al. Skin-depigmenting agent monobenzone induces potent T-cell autoimmunity toward pigmented cells by tyrosinase haptenation and melanosome autophagy. *J Invest Dermatol* 2011;**131**:1240–51.
17. Toosi S, Orlow SJ, Manga P. Vitiligo-inducing phenols activate the unfolded protein response in melanocytes resulting in upregulation of IL6 and IL8. *J Invest Dermatol* 2012;**132**:2601–9.
18. Yu R, Broady R, Huang Y, Wang Y, Yu J, Gao M, et al. Transcriptome analysis reveals markers of aberrantly activated innate immunity in vitiligo lesional and non-lesional skin. *PLoS One* 2012;**7**:e51040.
19. Kroll TM, Bommiasamy H, Boissy RE, Hernandez C, Nickoloff BJ, Mestril R, et al. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: relevance to vitiligo. *J Invest Dermatol* 2005;**124**:798–806.
20. Denman CJ, McCracken J, Hariharan V, Klarquist J, Oyarbide-Valencia K, Guevara-Patiño JA, et al. HSP70i accelerates depigmentation in a mouse model of autoimmune vitiligo. *J Invest Dermatol* 2008;**128**:2041–8.
21. Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res* 2003;**16**:90–100.
22. Norris DA, Kissinger RM, Naughton GM, Bystryn JC. Evidence for immunologic mechanisms in human vitiligo: patients' sera induce damage to human melanocytes in vitro by complement-mediated damage and antibody-dependent cellular cytotoxicity. *J Invest Dermatol* 1998;**90**:783–9.
23. Gilhar A, Zelickson B, Ulman Y, Etzioni A. *In vivo* destruction of melanocytes by the IgG fraction of serum from patients with vitiligo. *J Invest Dermatol* 1995;**105**:683–6.
24. Kroon MW, Kemp EH, Wind BS, Krebbers G, Bos JD, Gawkrodger DJ, et al. Melanocyte antigen-specific antibodies cannot be used as markers for recent disease activity in patients with vitiligo. *J Eur Acad Dermatol Venereol* 2013;**27**:1172–5.
25. van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W, Das P. Local immune response in skin of generalized vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. *Lab Invest* 2000;**80**:1299–309.
26. Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J Pathol* 1996;**148**:1219–28.
27. Ogg GS, Rod Dunbar P, Romero P, Chen JL, Cerundolo V. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J Exp Med* 1998;**188**:1203–8.
28. Lang KS, Caroli CC, Muhm A, Wernet D, Moris A, Schittek B, et al. HLA-A2 restricted, melanocyte-specific CD8(+) T lymphocytes detected in vitiligo patients are related to disease activity and are predominantly directed against MelanA/MART1. *J Invest Dermatol* 2001;**116**:891–7.
29. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci Transl Med* 2014;**6**:223ra223.
30. van den Boorn JG, Konijnenberg D, Dellemijn TA, van der Veen JP, Bos JD, Melief CJ, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. *J Invest Dermatol* 2009;**129**:2220–32.
31. Grimes PE, Morris R, Avaniss-Aghajani E, Soriano T, Meraz M, Metzger A. Topical tacrolimus therapy for vitiligo: therapeutic responses and skin messenger RNA expression of proinflammatory cytokines. *J Am Acad Dermatol* 2004;**51**:52–61.
32. Spritz RA. Modern vitiligo genetics sheds new light on an ancient disease. *J Dermatol* 2013;**40**:310–8.
33. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. *Nature* 2007;**445**:843–50.
34. Tobin DJ. The cell biology of human hair follicle pigmentation. *Pigment Cell Melanoma Res* 2011;**24**:75–88.
35. Boissy RE, Sakai C, Zhao H, Kobayashi T, Hearing VJ. Human tyrosinase related protein-1 (TRP-1) does not function as a DHICA oxidase activity in contrast to murine TRP-1. *Exp Dermatol* 1998;**7**:198–204.
36. Hook Jr RR, Berkelhammer J, Oxenhandler RW. Melanoma: Sinclair swine melanoma. *The American journal of pathology* 1982;**108**:130–3.
37. Lentz KJ, Burns RP, Loeffler K, Feeney-Burns L, Berkelhammer J, Hook Jr RR. Uveitis caused by cytotoxic immune response to cutaneous malignant melanoma in swine: destruction of uveal melanocytes during tumor regression. *Invest Ophthalmol Vis Sci* 1983;**24**:1063–9.
38. Misfeldt ML, Grimm DR. Sinclair miniature swine: an animal model of human melanoma. *Vet Immunol Immunopathol* 1994;**43**:167–75.
39. Erf GF. Animal models of autoimmune vitiligo. In: Picardo M, Taieb A, editors. *Vitiligo*. Berlin, Germany: Springer; 2010. p. 205–18.
40. Rosengren Pielberg G, Golovko A, Sundström E, Curik I, Lennartsson J, Seltenhammer MH, et al. A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nat Genet* 2008;**40**:1004–9.
41. Curik I, Druml T, Seltenhammer M, Sundström E, Pielberg GR, Andersson L, et al. Complex inheritance of melanoma and pigmentation of coat and skin in grey horses. *PLoS genetics* 2013;**9**:e1003248.
42. Naughton GK, Mahaffey M, Bystryn JC. Antibodies to surface antigens of pigmented cells in animals with vitiligo. *Proc Soc Exp Biol Med* 1986;**181**:423–6.
43. McKeever P, Nuttall T, Harvey RG. Pigmentary abnormalities. In: Beynon P, editor. *A colour handbook of skin diseases of the dog and cat*. CRC Press; 2009. p. 211–6.
44. Cyranoski D. Genetics: pet project. *Nature* 2010;**466**:1036–8.
45. Wick G, Andersson L, Hala K, Gershwin ME, Selmi C, Erf GF, et al. Avian models with spontaneous autoimmune diseases. *Adv Immunol* 2006;**92**:71–117.
46. Boissy RE, Smyth Jr JR, Fite KV. Progressive cytologic changes during the development of delayed feather amelanosis and associated choroidal defects in the DAM chicken line. A vitiligo model. *Am J Pathol* 1983;**111**:197–212.
47. Shi F, Erf GF. IFN-gamma, IL-21, and IL-10 co-expression in evolving autoimmune vitiligo lesions of Smyth line chickens. *J Invest Dermatol* 2012;**132**:642–9.
48. Erf GF, Trejo-Skalli AV, Smyth Jr JR. T cells in regenerating feathers of Smyth line chickens with vitiligo. *Clin Immunol Immunopathol* 1995;**76**:120–6.
49. Shresta S, Smyth Jr JR, Erf GF. Profiles of pulp infiltrating lymphocytes at various times throughout feather regeneration in Smyth line chickens with vitiligo. *Autoimmunity* 1997;**25**:193–201.
50. Austin LM, Boissy RE. Mammalian tyrosinase-related protein-1 is recognized by autoantibodies from vitiliginous Smyth chickens. An avian model for human vitiligo. *Am J Pathol* 1995;**146**:1529–41.
51. Lamont SJ, Smyth Jr JR. Effect of bursectomy on development of a spontaneous postnatal amelanosis. *Clin Immunol Immunopathol* 1981;**21**:407–11.
52. Wong GK, Liu B, Wang J, Zhang Y, Yang X, Zhang Z, et al. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. *Nature* 2004;**432**:717–22.
53. Lerner AB, Shiohara T, Boissy RE, Jacobson KA, Lamoreux ML, Moellmann GE. A mouse model for vitiligo. *J Invest Dermatol* 1986;**87**:299–304.
54. Lamoreux ML, Boissy RE, Womack JE, Nordlund JJ. The vit gene maps to the mi (microphthalmia) locus of the laboratory mouse. *J Hered* 1992;**83**:435–9.
55. Ferguson CA, Kidson SH. The regulation of tyrosinase gene transcription. *Pigment Cell Res* 1997;**10**:127–38.
56. Tassabehji M, Newton VE, Liu XZ, Brady A, Donnai D, Krajewska-Walasek M, et al. The mutational spectrum in Waardenburg syndrome. *Hum Mol Genet* 1995;**4**:2131–7.
57. Tripathi RK, Flanders DJ, Young TL, Oetting WS, Ramaiah A, King RA, et al. Microphthalmia-associated transcription factor (MITF) locus lacks linkage to human vitiligo or osteopetrosis: an evaluation. *Pigment Cell Res* 1999;**12**:187–92.
58. Zhu Y, Wang S, Xu A. A mouse model of vitiligo induced by monobenzone. *Exp Dermatol* 2013;**22**:499–501.
59. Overwijk WW, Lee DS, Surman DR, Irvine KR, Touloukian CE, Chan CC, et al. Vaccination with a recombinant vaccinia virus encoding a "self" antigen induces autoimmune vitiligo and tumor cell destruction in mice: requirement for CD4(+) T lymphocytes. *Proc Natl Acad Sci USA* 1999;**96**:2982–7.
60. Bowne WB, Srinivasan R, Wolchok JD, Hawkins WG, Blachere NE, Dyall R, et al. Coupling and uncoupling of tumor immunity and autoimmunity. *J Exp Med* 1999;**190**:1717–22.
61. Steitz J, Wenzel J, Gaffal E, Tüting T. Initiation and regulation of CD8+T cells recognizing melanocytic antigens in the epidermis: implications for the pathophysiology of vitiligo. *Eur J Cell Biol* 2004;**83**:797–803.
62. Mosenson JA, Zloza A, Nieland JD, Garrett-Mayer E, Eby JM, Huelsmann EJ, et al. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. *Science Transl Med* 2013;**5**:174ra128.
63. Gregg RK, Nichols L, Chen Y, Lu B, Engelhard VH. Mechanisms of spatial and temporal development of autoimmune vitiligo in tyrosinase-specific TCR transgenic mice. *J Immunol* 2010;**184**:1909–17.
64. Mehrotra S, Al-Khami AA, Klarquist J, Husain S, Naga O, Eby JM, et al. A coreceptor-independent transgenic human TCR mediates anti-tumor and anti-self immunity in mice. *J Immunol* 2012;**189**:1627–38.
65. Lambe T, Leung JC, Bouriez-Jones T, Silver K, Makinen K, Crockford TL, et al. CD4 T cell-dependent autoimmunity against a melanocyte neoantigen induces spontaneous vitiligo and depends upon Fas-Fas ligand interactions. *J Immunol* 2006;**177**:3055–62.
66. Muranski P, Boni A, Antony PA, Cassard L, Irvine KR, Kaiser A, et al. Tumor-specific Th17-polarized cells eradicate large established melanoma. *Blood* 2008;**112**:362–73.
67. Antony PA, Piccirillo CA, Akpinarli A, Finkelstein SE, Speiss PJ, Surman DR, et al. CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells. *J Immunol* 2005;**174**:2591–601.
68. Kunisada T, Lu SZ, Yoshida H, Nishikawa S, Nishikawa S, Mizoguchi M, et al. Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. *J Exp Med* 1998;**187**:1565–73.
69. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-gamma for autoreactive CD8(+) T-cell accumulation in the skin. *J Invest Dermatol* 2012;**132**:1869–76.
70. Chatterjee S, Eby JM, Al-Khami AA, Soloshchenko M, Kang HK, Kaur N, et al. A quantitative increase in regulatory T cells controls development of vitiligo. *J Invest Dermatol* 2014;**134**:1285–94.