

## MINIREVIEW

# Autoimmune Melanocyte Destruction in Vitiligo

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Vitiligo is a disorder of pigmentation characterized by the presence of milk-white skin macules. The term vitiligo may have evolved from either the Latin word *vitium*, meaning fault, or *vitelius* meaning spotted calf (Kovacs, 1998). Pigment-producing cells (melanocytes) are absent from vitiligo lesions (Le Poole et al, 1993b); their loss represents a key event in the pathogenesis of the disease. Vitiligo lesions can change in size and shape over time and can develop at any age, but in approximately half of all cases the disease onset is before the age of twenty (Lerner, 1959). Clinical presentations include (a) segmental vitiligo, characterized by lesions that occur in a dermatomal, asymmetric distribution (of limited clinical significance); (b) focal vitiligo, characterized by a limited number of small lesions; (c) generalized vitiligo, the most common type of vitiligo, where lesions occur with bilateral, symmetrical distribution; and (d) universal vitiligo, indicating complete or almost complete depigmentation (Mosher et al, 1993). The incidence of this disease varies greatly between populations (from 0.14% to 8.8%), but the worldwide incidence is considered to be between 1% and 2% (Mosher et al, 1993). As well as the enhanced ultraviolet (UV) sensitivity of depigmented spots, vitiligo also is a cosmetically disabling disorder that often leads to psychologic stress (Porter et al, 1979). In addition, due to the occurrence of similar hypopigmented skin lesions in the early phase of leprosy, vitiligo is a true social stigma in countries in which leprosy is endemic. This problem is probably best emphasized by the terminology used in southern India, where vitiligo is known as *ven kushtam*, meaning "white leprosy" (Mosher et al, 1993).

There are three main hypotheses for the pathogenesis of vitiligo: self destruction, neural, and autoimmune (Kovacs, 1998). In a number of recent studies,

strong evidence in favor of the autoimmune hypothesis has been obtained. This review will discuss the relevant new evidence for autoimmune melanocyte destruction in vitiligo.

### Ontogeny and Function of Normal Human Melanocytes

Absorption of photons by cellular DNA may cause DNA damage (McGregor, 1999). Therefore, exposure to UV light is a major risk factor for the development of skin cancer (Lim and Cooper, 1999). In addition, oxygen radicals that are generated by UV irradiation (Danno et al, 1984) may significantly alter the properties of proteins, thus impairing their function in the control of cell growth and survival (Riley, 1994). Protection from these harmful effects of UV light is mediated by melanin, which is capable of directly absorbing UV light (Hearing, 1999) and functions as a scavenger of free oxygen radicals (Bustamante et al, 1993). In normal healthy skin, melanin is synthesized by melanocytes that are located in the basal layer of the epidermis. Melanin synthesis, a multistep process involving tyrosinase as a key enzyme (Sanchez-Ferrer et al, 1995), takes place in specialized organelles called melanosomes (Hearing, 1999). Through dendrite-like processes, melanin is transferred to the keratinocytes (Yamamoto and Bhawan, 1994) in which it forms supranuclear caps that reduce UV transmission to the nuclear content (Kobayashi et al, 1998).

Melanocytes are not found exclusively in the epidermal basal layer and hair roots, but also in the uvea and retinal pigment epithelium of the eye, and in the stria vascularis of the inner ear (Jimbow et al, 1993). This may explain why vitiligo is associated with ophthalmological and auditory defects (Ardic et al, 1998). Melanocytes originate from the neural crest (Jimbow et al, 1993); during ontogenesis they migrate from the neural tube toward the epidermal basal layer (Wehrle-Haller and Weston, 1995). This migration involves stem cell factor (SCF) and the SCF-receptor encoded by the *c-Kit* proto-oncogene (Kunisada et al, 1998; Wehrle-Haller and Weston, 1995). The final entry of melanocyte progenitors from dermis into the epidermis presumably is facilitated by high E-cadherin ex-

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pression levels on the melanoblasts (Nishimura et al, 1999). In the adult human skin, melanocytes constitute about 3% to 5% of the total epidermal cell population. Racial differences in pigmentation intensity are not due to variations in melanocyte density but rather to differences in the number of melanosomes per cell and the melanin content per melanosome (Jimbow et al, 1993).

Although protection from UV irradiation is the primary function of melanocytes, a newly recognized role is their participation in the immune system. Melanocytes can express major histocompatibility complex (MHC)-class I and II molecules and adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (al Badri et al, 1993; Le Poole et al, 1993a; Smit et al, 1993). In addition, they can produce several cytokines, such as interleukin (IL)-1, IL-6 (Smit et al, 1993), IL-8 (Zachariae et al, 1991), and transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) (Le Poole and Boyce, 1999). Furthermore, melanocytes are not only capable of phagocytosis (Le Poole et al, 1993c), but can also process antigen and present antigenic peptides to T cells (Le Poole et al, 1993a). Melanocytes that present peptides from the 65-kd heat shock protein (HSP) of *Mycobacterium leprae* not only induce T cell proliferation, but also become the target of these cytotoxic T cell clones. These studies indicate that melanocytes are nonprofessional antigen-presenting cells (APC), which leads to a new hypothesis on the destruction of melanocytes in vitiligo.

## Immune Mechanisms in the Pathogenesis of Vitiligo

### Genetic Factors

The extent of familial aggregation of vitiligo is statistically significant. The pattern of relationship between relative risk and degree of kinship indicates that the disease is associated with genetic loci on different chromosomes, which points to a polygenic nature of the disease (Das et al, 1985; Majumder et al, 1993). In relation to this, it is known that vitiligo is frequently associated with several other autoimmune diseases including uveitis (Park et al, 1992), diabetes mellitus (Gould et al, 1985), autoimmune thyroid disease (Hegedus et al, 1994), and atrophic gastritis (Zauli et al, 1986). Further, several positive human leukocyte antigen (HLA)-associations for vitiligo have been reported: HLA-A2, HLA-Dw7 (Buc et al, 1996), HLA-DR4 (Dunston and Halder, 1990; Foley et al, 1983), B13, Bw35 (Metzker et al, 1980), Cw7, and DR6 (Venneker et al, 1993).

The initiation of antigen-specific T-cell-dependent immune responses generally requires costimulation through CD28/B7 (CD80 or CD86) ligation (McCoy and Le Gros, 1999). In addition, it was shown that interaction of APC expressed B7 with T-cell-expressed cytotoxic T-lymphocyte-associated molecule-4 (CTLA-4) delivers a negative signal for T cell response (McCoy and Le Gros, 1999). CTLA-4 gene polymorphisms were de-

scribed in autoimmune diseases like Graves disease (Kotsa et al, 1997), autoimmune hypothyroidism (Kotsa et al, 1997), and Addison's disease (Kemp et al, 1998a). Therefore, it was suggested that aberrant CTLA-4 expression or function, based on such polymorphisms, may be related to the occurrence of these diseases. Recently, CTLA-4 gene polymorphism has been described in a group of vitiligo patients who also presented with other autoimmune diseases (Kemp et al, 1999). It was suggested that this polymorphism may confer susceptibility to autoimmune-mediated mechanisms of melanocyte destruction in vitiligo through inadequate self tolerance.

### Humoral Immune Response

Antibodies reactive against melanocyte-derived antigens have been demonstrated in the serum of vitiligo patients. Several authors reported that the principle antigen recognized by these autoantibodies is tyrosinase (Fishman et al, 1997; Kemp et al, 1997; Song et al, 1994). Other melanocyte differentiation antigens that are recognized are gp100/Pmel17, and tyrosinase-related proteins (TRP)-1 and TRP-2 (Kemp et al, 1998b, 1998c). These differentiation antigens all localize primarily to the melanosomes (Hearing, 1999), whereas it has been well established that antibody-mediated killing requires membrane expression of the target antigens. Surprisingly, however, it was observed that patients' sera can induce in vitro damage to melanocytes via the classical pathway of complement activation and by antibody-dependent cellular cytotoxicity (Norris et al, 1988), and that at least some of the antibodies recognize membrane-expressed melanocyte antigens (Naughton et al, 1983a, 1983b; Yu et al, 1993). Further, it was shown that TRP-1 can be expressed on the cell surface of human and mouse melanoma cells (Takechi et al, 1996), suggesting that this differentiation antigen may represent a target for melanocyte destruction in vivo. Passive transfer of a monoclonal antibody against TRP-1 was found to induce melanoma regression as well as a patchy vitiligo-like depigmentation in mice (Hara et al, 1995; Takechi et al, 1996). Furthermore, it was shown that immunologic tolerance for TRP-1 in mice can be broken by active immunization with syngeneic TRP-1, when expressed in allogeneic cells or recombinant vaccinia virus (Naftzger et al, 1996; Overwijk et al, 1999), and also with "naked" plasmid DNA-encoding xenogeneic TRP-1 (Weber et al, 1998). In these melanoma models, vaccination-induced coat depigmentation was mediated by IgG class of anti-TRP-1 antibodies and occurred in the absence of detectable CTL responses.

The strongest evidence of a role for autoantibodies in melanocyte destruction in vitiligo was obtained in an in vivo model using nude mice grafted with normal human skin. When these mice were injected with purified IgG of vitiligo patients, it was observed that epidermal immunoglobulin deposits coincided with melanocyte destruction (Gilhar et al, 1995). However, reports on in situ immunoglobulin and complement

deposits in human vitiligo are limited and contradictory (Abdel-Naser et al, 1994; Uda et al, 1984; Yu et al, 1993). Furthermore, these deposits were observed on keratinocytes and not melanocytes. Therefore, it is possible that these autoreactive antibodies are secondary to melanocyte destruction. Nevertheless, although they may not be of prime importance for the autoimmune reaction, autoantibodies may serve as a good prognostic marker for disease activity. Indeed, a correlation was shown between the presence and titer of antipigment cell antibodies and disease activity (Harning et al, 1991).

### **Cellular Immune Response**

The first indications for a possible role for T cells in the pathogenesis of vitiligo came from case reports on inflammatory vitiligo (Buckley, 1953; Garb and Wise, 1948; Michaelsson, 1968). In this rare disorder the depigmented lesion is surrounded by a raised red rim. Histopathologic investigations of the perilesional skin suggested the involvement of lymphocytes in the depigmentation process. Immunohistochemical studies have now confirmed the presence of infiltrating T cells and their frequent apposition to perilesional melanocytes in this type of vitiligo (Le Poole et al, 1996; Yagi et al, 1997). Importantly, similar *in situ* T cell infiltrates with a predominant presence of CD8<sup>+</sup> T cells have also been detected in common generalized vitiligo (Abdel-Naser et al, 1994; Badri et al, 1993; Gross et al, 1987; van den Wijngaard et al, 2000).

Recently, Melan-A-specific CD8<sup>+</sup> T cells were identified in the peripheral blood of vitiligo patients by the tetramer technique (Ogg et al, 1998). Melan-A/MART-1 is one of the melanocyte-specific differentiation antigens that is recognized by CTL in melanoma (Coulie et al, 1994). High numbers of Melan-A-specific CD8<sup>+</sup> T cells were observed in 7 of 9 patients with HLA-A\*0201-positive vitiligo (Ogg et al, 1998). The frequency of these cells among the total T cell pool correlated with disease activity, and isolated Melan-A tetramer-positive T cells were able to lyse HLA-matched melanoma cells *in vitro*. Moreover, the blood-derived Melan-A-recognizing cells expressed the skin-homing receptor cutaneous lymphocyte-associated antigen (CLA) (Picker et al, 1990). The importance of skin-homing CD8<sup>+</sup> T cells was confirmed in an *in situ* immunohistochemical investigation of perilesional skin samples (van den Wijngaard et al, 2000). Most of the melanocyte-apposing T cells were CD8<sup>+</sup>/CLA<sup>+</sup>, and the majority of these T cells also expressed the cytotoxic effector molecules perforin and granzyme. The latter finding is of particular importance because *in vitro* experiments have indicated that melanocytes are sensitive to the granule exocytosis pathway of apoptosis induction (perforin/granzyme), but resistant to the Fas-ligand-mediated pathway (Rivoltini et al, 1998; R. M. van den Wijngaard, A. Wankowicz-Kalinska, J. Aten, M. van den Bergh-Weerman, E. de Vries, K. Dingemans, J. Borst, P. K. Das, unpublished observation). Similarly, in a mouse melanoma regression model, autoimmune destruction

of melanocytes could only proceed in the presence of perforin (Bowne et al, 1999).

Additional evidence for the importance of cell-mediated immunity in the pathogenesis of vitiligo has come from the field of melanoma research. Early clinical observations indicated that the presence of vitiligo has a favorable influence on the average five-year survival rate of patients with malignant melanoma (Bystryk et al, 1987; Nordlund et al, 1983). Furthermore, IL-2 based immunotherapy studies in melanoma patients suggested a role for autoreactive CTL in vitiligo (Rosenberg, 1997). In these studies, 26% of responding patients developed vitiligo in conjunction with melanoma regression, whereas no depigmentation was observed in nonresponding patients and in patients with unrelated malignancy. It should be emphasized that *in vitro* experiments demonstrated that CTL generated from melanoma tissue also recognize differentiation antigens expressed by normal human melanocytes (Anichini et al, 1993). Moreover, clonally-expanded T cells with identical T cell receptor V $\beta$  regions were simultaneously demonstrated in the vitiligo-like halo around a regressing melanoma and within the melanoma (Becker et al, 1999). High numbers of Melan-A-specific CTL have been observed in the peripheral blood of vitiligo patients (Ogg et al, 1998) and in melanoma patients with concurrent vitiligo, but not in melanoma patients without vitiligo (Pittet et al, 1999). Moreover, regression of metastatic melanoma and the occurrence of vitiliginous skin lesions in response to immunization with Melan-A peptide were both associated with oligoclonal expansion of Melan-A-reactive CTL (Jager et al, 2000). Also, when a patient with metastatic melanoma was infused with Melan-A-specific CD8<sup>+</sup> T cells, this patient developed inflammatory skin lesions and depigmented spots that were associated with the local presence of these T cell clones (Yee et al, 2000).

Taken together, the accumulated data indicate that CD8<sup>+</sup> CTL play an important role in immune-mediated destruction of autologous melanocytes. However, cellular cytotoxicity against melanocyte differentiation antigens may not be solely dependent on MHC class-I restricted T cells. Melan-A-specific CD4<sup>+</sup>-CTL have been generated from patients with melanoma as well as from normal control donors (Rivoltini et al, 1998; Zarour et al, 2000). The *in vivo* activation of such CD4<sup>+</sup> CTL may occur when professional APC, after endocytosis of dying melanocytes, migrate to skin-draining lymph nodes to present peptides derived from melanosomal antigens (Das et al, 2001). However, actual killing of melanocytes by MHC class-II restricted CTL also requires that target cells present endogenous melanosomal proteins in the context of MHC class-II molecules (Rivoltini et al, 1998). It is known that melanosomal glycoproteins are transported to the melanosomes by a dileucine-based sorting and retention motif, called the melanosomal transport signal (MTS) (Vijayasaradhi et al, 1995; Xu et al, 1998). Recently it was shown that this MTS motif can also target proteins to the endocytic pathway, leading to peptide presentation by MHC-class II molecules to

helper T cells (Wang et al, 1999). This may provide an explanation for the observed *in vitro* recognition of endogenous melanocyte differentiation antigens by cytotoxic CD4<sup>+</sup> CTL. Moreover, because enhanced numbers of MHC class-II-expressing melanocytes were reported in perilesional skin (al Badri et al, 1993; Le Poole et al, 1996), quantitative changes in MTS trafficking may be an additional cause of CTL-mediated melanocyte destruction in vitiligo.

A further role for CD4<sup>+</sup> T cells in the autoimmune killing of melanocytes was indicated when melanocyte destruction in mice was evoked by active immunization against the TRP-2 melanocyte differentiation antigen. This response was, although mediated by CD8<sup>+</sup> T cells, also dependent on the presence of CD4<sup>+</sup> T cells (Bowne et al, 1999). The importance of cell-mediated responses was further emphasized by the predominant presence of type-1 T cells at the perilesional site of active lesions. Type-1 T cells are involved in cell-mediated inflammatory reactions, whereas type-2 T cells are found in association with strong antibody responses. In a recent study, *in vitro* cytokine profiles of nonspecifically stimulated T cell clones, derived from the border of the depigmented lesion, were compared with those of nonlesional skin. It was found that active vitiligo lesions are mainly infiltrated with type-1 T cells, whereas uninvolved skin contained type-2 and type-0 subsets. Furthermore, some of these perilesional type-1-CD8<sup>+</sup> T cells were able to kill autologous melanocytes in an HLA class-I restricted manner (Wankowicz-Kalinska et al, submitted for publication). Although these findings provide further evidence for the importance of autoreactive T cells in skin depigmentation (type-1 response in the border area), the observed type-2 response at the nonlesional site may play a role in restricting the exacerbation of disease (D'Ambrosio et al, 2000).

## Conclusion

To date, three important hypotheses have been formulated to explain the etiology of vitiligo: the autoimmune, the self-destruction, and the neural theory. Although it cannot be excluded that the self-destruction and neural hypotheses are applicable in subsets of vitiligo patients, recently obtained evidence strongly indicates that this pigmentary disorder is an autoimmune disease. This statement is further appreciated when regarding the current clinical practice of vitiligo management; all of the nonsurgical vitiligo treatments with proven efficacy (Njoo et al, 1998) are based on immunosuppression (Cato and Wade, 1996; Hill et al, 1999; Krutmann and Morita, 1999; Ozawa et al, 1999). Thus, future investigations in vitiligo etiology should focus on finding the exact conditions that trigger and sustain this melanocyte-specific autoimmune response. In relation to this, it is important to note that MHC class-I as well as MHC class-II restricted T cells that recognize melanocyte differentiation antigens can be cultured from normal control donors (Ogg et al, 1998; Visseren et al, 1995; Zarour et al, 2000). Establishing the triggers that awaken this

latent mechanism in vitiligo will not only benefit patients with this autoimmune disease, but may also be of considerable importance for melanoma therapy, where an effective immune response against melanocyte differentiation antigens is lacking.

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