Published in final edited form as:
Crit Rev Immunol. 2000 ; 20(6): 433–450.

Autoimmunity and the Immunotherapy of Cancer: Targeting the "Self" to Destroy the "Other"

Willem W. Overwijk* and **Nicholas P. Restifo**
Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD

Abstract

It is increasingly clear that immunity to "self"-antigens may result in tumor destruction in mouse and man. But which antigens should be targeted with therapeutic cancer vaccines? In the case of melanoma, recognition of melanocyte differentiation antigens (MDA) can be associated with autoimmune depigmentation (vitiligo). We propose that intersection of protein transport to melanosomes and endosomes allows for the loading of MDA-derived peptides on MHC class II molecules, resulting in the activation of MDA-specific $CD4^+$ "helper" T cells that aid the induction of melanoma-specific $CD8^+$ T cells. Thus, the immunogenicity of MDA may be a consequence of their unique cell biology. Studies of MDA-based vaccines can provide new insight into the development of more effective cancer vaccines.

Keywords

melanocyte differentiation antigen; gp100; TRP-1; vitiligo; autoimmune disease; melanoma; melanosome; vaccine; T lymphocyte

I. INTRODUCTION

For decades, the occasional presence of immune-mediated skin depigmentation, or vitiligo, in melanoma patients has been linked with a favorable prognosis,[1,2] It was speculated that a limited immune reactivity against a growing melanoma resulted in some destruction of tumor cells as well as normal cutaneous melanocytes. With the advent of immunotherapy, in which a substantial portion of melanoma patients experienced tumor regression after their immune systems had been stimulated to attack their tumor, it became possible to look at this association more carefully. The results of this analysis revealed that upon treatment with the T-lymphocyte growth factor interleukin-2 (IL-2), approximately 20% of responding melanoma patients developed vitiligo (Figure 1*a*, 1*b*, 1*c*).[3] The relation between vitiligo and melanoma regression was highly significant ($p = 0.0002$); although tumor regression could be observed without vitiligo, virtually every patient that developed vitiligo experienced at least a partial tumor regression (defined as greater than 50% reduction of all lesions) (Table 1).[3] Importantly, none of 104 renal cell cancer patients treated with IL-2 developed vitiligo, suggesting that tumor-derived "self"-antigens were required for the induction of vitiligo. Together, these results suggested that normal proteins, expressed on both normal and malignant melanocytes, could act as targets for the immune system.

Address all correspondence to: Willem W. Overwijk, National Cancer Institute, Building 10, Room 2B42, Bethesda, MD 20892-1502.
Referee: Dr. J. Paul Zoeteweij, Dermatology Branch/AIDS Lab., Building 10, Room 12N238, NCI/NIH, 10 Center Drive MSC 1908, Bethesda, MD 20892
*This manuscript was written in partial fulfillment of Willem W. Overwijk's requirements toward a Ph.D. degree at George Washington University.

In the last decade, improved techniques to expand T cells in vitro has enabled the identification of human melanoma-specific T lymphocytes that also recognized antigens expressed by normal melanocytes.[4] Identification of these antigens was made possible by the application of techniques developed in the fields of biochemistry and molecular biology (Figure 2). Tumor-specific T lymphocytes can g used to screen tumor cDNA libraries transfected into cells that are themselves not recognized by the T lymphocytes. Transfected cDNAs that confer recognition are isolated and sequenced to identify the gene encoding the antigen.[5–8] Alternatively, peptides can be eluted from MHC molecules on the surface of tumor cells, and after fractionation by high-performance liquid chromatography, small peptide pools can be tested for recognition by tumor-reactive T cells. Further fractionation of recognized pools can lead to the isolation and sequencing of a single recognized peptide.[9,10] Finally, peptides from candidate antigens can be selected based on predicted affinity for MHC molecules, synthesized, and used to induce T cells that are then tested for recognition of tumor cells.[11–13] Each of these methods has led to identification of tumor-associated antigens.

One large class of antigens stands out in their potential for cancer treatment, and these are the shared tumor antigens.[5,7] Unlike mutated proteins, these molecules are present in unaltered form in cancer cells from many patients, pointing the way to the development of “off-the-shelf” reagents for cancer therapy. One large group of antigens consists of the cancer testis antigens, which are exclusively expressed in tumors of many different histologies as well as normal testis. The main members of this group belong to the MAGE family, cloned in large part by Boon and coworkers.[5] A second family of shared antigens intensively studied by many laboratories, including our own, contains the melanocyte differentiation antigens (MDA), mostly enzymes responsible for the production of melanin pigment[7] (Figures 3*a* and 3*b*).

The vast majority of melanoma-reactive T lymphocytes grown from fresh tumor explants recognize MDA. These antigens are expressed by normal melanocytes as well as by malignant melanoma cells and include gp100, MART-1, tyrosinase, tyrosinase related protein (TRP)-1, and TRP-2.[6,7,14–18] Interestingly, tyrosinase and TRP-1 had been identified earlier, by serological studies, as targets for antibodies in autoimmune vitiligo,[19–21] Many melanoma patients possess elevated levels of antibodies and/or T lymphocytes that are specific for one or more of these five antigens, and preliminary results indicate that two additional melanocyte-specific proteins, ocular albinism (OA-1) and P. polypeptide,[22,23] can likewise be recognized by $CD8^+$ T cells (C.E, Touloukian, Surgery Branch, NCI, unpublished results). However, until recently it was unclear whether it was possible to actively immunize patients to mount therapeutic immune responses against MDA. In such attempts to target MDA with cancer vaccines, one of the pitfalls may be the different immunologic nature of self-antigens when compared with “foreign” antigens, such as viral proteins.

T lymphocytes with strong “self”-reactivity are often physically deleted because of negative selection during maturation in the thymus, a process termed “central” tolerance.[24,25] However, a low level of autoreactivity is required for positive selection in the thymus, and T lymphocytes with low reactivity to autoantigens thus persist.[26,27] Mature self-reactive T lymphocytes that encounter antigen on normal tissues in the absence of an activating costimulatory microenvironment can be functionally eliminated by anergization or physically by deletion, thus effecting extrathymic or “peripheral” tolerance,[25,28,29] Alternatively, mature T lymphocytes with reactivity to self-antigens may remain in a functionally tolerant state, termed “ignorant,” if they do not encounter antigen bearing cells or if the target antigen is not processed and presented to a level that can trigger the specific T-cell receptor (TCR).[25,30,31] It is the challenge of the immunotherapist to identify those antigens for which specific T cells are present in patients and to then activate these T cells and expand their numbers to the point where they can effect tumor regression.

II. PICKING THE "RIGHT" ANTIGEN: "SELF" *VERSUS* NON-"SELF"

Until recently, most experimental data suggested that tumor antigens leading to tumor regression in animals were "unique" neoantigens, such as mutations or viral antigens. Classic experiments with chemically induced sarcomas had demonstrated that vaccination with irradiated tumor cells typically induced immunity that was uniquely specific for the tumor that had been used to vaccinate.[32,33] From a theoretical viewpoint, it seemed likely that tolerance to neoantigens would not exist, making them better targets for immunotherapy than constitutively expressed self-antigens. Yet recent data from animal models and clinical trials have begun to present a more complicated picture.

Most animal studies on unique antigens, such as the viral or mutated neoantigens expressed by tumors, suffer from a problem inherent to animal tumor models, which is the use of rapidly growing tumors that kill untreated animals within weeks. In contrast, human tumors typically grow slowly for years before clinical detection. The patient's immune system has therefore been exposed to the tumor-derived neoantigens for years. Since tumor cells lack costimulatory molecules, typically do not express MHC Class II to induce T-lymphocyte help, and are generally not inflamed or grossly necrotic in their preclinical stages, neoantigen-specific T lymphocytes have likely been thoroughly deleted or anergized by the time the tumor becomes clinically manifest. Even then, the large amount of "normal" tumor cells presenting the antigen in a noninflammatory fashion may well overcome and tolerize those lymphocytes that have been primed by the few poorly activated antigen-preventing cells (APC) picking up shed neoantigens.[34] Several reports have now convincingly demonstrated the ability of tumors to passively or even actively prevent or terminate immune responses to powerful foreign antigens, such as viral proteins.35–38 This quality of tumor cells may thus decrease the immunogenicity of foreign antigens, such as mutations or viral proteins to a level similar to that of true self-antigens, such as MDA.

As for the perceived lack of immunogenicity of self tumor antigens, such as MDA, lymphocytes isolated from melanoma specimens and grown in vitro with no other stimulation than autologous whole melanoma cells and IL-2, consistently recognize MDA in a great majority of cases, to the extent that it can become difficult to isolate lymphocytes with any other reactivities from such cultures.[39] The observation that a sizeable fraction of melanoma patients that respond to IL-2 therapy develop vitiligo also points to the ability of tumor-derived self-antigens to stimulate the immune system.[3] To explore the immunogenicity of self-antigens further and explore the therapeutic benefit of autoimmune responses to these proteins, we have attempted to deliberately induce immune responses to MDA.

Using the mouse homologues of the human genes for gp100, MART-1, tyrosinase, TRP-1, and TRP-2, we constructed recombinant vaccinia viruses (rVV) that encoded each of these mouse (m) MDA. Intravenous immunization of mice with rVVmTRP-1, but not with rVV encoding any of the other four MDA, led to a dramatic, vitiligo-like depigmentation within weeks, which in some cases spread progressively over the entire body (Figure 4). Histology revealed that no melanocytes remained in affected skin.[40] Interestingly, melanocytes in the eye and inner ear were not destroyed, suggesting that there may be some selectivity to subclasses of melanocytes or anatomic site, though the basis for this selectivity is presently unknown.[41]

It was possible to induce immune reactivity to mTRP-1, but the most important question was whether mice capable of destroying their normal melanocytes were also able to recognize and eliminate tumor cells expressing mTRP-1. When mice with vitiligo-like depigmentation were challenged with the spontaneous murine melanoma B16, they reliably rejected the tumor cells that grew uninhibited in mice vaccinated with control rVV. Thus the deliberate induction of autoimmunity to mTRP-1 resulted in destruction of tumor cells.[40]

It is interesting that of the five MDA tested under identical circumstances, TRP-1 was uniquely capable of inducing vitiligo. It is possible that this reflects a differential level of tolerance to the MDA, with the least stringent tolerance existing to TRP-1.[42] Alternatively, the cell biology of TRP-1 synthesis and trafficking might make it a unique target for immune responses.[43,44] TRP-1 is produced in high levels in melanoma cells and melanocytes and secreted as well as expressed on the cell surface,[43,44] where it could become a target for class-switched IgG antibodies that were present in high liters in mice with vitiligo,[40] In addition, different combinations of MDA, vaccine vehicles, and animal strains or species appear to render MDA capable of inducing tumor protection and sometimes vitiligo. For example, we and others have recently induced protection against B16 melanoma challenge and localized vitiligo by vaccinating with plasmid DNA encoding mTRP-2.[45] It is remarkable that DNA immunization appears to elicit stronger autoimmunity when using mTRP-2, while rVV is superior when mTRP-1 is the antigen. One possibility is the difference in mouse strains used in both studies, pointing to a possible impact of non-MHC genes on the induction of autoimmunity to MDA. Another study examined the use of murine DC pulsed with mgp100 peptides in combination with anti-CTLA4 antibody treatment. CTLA4 is a receptor for B7-1 and B7-2 and is thought to inhibit T-cell activation. Preliminary results show that presentation of gp100 peptides on dendric cells (DC) in combination with CTLA4 blockade induces vitiligo and shrinkage of established B16 melanoma (J. P. Allison, personal communication). Yet another approach to induce autoimmunity and anti-tumor immunity is to use altered forms of self-proteins, such as self-proteins that have undergone alternative post-translational modifications during expression in xenogeneic host cells or even by immunizing with genes encoding xenogeneic protein homologues.[46–50]

The importance of antigen choice is further highlighted by the results of immunization with rVV encoding the MDA mgp100. In repeated experiments we never found any evidence of induction of cytotoxic T cells, autoimmunity, or protection from B16 challenge, even though the virus was constructed identically to the one encoding mTRP-1. Guided by reports on the induction of autoimmunity by xenoimmunization,[51,52] we immunized mice with rVV encoding hgp100. Human gp100 and mgp100 share 76% homology at the amino acid level, allowing potential xeno (human)-reactive T cells to be crossreactive with the mouse sequence. Indeed, using human gp100 it was possible to induce $CD8^+$ T cells that recognized both hgp100 and mgp100, as well as B16 melanoma.[49] Detailed studies of the minimal epitope recognized demonstrated that a peptide from hgp100, KVPRNQDWL, displayed dramatically enhanced binding to $H\text{-}2D^b$, the restricting MHC Class I molecule. The mouse peptide, EGSRNQDWL, bound less well, although sufficiently to be recognized by crossreactive T cells. These T cells were therapeutic in vivo in a 3-day B16 lung metastases model, showing that they could effectively recognize tumor cells expressing endogenous mgp100 in established tumor.[49]

It is thus difficult but not impossible to induce powerful de novo immune responses to genuine self-antigens that can specifically destroy normal tissues as well as tumors.[40,46,49,31,33,34] Human trials are beginning to demonstrate the ability to vaccinate against self-antigens, with the induction of strong T-lymphocyte responses and even some tumor regression.[55–57] In addition, the recent identification of MDA-specific T lymphocytes in vitiligo lesions suggests that an active MDA-specific immune response is also induced in patients with "spontaneous" vitiligo.[58] Using tetramers of HLA-A201, complexed with the immunodominant MART-1 peptide, cytotoxic $CD8^+$ T lymphocytes that were specific for MART-1 and could lyse melanomas in vitro were found to be highly elevated in vitiligo patients compared with controls.[58] Although there likely is immunological tolerance to MDA, this tolerance is not absolute and can be broken.

Taken together, these observations suggest that there may not be a big difference in the immunogenicity between unique and self-antigens when they are expressed by tumors. The

experimental evidence suggests that it is difficult but possible to induce powerful, therapeutic immune responses to tumor-associated antigens. An obvious advantage of the use of self-antigens is that they are shared among patients, precluding the need to identify each patients' individual tumor-associated (neo)antigen. Therefore, the application of self-antigens can be tailored to groups of patients based on their HLA, allowing development of off-the-shelf cancer vaccines. In addition, melanomas typically express multiple MDA, allowing for the development of combination vaccines to counter possible immune escape by heterogeneous antigen expression or antigen loss by tumor cells. We are currently preparing to test the activity of full-length human TRP-1 encoded by recombinant fowlpox virus in melanoma patients. In addition, new vectors are being developed.[59,60] If self-antigens indeed prove to be useful targets for cancer immunotherapy, it may be possible to apply principles that are currently being learned from melanoma vaccines to cancers of other nonvital organs, such as the prostate, breast, ovary, and thyroid gland.

III. CD4+ T CELLS IN THE IMMUNE RESPONSE TO SELF-ANTIGENS

Since mouse models revealed a clear antitumor effect of autoimmunity against mTRP-1, it became important to understand which immune compartments were responsible for both phenomena. We studied the contribution of MHC Class I and II, using knockout mice as well as in vivo depletion of $CD8^+$ and $CD4^+$ T lymphocytes. The results indicated that both rVVmTRP-1 -induced vitiligo and tumor destruction relied critically on $CD4^+$ T lymphocytes but not on $CD8^+$ T lymphocytes.[40] This was somewhat surprising since, in recent years, most efforts (including our own) have focused primarily on the development of cancer vaccines stimulating $CD8^+$ T lymphocytes.[42] One reason for this skewed interest may be the relative ease with which MHC Class I-restricted antigens can be cloned, compared with the much more laborious and technically challenging biochemical approaches needed to isolate the MHC Class II-restricted antigens recognized by $CD4^+$ T lymphocytes. [16,61,62] The recent development of a cDNA library-based method for cloning MHC Class II-restricted antigens may expedite the identification of these antigens.[62,63] Alternatively, transgenic mice expressing human MHC Class II molecules can be immunized with candidate antigens to induce antigen-specific $CD4^+$ T cells that can then be used to identify the exact peptides that are recognized.[13] Many classic animal studies had already demonstrated crucial roles for $CD4^+$ T lymphocytes in tumor immunity and treatment, and more recent, refined studies have confirmed those observations.[64–68]

The recent revival of appreciation for the role of $CD4^+$ T lymphocytes in antitumor immunity has coincided with the identification of new molecular mechanisms through which $CD4^+$ T lymphocytes aid the initiation and maintenance of the antitumor immune response.[40,64,65,69,70] $CD4^+$ T lymphocytes can activate APC through engagement of CD40, secrete proinflammatory cytokines such as TNF-α, interferon-γ, and IL-2, and induce the production of chemokines to attract a host of other cells to the site of immune reactivity.[71–73] Some groups have looked in more detail at the specific role of $CD4^+$ T lymphocytes in tumor immunity and found that $CD4^+$ T lymphocytes can attract, activate, and differentiate $CD4^+$ and $CD8^+$ T cells as well as nonspecific leukocytes, such as eosinophils, macrophages, and B cells.[46,74,75] More recent studies show that $CD4^+$ T cells can activate endogenous, tumor-specific $CD8^+$ T cells that subsequently induce tumor regression.[67] In addition, some melanoma patients have significant levels of class-switched, IgG-type antibodies against melanosomal proteins in their serum, suggesting activation of melanocyte-specific $CD4^+$ T lymphocytes in human cancers as well.[76,77]

The most dramatic examples of the powerful steering function of $CD4^+$ T lymphocytes in the immune response to self-proteins are found in models of autoimmune diseases, such as experimental allergic encephalomyelitis, systemic lupus erythematosus, and diabetes. In both

mouse and man, these diseases are associated with particular MHC Class II haplotypes, and disease can often be transferred to healthy animals with purified, self-reactive $CD4^+$ splenocytes or specific $CD4^+$ T lymphocyte clones.[42,78–80] Conversely, active disease can sometimes be suppressed with regulatory $CD4^+$ T-lymphocyte populations, demonstrating the pivotal role of $CD4^+$ T lymphocytes in the induction and progression of immune responses to self-antigens.[81–83]

Together with the evidence for the existence and involvement of functional, melanoma-specific $CD4^+$ T lymphocytes in melanoma patients,[13,16] these observations point to $CD4^+$ T-cell "help" as a component that may have been underemphasized in many of the current clinical vaccine approaches. Though some have experienced an encouraging degree of success, results from many cancer vaccines currently in clinical trials are demonstrating varying degrees of vaccine-induced tumor-specific $CD8^+$ T lymphocytes but only occasional objective tumor regressions.[56,84] Clearly inducing self-reactive, tumor-specific T cells alone is no guarantee for tumor control. Indeed, mice vaccinated with rVVhgp100 develop clearly elevated levels of mgp100-specific T-cell precursors; yet this treatment does not treat even tiny, 3-day B16 pulmonary nodules (Overwijk et al., unpublished results). In addition, despite the clear self-reactivity of mgp100-reactive $CD8^+$ T cells, we have never observed vitiligo in mice immunized with rVVhgp100 or adoptively transferred with therapeutic, cultured $CD8^+$ T-cell clones with or without IL-2. Only transfer of in vitro activated mgp100-specific T cells could destroy established pulmonary micrometastases. It is possible that a lack of mgp100-specific $CD4^+$ T cells is limiting for in vivo activation of mgp100-specific $CD8^+$ T cells, especially since $CD4^+$ T cells were critical for induction of vitiligo and tumor protection in mice vaccinated with rVVmTRP-1. It is possible that concurrent induction of $CD4^+$ and $CD8^+$ tumor-specific T cells will enhance the therapeutic activity of anticancer vaccines. The availability of model systems that allow targeting of the murine homologues of truly endogenously expressed self-antigens in human tumors will be of great value in addressing this important question.

IV. THE MELANOCYTE AND ITS ANTIGENS: DOES UNIQUE BIOLOGY CONFER UNIQUE IMMUNOGENICITY?

A question that remains is why melanomas are so exquisitely immunogenic among human tumors, and, more specifically, why MDA are identified so frequently as the principal targets of T lymphocytes grown from melanoma lesions. One reason for this enigmatic quality of MDA may reside in their unique cellular biology.

It has been noted that all MDA frequently recognized by T cells reside in the melanosome. This organelle where pigment biosynthesis occurs has long been hypothesized to be biochemically and developmentally related to organelles in the endosome and lysosome lineages. In fact, much of the work leading to a deeper understanding of MHC Class II peptide loading has been centered around melanosome vesicle fractions isolated from the melanoma cell line Mel JuSo, which constitutively expresses MHC Class II and is highly efficient in presenting antigens on MHC Class II.[85–86] These vesicles bear remarkable similarity to the MIIC compartment for peptide loading originally isolated from professional APC, such as activated B-lymphoblastoid cells and DC.[86–88] The vesicles are prominently present in Mel JuSo and contain invariant chain, HLA-DM, and the endosomal proteases cathepsin-L and -S — all molecules critically involved in MHC Class II-restricted antigen presentation.[89] Additional evidence that suggests a close relationship between melanosomes and endosomes/lysosomes includes the following (Table 2):

1. Both melanosomes and endosomes/lysosomes have an acidic content characterized by the presence of acid hydrolases and "lysosome-specific" proteins such as lysosome associated membrane protein-1 (LAMP-1).[90–92]

2. Several mutations in genes that regulate formation of endosomes and/or lysosomes also affect melanosome formation. Examples include mice with the *mocha* mutation, which lack a functional adaptor protein complex AP-3 associated with coated vesicles budding from the trans-Golgi network. Mice carrying the *mocha* mutation display, among other defects, coat and eye color dilution as well as lysosomal abnormalities, suggesting a joint pathway of cargo transport to melanosomes and lysosomes.[93] Another mouse pigmentation-associated mutation, *light ear*, likewise is characterized by concurrent lysosomal dysfunction.[94] Humans homozygous for the recessive autosomal mutation that causes the Chediak-Higashi syndrome are hypopigmented and display giant, fused melanosomes as well as giant lysosomes in nonpigmented cells, including the resident Langerhans cells of the skin, which are normally responsible for antigen presentation.[95] Perhaps as a result of this melanosomal/lysosomal dysfunction, peptide loading onto MHC Class II and antigen presentation are strongly delayed in these patients.[96]

3. Electron microscopic studies indicate that cultured choroidal melanocytes from cattle can phagocytose gold-labeled albumin, which then appears in melanin granules. Melanin granules associated with gold particles are subsequently extruded into the culture medium, confirming physical mixing of phagocytosed material and melanocyte contents.[97] A similar study indicates that latex beads phagocytosed by melanocytes appear in phagosomes that subsequently fuse with melanosomes, suggesting that the melanosome intersects with a degradative pathway for phagocytosed materials.[98]

4. With specific regard to MDA, which are typically found within the melanosome, a sizeable portion of TRP-1 in melanocytes is transported from the terminal trans-Golgi apparatus to an acidic, nonmelanosomal, endosome-like compartment.[44] Fibroblasts, which lack melanosomes that are transfected with TRP-1 cDNA, show accumulation of TRP-1 protein in endosomes, suggesting that the targeting signal in the TRP-1 amino acid sequence discriminates little, if at all, among the two organelles.[91,99]

5. Vaccination with a DNA construct encoding the model tumor antigen ovalbumin fused to the melanosomal transport signal (MTS) of TRP-1 resulted in greatly enhanced tumor-specific immune responses when compared with vaccination with DNA encoding the antigen fused to a nonfunctional MTS. The antitumor response was dependent on the MTS as well as on $CD4^+$ T cells, suggesting that the MTS targeted the antigen into the MHC Class II processing pathway of DNA-transfected APC [43]

Together, these observations strongly suggest that the endocytic compartment may well intersect with the transport routes of proteins targeted to the melanosome. We speculate that a fraction of translated MDA constitutively localizes to the endocytic compartment,[100] to be transported along with proteins destined for the dense endocytic compartment for peptide loading (MIIC), the site in professional APC and also melanoma cells where MHC Class II molecules associate with peptides.[87,101,102]

The result of such a targeting may be direct availability of high levels of melanosomal proteins for processing and loading on MHC Class II molecules at any time that MHC Class II expression would be induced on melanocytes or melanoma cells. Some human melanomas express MHC Class II constitutively, and a large fraction of melanomas as well as normal melanocytes can be readily induced with IFN-γ to express substantial levels of MHC Class II.

[16,103,104] Likewise, stimulation of the spontaneous murine B16 melanoma, but not several other histologies, with IFN-γ rapidly induces expression of MHC Class II on the surface[105] (D. Surman, unpublished results). Melanocytes in active human vitiligo lesions, but not in inflammatory skin lesions from patients with psoriasis, also display a marked upregulation of MHC Class II and ICAM-I, suggesting that melanocytes are active contributors to the inflammation rather than mere bystanders or target cells.[106] Vitiligo lesions are also typically infiltrated by IL-2-producing cells — likely MHC Class II-restricted $CD4^+$ T lymphocytes.[107–109] Together this suggests that MDA are immunologically unique in that they are expressed exclusively in a relatively small-cell population, resulting in low levels of antigen during central and peripheral T-cell selection and thus effecting relatively mild tolerization.[34,110] Yet high amounts of MDA can be presented on MHC Class I and MHC Class II upon triggering of melanocytes or melanoma cells by inflammatory stimuli such as IFN-γ, allowing for recognition by those T cells that escaped tolerance.

Melanocytes and melanoma cells may not be equal to professional APC, such as DC, in their ability to provide costimulation through molecules such as B7-1/CD80, B7-2/CD86, and CD40, though a fraction of melanomas reportedly express these costimulatory molecules.[111–114] Several studies indicate an important role for ICAM-1/CD54 in MHC Class II-restricted antigen presentation by melanocytes and melanoma cells.[103,115] It is possible that a lack of expression of certain costimulatory molecules, such as B7-1 and B7-2, on melanocytes and melanoma cells can be partially compensated for by other costimulatory and adhesion molecules.

In addition to the direct presentation of MDA by melanocytes, the cellular biology of these proteins may make them uniquely available to presentation by more professional APC, such as DC. Normal pigmentation occurs through the uptake by keratinocytes of intact melanosomes that are secreted by melanocytes.[116] Besides the melanin that confers the pigmentation, these melanosomes also contain high quantities of MDA.[99,117,118] It is conceivable that a fraction of extruded melanosomes are available for uptake by APC, such as DC-like Langerhans cells of the skin, which can then process and present MDA-derived peptides on MHC Class I and MHC Class II. Since both melanocytes and Langerhans cells, as well as MDA-specific T cells are relatively rare, a productive interaction between the three cell types may occur so infrequently that autoimmune disease does not ensue, especially if antigen presentation takes place in a noninflammatory, tolerizing environment in a healthy individual. However, within a growing melanoma, large numbers of tumor cells present MDA on MHC Class I and often MHC Class II, whereas high levels of MDA are simultaneously released in an environment with local tissue damage. Indeed, tumor infiltration by APC and T cells is routinely observed upon melanoma biopsy. This may allow the interaction between MDA-specific T cells and (partially)-activated APC that present MDA to such a degree that mild, localized T-cell activation and proliferation can occur. These low but significant numbers of MDA-specific T cells can proliferate when melanoma biopsies are cultured in IL-2. In vivo, they may mediate the spontaneous regression of tumor in a very small but significant fraction of patients, a fraction increased to 17% upon the administration of high-dose IL-2.[119,120] Coadministration of IL-2 with specific T cells in the form of ex vivo-expanded tumor-infiltrating lymphocytes doubles this response rate to 34%.[121] Even stronger T-cell stimulation with both interleukin-2 and specific antigen through gp100 peptide vaccination increases the fraction of patients that responds to 42%.[57]

Thus, although far from therapeutic in itself, the weak endogenous immune response triggered by MDA may provide a substrate for therapeutic intervention and augmentation that can lead to complete regression of the tumor. Interestingly, some patients treated with MHC Class I-restricted gp100 peptide and IL-2 experience a very localized autoimmune destruction of normal melanocytes around regressing melanoma lesions (Figure 5b). This may suggest that

proinflammatory “danger” signals in the tumor microenvironment sustain the immune responses to self-antigens. It is also possible that it is mainly the high concentration of gp100 antigen in the tumor that provides the trigger for immune destruction, especially since inflammation and depigmentation upon treatment can also occur in distant normal nevi (Figure 5a).

V. CONCLUSION

The development of new mouse models for the treatment of cancer and the study of basic aspects of vaccine development using self-antigens are opening new avenues toward the rational design of more effective cancer vaccines. In addition, the first results from clinical vaccine trials are providing the necessary “reality check” in the application of strategies gleaned from animal studies. It is becoming apparent that self-antigens can be effective in anticancer vaccines, providing opportunities for the development of standardized therapies for melanoma patients.

The unique cellular biology of MDA provides a clue to the riddle of the unusual immunogenicity of melanoma among human cancers. Human and animal studies of MDA-based cancer vaccines may help shed more light on important aspects of cancer-vaccine design, such as choosing the “correct” antigen, vaccine vehicle, and adjuvant, and ensure the induction of both $CD4^+$ and $CD8^+$ T-lymphocyte responses. Lessons learned during the development of vaccines for melanoma may ultimately aid in the design and clinical application of vaccines for other malignancies.

Acknowledgements

The authors wish to thank Dr. Steven A. Rosenberg for helpful discussion, Dr. Christopher E. Touloukian for creating Figure 3*b*, and Ms. Martha Blalock for expert assistance with graphics.

References

1. Bystryn JC, Rigel D, Friedman RJ, Kopf A. Prognostic significance of hypopigmentation in malignant melanoma. Arch Dermatol 1987;123:1053–1055. [PubMed: 3631983]
2. Nordlund JJ, Kirkwood JM, Forget BM, Milton G, Albert DM, Lerner AB. Vitiligo in patients with metastatic melanoma: a good prognostic sign. J Am Acad Dermatol 1983;9:689–696. [PubMed: 6643767]
3. Rosenberg SA, White DE. Vitiligo in patients with melanoma: normal tissue antigens can be targets for cancer immunotherapy, J. Immunother Emphasis Tumor Immunol 1996;19:81–84.
4. O’Neil BH, Kawakami Y, Restifo NP, Bennink JR, Yewdell JW, Rosenberg SA. Detection of shared MHC-restricted human melanoma antigens after vaccinia virus-mediated transduction of genes coding for HLA. J Immunol 1993;151:1410–1418. [PubMed: 8335937]
5. Boon T, Coulie PG, Van DE. Tumor antigens recognized by T cells. Immunol, Today 1997;18:267–268. [PubMed: 9190110]
6. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA 1994;91:3515–3519. [PubMed: 8170938]
7. Rosenberg SA. Cancer vaccines based on the identification of genes encoding cancer regression antigens. Immunol, Today 1997;18:175–182. [PubMed: 9136454]
8. Rosenberg SA. A new era for cancer immunotherapy based on the genes that encode cancer antigens. Immunity 1999;10:281–287. [PubMed: 10204484]
9. Cox AL, Skipper J, Chen Y, Henderson RA, Darrow TL, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CLJ. Identification of a peptide recognized by five melanoma-specific human cytotoxic T-cell lines. Science 1994;264:716–719. [PubMed: 7513441]

10. Hunt DF, Henderson RA, Shabanowitz J, Sakaguchi K, Michel H, Sevilir N, Cox AL, Appella E, Engelhard VH. Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science 1992;255:1261–1263. [PubMed: 1546328]
11. Parkhurst MR, Fitzgerald EB, Southwood S, Sette A, Rosenberg SA, Kawakami Y. Identification of a shared HLA-A*0201-restricted T-cell epitope from the melanoma antigen tyrosinase-related protein 2 (TRP2). Cancer Res 1998;58:4895–4901. [PubMed: 9809996]
12. Salazar-Onfray F, Nakazawa T, Chhajlani V, Petersson M, Karre K, Masucci G, Celis E, Sette A, Southwood S, Appella E, Kiessling R. Synthetic peptides derived from the melanocyte-stimulating hormone receptor MC1R can stimulate HLA-A2-restricted cytotoxic T lymphocytes that recognize naturally processed peptides on human melanoma cells. Cancer Res 1997;57:4348–4355. [PubMed: 9331097]
13. Touloukian CE, Leitner WW, Topalian SL, Li YF, Robbins PF, Rosenberg SA, Restifo NP. Identification of a MHC class II-restricted human gp100 epitope using DR4-IE transgenic mice. J Immunol 2000;164:3535–3542. [PubMed: 10725708]
14. Bakker AB, Schreurs MW, de BA, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med 1994;179:1005–1009. [PubMed: 8113668]
15. Brichard V, Van PA, Wolfel T, Wolfel C, De PE, Lethe B, Coulie P, Boon T. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 1993;178:489–495. [PubMed: 8340755]
16. Topalian SL, Gonzales MI, Parkhurst M, Li YF, Southwood S, Sette A, Rosenberg SA, Robbins PF. Melanoma-specific $CD4^+$ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. J Exp Med 1996;183:1965–1971. [PubMed: 8642306]
17. Wang RF, Appella E, Kawakami Y, Kang X, Rosenberg SA. Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. J Exp Med 1996a;184:2207–2216. [PubMed: 8976176]
18. Wang RF, Parkhurst MR, Kawakami Y, Robbins PF, Rosenberg SA. Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. J Exp Med 1996b; 183:1131–1140. [PubMed: 8642255]
19. Cui J, Arita Y, Bystryn JC. Cytolytic antibodies to melanocytes in vitiligo. J Invest Dermatol 1993;100:812–815. [PubMed: 8496621]
20. Mattes MJ, Thomson TM, Old LJ, Lloyd KO. A pigmentation-associated, differentiation antigen of human melanoma defined by a precipitating antibody in human serum. Int J Cancer 1983;32:717–721. [PubMed: 6197381]
21. Song YH, Connor E, Li Y, Zorovich B, Balducci P, Maclaren N. The role of tyrosinase in autoimmune vitiligo. Lancet 1994;344:1049–1052. [PubMed: 7934446]
22. Rinchik EM, Bultman SJ, Horsthemke B, Lee ST, Strunk KM, Spritz RA, Avidano KM, Jong MT, Nicholls RD. A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. Nature 1993;361:72–76. [PubMed: 8421497]
23. Schiaffino MV, Baschirotto C, Pellegrini G, Montalti S, Tacchetti C, De Luca M, Ballabio A. The ocular albinism type 1 gene product is a membrane glycoprotein localized to melanosomes. Proc Natl Acad Sci USA 1996;93:9055–9060. [PubMed: 8799153]
24. Kappler JW, Roehm N, Marrack P. T-cell tolerance by clonal elimination in the thymus. Cell 1987;49:273–280. [PubMed: 3494522]
25. Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. Science 1998;280:243–248. [PubMed: 9535647]
26. Hu Q, Bazemore WC, Girao C, Opferman JT, Sun J, Shabanowitz J, Hunt DF, Ashton-Rickardt PG. Specific recognition of thymic self-peptides induces the positive selection of cytotoxic T lymphocytes. Immunity 1997;7:221 –231. [PubMed: 9285407]
27. Sant'Angelo DB, Waterbury PG, Cohen BE, Martin WD, Van Kaer L, Hayday AC, Janeway CAJ. The imprint of intrathymic self-peptides on the mature T-cell receptor repertoire. Immunity 1997;7:517–524. [PubMed: 9354472]

28. Kurts C, Heath WR, Kosaka H, Miller JF, Carbone FR. The peripheral deletion of autoreactive CD8 $^{+}$ T cells induced by cross-presentation of self-antigens involves signaling through CD95 (Fas, Apo-1). J Exp Med 1998;188:415–420. [PubMed: 9670055]
29. Schwartz RH. T-cell clonal anergy. Curr Opin Immunol 1997;9:351–357. [PubMed: 9203408]
30. Liu GY, Fail-child PJ, Smith RM, Prowle JR, Kioussis D, Wraith DC. Low avidity recognition of self-antigen by T cells permits escape from central tolerance. Immunity 1995;3:407–415. [PubMed: 7584132]
31. von HM, Guerder S, Lewicki H, Flavell RA, Oldstone MB. Coexpression of B7-1 and viral (“self”) transgenes in pancreatic beta cells can break peripheral ignorance and lead to spontaneous autoimmune diabetes. Immunity 1995;3:727–738. [PubMed: 8777718]
32. Dudley ME, Roopenian DC. Loss of a unique tumor antigen by cytotoxic T-lymphocyte immunoselection from a 3-methylcholanthrene-induced mouse sarcoma reveals secondary unique and shared antigens. J, Exp Med 1996;184:441–447. [PubMed: 8760797]
33. Prehn RT, Main JM. Immunity to methyl-cholanthrene-induced sarcomas. J Natl Cancer Inst 1957;18:769–774. [PubMed: 13502695]
34. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991–1045. [PubMed: 8011301]
35. Bogen B. Peripheral T-cell tolerance as a tumor escape mechanism: deletion of CD4^{+} T cells specific for a monoclonal immunoglobulin idiotype secreted by a plasmacytoma. Eur J Immunol 1996;26:2671–2679. [PubMed: 8921954]
36. Hermans IF, Daish A, Yang J, Ritchie DS, Ronchese F. Antigen expressed on tumor cells fails to elicit an immune response, even in the presence of increased numbers of tumor-specific cytotoxic T-lymphocyte precursors. Cancer Res 1998;58:3909–3917. [PubMed: 9731502]
37. Prevost-Blondel A, Zimmermann C, Stemmer C, Kulmburg P, Rosenthal FM, Pircher H. Tumor-infiltrating lymphocytes exhibiting high ex vivo cytolytic activity fail to prevent murine melanoma tumor growth in vivo. J Immunol 1998;161:2187–2194. [PubMed: 9725210]
38. Staveley-O’Carroll K, Sotoraayor E, Montgomery J, Borrello I, Hwang L, Fein S, Pardoll D, Levitsky H. Induction of antigen-specific T cell anergy: an early event in the course of tumor progression. Proc Natl Acad Sci USA 1998;95:1178–1183. [PubMed: 9448305]
39. Curtsinger JM, Schmidt CS, Mondino A, Lins DC, Kedl RM, Jenkins MK, Mescher MF. Inflammatory cytokines provide a third signal for activation of naive CD4^{+} and CD8^{+} T cells. J Immunol 1999;162:3256–3262. [PubMed: 10092777]
40. Overwijk WW, Lee DS, Surman DR, Irvine KR, Touloukian CE, Chan CC, Carroll MW, Moss B, Rosenberg SA, Restifo NP. 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;96:2982–2987. [PubMed: 10077623]
41. Kim CJ, Chan CC, Rosenberg SA. Ophthalmologic evaluation in patients undergoing immunization with melanoma-associated antigens. J Immunother 1999;22:182–183. [PubMed: 10093043]
42. Pardoll DM. Inducing autoimmune disease to treat cancer. Proc Natl Acad Sci USA 1999;96:5340–5342. [PubMed: 10318881]
43. Wang S, Bartido S, Yang G, Qin J, Moroi Y, Panageas KS, Lewis JJ, Houghton AN. A role for a melanosome transport signal in accessing the MHC class II presentation pathway and in eliciting CD4^{+} T-cell responses. J Immunol 1999;163:5820–5826. [PubMed: 10570265]
44. Xu Y, Setaluri V, Takechi Y, Houghton AN. Sorting and secretion of a melanosome membrane protein, gp75/TRPl. J Invest Dermatol 1997;109:788–795. [PubMed: 9406822]
45. Bronte V, Apolloni E, Ronca R, Zamboni P, Overwijk WW, Surman DR, Restifo NP, Zanovello P. Genetic vaccination with “self tyrosinase-related protein 2 causes melanoma eradication but not vitiligo. Cancer Res 2000;60:253–258. [PubMed: 10667570]
46. Naftzger C, Takechi Y, Kohda H, Hara I, Vijayasaradhi S, Houghton AN. Immune response to a differentiation antigen induced by altered antigen: a study of tumor rejection and autoimmunity. Proc Natl Acad Sci USA 1996;93:14809–14814. [PubMed: 8962137]
47. Weber LW, Bowne WB, Wolchok JD, Srinivasan R, Qin J, Moroi Y, Clynes R, Song P, Lewis JJ, Houghton AN. Tumor immunity and autoimmunity induced by immunization with homologous DNA. J Clin Invest 1998;102:1258–1264. [PubMed: 9739060]

48. Dyall R, Bowne WB, Weber LW, LeMaoult J, Szabo P, Moroi Y, Piskun G, Lewis JJ, Houghton AN, Nikotic-Zugic J. Heteroclitic immunization induces tumor immunity. J Exp Med 1998;188:1553–1561. [PubMed: 9802967]
49. Overwijk WW, Tsung A, Irvine KR, Parkhurst MR, Goletz TJ, Tsung K, Carroll MW, Liu C, Moss B, Rosenberg SA, Restifo NP. gp100/pmel 17 is a murine tumor rejection antigen: induction of "self-reactive, tumoricidal T cells using high-affinity, altered peptide ligand. J Exp Med 1998;188:277–286. [PubMed: 9670040]
50. Steitz J, Bruck J, Steinbrink K, Enk A, Knop J, Tuting T. Genetic immunization of mice with human tyrosinase-related protein 2: implications for the immunotherapy of melanoma. Int J Cancer 2000;86:89–94. [PubMed: 10728600]
51. Fong L, Ruegg CL, Brockstedt D, Engleman EG, Laus R, Abati A. Induction of tissue-specific autoimmune prostatitis with prostatic acid-phosphatase immunization — implications for immunotherapy of prostate-cancer. J Immunol 1997;159:3113–3117. [PubMed: 9317107]
52. Mamula MJ, Lin RH, Janeway CAJ, Hardin JA. Breaking T-cell tolerance with foreign and self co-immunogens. A study of autoimmune B-and T-cell epitopes of cytochrome c. J Immunol 1992;149:789–795. [PubMed: 1321851]
53. Bowne WB, Srinivasan R, Wolchok JD, Hawkins WG, Blachere NE, Dyall R, Lewis JJ, Houghton AN. Coupling and uncoupling of tumor immunity and autoimmunity. J Exp Med 1999;190:1717–1722. [PubMed: 10587362]
54. Kozhich AT, Caspi RR, Berzofsky JA, Gery I. Immunogenicity and immunopathogenicity of an autoimmune epitope are potentiated by increasing MHC binding through residue substitution. J Immunol 1997;158:4145–4151. [PubMed: 9126974]
55. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 1998;4:328–332. [PubMed: 9500607]
56. Restifo NP, Rosenberg SA. Developing recombinant and synthetic vaccines for the treatment of melanoma. Curr Opin Oncol 1999;11:50–57. [PubMed: 9914879]
57. Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola F, Topalian SL, Restifo NP, Dudley ME, Schwarz SL, Spiess PJ, Wunderlich JR, Parkhurst MR, Kawakami Y, Seipp CA, Einhorn JH, White DE. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 1998;4:321–327. [PubMed: 9500606]
58. Ogg GS, Rod DP, Romero P, Chen JL, Cerundolo V. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. J Exp Med 1998;188:1203–1208. [PubMed: 9743539]
59. Leitner WW, Ying H, Driver DA, Dubensky TW, Restifo NP. Enhancement of tumor-specific immune response with plasmid DNA replicon vectors. Cancer Res 2000;60:51–55. [PubMed: 10646851]
60. Ying H, Zaks TZ, Wang RF, Irvine KR, Kammula US, Marincola FM, Leitner WW, Restifo NP. Cancer therapy using a self-replicating RNA vaccine. Nat Med 1999;5:823–827. [PubMed: 10395329]
61. Pieper R, Christian RE, Gonzales MI, Nishimura MI, Gupta G, Settlage RE, Shabanowitz J, Rosenberg SA, Hunt DF, Topalian SL. Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T cells. J Exp Med 1999;189:757–766. [PubMed: 10049939]
62. Wang RF, Wang X, Atwood AC, Topalian SL, Rosenberg SA. Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen. Science 1999a;284:1351–1354. [PubMed: 10334988]
63. Wang RF, Wang X, Rosenberg SA. Identification of a novel major histocompatibility complex class II-restricted tumor antigen resulting from a chromosomal rearrangement recognized by CD4(+) T cells. J Exp Med 1999b;189:1659–1668. [PubMed: 10330445]
64. Greenberg PD, Cheever MA, Fefer A. Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-l $^{+}$2-lymphocytes. J Exp Med 1981;154:952–963. [PubMed: 6974221]
65. Pardoll DM, Topalian SL. The role of CD4^{+} T-cell responses in antitumor immunity. Curr Opin Immunol 1998;10:588–594. [PubMed: 9794842]

66. Schild HJ, Kyewski B, Von Hoegen P, Schirrmacher V. $CD4^+$ helper T cells are required for resistance to a highly metastatic murine tumor. Eur J Immunol 1987;17:1863–1866. [PubMed: 2961578]

67. Surman DR, Dudley ME, Overwijk WW, Restifo NP. Cutting edge: $CD4^+$ T-cell control of $CD8^+$ T-cell reactivity to a model tumor antigen. J Immunol 2000;164:562–565. [PubMed: 10623795]

68. Toes RE, Ossendorp F, Offringa R, Melief CJ. CD4 T cells and their role in antitumor immune responses. J Exp Med 1999;189:753–756. [PubMed: 10049938]

69. Greenberg PD, Cheever MA. Effector mechanisms operative in adoptive therapy of tumor-bearing animals: implications for the use of interleukin-2. J Biol Response Mod 1984;3:455–461. [PubMed: 6239012]

70. Ossendorp F, Mengede E, Camps M, Filius R, Melief CJ. Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumors. J Exp Med 1998;187:693–702. [PubMed: 9480979]

71. Lu Z, Yuan L, Zhou X, Sotomayor E, Levitsky HI, Pardoll DM. CD40-independent pathways of T-cell help for priming of CD8(+) cytotoxic T lymphocytes. J Exp Med 2000;191:541–550. [PubMed: 10662799]

72. Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a $CD4^+$ T-helper and a T-killer cell. Nature 1998;393:474–478. [PubMed: 9624003]

73. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 1998;393:480–483. [PubMed: 9624005]

74. Hara I, Takechi Y, Houghton AN. Implicating a role for immune recognition of self in tumor rejection: passive immunization against the brown locus protein. J Exp Med 1995;182:1609–1614. [PubMed: 7595233]

75. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4(+) T cells in the antitumor immune response. J Exp Med 1998;188:2357–2368. [PubMed: 9858522]

76. Chen YT, Gure AO, Tsang S, Stockert E, Jager E, Knuth A, Old LJ. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc Natl Acad Sci USA 1998;95:6919–6923. [PubMed: 9618514]

77. Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, Karbach J, Arand M, Knuth A, Old LJ. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 1998;187:1349–1354. [PubMed: 9547346]

78. Buc M, Fazekasova H, Cechova E, Hegyi E, Kolibasova K, Ferencik S. Occurrence rates of HLA-DRB1, HLA-DQB1, and HLA-DPB1 alleles in patients suffering from vitiligo. Eur J Dermatol 1998;8:13–15. [PubMed: 9653015]

79. McDevitt HO. The role of MHC class II molecules in susceptibility and resistance to autoimmunity. Curr Opin Immunol 1998;10:677–681. [PubMed: 9914229]

80. Whitham RH, Bourdette DN, Hashim GA, Herndon RM, Ilg RC, Vandenbark AA, Offner H. Lymphocytes from SJL/J mice immunized with spinal cord respond selectively to a peptide of proteolipid protein and transfer relapsing demyelinating experimental autoimmune encephalomyelitis. J Immunol 1991;146:101–107. [PubMed: 1701788]

81. Akkaraju S, Ho WY, Leong D, Canaan K, Davis MM, Goodnow CC. A range of CD4 T-cell tolerance: partial inactivation to organ-specific antigen allows nondestructive thyroiditis or insulitis. Immunity 1997;7:255–271. [PubMed: 9285410]

82. Kumar V, Stellrecht K, Sercarz E. Inactivation of T-cell receptor peptide-specific CD4 regulatory T cells induces chronic experimental autoimmune encephalomyelitis (EAE). J Exp Med 1996;184:1609–1617. [PubMed: 8920851]

83. Olivares-Villagomez D, Wang Y, Lafaille JJ. Regulatory CD4(+) T cells expressing endogenous T-cell receptor chains protect myelin basic protein-specific transgenic mice from spontaneous autoimmune encephalomyelitis. J Exp Med 1998;188:1883–1894. [PubMed: 9815266]

84. Restifo NP, Ying H, Hwang L, Leitner WW. The promise of nucleic acid vaccines. Gene Ther 2000;7:89–92. [PubMed: 10673713]

85. Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, Paradise C, Kunkel L, Rosenberg SA. High-dose recombinant interleukin 2 therapy

for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999;17:2105. [PubMed: 10561265]

86. Tulp A, Verwoerd D, Dobberstein B, Ploegh HL, Pieters J. Isolation and characterization of the intracellular MHC class II compartment. Nature 1994;369:120–126. [PubMed: 8177317]
87. Peters PJ, Raposo G, Neefjes JJ, Oorschot V, Leijendekker RL, Geuze HJ, Ploegh HL. Major histocompatibility complex class II compartments in human B lymphoblastoid cells are distinct from early endosomes. J Exp Med 1995;182:325–334. [PubMed: 7629497]
88. West MA, Lucocq JM, Watts C. Antigen processing and class II MHC peptide-loading compartments in human B-lymphoblastoid cells. Nature 1994;369:147–151. [PubMed: 8177319]
89. Riese RJ, Wolf PR, Bromme D, Natkin LR, Villadangos JA, Ploegh HL, Chapman HA. Essential role for cathepsin S in MHC class II-associated invariant chain processing and peptide loading. Immunity 1996;4:357–366. [PubMed: 8612130]
90. Diment S, Eidelman M, Rodriguez GM, Orlow SJ. Lysosomal hydrolases are present in melanosomes and are elevated in melanizing cells. J Biol Chem 1995;270:4213–215. [PubMed: 7876179]
91. Orlow SJ. Melanosomes are specialized members of the lysosomal lineage of organelles. J Invest Dermatol 1995;105:3–7. [PubMed: 7615972]
92. Zhou BK, Boissy RE, Pifko-Hirst S, Moran DJ, Orlow SJ. Lysosome-associated membrane protein-1 (LAMP-1) is the melanocyte vesicular membrane glycoprotein band II. J Invest DermatoL 1993;100:110–114. [PubMed: 8429232]
93. Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, Carskadon SL, Kapfhamer D, Sufalko D, Robinson MS, Noebels JL, Burmeister M. Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. Neuron 1998;21:111–122. [PubMed: 9697856]
94. Meisler M, Levy J, Sansone F, Gordon M. Morphologic and biochemical abnormalities of kidney lysosomes in mice with an inherited albinism. Am J Pathol 1980;101:581–593. [PubMed: 7446708]
95. Carrillo-Farga J, Gutierrez-Palomera G, Ruiz-Maldonado R, Rondan A, Antuna S. Giant cytoplasmic granules in Langerhans cells of Chediak-Higashi syndrome. Am J Dermatopathol 1990;12:81–87. [PubMed: 2316818]
96. Faigle W, Raposo G, Tenza D, Pinet V, Vogt AB, Kropshofer H, Fischer A, de Saint-Basile G, Amigorena S. Deficient peptide loading and MHC class II endosomal sorting in a human genetic immunodeficiency disease: the Chediak-Higashi syndrome. J Cell Biol 1998;141:1121–1134. [PubMed: 9606205]
97. Schraermeyer U. Transport of endocytosed material into melanin granules in cultured choroidal melanocytes of cattle — new insights into the relationship of melanosomes with lysosomes. Pigment Cell Res 1995;8:209–214. [PubMed: 8610072]
98. Le Poole IC, van den Wijngaard RM, Westerhof W, Verkruisen RP, Dutrieux RP, Dingemans KP, Das PK. Phagocytosis by normal human melanocytes in vitro. Exp Cell Res 1993;205:388–395. [PubMed: 8482344]
99. Vijayasaradhi S, Doskoch PM, Houghton AN. Biosynthesis and intracellular movement of the melanosomal membrane glycoprotein gp75, the human b (brown) locus product. Exp Cell Res 1991;196:233–240. [PubMed: 1832641]
100. Calvo PA, Frank DW, Bieler BM, Berson JF, Marks MS. A cytoplasmic sequence in human tyrosinase defines a second class of di-leucine-based sorting signals for late endosomal and lysosomal delivery. J Biol Chem 1999;274:12780–12789. [PubMed: 10212263]
101. Nijman HW, Kleijmeer MJ, Ossevoort MA, Oorschot VM, Vierboom MP, van de Keur M, Kenemans P, Kast WM, Geuze HJ, Melief CJ. Antigen capture and major histocompatibility class II compartments of freshly isolated and cultured human blood dendritic cells. J Exp Med 1995;182:163–174. [PubMed: 7790816]
102. Rudensky AY, Marie M, Eastman S, Shoemaker L, DeRoos PC, Blum JS. Intracellular assembly and transport of endogenous peptide-MHC class II complexes. Immunity 1994;1:585–594. [PubMed: 7600287]
103. Brady MS, Eckels DD, Ree SY, Schultheiss KE, Lee JS. MHC class II-mediated antigen presentation by melanoma cells. J Immunother Emphasis Tumor Immunol 1996;19:387–397. [PubMed: 9041456]

104. Hersey P, Jamal O. Immunohistological relation between DR antigen expression on melanoma cells and infiltration by $CD8^+$ T cells. Pathology 1990;22:133–139. [PubMed: 2147056]

105. Bohm W, Thoma S, Leithauser F, Moller P, Schirmbeck R, Reimann J. T-cell-mediated, IFN-gamma-facilitated rejection of murine B16 melanomas. J Immunol 1998;161:897–908. [PubMed: 9670968]

106. al Badri AM, Foulis AK, Todd PM, Gariouch JJ, Gudgeon JE, Stewart DG, Gracie JA, Goudie RB. Abnormal expression of MHC class II and ICAM-1 by melanocytes in vitiligo. J Pathol 1993;169:203–206. [PubMed: 8095298]

107. Badri AM, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG, Goudie RB. An immunohistological study of cutaneous lymphocytes in vitiligo. J Pathol 1993;170:149–155. [PubMed: 8345407]

108. Hann SK, Park YK, Lee KG, Choi EH, Im S. Epidermal changes in active vitiligo. J Dermatol 1992;19:217–222. [PubMed: 1607483]

109. 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;148:1219–1228. [PubMed: 8644862]

110. Ohashi PS. T-cell selection and autoimmunity: flexibility and tuning. Curr Opin Immunol 1996;8:808–814. [PubMed: 8994860]

111. Denfeld RW, Dietrich A, Wuttig C, Tanczos E, Weiss JM, Vanscheidt W, Schopf E, Simon JC. In situ expression of B7 and CD28 receptor families in human malignant melanoma: relevance for T-cell-mediated anti-tumor immunity. Int J Cancer 1995;62:259–265. [PubMed: 7543078]

112. Thomas WD, Smith MJ, Si Z, Hersey P. Expression of the co-stimulatory molecule CD40 on melanoma cells. Int J Cancer 1996;68:795–801. [PubMed: 8980186]

113. van den Oord JJ, Maes A, Stas M, Nuyts J, Battocchio S, Kasran A, Garmyn M, De Wever I, De Wolf-Peeters C. CD40 is a prognostic marker in primary cutaneous malignant melanoma. Am J Pathol 1996;149:1953–1961. [PubMed: 8952530]

114. von Leoprechting A, van der Bruggen P, Pahl HL, Aruffo A, Simon JC. Stimulation of CD40 on immunogenic human malignant melanomas augments their cytotoxic T lymphocyte-mediated lysis and induces apoptosis. Cancer Res 1999;59:1287–1294. [PubMed: 10096561]

115. Alexander MA, Bennicelli J, Guerry D. Defective antigen presentation by human melanoma cell lines cultured from advanced, but not biologically early, disease. J Immunol 1989;142:4070–4078. [PubMed: 2469732]

116. Wolff K, Konrad K. Melanin pigmentation: an in vivo model for studies of melanosome kinetics within keratinocytes. Science 1971;174:1034–1035. [PubMed: 5120089]

117. Jimbow K, Gomez PF, Toyofuku K, Chang D, Miura S, Tsujiya H, Park JS. Biological role of tyrosinase related protein and its biosynthesis and transport from TGN to stage I melanosome, late endosome, through gene transfection study. Pigment Cell Res 1997;10:206–213. [PubMed: 9263327]

118. Vijayasaradhi S, Xu Y, Bouchard B, Houghton AN. Intracellular sorting and targeting of melanosomal membrane proteins: identification of signals for sorting of the human brown locus protein, gp75. J Cell Biol 1995;130:807–820. [PubMed: 7642699]

119. Overwijk WW, Theoret MR, Restifo NP. The future of interleukin-2: enhancing therapeutic anticancer vaccines. Cancer J Sci Am 2000;6(Suppl 1):S76–S80. [PubMed: 10685664]

120. Rosenberg SA, Lotze MT, Yang JC, Topalian SL, Chang AE, Schwartzentruber DJ, Aebersold P, Leitman S, Linehan WM, Seipp CA. Prospective randomized trial of high-dose interleukin-2 alone or in conjunction with lymphokine-activated killer cells for the treatment of patients with advanced cancer. J Natl Cancer Inst 1993;85:622–632. [PubMed: 8468720]

121. Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwartzentruber DJ, Weber JS, Parkinson DR, Seipp CA, Einhorn JH, White DE. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst 1994;86:1159–1166. [PubMed: 8028037]

122. Kobayashi T, Urabe K, Winder A, Jimenez-Cervantes C, Imokawa G, Brewington T, Solano F, Garcia-Borron JC, Hearing VJ. Tyrosinase related protein 1 (TRP1) functions as a DHICA oxidase in melanin biosynthesis. EMBO J 1994;13:5818–5825. [PubMed: 7813420]

123. Murata K, Garcia-Sastre A, Tsuji M, Rodrigues M, Rodriguez D, Rodriguez JR, Nussenzweig RS, Palese P, Esteban M, Zavala F. Characterization of in vivo primary and secondary $CD8^+$ T-cell responses induced by recombinant influenza and vaccinia viruses. Cell Immunol 1996;173:96–107. [PubMed: 8871605]

124. Schiaffino MV, d'Addio M, Alloni A, Baschirotto C, Valetti C, Cortese K, Puri C, Bassi MT, Colla C, De Luca M, Tacchetti C, Ballabio A. Ocular albinism: evidence for a defect in an intracellular signal transduction system. Nat Genet 1999;23:108–112. [PubMed: 10471510]

125. Orlow SJ, Brilliant MH. The pink-eyed dilution locus controls the biogenesis of melanosomes and levels of melanosomal proteins in the eye. Exp Eye Res 1999;68:147–154. [PubMed: 10068480]

126. Gahl WA, Potterf B, Durham-Pierre D, Brilliant MH, Hearing VJ. Melanosomal tyrosine transport in normal and pink-eyed dilution murine melanocytes. Pigment Cell Res 1995;8:229–233. [PubMed: 8789196]

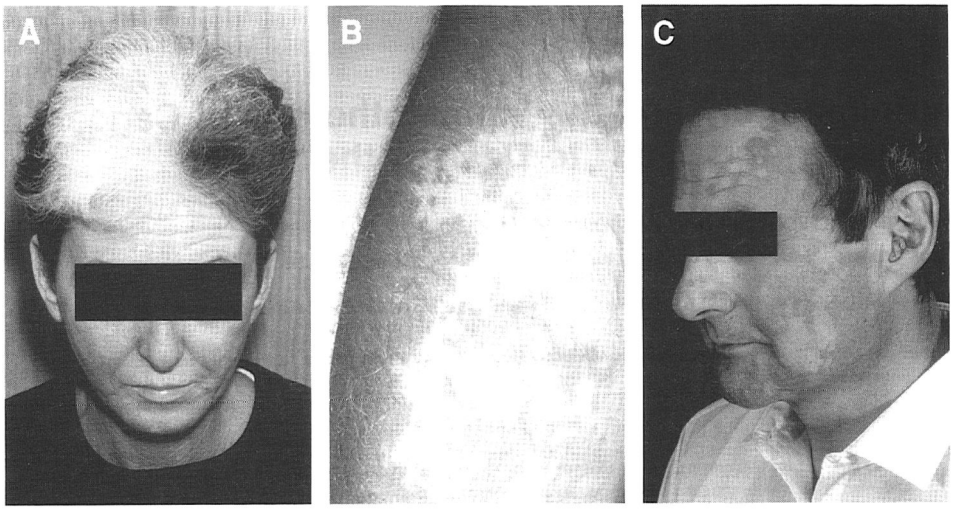


FIGURE 1.

Vitiligo in melanoma patients treated with high-dose interleukin-2. Patients were treated with infusions of recombinant human interleukin-2 and experienced tumor regression, together with profound depigmentation of hair (a) and skin (*a*,*b*,*c*).[3]

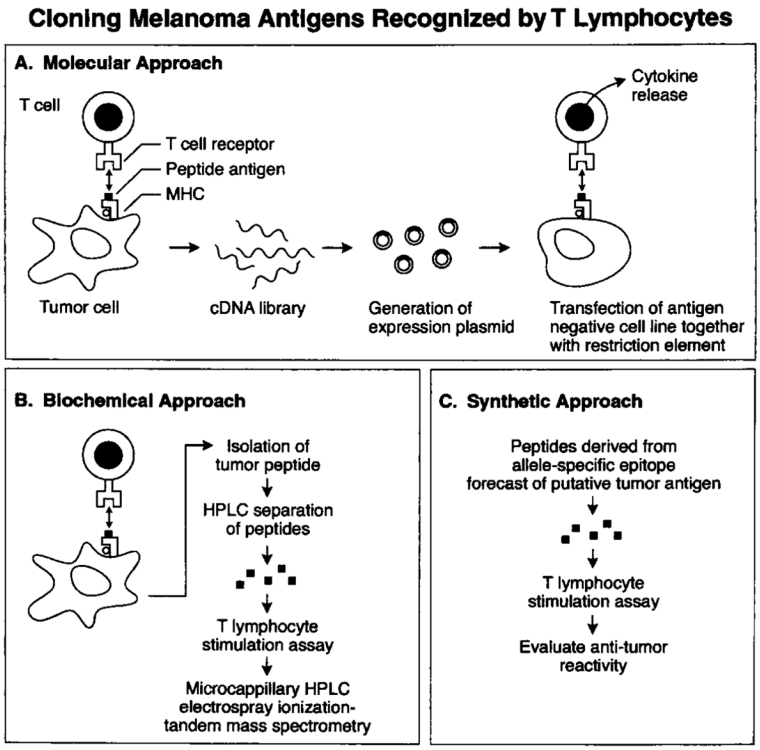
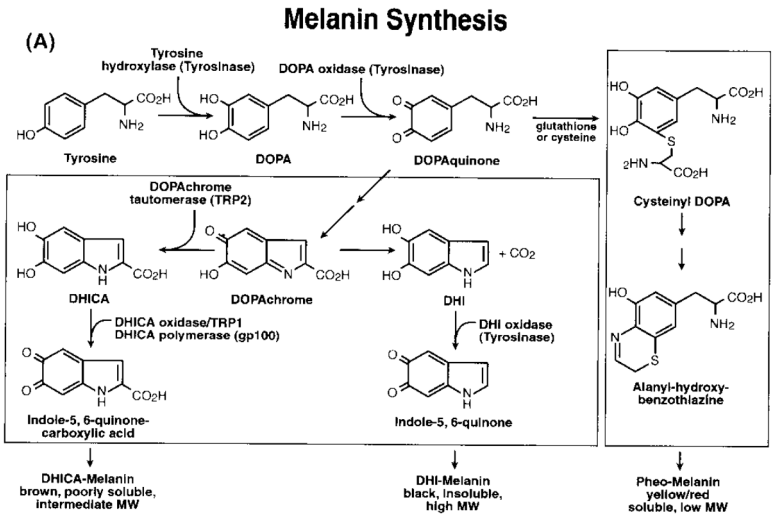


FIGURE 2.

Strategies for cloning tumor-associated antigens, (a) Genetic approach: mRNA extracted from tumor cells that are recognized by T cells is reverse transcribed into cDNA and cloned into an expression library. Pools of 10 to 50 cDNAs are transfected into antigen-negative cells expressing the correct restriction element. cDNAs conferring recognition by tumor-reactive T cells (as measured by cytokine secretion) are retested individually and sequenced to identify the protein recognized.[5–8] (*b*) Biochemical approach: peptides bound to MHC I or II molecules on the surface of tumor cells that are recognized by T cells are isolated, fractionated using microcapillary high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry[10] and tested for recognition by tumor-specific T cells by pulsing onto antigen-negative cells such as T2 cells.[9] (*c*) Synthetic approach: candidate antigen-derived peptides that are predicted to bind to MHC molecules are synthesized and used to induce de novo T-cell reactivity in vitro. The resulting T cells are tested for specific recognition of tumor cells expressing the candidate antigen.[11,12]



Melanoma Associated Antigen Profile

(B)

Antigen	Other name	Murine locus	Human Disease Mutation
Tyrosinase	Tyrosine hydorxylase DOPA oxidase DHI oxidase	*Albino c locus*	OCA I Oculocutaneous Albinism
TRP-1	gp-75 b-protein catalase-b DHICA oxidase	*brown b locus*	OCA III
TRP-2	DOPAchrome tautomerase	*slaty sit locus*	OCA IV
gp100	Pmel 17 DHICA polymerase	*silver si locus*	Premature grey hair?
MART-1	Melan-A	*none*	Vogt-Koyanagi-Harada
P polypeptide	none	*pink-eyed dilution*	OCA II
Ocular Albinism	none	*OA-1*	Ocular Albinism I

FIGURE 3.

(a) Function of melanocyte differentiation antigens in the synthesis of melanin pigment. With the possible exception of MART-1. for which a clear role has yet to be identified, all MDA that are recognized by melanoma-specific T cells fulfill an enzymatic function in melanogenesis.[122,123] The putative melanoma antigen OA-1 is an exclusively intracellular, G-protein linked receptor that is integral to the melanosomal membrane;[23,124] a second putative melanoma antigen. P. polypeptide, is implicated in melanosome biogenesis and possibly in melanosomal tyrosine transport, although the latter function remains controversial.[22,125,120] (*b*) Implication of human and mouse MDA in phenotypical appearance and disease states.

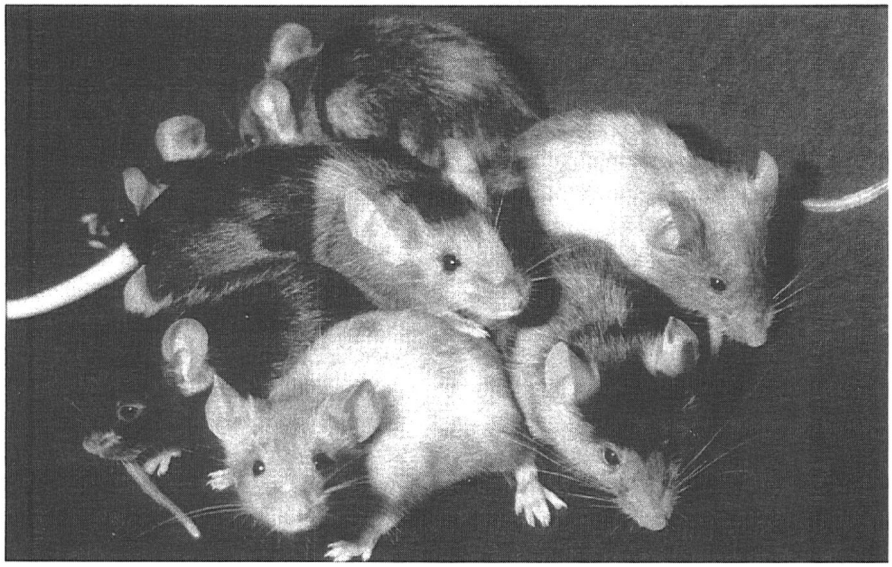


FIGURE 4.

Vaccine-induced vitiligo in mice. C57BU6 mice, which were vaccinated with recombinant vaccinia virus encoding normal mouse TRP-1 (rVVmTRP-1), developed striking vitiligo. Mice developing vitiligo were fully protected from a challenge with the spontaneous murine melanoma B16.[40]

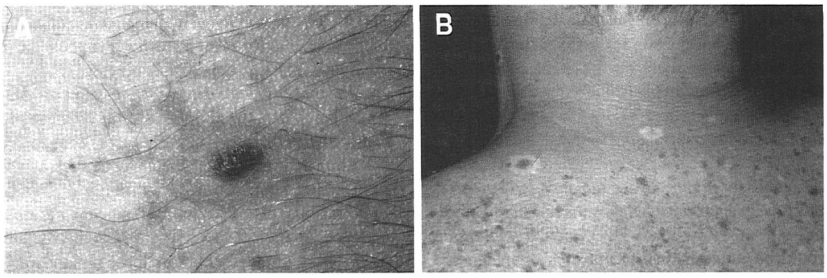


FIGURE 5.

Vaccine-induced vitiligo and antitumor effects in melanoma patients, (a) Patients receiving a HLA-A2-restricted, modified gp100 peptide spanning amino acids 209 through 217 in conjunction with interleukin-2 occasionally developed a striking, halo-like inflammation around normal nevi, suggesting that the vaccine induced a localized immune aggression against normal melanocytes expressing gp100.[57] (*b*) A fraction of patients who responded to gp100 peptide vaccination and underwent tumor regression experienced autoimmune destruction of normal cutaneous melanocytes in the direct vicinity of regressing tumor lesions. The dark color that remains reflects the presence of macrophages laden with degradation-resistant melanin pigment; upon biopsy the lesions contained no live tumor cells.[57]

TABLE 1

TABLE 1A. Vitiligo in Cancer Patients Treated with Interleukin-2

	Number of patients*	
	Total	**With vitiligo (%)**
Metastatic renal cancer	104	0 (0%)
Metastatic melanoma	73	12 (16%) ($p_2 < 0.0001$)

TABLE 1B. Vitiligo in Patients with Metastatic Melanoma Treated with Interleukin-2

	Number of patients*	
	Total	**With vitiligo (%)**
Non-responder	27	0 (0%)
Responder	42	12 (29%) ($p_2 < 0.002$)

* All patients assessed for vitiligo at least 1 year after receiving interleukin-2.

TABLE 2

Close Relationship Between Melanosomes and Endosomes/Lysosomes

1	Both melanosomes and endosomes/lysosomes are acidic and contain “lysosome-specific” proteins, such as acid hydrolases and LAMP-1.
2	Mutations in genes that regulate formation of endosomes and/or lysosomes also affect melanosome formation and pigmentation: *mocha* mutation, *light ear* mutation, ChediakHigashi syndrome.
3	Extracellular material phagocytosed by melanocytes is transported to both lysosomes and melanosomes.
4	In melanocytes, a fraction of translated TRP-1 is routed to an acidic, non-melanosomal, endosome-like compartment. TRP-1 produced in melanosome-deficient fibroblasts accumulates in the endosome.
5	MHC Class II-dependent, antigen-specific T-cell responses to DNA vaccination can be greatly enhanced by fusing the antigen to the melanosomal transport signal of TRP-1.