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Adoptive T Cell Immunotherapy of Human Uveal Melanoma Targeting gp100

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HLA-A*0201-restricted CTL against human gp100 were isolated from HLA-A*0201/K b (A2/K b)-transgenic mice immunized with recombinant canarypox virus (ALVAC-gp100). These CTL strongly responded to the gp100 154–162 epitope, in the context of both the chimeric A2/K b and the wild-type HLA-A*0201— molecule, and efficiently lysed human HLA-A*0201+, gp100+ melanoma cells in vitro. The capacity of the CTL to eradicate these tumors in vivo was analyzed in A2/K b-transgenic transgenic mice that had received a tumorigenic dose of human uveal melanoma cells in the anterior chamber of the eye. This immune-privileged site offered the unique opportunity to graft xenogeneic tumors into immunocompetent A2/K b-transgenic mice, a host in which they otherwise would not grow. Importantly, systemic (i.v.) administration of the A2/K b-transgenic gp100 154–162-specific CTL resulted in rapid elimination of the intraocular uveal melanomas, indicating that anti-tumor CTL are capable of homing to the eye and exerting their tumoricidal effector function. Flow cytometry analysis of ocular cell suspensions with HLA-A*0201-gp100 154–162 tetrameric complexes confirmed the homing of adoptively transferred CTL. Therefore, the immune-privileged state of the eye permitted the outgrowth of xenogeneic uveal melanoma cells, but did not protect these tumors against adoptive immunotherapy with highly potent anti-tumor CTL. These data constitute the first direct indication that immunotherapy of human uveal melanoma may be feasible. The Journal of Immunology, 2000, 165: 7308–7315.

In the present study, we isolated HLA-A*0201-restricted CTL specific for the human melanoma Ag gp100 from HLA-A*0201/K b (A2/K b)3-transgenic mice (13) and examined the capacity of these CTL to eradicate human melanoma cells in vitro as well as in vivo. We were particularly interested in the in vivo eradication of intraocular human uveal melanomas. This type of melanoma is the most common primary intraocular malignancy in adults. Although treatments of the primary tumor that allow preservation of the eye have progressed greatly, these approaches have severe side effects. Gunduz et al. (14) recently showed that 42% of eyes successfully treated with radiation subsequently developed radiation retinopathy within the first 5 years after treatment. As a result of the high incidence of metastases, which are largely refractory to conventional treatment, uveal melanoma has a high mortality rate (reviewed by Pyrhönen in Ref. 15). Because gp100 was found expressed in the majority of human uveal melanomas (16–18), this Ag would constitute an appropriate target for immunotherapy of such tumors. A potential hurdle for successful immunotherapy of such tumors is the fact that the eye is, at least to a certain extent, an immune-privileged site (19, 20). Furthermore, melanoma cells have been reported to exert lymphocyte-inhibitory properties within the ocular microenvironment (21). To test the feasibility of immunotherapy of intraocular human uveal melanomas, we engrafted human HLA-A*0201+, gp100+-uveal melanoma cells in the anterior chamber of the eye of A2/K b-transgenic mice. The xenogeneic melanoma cells, which are rapidly eliminated in such mice when grafted into other sites of the body, were capable of forming tumors in this site. We employed this tumor model to test whether systemic administration of gp100-specific CTL, isolated from A2/K b mice and expanded in vitro, would result in elimination of these intraocular tumors.

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3 Abbreviations used in this paper: A2/K b, HLA-A*0201/K b; TIL, tumor-infiltrating lymphocyte; Ad5, adenovirus type 5.
M1B-gp100 was derived from a nontransformed mouse embryo cell line of C57BL/6 (B6) origin through transfection with the genes for murine B7.1, HLA-A*0201/K b (A2/K b ), and human gp100. M1B-MAGE was generated in a similar manner by transfection with the gene for human MAGE-2. BA-gp100 and BA-MART were derived from murine melanoma cell line B16-F10 through the transfection of the genes for A2/K b and human gp100 or human MART-1, respectively. Previously described cell lines in these experiments included A2/K b (13), human melanoma cell lines BLM, BLM-gp100 (22), Mel 397 (23), and Mel 397 A2/K b (kindly provided by J. J. Adema, University Hospital, Nijmegen, The Netherlands). Human uveal melanoma cell lines OMM-1 (17), OC-M-3, and 92–1 (24), and human osteosarcoma cell line SAOS (25). Infection of SAOS with recombinant ALVAC (described below) was performed by infection of 10^5 cells in 0.5 ml serum-free medium with a multiplicity of infection of 10 during 3 h at 37°C, followed by washing of the cells and culturing for 2 days. Murine CTL clone 100B6 specifically recognizes a peptide epitope derived from the adenosine type 5 EIB protein (26). All cell lines were maintained in IMDM (BioWhittaker, Walkersville, MD), supplemented with 8% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 μg/ml 2-ME. Murine CTL lines and clones were cultured in IMDM supplemented with 8% heat-inactivated FCS, 10 cts units IL-2/ml, 100 U/ml penicillin, 100 μg/ml streptomycin, 20 μg/ml 2-ME, and 2 mM l-glutamine. WEHI 164 clone 13 cells were cultured in RPMI 1640 supplemented with 8% heat-inactivated FCS, penicillin, l-glutamine (216 mg/ml), l-asparagine (36 mg/ml), and l-arginine-HCl (116 mg/ml).

Immunization of HLA-A*0201/K b -transgenic mice

HLA-A*0201/K b (A2/K b )-transgenic mice were kindly provided by Dr. L. Sherman (Scirpps Laboratories, San Diego, CA). These mice express a chimeric class I MHC molecule composed of the α1 and α2 domains of HLA-A*0201 and the α3, cytoplasmic, and transmembrane domains of the mouse H-2K b molecule (13). A2/K b mice were vaccinated with different recombinant ALVAC canary poxviruses (Virogenetics, Troy, NY). ALVAC-gp100 encoded the full-length human gp100 sequence. ALVAC-AAA (PE03) encoded four epitopes (MART-127–35, gp100 154 –162, gp100 209 –217, and gp100 280 –288) separated by a triple alanine spacer (AAA-AAGIGILTV-AAA-AAGIGILTV-AAA-AAGIGILTV-AAA-AAGIGILTV-AAA). ALVAC-NKRK (PE02) contained the epitopes separated by an NKRK spacer (NKRK-AAGIGILTV-NKRK-KTWGQYWQV-NKRK-IT). ALVAC-SGPV-SGPV-NKRK-AAGIGILTV-NKRK-KTWGQYWQV-NKRK-IT. This spacer sequence has successfully been employed for other multiepitope constructs (27). ALVAC-NKRK (PE02) contained the epitopes separated by an NKRK spacer (NKRK-AAGIGILTV-NKRK-KTWGQYWQV-NKRK-IT). This spacer sequence is predicted to contain three trypsin cleavage sites. Trypsin-like activity has been associated with the proteasome. The parental ALVAC virus was used as a negative control.

Mice received three i.v. doses of 10^5 PFU of the same ALVAC. Ten days after the last vaccination, the mice were sacrificed, and splenocytes were isolated. Different irradiated stimulator cells, sharing expression of A2/K b and gp100, were used in the following order: 1) A2/K b LPS blasts loaded with the three human gp100 peptides (to prevent competition between these peptides for binding to A2/K b); three portions of LPS blasts were separately loaded with the different peptides and washed three times, after which they were pooled; 2) Mel 397 A2/K b; and 3) MIB-gp100. For anti-tumor vaccination studies, mice received two i.v. doses of 10^5 PFU with a 10-day interval. Ten days after the last vaccination, the mice were challenged with 3 x 10^6 OMM-1 cells in the anterior chamber of the eye, and tumor development was monitored.

Analysis of CTL activity in vitro

Cell-mediated lymphocyte cytotoxicity was measured with a 51Cr release assay as described previously (2). The mean percentage of specific lysis in triplicate wells was calculated as follows: % specific lysis = [(cpm experimental release - cpm spontaneous release)/(cpm maximum release - cpm spontaneous release)] x 100. For TNF-α release assays, Jurkat A2/K b cells were loaded with peptide (10 μg/ml) for 1 h at 37°C, washed three times, and used as stimulator cells. In short, 5 x 10^5 murine T cells were added to 10^5 peptide-loaded Jurkat A2/K b cells. After 24 h, the supernatant was harvested, and its TNF-α content was determined by its cytolytic effect on mouse fibrosarcoma cell line WEHI 164 clone 13, measured by the intensity of vital staining on an ELISA plate reader. The maximum cytolytic effect was estimated by adding 500 pg/ml human rTNF-α.

Adaptive immunotherapy experiments

A modified quantitative technique for deposition of a definitive number of tumor cells into the anterior chamber of the mouse eye was used (28). Mice were deeply anesthetized with a mixture (ratio 1:1) of xylazine (Rompun 2%; Bayer, Leverkusen, Germany) and ketamine hydrochloride (Aescoket, Aesculaap BV, Bockel, The Netherlands) given i.p. The eye was viewed through a low-power (8 x) of a dissecting microscope, and a sterile 30-gauge needle was used to puncture the cornea at the corneoscleral junction, parallel and anterior to the iris. A glass micropipet (~80 μm in diameter) was fitted into a sterile infant-feeding tube, which was mounted onto a sterile 0.1 Hamilton syringe (Hamilton, Whittier, CA). The pipette, loaded with OMM-1 cell suspension (7.5 x 10^6/ml), was introduced through the puncture site of the cornea, and 4 μl of the OMM-1 cell suspension was delivered into the anterior chamber. The eyes were examined three times per week with a dissecting microscope to observe and document tumor growth. Subcutaneous induction of tumors, as well as i.v. administration of CTL clones, was performed as described previously (28, 29). Tumor-challenged mice were assigned randomly to treatment protocols with relevant CTL or control CTL. All CTL treatments were performed in combination with a s.c. injection of 10^5 Cetus units of IL-2 in an IFA depot. Histological analysis of the treated and control mice was performed as described previously (28).

Flow cytometry of tumor-infiltrating lymphocytes (TIL)

Eyes were mashed through a nylon filter to obtain single-cell suspensions, passed over a Ficoll gradient, washed, and triple stained with propidium iodide (1 μg/ml), anti-mCD8-FITC (1:200 dilution, Pharmingen, San Diego, CA), and HLA-A*0201-allophycocyanin tetrameric complexes harboring the gp100[154–162] peptide (1:10 dilution; a kind gift from Dr. H. Spits, The Netherlands Cancer Institute, The Netherlands). Analysis by flow cytometry (FACScalibur; Becton Dickinson, Mountain View, CA) of propidium iodide-negative cells was performed immediately after staining.

Results

Induction of HLA-restricted, human gp100-specific immunity in HLA-A*0201/K b -transgenic mice

Human gp100-specific CTL immunity was induced in HLA-A*0201/K b (A2/K b )-transgenic mice through immunization with recombinant canarypox viruses (ALVAC) that encode the three known HLA-A*0201-restricted epitopes gp100[154–162] (22), gp100[209–217], and gp100[280–288] (30). The full-length human gp100 protein is known to harbor, in addition to the three epitopes of interest, at least one and possibly more epitopes that are immunogenic in the context of murine class I MHC (31), while the A2/K b-transgenic mice coexpress the H-2D b and K b molecules (13). To skew the CTL response of the mice toward the HLA-restricted epitopes of interest, we immunized not only with ALVAC-gp100, encoding the full-length human gp100 Ag, but also with multiepitope ALVAC, encoding synthetic polypeptides that comprise a string-of-beads arrangement of the three epitopes (see Materials and Methods for details). Similar multiepitope vaccines have previously been employed successfully by several laboratories for the induction of effective anti-tumor CTL immunity in mice (e.g., Refs. 27 and 32).

Mice received three subsequent i.v. doses of the same ALVAC vaccine, after which the splenocytes were restimulated in vitro in the presence of cells positive for the three human gp100 epitopes and HLA-A*0201. We submitted the splenocytes to three sequential rounds of stimulation with different stimulator cells in the following order: A2/K b-transgenic LPS blasts loaded with the three gp100 epitopes (see Materials and Methods for details), an A2/K b-transfectant of the gp100-positive human melanoma cell line Mel397, and A2/K b-transgenic mouse embryonic cells transfected with the genes for human gp100 and murine B7.1 (MIB-gp100). After this three-step restimulation protocol, the T cell cultures were tested for their reactivity against a panel of target cells in a TNF-α release assay. The T cell cultures responded against human and murine cell lines expressing both human gp100 and...
A2/Kb-transgenic CTL clones efficiently lyse human melanoma cells in vitro

After initial analysis of the polyclonal T cell cultures, CTL clones were isolated through limiting dilution using BLM-gp100 tumor cells (HLA-A*0201) as stimulator cells. These clones showed a reactivity pattern identical with that of the bulk cultures from which they were derived, in that they recognized human and murine cells that presented the gp100154–162 epitope in the context of the A2/Kb molecule (Fig. 2). Importantly, the CTL were also capable of lysing gp100-positive human melanoma cells expressing the wild-type HLA-A*0201 restriction element (Fig. 2C). Because the murine CD8 molecule on these CTL cannot efficiently associate with the α3 domain of the HLA-A*0201 molecule (13), this interaction is apparently not required, implying that the TCRs of these CTL exhibit high affinity for their target Ag. This notion was confirmed by measuring the reactivity of the A2/Kb-transgenic CTL clones against titered amounts of exogenously loaded gp100154–162 peptide. The CTL exhibited efficient lysis of peptide-loaded target cells, reaching 50% of their maximal lytic activity at peptide concentrations of 10 ng/ml (Fig. 3A). This sensitivity, which is similar to that found for TIL1200 cells, a human TIL line recognizing the same epitope (22), is commonly found for CTL that are capable of responding to physiological quantities of naturally processed Ag (33).

The specificity of the A2/Kb-transgenic CTL was compared in more detail to that of the TIL1200 cells by testing the reactivity of these responders to a series of variants of the gp100154–162 peptide in which residues at the different positions were replaced by alanine. This revealed that, at least for the A2/Kb-transgenic CTL clone tested, the fine specificity with respect to the gp100154–162 epitope was very similar, if not identical, to that of the human-derivated TIL1200 cells (Fig. 3B, and Ref. 22). Taken together, our data show that immunization of A2/Kb-transgenic mice with recombinant ALVAC vaccines encoding human gp100 CTL epitopes can elicit potent HLA-A*0201-restricted CTL immunity against the gp100154–162 epitope.

Gp100-specific CTLs control the growth of intraocular human uveal melanomas in A2/Kb-transgenic mice

At present, no effective treatment is available for metastatic uveal human melanoma (15). In contrast, a majority of the human uveal melanomas tested were found to express both gp100 and class I HLA molecules (16–18, 34). Furthermore, it has been shown that uveal melanoma cells can be lysed by tyrosinase and MAGE-specific CTL clones in vitro (35). Therefore, we set out to analyze the sensitivity of human uveal melanoma cells for our A2/Kb-transgenic gp100-specific CTL in vitro as well as for adoptive immunotherapy with these CTL in vivo. We employed the human uveal melanoma cell line OMM-1, which is positive for both HLA-A*0201 and gp100 (17). In accordance with the expression of these Ags, OMM-1 was shown to be an excellent target for lysis by A2/Kb-transgenic gp100154–162-specific CTL in vitro (Fig. 4). We subsequently performed adoptive transfer experiments in A2/Kb mice that were challenged with an intraocular dose of this human uveal melanoma cell line. We have recently found that highly immunogenic adenovirus type 5 (Ad5)-transformed cells, which otherwise fail to grow in syngeneic immunocompetent mice (29), do form tumors when inoculated in the anterior chamber of the eye.

**FIGURE 1.** T cell cultures derived from A2/Kb-transgenic mice immunized with different ALVAC types exhibit similar target specificity. A. Reactivity of polyclonal T cell cultures was examined in a TNF-α release assay against A2/Kb-transgenic mouse embryo cells expressing murine B7.1 and human gp100 (MIB-gp100), a control cell expressing human MAGE-2 (MIB-MAGE), an A2/Kb transfectant of the gp100-positive human melanoma cell line Mel397 as well as the parental cell, and against human HLA-A*0201-positive osteosarcoma cells (SAOS) infected with either ALVAC-gp100 or control ALVAC. B. Reactivity was determined against A2/Kb-transfected Jurkat T cells loaded with either of the indicated gp100 peptides. The T cell bulks were derived from A2/Kb-transgenic mice that had been vaccinated with ALVAC encoding full-length human gp100 (ALVAC-gp100), or encoding multiepitope polypeptide in which the three gp100-epitopes were spaced by triple alanine sequences (ALVAC-AAA) or by NKRK sequences (ALVAC-NKRK). See Materials and Methods for details on the multiepitope constructs.
Reactivity of CTL (clone 8J) derived from the bulk culture of ALVAC-gp100-immunized A2/Kb mice (see Fig. 1) was tested in a cytolytic assay against a target cell panel. A, B16 murine melanoma cells transfected with the genes for A2/Kb and human gp100 (BA-gp100), a control cell expressing human MART-1 (BA-MART). B, A2/Kb-transfected Jurkat T cells loaded with either the gp100154–162 peptide or a control peptide. C, An A2/Kb-transfectant of the gp100-positive, HLA-A*0201-negative human melanoma cell line Mel397, the HLA-A*0201-positive, gp100-negative human melanoma cell line BLM, and a gp100-transfected counterpart (BLM-gp100). All data were obtained in one single representative experiment, but are divided over three panels for clarity. Similar data was obtained for other CTL clones derived from T cell bulks of A2/Kb mice that were immunized with ALVAC-gp100 or the multiepitope vaccines ALVAC-AAA and ALVAC–NKRK (not shown, see Fig. 1 for bulks).

In conclusion, A2/Kb-transgenic mice can be employed to raise highly effective HLA-restricted anti-melanoma CTL that are capable of eradicating human melanoma cells in vitro as well as in vivo. Furthermore, our data demonstrate that the anterior chamber of the eye can be employed as a unique location permitting the outgrowth of, and the testing of immunotherapeutic approaches against, human tumors in an HLA-transgenic host. Finally, the efficient eradication of human uveal melanoma cells in their physiological context by systemically administered CTL constitutes the first indication that adoptive immunotherapy of such tumors may be feasible.

**Discussion**

The present manuscript describes the isolation from A2/Kb mice of HLA-A*0201-restricted gp100 CTL that are capable of efficiently eliminating human melanoma cells in vitro and in vivo. An advantage of using A2/Kb mice instead of mice expressing the wild-type HLA-A*0201 molecule is that the H-2Kb-derived α3 domain permits efficient interaction of HLA-restricted murine CTL with their target through their CD8 receptor (13). However, a drawback may be that the HLA-restricted CTL generated in A2/Kb-transgenic mice, especially in mice that have been immunized with considerable quantities of synthetic peptides, do not exhibit very high affinity for their target epitope and therefore depend for their reactivity on the interaction with CD8. The consequence of this CD8 dependency would be that such CTL, although capable of recognizing their target epitope in the context of the A2/Kb molecule, would fail to respond to this epitope in the context of the physiologically relevant HLA-A*0201 molecule (36). To prevent the induction of CTL of insufficient affinity, we avoided immunization with synthetic peptides and instead immunized with recombinant canarypox viruses (ALVAC) encoding these epitopes. To ensure induction of HLA-A*0201-restricted gp100-specific CTL instead of CTL directed against epitopes restricted by the endogenous H-2Dk and Kk molecules, we immunized not only with ALVAC-gp100, encoding the full-length human gp100 Ag, but also with multiepitope ALVAC encoding synthetic polypeptides, which comprises a string-of-beads arrangement of the three gp100 epitopes. In all cases, CTL specific for the gp100154–162 peptide were obtained, suggesting that this epitope is the immunodominant HLA-A*0201-restricted gp100 epitope in A2/Kb mice. This can most readily be explained by the fact that the gp100154–162 and gp100280–286 epitopes, in contrast to the gp100259–217 epitope, are not conserved between mice and humans (10), whereas the
gp100^{154–162} peptide exhibits the strongest binding to HLA-A*0201 (37).

Our adoptive immunotherapy experiments in A2/K^b mice challenged by intraocular injection with human uveal melanoma cells show that systemic (i.v.) administration of the A2/K^b-transgenic anti-gp100 CTL can completely control the outgrowth of these tumors. Moreover, we demonstrated that these CTL can be detected in eyes bearing an OMM-1 tumor in the anterior chamber. As noted before, mice receiving control treatment do exhibit spontaneous regression of established intraocular tumors (Ref. 28, and this paper). Additional studies have indicated that this phenomenon largely depends on the activity of endogenous CD4^+ T cells (L.R.H.M.S. and R.E.M.T., unpublished observations). Despite spontaneous regression, a sufficient time-window is available for evaluation of the tumoricidal efficacy of adoptively transferred CTL. In two previous papers, HLA-A*0201-restricted CTL isolated from HLA-transgenic mice were shown to partially control the outgrowth of human tumors in SCID mice (11, 12).
FIGURE 6. Effective adoptive immunotherapy of intraocular tumors consisting of human uveal melanoma cells. A2/Kb-transgenic mice received an intraocular injection of 3 × 10^5 OMM-1 human uveal melanoma cells. One day later, mice (seven per group) were treated by adoptive transfer with either A2/Kb-transgenic gp100\textsuperscript{154–162}-specific CTL (clone 8J, panels A–G) or Ad5-specific CTL (clone 100B6, panels H–N), both in combination with a s.c. depot of rIL-2 in IFA. Graphs show growth of tumors in individual mice as expressed by the percentage of the iris that was covered by the tumor (see example in Fig. 5A).

studies with xenogeneic models in which in vivo efficacy of HLA-restricted anti-tumor CTL was demonstrated involved either peritumoral injection of effector cells (e.g., Refs. 38–40) or injection of a mixture of tumor and effector cells in a Winn-type assay (e.g., Refs. 41–43). In these latter experiments, homing of the effector cells to the tumor site is not a prerequisite. Notably, the requirement of homing involves not only the ability of the CTL to travel through the periphery of the host and track down their target, but also the capacity of a sufficient proportion of these cells to survive this journey as well as the power of this fraction to launch a ferocious tumoricidal attack. This implies that adoptive immunotherapy involving systemic injection of CTL into mice bearing xenogeneic tumors requires high-quality CTL.

In addition to the adoptive transfer experiments in A2/Kb-transgenic mice, we performed parallel experiments in C57BL/6 (A2/Kb-negative) nude mice. As mentioned above, immunodeficient mice such as nude or SCID mice are commonly used as hosts in xenogeneic tumor models. However, in the latter setting, our gp100-specific A2/Kb-transgenic CTL failed to eradicate the intraocular OMM-1 tumors (data not shown). This suggests that expression of the A2/Kb molecule in the host is essential for the in vivo efficacy of the A2/Kb-transgenic CTL. The mechanistic aspects of this phenomenon lie outside the scope of the present study. Importantly, we demonstrate that the in vivo efficacy of the A2/Kb-transgenic CTL against a xenogeneic tumor can be tested in an immunocompetent syngeneic host by engrafting the xenogeneic human tumor in the anterior chamber of the eye. Because we and others have shown that intraocular engraftment enables the in vivo growth of tumors that otherwise fail to grow (28, 44), this experimental setting will most likely be applicable for analyzing the efficacy of adoptive immunotherapy with HLA-transgenic T cells against a variety of human tumors.

Metastatic uveal melanoma constitutes a formidable therapeutic challenge. At first sight, these tumors seem an equally poor target for immunotherapeutic approaches. They arise in an immune-privileged site that can sustain the growth of foreign tissues and in which different mechanisms for suppression of immune responses are operational (19, 20, 45, 46). Furthermore, the microenvironment of the eye was shown to endow human uveal melanoma cells with lymphocyte-inhibitory properties (21). Another compelling observation is that lack of expression of class I HLA-Ags on human uveal melanoma was found to be correlated with a better, rather than poor, patient survival (34). This suggests that NK cells have a protective role in the development of metastatic disease, whereas, in contrast to what has been observed for skin melanoma (47–49), CTL-mediated immunity does not impose a selective pressure for loss of HLA-expression by these tumors. Paradoxically, work with animal models for intraocular tumors has demonstrated that adoptively transferred T cells can in principle be effective against such tumors (28, 50). In the latter study, we demonstrated that adoptive transfer of human Ad5-specific CTL resulted in rapid and complete eradication of intraocular tumors of Ad5-transformed cells. An explanation for this paradox could be that these murine studies made use of model tumors that had not originally developed in the eye and that therefore lacked certain features typical of uveal melanosomas. Importantly, our present work in A2/Kb mice demonstrates that adoptive immunotherapy is effective not only against such model tumors but also against the intraocular engraftment of human uveal melanoma. This is in accordance with the fact that OMM-1 and many human uveal melanosomas express class I HLA as well as a variety of melanoma Ags (16–18, 34), and that such cells are excellent targets for lysis by tumor-specific CTL in vitro (as shown by this study and previous studies (35)). Although we showed that adoptively transferred CTL eliminate the intraocular tumor in the absence of detectable damage to the eye, it should be noted that the CTL used so far were directed against foreign Ags. The gp100\textsuperscript{154–162} epitope is not conserved between mice and humans (10). Therefore, it is important to test the effect of CTL against autologous Ags, especially epitopes that are also expressed in the eye. An exquisite opportunity to address this issue is offered by the recent observation that potent anti-tumor CTL immunity against the gp100\textsuperscript{209–217} epitope, which is conserved between mice and humans, can be raised through immunization with recombinant virus encoding a variant of this peptide exhibiting improved binding to HLA-A*0201 (10). Although in these experiments, involving vaccination of A2/Kb mice
suspensions.

Therefore, we evaluated whether vaccination of A2/Kb will be whether such side effects would result in damage that would where they exert their tumoricidal effects. The prime question will be long-standing problem.

tastases (15), T cell immunotherapy may offer a solution for a uveal melanoma is associated with a high incidence of fatal me-
situations of minimal residual disease, which in the case of human noma, the most important application would probably be to use such therapy appears to be effective against primary uveal mel-
ation is a valid option, because others have shown that vaccina-
tion induced only a limited number of CTL capable of reacting in con-
trast, Ag-specific vaccination of A2/Kb mice is known primar-
y to recognize the gp100154–162 epitope in the context of the HLA-A*0201-restricted peptide epitope derived from the gp100 antigen as the dominant epitope recognized by an HLA-A2.1-restricted anti-melanoma CTL line.

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response in transgenic mice carrying a chimeric human-mouse class I major his-


