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Detection of Shared MHC-Restricted Human Melanoma Antigens after Vaccinia Virus-Mediated Transduction of Genes Coding for HLA

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Abstract

To detect shared human melanoma Ag that are recognized by HLA-A2 restricted, melanoma-specific CTL derived from tumor infiltrating lymphocytes, we have developed a convenient method to insert and express foreign HLA genes capable of presenting Ag on target cell lines. Seventeen melanoma cell lines and 11 nonmelanoma cell lines were infected with recombinant vaccinia virus containing the HLA-A2.1 gene. Infection by the vaccinia virus resulted in expression of functional HLA-A2 molecules on the cell surface of virtually 100% of infected cells within a 3.5-h period. The results showed that 11 of 17 (65%) naturally HLA-A2^{-} melanoma cell lines were specifically lysed by the HLA-A2-restricted, melanoma-specfic TIL after infection with the vaccinia-HLA-A2.1 virus. None of the nine human nonmelanoma cell lines tested (three colon cancer, four breast cancer, or two immortalized non-tumor cell lines) or two murine melanoma cell lines were lysed by the HLA-A2-restricted TIL after vaccinia-HLA-A2.1 infection. Coinfection of the vaccinia virus containing the β2-microglobulin gene with the vaccinia-HLA-A2.1 virus increased the surface expression of HLA-A2 and subsequent lysis by melanoma-specific tumor infiltrating lymphocytes. With this new method we could extend previous findings demonstrating that shared melanoma Ag recognized by HLA-A2-restricted tumor infiltrating lymphocytes exist among melanoma cells from different patients regardless of HLA type. These Ag represent excellent candidates for the development of vaccines to induce T cell responses for the immunotherapy of patients with melanoma.

Adoptive transfer of tumor-specific CTL derived from TIL[3] along with the administration of IL-2 has been shown to mediate tumor regression in murine tumor models and in humans with metastatic melanoma (1–3). Recognition by TIL of melanoma cells occurs through the TCR (4–6) that recognizes a complex consisting of a processed peptide, which is cleaved from an antigenic protein, and a restriction element or MHC molecule, which presents the peptide in a groove formed by two opposing α-helical loops and a β-sheet floor in the α1 and α2 domains of the class I molecule (7–10). Human melanoma Ag recognized by T cells, particularly Ag relevant to in vivo tumor rejection, have not been well characterized. The recently cloned MAGE-1 gene, whose product is recognized by a melanoma-specific CTL clone in the context of HLA-A1, represents the first candidate for a possible tumor rejection Ag, although it's in vivo relevance to tumor rejection is unknown (11). In our studies, a panel of TIL, some of which were effective in clinical trials, did not recognize MAGE-1 (12).

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[3] Abbreviations used in this paper: TIL, tumor infiltrating lymphocytes; TK, thymidine kinase.

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We have focused particularly on the study of HLA-A2-restricted CTL not only because the HLA-A2 molecule is expressed on nearly one-half of patients, but also because HLA-A2-restricted, melanoma-specific CTL may be preferentially induced in melanoma compared to CTL-restricted by other HLA molecules (13,14). These HLA-A2-restricted, melanoma-specific CTL can recognize allogeneic melanomas expressing HLA-A2 molecules in a TCR-dependent, MHC class I-restricted manner, suggesting the existence of common melanoma Ag (6,15–17). In addition, we have shown that these CTL are capable of recognizing naturally HLA-A2^{-} melanoma cell lines after transfection with the HLA-A2.1 gene (22). It is possible that one protein contains peptides capable of binding to a number of HLA molecules, and that such a protein can thus be recognized by T cells in the context of a number of HLA types (18). If this is the case, it may be possible to use such common antigenic proteins as vaccines to induce T cell responses in patients of any HLA type.

In our previous study of HLA-A2.1 transfected melanoma lines (6), three technical problems made it difficult to use the transfection technique for a more comprehensive study. First, the transfection and selection of cells is a long process, limiting the number of lines that one can study. Second, many cell lines were difficult to transfect, and thus impossible to study. Finally, by transfecting and selecting, we could look only at clones from within a population which might be selected for specific characteristics, and therefore were not necessarily representative of the entire population.

In this study we have used a rapid method for detecting melanoma Ag presented by the HLA-A2.1 molecule in naturally HLA-A2^{-} cells. To do so we have used a vaccinia virus expression vector to insert and express exogenous HLA-A2.1 molecules on the surface of cells. Vaccinia virus is a large DNA virus that replicates entirely within the cell cytoplasm, carrying its own DNA and RNA polymerases. The virus is capable of transcribing and translating inserted foreign genes under the control of a vaccinia promoter, with posttranslational modifications occurring faithfully (19–21). Vaccinia virus has a broad host range, infects nearly 100% of cells, and expresses inserted genes rapidly at a high level. We have constructed a recombinant vaccinia virus containing the HLA-A2.1 gene driven by a vaccinia tandem early-late promoter. Infection by the virus induces expression of functional HLA-A2.1 molecules on virtually 100% of tumor cells within a 3- to 4-h period. Thus we can potentially detect melanoma Ag within the entire population of cells. Using this rapid assay, we evaluated a variety of cell lines for the existence of melanoma Ag recognized by several allogeneic HLA-A2-restricted TIL.

Materials and Methods

Culture of TIL and melanoma cell lines

TIL were prepared and grown in culture as previously described (22). TIL are cultured in RPMI 1640 + 10% human AB serum containing glutamine, antibiotics, and 1000 U/ml IL-2 (kindly provided by Cetus Corp. Emeryville, CA). Melanoma cell lines were cultured in RPMI 1640 + 10% FCS, colon cancer cell lines in DMEM + 10% FCS, and breast cancer lines in Eagle's improved minimum essential medium (Biofluid, Rockville, MD) + 10% FCS.

Generation of H-2K^{d}-restricted, vaccinia virus-specific murine CTL and HLA-A2-restricted, influenza M1-specific human CTL

The vaccinia-specific murine CTL were generated by in vivo priming of female 6 to -8-wk-old BALB/C mice by i.v. injection of 100 plaque-forming units of vaccinia virus. After 2 wk, spleens were removed, dispersed to single cell suspensions, and stimulated in vitro with vaccinia virus infected BALB/C splenocytes at a ratio of 2:1. Cells were then cultured for 7 days in Iscove's modified medium with 7.5% heat-inactivated FCS to generate CTL.

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The HLA-A2.1-restricted, influenza M1-specific human CTL were generated by culturing 3 $\times 10^6$ PBMC from a HLA-A2 donor in a 2 ml of Iscove's modified medium + 10% AB serum containing 1 μM of M1 58–66 peptide (GILGFVFTL). On day 3, IL-2 was added at a final concentration of 5 U/ml. On day 7, the responding cells were harvested and mixed with 2000 rad irradiated stimulator cells that were prepared by 3-h incubation of PBMC from the same donor with 1 μM of the M1 58–66 peptide. IL-2 was added in culture on the next day at a concentration of 10 U/ml. After three to four restimulations, cultured T cells exhibited HLA-A2-restricted, M1-specific cytolysis.

Vaccinia virus constructs

cDNA for the HLA-A2.1 gene, the human β2-microglobulin gene, and the murine H-2K^d gene were inserted into the TK gene in the plasmid pSCII, allowing homologous recombination to occur with the viral TK gene. The inserted genes are transcribed by a vaccinia tandem early and late promoter. As a result, viral particles containing the gene of interest display a TK$^-$ phenotype, and can be selected in media containing bromodeoxyuridine. Virus stocks were amplified in the TK$^-$ human sarcoma line 143B, by infection at a multiplicity of infection of 0.1 for 1.5 h, followed by incubation for 3 days in RPMI 1640 + 10% FCS. Infected cells were collected, suspended in RPMI 1640 + 0.1% BSA, and three times freeze-thawed. Virus was titered on a monolayer of the TK$^-$ 143B cell line.

Vaccinia virus infection of melanoma cells

Target cells at a concentration of 10^7/ml were incubated for 1.5 h at 37°C in RPMI 1640 + 0.1% BSA with an equivalent volume of vaccinia virus (10^8 plaque-forming units/ml) in RPMI + 0.1% BSA. After this initial period of infection, cells were brought to a concentration of approximately 5×10^5/ml in RPMI 1640 + 10% FCS and incubated for 1.5 to 2 h at 37°C. Coinfection of vaccinia-HLA-A2.1(vac-A2) and vaccinia-β2-microglobulin(vac-β2 m) or vaccinia-H-2K^d(vac-K^d) and vac-β2 m were performed at multiplicity of infection of 10:1 and 5:1, respectively.

Cytotoxicity assay

Cytotoxicity assays were performed as previously described (22). Briefly, target cells were incubated with $Na^{51}CrO_4$ for 1.5 h and washed. Effector cells were then added to 5×10^3 target cells/well in a triplicate fashion in 96-well U-bottom tissue culture plates and incubated at 37° C for 5 h. After measuring radioactivity of harvested supernatants, % specific lysis was calculated by the formula:

$$\left(\frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{maximum cpm} - \text{spontaneous cpm}}\right) \times 100$$

Significance of lysis was statistically analyzed by two-tailed *t*-test.

mAb and flow cytometric analysis

Culture supernatants containing anti-HLA-A2 mAb (BB7.2) (23) and anti-H-2K^d mAb (SF-1.1.1) were prepared from the corresponding hybridomas (American Type Tissue Culture Collection, Rockville, MD). Anti-Thy-1.2 mAb was purchased from Beckton Dickson, Mountain View, CA. Antivaccinia virus mAb (TW2.3) was prepared in our laboratory. One million cells per sample were incubated in heat-inactivated human AB serum for 20 min at 4° C then washed with HBSS + 10% FCS and 0.01% NaN_2. Cells were incubated with unconjugated mAb followed by a FITC-conjugated goat anti-mouse IgG Ab for 1 h at 4°C. Cells were washed and fixed in 1% paraformaldehyde solution for 10 min, then analyzed by FACScan (Becton Dickson).

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Results

Characterization of HLA-A2.1-restricted melanoma-specific CTL generated from TIL

TIL lines 620, 660, 1074, 1128, and 1143 were generated as previously reported by culturing TIL in 1000 U/ml IL-2 (22). After approximately 30 to 40 days in culture, these TIL lysed not only the autologous melanoma but also a number of allogeneic melanoma cell lines expressing HLA-A2 as previously described (6). The TIL did not, however, lyse HLA-A2$^-$ melanoma cell lines. In addition, TIL lines 620, 660, 1074, and 1128 did not lyse a number of HLA-A2-expressing nonmelanoma cell lines including EBV-transformed B cells, Burkitt's lymphoma cell lines, breast cancer cell lines, tumor lines of neuroectodermal origin, PHA lymphoblasts, and fibroblasts. Lysis occured in a TCR-dependent and MHC class I-restricted manner, as demonstrated by inhibition by anti-CD3 or anti-MHC class I mAb. Thus we considered these TIL to be HLA-A2-restricted and relatively melanoma-specific CTL (6).

TIL 1143 behaved somewhat differently from the others, lysing the HLA-A2$^+$ 501 EBV-B cell line, but not lysing the HLA-A2+ 697 EBV-B cell line or the HLA-A2$^+$ 501 or M1A2.1 fibroblast cell line. This would seem to indicate that TIL 1143 contained CTL that recognized some Ag not associated with melanoma as well as containing melanoma-specific CTL. The batch of TIL 620 that we used in this study also lysed some HLA-A2$^-$ cell lines including 836 mel, 888 mel, and 1182 mel. TIL 620 may contain a sub-population of CTL restricted by HLA-Cw7 that is shared with 836 and 888 mel.

Kinetics of surface expression of HLA-A2.1 molecule after Vac-A2 infection

HLA-A2$^-$ melanoma cell lines 397 and 928 mel were infected with vaccinia virus containing the HLA-A2.1 gene (vac-A2) and incubated for 1.5 h. Cells were stained with BB 7.2 (anti-HLA-A2) or irrelevant anti-Thy-1.2 mAb after 0, 1.5, 3.5, 5.5, and 16 h then analyzed by flow cytometry (Fig. 1). Both cell lines clearly demonstrated HLA-A2 expression at the 3.5 h time point. Expression at 3.5 h appeared equal to that seen at both the 5.5 and 16 h time points indicating very rapid surface expression of the molecule. Virtually 100% of cells expressed HLA-A2 molecules. HLA-A2 expression on cell lines infected with vac-A2 varied and was usually 10 to 50% as high as that of the positive control HLA-A2$^+$ 501 mel.

HLA-A2.1 molecules provided by vaccinia virus can present antigenic peptide to T cells

To determine the ability of the HLA-A2.1 molecule to present peptide Ag to the HLA-A2-restricted T cells, the vac-A2-infected melanoma cell line 397 mel was pulsed with the influenza virus M1 antigenic peptide fragment M1 58–66 and lysability by the HLA-A2-restricted, M1-specific CTL was tested (Table I). The vac-A2-infected 397 mel expressing HLA-A2 and the naturally HLA-A2$^+$ 501 mel cell line were both lysed by the HLA-A2-restricted, M1-specific CTL after incubation with the peptide, although 397 mel cells expressing HLA-A2 but not pulsed with peptide and cells not expressing HLA-A2.1 pulsed with the peptide were not lysed. Thus the HLA-A2.1 expressed on the cell surface of 397 mel was able to present Ag to HLA-A2-restricted CTL.

Vac-A2 infection made some HLA-A2 negative melanoma cell lines lysable by HLA-A2-restricted, melanoma-specific CTL

Three HLA-A2$^-$ melanoma cell lines, 888 mel, 928 mel, and G361 were infected with vac-A2. Two of three cell lines, 928 mel and G361, have been previously shown to be lysed by TIL after stable transfection of the HLA-A2.1 gene (6). After infection by vac-A2, all three HLA-A2$^-$ lines were lysed by TIL 620, 660, and 1074 but cells infected with wild-type vaccinia virus, vaccinia virus containing the human β2-microglobulin gene (vac-β2 m), or vaccinia virus containing the murine class I MHC, H-2K^d gene (vac-K^d) were not lysed (Table II). The level

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of lysis was usually less than that of HLA-A2$^+$ 501 mel as shown in experiment 2 in Table II, which might result from less surface expression of HLA-A2 on vac-A2-infected cells. Specific expression of the HLA-A2 molecule on the cell only after vac-A2 infection was confirmed by flow cytometry using the anti-HLA-A2 mAb, BB7.2. Infection and replication of vaccinia virus in all of the cell lines was confirmed by flow cytometry using either the antivaccinia mAb, TW2.3, recognizing a vaccinia protein, or by the mAb HB159 recognizing H-2K^d (data not shown). These results suggest that lysis of HLA-A2-expressing melanoma cell lines was neither a result of vaccinia virus infection per se, nor was it a result of the introduction onto the surface of a foreign MHC gene, somehow resulting in an increased susceptibility to lysis by TIL. The lysis observed is thus likely due to specific interaction between the HLA-A2 molecule on the melanoma cell surface and the HLA-A2-restricted, melanoma-specific CTL.

As a further control for specificity, and additionally to confirm that the target cells were capable of processing and presenting Ag, we used a chimeric murine-human system that we have recently used to evaluate the Ag processing ability of a variety of human tumor lines (24). The H-2K^d-restricted, vaccinia-specific murine CTL lyse all targets infected with the vac-K^d. However, none of the vac-A2-infected targets were lysed by these murine CTL. These results further support our contention that lysis of targets was specific to expression of the correct restriction element on the target cell surface, and confirm that the cells were capable of processing Ag for presentation by an exogenously supplied restriction molecule.

Coinfection of cells with vac-A2 and vac-β2 m led to increased cell surface expression of HLA-A2.1 molecules, as well as increased specific lysis by TIL in some cases (Fig. 2 and Table II). HLA-A2 expression that was 50 to 100% as high as that of 501 mel could occasionally be observed. Providing more β2-microglobulin may increase the sensitivity of our assay by rapidly increasing the expression of exogenously supplied HLA-A2.1 molecules on the surface of the cell. It has been shown that β2-microglobulin association with the MHC class I H chain and peptide is necessary for transportation and surface expression of the MHC class I trimolecular complex (25).

To determine whether increasing the incubation time would increase the sensitivity of our assay, we compared overnight incubation after infection with vac-A2 or vac-K^d to an incubation of 3.5 h (Table III). The overnight incubation led to a decrease in specific lysis by the TIL, as well as to an increase in the nonspecific lysis of the control-(vac-K^d) infected cells in the case of G361 and 928 mel. In fact, cells began dying during the longer incubation. HLA-A2 expression of 928 mel decreased after 16 h. This could result in decreased lysis by TIL. In the case of 397 mel and G361, HLA-A2 expression was minimally changed. Vaccinia virus might prevent synthesis of endogenous proteins including melanoma Ag in the cell, and might also produce an abundance of vaccinia proteins mainly driven by the vaccinia late promoter. Such abundant vaccinia proteins in the cell could competetively inhibit binding of endogenous peptides to HLA-A2.1 molecules within the endoplasmic reticulum. In fact, H-2K^d- restricted, vaccinia-specific murine CTL could lyse vaccinia-infected cells at 16 h as well as at 3.5 h, probably because both vaccinia Ag and H-2K^d were abundantly synthesized. These may also be the reasons for the low level of lysis of Vac-A2-infected cells compared to that of HLA-A2$^+$ 501 mel. For these reasons we chose to assay for lysis after the shortest period (3.5 h) of incubation at which we saw expression of the HLA-A2.1 molecule. This we hoped would allow newly synthesized HLA-A2.1 molecules to encounter already existing peptides within the endoplasmic reticulum, and provide the best chance for specifically detecting melanoma Ag. HLA-A2.1 molecules should also continue to be produced in the cells throughout the period of the chromium release assay, which should make a short incubation time sufficient for detection of lysis.

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Screening of melanoma and nonmelanoma cell lines for expression of shared melanoma Ag recognized by HLA-A2-restricted TIL using vaccinia virus

With this new method we screened various melanoma and nonmelanoma cell lines for the existence of melanoma Ag recognizable by TIL in the context of the HLA-A2.1 molecule. We defined a cell line as melanoma Ag^+ if that cell line expressed the HLA-A2.1 on its cell surface and was specifically lysed by HLA-A2-restricted melanoma-specific TIL (more than 10% specific lysis with statistical significance) after vac-A2 infection. We defined a cell line as melanoma Ag^- if that cell line was lysable by H-$2K^d$-restricted, vaccinia-specific murine CTL after vac-K^d infection and expressed HLA-A2.1 on its cell surface but was not lysed by HLA-A2-restricted TIL after vac-A2 infection.

A total of 17 HLA-$A2^-$ melanoma cell lines were tested by infection with vac-A2 and vac-β2 m. Although lysis was not as high as that of 501 mel in many cases, 11 of these cell lines (11/17 = 65%) were significantly lysed by all or most of the 5 HLA-A2-restricted TIL. None of three colon cancer lines, four breast cancer lines, or two murine melanoma cell lines were lysed after vac-A2 infection. Representative data are shown in Tables IV and V, and a summary of the data is shown in Table VI. In Table V, it can be seen that some targets were lysed above 10%; however, none of these values proved significant ($p > 0.05$). In only one of the repeated experiments colon cancer cell line, HT-29 was significantly lysed by only TIL620 (data not shown). In Table IV, melanoma cell lines lysed by HLA-A2- restricted TIL were not lysed by H-$2K^d$-restricted murine CTL or by TIL888 that were restricted by class I HLA other than HLA-A2. Therefore, the lysis was not due to nonspecific increase of susceptibility to T cell lysis. These results indicate that the melanoma Ag recognized by our HLA-A2-restricted allogeneic TIL did not appear to be expressed by the colon and breast cancer cell lines or the murine melanoma cell lines that we tested. In addition, five HLA-$A2^-$ tumors of neuroectodermal origin, including one astrocytoma, one retinoblastoma, one glioma, and two neuroblastomas were not lysed after vac-A2 infection (26). The fact that some cell lines expressing the HLA-A2.1 molecules were not lysed by TIL suggests that vaccinia viral Ag was not the target molecule for TIL, although most adult individuals have been previously immunized against this virus.

Discussion

We have developed a new, convenient assay using recombinant vaccinia virus to detect the expression of human melanoma Ag recognizable by T cells in the context of the HLA-A2.1 molecule in a variety of cell lines. Recombinant vaccinia virus has a wide host range, virtually 100% infection efficiency, and a rapid and high level of expression of the inserted foreign gene. In this study these features were confirmed with a variety of cell lines including melanoma, colon, and breast cancer lines. Although vaccinia virus infection is cytopathic, making expression transient, we identified a time window in which we could test susceptibility to TIL lysis with high specificity. With this method we could rapidly express foreign class I MHC molecules on the cell surface. The HLA molecule provided by vaccinia virus functioned properly to present exogeneously added or endogeneously synthesized antigenic peptides to T cells. This technique is useful for the study of Ag-T cell interactions in humans, becauase any class I molecule used by a particular T cell line or clone could potentially be inserted into a cell to determine whether particular Ag are present within the cell, regardless of the cell's natural HLA type.

We have provided further evidence that Ag recognized by HLA-A2- restricted melanoma-specific CTL derived from melanoma deposits are shared among melanomas, and not among nonmelanoma tumors and murine melanomas tested. To our knowledge, a comprehensive study of the existence of Ag recognized by the HLA-A2-restricted melanoma-specific CTL among HLA-A2 melanomas has not previously been performed. In this study, 11 of 17 (65%)

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melanoma cell lines were recognized by the HLA-A2-restricted, melanoma-specific TIL after expressing vaccinia-HLA-A2.1 -derived HLA-A2.1 molecules. In our previous study 17 stable HLA-A2.1-transfected melanoma clones from six melanoma cell lines were all lysed by the HLA-A2-restricted TIL (6). Five melanoma cell lines, 397 mel, 894 mel, 928 mel, G361, and SKmel2 were used for both studies and were confirmed as melanoma Ag^+ using the vaccinia method.

In our previous studies many cell lines were difficult to transfect with the genomic HLA-A2.1 gene. Infection with vac-A2, especially when combined with vac-β2 m, led to rapid expression of the HLA-A2.1 molecules on the cell surface of almost all cells that we tested. In addition, expression of HLA-A2 on most cells after vaccinia allowed us to see representative results from a heterogenous population of a cell line, rather than from the particular clones that were selected during the transfection procedure of the previous study. It is interesting that all six of the highly transfectable melanoma cell lines from five patients previously tested were found to express the melanoma Ag recognizable by HLA-A2-restricted melanoma-specific TIL, whereas four of seven vac-A2-infected melanoma cell lines that were previously not transfectable with the plasmid were lysed by the HLA-A2-restricted TIL. The same TIL, including TIL620 and TIL660, could lyse 12 of 18 (67%) naturally HLA-$A2^+$ melanoma cell lines (Y. Kawakami, and S. A. Rosenberg, manuscript in preparation). Therefore, the frequency of melanoma Ag expression on melanoma cells appeared to be equivalent among HLA-$A2^+$ and HLA-$A2^-$ melanomas. Based on the patterns of individual TIL reactivity with HLA-A2.1-transfected melanomas, at least two shared melanoma Ag seem to exist, because HT144 or Skmel28 transfectants were not lysed by TIL660 or TIL 1128, respectively.

It is still possible that six human melanoma cell lines were not lysed by HLA-A2-restricted TIL for reasons other than a lack of shared melanoma Ag. Lack of lysis could be due to insufficient sensitivity of this assay. The optimal time for expression of the melanoma Ag in the groove of HLA-A2.1 molecule on the surface may be different among cell lines. The low HLA-A2.1 surface expression of HLA-A2.1 on surface seen in some cases might result in absence of lysis by TIL; however, low HLA-A2 expression (around 10% of HLA-A2 expression of 501 mel) was sufficient for lysis by TIL in many Ag^+ melanoma cell lines including 888 mel. All resistant cell lines were lysable and had normal Ag processing ability as measured by the ability of H-$2K^d$-restricted vaccinia-specific CTL to lyse them after vac-K^d infection. Two of six resistant melanoma cell lines, 586 and 537 mel were lysed by autologous TIL that were restricted by class IMHC other than HLA-A2. These autologous CTL may recognize unique melanoma Ag.

The vaccinia-HLA method thus is a powerful tool to study the presence of Ag recognized by T cells in humans with any HLA type. The fact that 65% of melanoma cell lines derived from different patients expressed common antigenic proteins that were recognized by TIL, some of which caused tumor regression when adoptively transfered into patients, suggests the possibility of developing immunization strategies using these Ag to induce antimelanoma T cell responses. The isolation and identification of such melanoma Ag is in progress at both the gene and peptide level.

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References

1. Rosenberg SA, Speiss P, Lafreniere RA. New approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 1986;233:1318. [PubMed: 3489291]

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2. Barth RJ, Bock SN, Mulé JJ, Rosenberg SA. Unique murine tumor-associated antigens identified by tumor infiltrating lymphocytes. J Immunol 1990;144:1531. [PubMed: 2303716]
3. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, Simpson C, Carter C, Bock S, Schwartzentruber D, Wei JP, White DE. Use of tumor infiltrating lymphocytes and interleukin 2 in the immunotherapy of patients with metastatic melanoma: preliminary report. N Engl J Med 1988;319:1676. [PubMed: 3264384]
4. Topalian SL, Solomonm D, Rosenberg SA. Tumor specific cytolysis by lymphocytes infiltrating human melanomas. J Immunol 1989;142:3714. [PubMed: 2785562]
5. Itoh K, Platsoucas CD, Balch CM. Autologous tumor-specific cytotoxic T lymphocytes in the infiltrate of human metastatic melanomas: activation by interleukin 2 and autologous tumor cells, and involvement of the T cell receptor. J Exp Med 1988;168:1419. [PubMed: 3262710]
6. Kawakami Y, Zakut R, Topalian SL, Stötter H, Rosenberg SA. Shared human melanoma antigens: recognition by TIL in HLA- A2.1 transfected melanomas. J Immunol 1992;148:638. [PubMed: 1729379]
7. Townsend A, Bodmer H. Antigen recognition by class I-restricted T lymphocytes. Annu Rev Immunol 1989;7:601. [PubMed: 2469442]
8. Bjorkman PJ, Saper MA, Samraoui B, Bennet WS, Strominger JL, Wiley DC. Structure of the human clas I histocompatibility antigen, HLA-A2. Nature 1987;329:506. [PubMed: 3309677]
9. Bjorkman PJ, Saper MA, Samraoui B, Bennet WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature 1987;329:512. [PubMed: 2443855]
10. Yewdell JW, Bennink JR. The binary logic of antigen processing and presentation to T-cells. Cell 1990;62:203. [PubMed: 1695549]
11. Van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991;254:1643. [PubMed: 1840703]
12. Zakut R, Topalian SL, Kawakami Y, Mancini M, Eliyahu S, Rosenberg SA. Differential expression of MAGE-1, -2, -3 mRNA in transformed and normal human cell lines. Cancer Res 1993;53:5. [PubMed: 8416750]
13. Crowley NJ, Darrow TL, Quinn-Aleen MA, Seigler HF. MHC-restricted recognition of autologous melanoma by tumor specific cytotoxic T-cells: evidence for restriction by a dominant HLA-A allele. J Immunol 1991;146:1692. [PubMed: 1671580]
14. Wölfel T, Klehmann E, Muller C, Schütt KH, Meyer zum Buschenfelde KH, Knuth A. Lysis of human melanoma cells by autologous cytolytic T-cell clones: identification of human histocompatibility leukocyte antigen A2 as a restriction element for three different antigens. J Exp Med 1989;170:797. [PubMed: 2788708]
15. Darrow TL, Slingluff CL, Siegler HF. The role of HLA class I antigen in recognition of melanoma cells by tumor- specific cytotoxic T lymphocytes: Evidence for shared tumor antigens. J Immunol 1989;142:3329. [PubMed: 2785141]
16. Hom SS, Topalian SL, Simonis T, Mancini M, Rosenberg SA. Common expression of melanoma tumor-associated antigens recognized by human tumor infiltrating lymphocytes: analysis by HLA restriction. J Immunother 1991;10:153. [PubMed: 1868040]
17. Hom SS, Schwartzentruber DJ, Rosenberg SA, Topalian SL. Specific release of cytokine by lymphocytes infiltrating human melanoma in response to shared melanoma antigens. J Immunother 1993;13:18.
18. Zamvil SS, Steinman L. The T lymphocyte in experimental allergic encephalomyelitis. Annu Rev Immunol 1990;8:879.
19. Moss B, Flexner C. Vaccinia virus expression vectors. Annu Rev Immunol 1987;5:305. [PubMed: 2439103]
20. Moss B. Vaccinia virus: a tool for research and vaccine development. Science 1991;252:1662. [PubMed: 2047875]
21. Bennink JR, Yewdell JW. Recombinant vaccinia viruses as vectors for studying T lymphocyte specificity and function. Curr Top Micro Immunol 1990;163:154.

22. Kawakami Y, Rosenberg SA, Lotze MT. Interleukin-4 promotes the growth of tumor infiltrating lymphocytes cytotoxic for human autologous melanoma. J Exp Med 1988;168:2183. [PubMed: 3264324]
23. Parham P, Brodsky FM. Partial purification and some properties of BB7.2: a cytotoxic monoclonal antibody with specificity for HLA-A2 and a variant of HLA-A28. Hum Immunol 1981;3:277. [PubMed: 7035415]
24. Restifo NP, Esquivel F, Kawakami Y, Yewdell JW, Mule JJ, Rosenberg SA, Bennink JR. Identification of human cancers deficient in antigen processing. J Exp Med 1993;177:65.
25. Yewdell JW, Bennink JR. Processing and presentation of viral proteins to MHC class I-restricted T lymphocytes. Year Immunol 1990;6:38. [PubMed: 2701910]
26. Shamamian P, Kawakami Y, Restifo NP, Rosenberg SA, Topalian SL. Recognition of neuroectodermal (NE) tumors by melanoma specific T-cells: evidence for shared tumor associated antigens (Ag). Proc AACR 1993;34:482.

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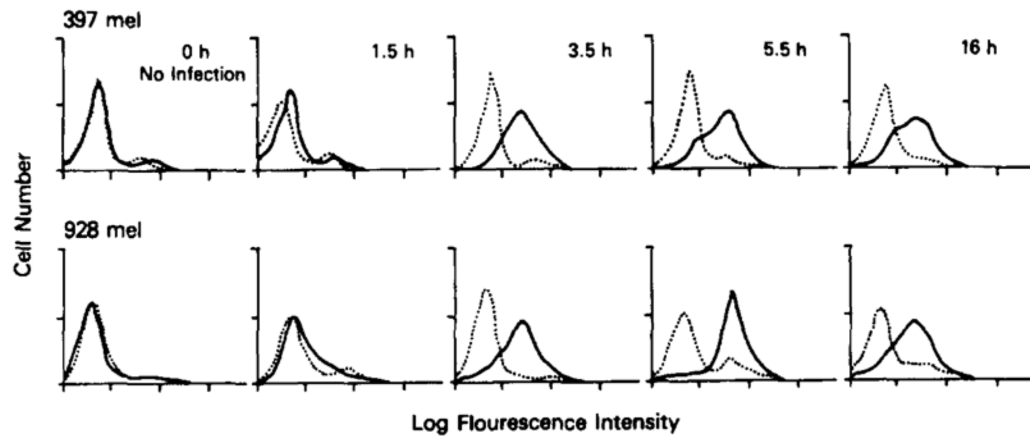

FIGURE 1.
Time kinetics of surface expression of HLA-A2 molecule after infection with vac-A2. Flow cytometric analysis of two melanoma cell lines, 397 and 928 mel, at various times after infection with vac-A2 shows expression of HLA-A2 on the cell surface (with anti-HLA-A2 mAb, BB7.2, *solid line*) as early as 3.5 h after the time of infection. Anti-Thy-1.2 mAb was used as a control (*dashed line*).

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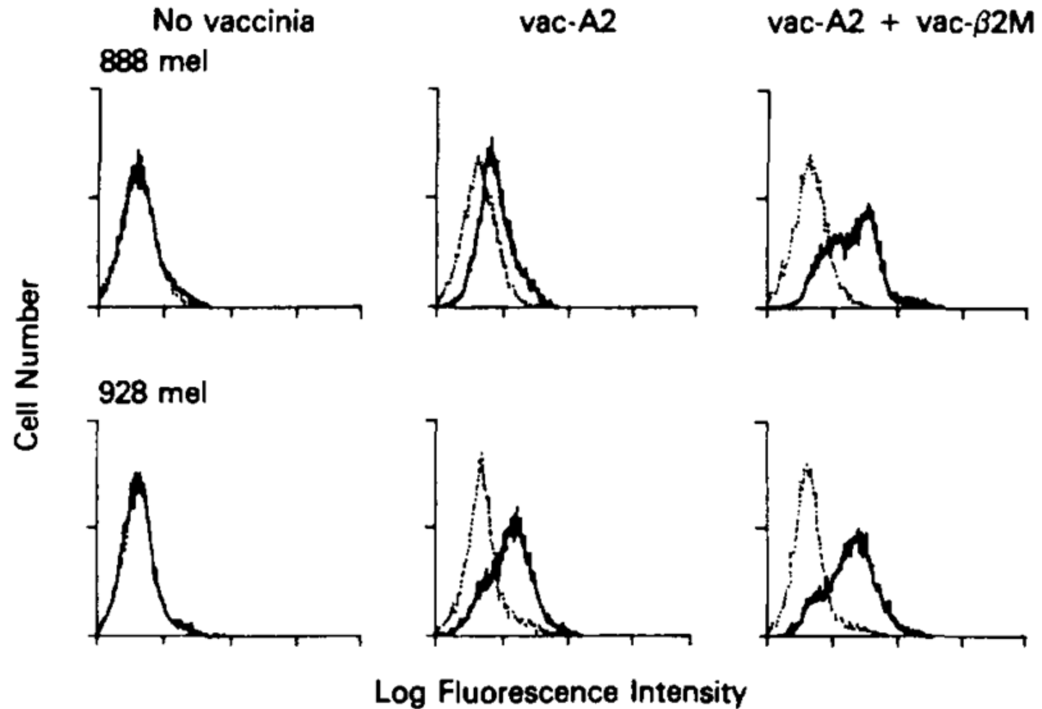

FIGURE 2.
Coinfection of melanoma cell lines with vac-A2 and vac-*β*2 m increased surface expression of HLA-A2. Two melanoma cell lines, 888 and 928 mel, were incubated either without vac, with vac-A2 or with vac-A2 and vac-*β*2 m for 3.5 h and stained with anti-HLA-A2 mAb (BB7.2, *solid line*) or anti-Thy-1.2 mAb (*dashed line*). Surface expression of HLA-A2 were analyzed by flow cytometry. Peak and mean channel number of cells stained with anti-HLA-A2 mAb are follows: 501 mel (HLA-$A2^+$ control, not shown) 64, 138; 888 mel, 4, 6; 888 mel + vac-A2, 8, 11; 888 mel + vac-A2 + vac-*β*2 m, 37, 33; 928 mel, 4, 6; 928 mel+vac-A2, 17, 18; 928 mel + vac-A2 + vac-*β*2 m, 26, 28. Coinfection of vac-*β*2 m with vac-A2 increased HLA-A2 surface expression.

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Table I

HLA-A2.1 molecules expressed on vac-A2 infected melanoma cell lines could present influenza virus M1 peptide to HLA-A2.1 restricted, M1-specific CTL[a]

Target	HLA-A2	M1 Peptide	
		−	+
		% specific lysis	
501mel	+	3	**41**
397mel	−	1	2
397mel+vac-A2	+	1	**22**

[a]397mel, an HLA-A2^{-} melanoma, was infected with vac-A2 at a moi of 10 for 1 h, then washed and incubated overnight. The next day, targets were incubated with the influenza virus M1 58–66 peptide at a concentration of 1 μM at 37 C for 1 h. A4-h ^{51}Cr release assay was performed at E:T ratio = 2.5:1. HLA-A2 surface expression was analyzed by flow cytometry. The HLA-A2.1-restricted M1-specific CTL lysed only 501 mel, an HLA-A2^{+} melanoma cell line, and the vac-A2 infected 397mel which were incubated with the M1 peptide. Bold number, Statistically significant lysis ($p < 0.05$).

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Table II

HLA-A2.1 restricted melanoma specific TIL can specifically lyse vac-A2 infected melanoma cell lines[a]

Expt. 1						Expt. 2				
Target	Vaccinia	HLA-A2	TIL660	TIL1074	Kd-CTL	Target	Vaccinia	HLA-A2	TIL660	TIL620
			% specific lysis						% specific lysis	
501mel	None	+	**29**	**24**	ND	501mel	None	+	**45**	**48**
928mel	None	−	−3	0	ND	888mel	None	−	14	7
	kDa	−	−2	−3	**67**		kDa	−	6	2
	β2m	−	5	1	ND		β2m	ND	ND	ND
	A2	+	**13**	**18**	1		A2	+	**28**	**19**
	A2, β2m	+	**24**	**34**	−3		A2, β2m	+	**28**	**28**
G361	None	−	4	2	ND	G361	None	−	7	2
	kDa	−	7	9	**47**		kDa	−	6	2
	A2	+	**28**	**30**	−2		A2	+	**30**	**22**
	A2, β2m	+	ND	ND	ND		A2, β2m	+	**32**	**28**

[a]HLA-A2 negative melanoma lines 928mel, 888mel, and G361 were infected with vac-A2, then tested for lysis by the HLA-A2-restricted TIL 660 and 1074. A 5-h ^{51}Cr release assay was performed. Results are shown as % specific lysis at E:T = 40:1. HLA-A2 surface expression was tested by flow cytometry. Only cells infected with vac-A2 were lysed, and some increase was seen in lysis upon coinfection by vac-A2 and vac-β2m. 501mel is an HLA-A2^{+} melanoma control, and K^{d}-CTL are H-2K^{d}-restricted vaccinia viral Ag-specific murine CTL. Lysis of 888mel by TIL660 in experiment 2 (14%) was not statistically significant because of the high standard deviation of the data. Bold numbers, Statistically significant lysis ($p < 0.05$).

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Table III

Comparison of lysis of three melanoma lines infected with vac-A2 after incubation periods of 3.5 or 16[a]

Target	Incubation duration (h)	Vaccinia virus	TIL620	TIL660	TIL1074	Kd-CTL	FACS
				% specific lysis			
501mel	0	None	**57**	**72**	**61**	3	7/76
397mel	3.5	A2	**29**	**29**	**35**	3	10/41
		kDa	−3	4	−7	**46**	6/56
	16	A2	**17**	**13**	**17**	−3	8/33
		kDa	0	3	0	**38**	10/33
928mel	3.5	A2	**44**	**41**	**31**	3	7/23
		kDa	4	7	2	**58**	7/23
	16	A2	5	9	6	−7	8/10
		kDa	−1	−3	17	**49**	8/22
G361	3.5	A2	**43**	**43**	**40**	−4	8/12
		kDa	10	7	3	**49**	8/28
	16	A2	0	**32**	2	−6	9/13
		kDa	4	**30**	**25**	**45**	8/20

[a] Lysis by three HLA-A2-restricted, melanoma-specific TIL and H-2K^d-restricted, vaccinia-specific CTL of three melanoma lines was tested by 5 h-^{51}Cr release assay. Results were expressed as % specific lysis at E:T = 40:1. Note that killing of vac-A2-infected targets by TIL decreased at the longer incubation, whereas nonspecific lysis by mouse CTL increased. FACS, Mean channel number of cells stained with control anti-Thy-1.2 mAb/mean channel number of cells stained with anti-HLA-A2 mAb for vac-A2-infected cells or with antivaccinia mAb for vac-K^d-infected cells. Bold number, Statistically significant lysis ($p < 0.05$).

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Table IV

Lysis of some melanoma cell lines after vac-A2 infection

Target	Vaccinia	FACS	TIL620	TIL660	TIL1143	Kd-CTL	TIL888	LAK
				% specific lysis				
Expt. 1								
501 mel	None	7/94	**11**	**28**	**26**	0	ND	ND
888mel	A2, (β2m	5/11	**15**	**22**	**48**	3	ND	ND
883mel	A2, β2m	5/10	**18**	**19**	**30**	6	ND	ND
	kDa, β2m	5/21	3	2	3	**90**	ND	ND
586mel	A2, β2m	5/14	2	0	9	−4	ND	ND
	kDa, β2m	5/19	0	−1	0	**56**	ND	ND
1173mel	A2, β2m	5/22	8	4	9	−1	ND	ND
	kDa, β2m	5/22	4	0	2	**20**	ND	ND
Expt. 2								
501 mel	None	5/60	**32**	**62**	**29**	−1	ND	ND
888mel	A2, β2m	5/35	**62**	**81**	**66**	3	ND	ND
SKmel 1	A2, β2m	8/37	**16**	**30**	**20**	2	ND	ND
	kDa, β2m	8/24	2	−2	1	**17**	ND	ND
537mel	A2, β2m	7/32	1	−3	−2	−3	ND	ND
	kDA, β2m	7/11	3	2	2	**24**	ND	ND
Expt. 3								
501 mel	None	6/37	**61**	**34**	**38**	ND	11	**79**
1182mel	A2, β2m	6/47	**31**	**17**	**15**	ND	0	**83**
	kDa, β2m	7/56	**22**	3	3	ND	6	**71**
SKmel28	A2, β2m	8/36	**14**	**11**	**16**	ND	4	**68**
	kDa, β2m	7/39	3	2	1	ND	−1	**68**
1174mel	A2, β2m	6/15	−2	−4	−5	ND	−5	**55**
	kDa, β2m	6/21	−1	−3	−4	ND	−2	**49**
1180mel	A2, β2m	6/10	0	−1	−1	ND	0	**83**
	kDa, β2m	6/12	0	−1	−2	ND	− 1	**75**
HS294T	A2, β2m	9/32	7	0	3	ND	2	**76**
	kDa, β2m	7/52	7	2	3	ND	2	**68**

[a] Melanoma cell lines were coinfected with either vac-A2 and vac-β2m or vac-K^d and vac-β2m. Four HLA-A2-restricted TIL, 620, 660, 1074, 1143, and TIL 888 that was restricted by class I MHC other than HLA-A2 were used for the 5-h-^{51}Cr release assay (E:T = 40:1) along with H-$2K^d$-restricted, antivaccinia murine CTL. FACS, Mean channel number of cells stained with control anti-Thy-1.2 mAb/mean channel number of cells stained with anti-HLA-A2 mAb for vac-A2-infected cells or with antivaccinia mAb for vac-K^d-infected cells. TIL620 lysed 1182mel with or without vaccinia virus infection. Bold numbers, Statistically significant lysis ($p < 0.05$).

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Table V

Lysis of HLA-A2⁻ breast and colon cancer cell lines after vac-A2 infection[a]

Target	Vaccinia	HLA-A2	TIL620	TIL660	TIL1074	TIL1128	TIL888
			% specific lysis				
Melanoma							
501 mel	None	+	**41**	**21**	**33**	**17**	**3**
888mel	A2, β2m	+	**56**	**42**	**48**	**24**	**52**
Breast cancer							
HS-578T	A2, β2m	+	3	10	5	0	1
	kDa, β2m	−	3	2	0	−2	−4
ZR-75–1	A2, β2m	+	2	1	7	5	−1
	kDa, β2m	−	5	9	−1	3	1
But	A2, β2m	+	−1	5	1	0	−1
	kDa, β32m	−	−1	−2	−3	−3	−5
Orn	A2, β2m	+	3	1	1	2	7
	kDa, β2m	−	−1	−8	−8	−8	−7
Colon cancer							
HT-29	A2, β2m	+	13	4	7	6	3
	kDa, βm	−	3	11	2	15	7
LS-174T	A2, β2m	+	0	4	6	2	9
	kDa, β2m	−	1	8	23	3	2
WIDR	A2, β2m	+	2	6	4	12	−2
	kDa, β2m	−	3	1	13	−2	9

[a] A 5-h-^{51}Cr release assay was performed (E:T = 40:1). TIL 888 is shown as a positive control for virus activity during this assay. Additionally cell lines were shown by flow cytometry to express HLA-A2 at the time of the assay. Bold numbers, Statistically significant lysis ($p < 0.05$).

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Table VI

Summary of lysis of cell lines by HLA-A2-restricted, melanoma-specific TIL after vac-A2 infection[a]

Cell Line	TIL620	TIL660	TIL1074	TIL1128	TIL1143
Melanoma					
397mel	+[b]	+	+	+	+
537mel	−	−	−	−	+
586mel	−	−	−	−	+
883mel	+	+	+	ND	+
888mel	[c]	+	+	+	+
894mel	+	+	+	ND	ND
928mel	+	+	+	ND	+
1173mel	−	−	−	−	−
1174mel	−	−	−	−	−
1180mel	−	−	−	ND	−
1182mel	[c]	+	+	ND	+
G361	+	+	+	ND	+
HS294T	−	−	−	−	−
HT144	+	−	+	+	+
SKmel1	+	+	+	ND	+
SKmel2	+	+	+	+	+
SKmel28	+	+	+	−	+
Colon cancer					
HT29	±	−	−	−	−
LS174T	−	−	−	−	−
WIDR	−	−	−	−	−
Breast cancer					
HS578T	−	ND	−	−	ND
ZR751	−	−	−	−	ND
But	−	−	−	−	ND
Orn	−	−	−	−	ND
Other human cell lines					
501EBV-B cell	−	−	−	ND	ND
M1A2.1 fibroblast	−	−	−	ND	ND
Murine melanoma					
B16	−	−	−	ND	ND
M3	−	−	−	ND	ND

[a] Targets are originally all HLA-A2 negative except 501 EBV-B cell and M1A2.1 fibroblast. Summary of a number of experiments using the TIL shown.

[b] + indicates lysis on average of > 10%, whereas - indicates ≤10% specific lysis. ± for HT29 withTIL620 indicates that one of the repeated experiments showed significant lysis >10%.

[c] indicates lysis by TIL620 without vac-A2 infection.