Detection of Naturally Processed and HLA-A1-presented Melanoma T-Cell Epitopes Defined by CD8+ T-Cells' Release of Granulocyte-Macrophage Colony-stimulating Factor but not by Cytolysis

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ABSTRACT

Several antigens, including the products encoded by the genes MAGE-1 and MAGE-3, are recognized on human melanoma cells by HLA-A1, HLA-A2, or HLA-Cw*1601*-restricted T cells on autologous or HLA-matched melanoma cell lines. T-cell recognition of naturally processed MHC class I-presented peptides, or alternatively synthetic peptides derived from MAGE-1 or MAGE-3, leads to cytokine release as well as to a cytotoxic T-cell response in these antimelanoma-directed polyclonal or clonal effector T-cell populations. Recent reports suggest that the activity of T lymphocytes infiltrating melanoma in vivo appears to be impaired. We report here the characterization of the in vitro (in the presence of 6000 IU interleukin 2) expanded tumor-infiltrating lymphocyte (TIL) T-cell line PM2-B2 derived from a patient with rapidly progressing and therapy-resistant head and neck melanoma. The TIL cell line PM2-B2 did not lyse, but instead released granulocyte-macrophage colony-stimulating factor in response to the autologous tumor or HLA-A1-matched allogeneic tumor cell lines. The TIL line PM2-B2 did not kill the MHC class I normal killer/lymphokine-activated killer target cell lines Daodu or K562. The fine specificity of the TIL line PM2-B2 restricted by HLA-A1 was further characterized by evaluating specific granulocyte-macrophage colony-stimulating factor release in response to MHC class I-eluted peptides derived from HLA-A1+ melanoma cell lines. TIL PM2-B2 failed to recognize the recently described HLA-A1-presented peptides derived from the gene products encoded by MAGE-1 or MAGE-3. PCR-based analysis of the freshly harvested tumor from patient PM2-B2 revealed the presence of message for the melanoma-associated gene products MAGE-1 and MAGE-3, but not for tyrosinase or MART-1/MELAN-A. Acid elution and high performance liquid chromatography fractionation of MHC class I-presented peptides from HLA-A1-matched melanoma cell lines 397 or 888 revealed that TIL PM2-B2 recognized at least three distinct peptide epitopes eluting in high performance liquid chromatography bioactive fractions 5/6, 36, and 51/52. These bioactive peaks appeared to be shared among HLA-A1+ melanoma cell lines. We suggest, based on this report, that HLA-A1-presented melanoma-derived peptides (other than those previously reported peptides derived from MAGE-1 or MAGE-3) may represent targets for TIL recognition as defined by cytokine release, but not cytotoxicity. Such an immune response differentially defined by cytokine release, but absent cytotoxic functions, may either reflect the impaired cytolytic function of the TIL population or reflect the inherent nature of HLA-A1-presented melanoma T-cell epitopes leading to cytokine release, but not to a cytotoxic T-cell response. Additionally, this report suggests that the individual T-cell immune response to melanoma may be rather complex, involving diverse T-cell effector functions (e.g., cytotoxicity or cytokine release), each of which should be evaluated in studies of antitumor-specific T-cell reactivity.

INTRODUCTION

Although 85% of melanoma patients are cured by surgical excision alone, melanoma lesions do progress in many patients, despite the presence of extensive infiltration by presumably antitumor-reactive T cells. Separation of TILs2 from tumor and subsequent expansion of these effector T cells in vitro in the presence of IL-2 results in effector cells capable of mediating tumor regression after adoptive transfer in vivo (1, 2). The efficacy of the host immune response and therapeutic interventions aimed at inducing an effective long-term antitumor-directed T-cell immunity may depend on the expression of immunogenic epitopes by autologous tumor and by the presence of host T cells capable of responding to these antigens.

T-cell epitopes presented by tumors may not be adequately or appropriately presented. This may be due to down-regulation in expression of molecules involved in antigen processing and presentation (3) or to down-regulation of tumor antigen(s) expression required for T-cell recognition in vivo (4, 5).2 The efficacy of TILs capable of infiltrating melanoma lesions may

2 The abbreviations used are: TIL, tumor-infiltrating lymphocyte; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; HPLC, high performance liquid chromatography; RT, room temperature; CM, complete medium; mAb, monoclonal antibody.

also be critically dependent on effector T-cell functions (e.g., proliferation or cytolysis) which appear to be frequently impaired as a result of decreased T-cell receptor ζ chain cell surface expression as described for TIL harvested from renal cell cancer (6), colorectal cancer (7), or melanoma patients. Impaired T-cell function, as measured by cytokine release or by specific proliferation in response to autologous tumor, may also be due to deficient expression of LFA-3 defects on melanoma cells (8).

At least six T-cell-defined naturally processed and presented T-cell epitopes presented by HLA-A2.1 have been described (9, 10). Genes encoding proteins such as these naturally presented T-cell epitopes have now been identified as tyrosinase (11, 12), gp100/Pmel17 (13-15), MART-1/MELAN-A (16-20), and MAGE-3 (21). Human TILs recognizing melanoma peptide epitopes in the context of HLA-A1 that derive from tumor antigens encoded by the genes MAGE-1 (22, 23) and MAGE-3 (24) have been described. These antigens were defined by tumor necrosis factor α release or cytolytic activity of in vitro expanded oligoclonal or clonal T lymphocytes (22-25). These antigens are expressed by established melanoma cell lines, as well as by freshly harvested tumor single-cell suspensions from early melanoma lesions and from regional or distant melanoma metastases (26).

We report here the identification of three naturally processed and HLA-A1-presented T-cell epitopes shared by two different HLA-A1+ melanoma cell lines and recognized by a HLA-A1-restricted T-cell line designated as PM2-B2. Of particular interest, the biological response of the TIL PM2-B2 line to specific recognition of these peptides presented by HLA-A1 is restricted to cytokine release as measured by GM-CSF release; no cytotoxic T-cell response occurs.

**MATERIALS AND METHODS**

**Cell Lines.** The human melanoma cell line 888 (HLA-A1+, A24+, B5+, B22+, and HLA-C undetermined), Mel 397 (HLA-A1+, A10+, B8+, B62+, and Cw4+), and Mel S26 (HLA-A2+, A3+, B501, B62+, and Cw4+), kindly provided by Dr. S. Rosenberg, National Cancer Institute, were maintained in RPMI 1640 (CM) and supplemented with 250 μg/ml hygromycin B.

**TILs.** TILs from patient PM2-B2 were generated as described previously (28) by mincing freshly harvested tumor, digestion with collagenase (Sigma, St. Louis, MO), and centrifugation over a Ficoll gradient. The gradient interface was collected, washed twice, and resuspended in AIM-V medium (GIBCO) supplemented with 10% heat-inactivated human pooled AB serum and 6000 IU/ml recombinant human IL-2 (Perkin Elmer/Cetus, Emeryville, CA), and seeded at 5 × 10^6 cells/ml in 24-well tissue culture plates. TIL PM2-B2 were split if the cell number exceeded 1.5 × 10^6 cells/ml and fed with IL-2 (6000 IU/ml) containing AIM-V medium. TIL PM2-B2 have never been restimulated with autologous or allogeneic tumor cells in vitro and have been determined to be 80% CD8+ by flow cytometric analysis after 4 weeks in culture. They were used that time as effector T cells in cytotoxicity or cytokine release assays, as indicated.

**Melanoma Peptide Extraction.** Peptides were extracted from the HLA-A1+ melanoma cell lines 888 or 397 using a pH 3.3 acid elution technique as recently described (9, 29). This procedure results in the denaturation of the ternary complex of the MHC I heavy chain with peptide and β2-microglobulin dissociation into the culture supernatant. Peptides were concentrated using a C18 SepPak column (Waters, Milford, MA), and then HPLC fractionated as previously described (29). Individual HPLC fractions were collected, lyophilized to remove organic solvents, and reconstituted in 200 μl AIM-V. Peptides were stored at −20°C until ready for use in biological assays.

**Synthetic Peptides.** MAGE-1-derived peptide EADPT- GHSY (23) and MAGE-3 (24) derived peptide EVDPIGHLY were synthesized by Fmoc chemistry in the Peptide Synthesis Facility (Shared Resource) of the Pittsburgh Cancer Institute and were found to be >80% pure by HPLC analysis.

**Cytolytic Assays.** A standard 4-h chromium release assay was used to assess recognition of HLA-A1+ melanoma tumor cells (including Mel 888, 397, and the autologous tumor Mel PM2-B2) by TIL PM2-B2 (E:T ratio = 10:1). Additional target cell lines included Daudi or K562 to assess lymphokine-activated killer cell or natural killer cell activity, respectively, within TIL line PM2-B2. In peptide-pulsing assays (using naturally eluted HPLC-fractionated peptides), 20 μl of individual HPLC fractions were added to wells containing chromium-labeled C1R-A1+ B-cell targets (100 μl of a cell suspension of 1 × 10^6 target cells/ml and 5 μg human β2-microglobulin/ml in complete RPMI 1640 medium), and the resulting peptide-target mixture was incubated for 2 h at RT to allow time for peptide charging of target cell class I complexes. The same procedure was applied using synthetic peptides, which had been reconstituted in DMSO and diluted to the appropriate concentrations as indicated. Finally, 100 μl TIL effector cells (E:T ratio = 10:1) were added to assay wells, and plates were incubated for 4 h at 37°C. Spontaneous release wells received 100 μl TIL medium (AIM-V), and maximum release wells received 100 μl Triton X-100 (10% v/v in water). One hundred μl aliquots were harvested from each well and counted in a gamma counter. Results are reported as percentage of specific chromium release calculated as: (Experimental cpm − spontaneous cpm)/(Maximum cpm − spontaneous cpm) × 100.

**Cytokine Release Assays.** Synthetic peptides: Serial 2-fold dilutions of synthetic peptides (1000 ng-3.9 ng) were used in peptide-pulsing assays. TILs were added to peptide-pulsed target cells at E:T ratios of 10:1 and incubated for 4 h at 37°C. Supernatants were harvested and assayed for IL-2 (Perkin Elmer/Bender), IL-4, and IL-10 (Genzyme). Values for IL-2 and IL-4 were expressed as pg/ml, and those for IL-10 as pg/10^6 target cells.

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4 T. Whiteside, personal communication.
diluted in RPMI 1640 containing 10% FCS (CM) and pipetted in a 96-well microtiter plate in a total volume of 10 μl/well for each individual peptide. Control wells received 10 μl CM without peptide. C1R-A1 recipient target cells were irradiated (3000 Gy) and adjusted to 10⁵ cells/ml in CM supplemented with 5 μg/ml β2-microglobulin (Sigma). One hundred μl of this single-cell suspension were added to individual wells and incubated 2 h at RT to allow peptide MHC class I charging, followed by two washing steps by adding 100 μl CM to individual wells and centrifugation (500 rpm) for 5 min. After careful removal of 200 μl medium in each individual well, the cell pellet was again resuspended in 200 μl medium to remove residual peptide in solution, followed by centrifugation and aspiration of the supernatant. The cell pellet was then resuspended in 100 μl CM, and a 100-μl single-cell suspension of TIL PM2-B2 (10⁶ cells/ml) in AIM-V was added to assay plates. As indicated, the TIL line PM2-B2 was maintained in AIM-V supplemented with 6000 IU tide/μg-microglobulin complexes were dissociated by incubation with Lambda Inc., and 2 ~11 mg/100-1μl solution were used for infected C1R cells plus T cells without peptide. After incubation for 2 min in acid buffer, pH 3.3 (total volume, 5 ml). In brief, C1R cells transfected with the HLA-A1 molecule were used as an isotype control. The protein A-purified mAb L243 (0289HA) specific for human HLA-A1 (directed against a monomorphic or conformational epitope in the class I heavy chain) was kindly provided by the University of Mainz, Germany) and has been shown to block MHC class II (DR)-directed T-cell responses (10 l~1 1.64 mg/ml anti-MHC class II DR) was kindly provided by Chomczynski and Sacchi (31). First-strand cDNA synthesis was performed by heating the reaction at 37°C for 1 h, followed by the addition of random primers (final concentration, 0.8 μg), and 2 μl Moloney murine leukemia virus reverse transcriptase (Perkin Elmer/Cetus PCR thermal cycler). The 40-μl reaction volume contained: 8 μl RNA in 16 μl H₂O, 8 μl 5 X RB, 4 μl DTT (final concentration, 10 mM), 2 μl dNTP (dATP, dCTP, dGTP, and dTTP final concentration 1 mM each), 3 μl RNase inhibitor (final concentration, 120 units), 1 μl actinomycin D (final concentration, 2 μg), 4 μl oligo(dT) random primers (final concentration, 0.8 μg), and 2 μl Moloney murine leukemia virus reverse transcriptase (Perkin Elmer/Cetus; final concentration, 400 IU/ml).

Amplification of Melanoma/Melanocyte-encoded Rejection Antigens. One hundred fifty ng cDNA were used in each individual PCR reaction throughout all experiments, and the integrity of individual cDNA samples was analyzed using
human β-actin-specific primers (5′-3′; forward: ATTTCCTGGTGAGATGGAGG; backward: GCACGCATGACTGAGGAGC) using the temperature profile 95°C for denaturation, annealing for 1 min at 60°C, followed by 1 min at 72°C for extension (30 cycles). The same protocol was used for amplification of tyrosinase-, MART-1/MELAN-A-, and MAGE-1 and MAGE-3-encoded gene products. Primers sequences were (5′-3′): MART-1/MELAN-A (18), forward: ACTGCTCATCGGCTGG; backward: TCAGCCATGTCTCAGGTG, MAGE-1, forward: CGGCCGAAGGAACTGACCCAG; backward: GCTGGAACCCTCAGGGTGC; and MAGE-3 (24), forward: TGGAGGACAGAGGCCCC; backward: GGACGATTATGGACG. The primer sequences for detection of tyrosinase were: forward: TTGTCAGATTTGTTGACC; backward: AGGCATTGTCATGCTGTTT. Generally, each reaction mixture (volume 50 µl) contained 25 µl H2O, 5 µl PCR 10× reaction buffer (500 µmol each primer), 1.5 µl cDNA (150 ng), and 1 µl Taq polymerase (5 units/µl; Perkin Elmer/Cetus). The respective PCR reactions were run on 1.5% ethidium bromide-stained agarose gels. In each PCR run, a positive (cDNA prepared from MEL 624) and a negative control (PCR mastermix without template cDNA) were included. Primers were synthesized at the DNA Synthesis Facility (University of Pittsburgh).

**RESULTS**

**TIL PM2-B2 Recognizes Autologous or HLA-A1-matched Allogeneic Melanoma as Measured by GM-CSF Release, but not by Cytotoxicity.** Tumor single-cell suspensions derived from autologous tumor Mel PM-NH or HLA-A1-matched allogeneic melanoma cell lines Mel 397 or Mel 888 were tested in a standard cytotoxicity assay for TIL PM-NH recognition. Additional targets included the cell lines Daudi or K562 to assess lymphokine-activated killer cell or natural killer cell activity. We reproducibly could not detect a cytotoxic T-cell response for any of the targets tested (data not shown). Therefore, we examined GM-CSF production of TIL PM2-B2 in response to tumor, which has been reported to represent the most sensitive assay detecting specific T-cell effector functions compared to γ-IFN or tumor necrosis factor α detection systems (32-34). To detect GM-CSF release of TIL PM2-B2 in response to autologous tumor cells or HLA-A1-matched (Mel lines 397 and 888) or -A1-nonmatched (Mel 526) melanoma cells, we performed a 16-h cytokine GM-CSF release assay (Table 1). Supernatants harvested from tumor cells or TIL single-cell suspensions alone did not exhibit any GM-CSF release. In contrast, coincubation of autologous tumor cells or HLA-A1-matched melanoma cell lines (397 and 888) with TIL PM2-B2 resulted in specific GM-CSF release in the range of 30–42 pg/ml in 16 h. Negative controls included coinoculation of TIL PM-NH with an A1+ melanoma cell line (Mel 526), incubation with HLA-A1+ matched nonmelanoma stimulator cells, or incubation of autologous tumor cells or Mel 397 in the presence of an HLA-A1 or MHC class I blocking mAb, resulting in 5–6 pg/ml GM-CSF release, respectively. GM-CSF release by TIL PM2-B2 in response to autologous tumor cells could not be blocked by an antibody directed against MHC class II (DR)-restraining molecules or with an irrelevant IgM (control) mAb (Table 1).

**Peptides EADPTGHSY (Derived from MAGE-1) or EVDPIGHLY (Derived from MAGE-3) Are Not Recognized by TIL PM2-B2 as Detected by Cytokine Release or Cytotoxic T-Cell Responses.** Since the gene products derived from MAGE-1 or MAGE-3 have been demonstrated to provide T-cell epitopes recognized by HLA-A1-restricted T cells from melanoma patients, we examined whether these peptides would represent T-cell epitopes capable of eliciting GM-CSF release by TIL PM2-B2. To ensure peptide binding to the antigen-presenting cell (C1R-A1), we performed an HLA-A1 reconstitution assay using the MAGE-1-derived peptide EADPTGHSY or the MAGE-3-derived peptide EVDPIGHLY (Fig. 1). Peptide EADPTGHSY is capable of reconstituting HLA-A1 to 83% at a concentration of 1000 ng titering to 34% of HLA-A1 reconstitution at 31.25 ng peptide (Fig. 1). Peptide EVDPIGHLY exhibited 52% HLA-A1 reconstitution at 1000 ng peptide titering to 5% MHC class I reconstitution at 62.5 ng peptide (Fig. 1). The same titration of both peptides was performed on C1R-HLA-A1 cells (105 TILs and 104 stimulator cells/well). Controls included TILs or tumor cells alone exhibiting no GM-CSF release.

**Table 1** TIL PM2-B2 recognize HLA-A1+ melanoma cells as detected by GM-CSF release

<table>
<thead>
<tr>
<th>Stimulatorsa</th>
<th>Tumor alone</th>
<th>Tumor + TILs</th>
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<td>HLA-A1</td>
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<td>Exp. 2</td>
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<td>70</td>
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<td>0</td>
<td>40</td>
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<tr>
<td>Mel 888</td>
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<td>32</td>
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<tr>
<td>Mel 526</td>
<td>A2, A3</td>
<td>0</td>
<td>5</td>
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<tr>
<td>EBV line K4B</td>
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<td>0</td>
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<td>C1R-HLA-A1</td>
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a TIL line PM2-B2 was incubated for 16 h with HLA-A1-matched or nonmatched melanoma cell lines, the HLA-A1+ human EBV+ B-cell line K4B, or C1R-HLA-A1 cells (105 TILs and 104 stimulator cells/well). Supernatants were harvested and tested for GM-CSF using ELISA as described. HLA-A1-restricted TIL specificity was demonstrated using an antibody (IgM) directed against the HLA-A1 molecule or the MHC class I-directed mAb W6/32. Incubation of tumor cells with an irrelevant IgM mAb (CSLEX1, anti-sialyl Le'x) did not significantly block melanoma cell recognition by TIL PM2-B2 measured by GM-CSF release (data not shown). Controls included TILs or tumor cells alone exhibiting no GM-CSF release.

b GM-CSF release/105 TILs/103 tumor cells/16 h (pg/ml)
Fig. 1. MAGE-1- and MAGE-3-derived peptides bind to HLA-A1 on C1R-A1. Binding of MAGE-1-derived peptide EADPTGHSY and the MAGE-3-presented peptide EVDPIGHLY to the MHC class I-presenting HLA-A1 molecule was validated using a flow cytometric peptide-binding assay as described in “Materials and Methods.” These peptides, presented by C1R-A1, were not recognized by the TIL line PM2-B2 as detected by cytotoxicity or GM-CSF release.

Detection of mRNA Expression of Melanoma Antigens Recognized by T Lymphocytes. Absence of anti-MAGE-1- or MAGE-3-directed T cells in TIL PM2-B2 could potentially be due to the absence of the gene products provided by the MAGE-1/3 genes. Since TIL PM2-B2 appears to recognize HLA-A1-presented T-cell epitopes (Table 1), which may not be related to the recently described MAGE-1 or MAGE-3 epitopes, we tested mRNA expression for two additional melanoma-associated antigens (MART-1/MELAN-A and tyrosinase), which have been demonstrated to be recognized by the MHC class I-restricted CD8+ T lymphocytes (11, 12, 17–20). It may well be that these (in the context of HLA-A2) immunodominant antigens may also provide peptide epitopes for HLA-A1-restricted T-cell recognition, since tyrosinase has been shown to serve as a peptide source for several MHC-restricting elements including HLA-A2, HLA-A24, HLA-B44, and MHC class II (11, 12, 35, 36). Control specimens included the melanoma cell lines 397 and 888, and the melanoma cell line 624, which has been described to encode the MART-1/MELAN-A antigen (17, 19, 20). Freshly harvested tumor single-cell suspension from patient PM2-B2 was used as a source for mRNA isolation to exclude any culture-induced artifacts modulating gene expression. PCR-based analysis demonstrated the presence of MAGE-1 and MAGE-3 mRNA in all specimens examined, except a considerably low expression of MART-3 in the control specimen Mel 624 (Fig. 2A). In contrast, tyrosinase tested positive in the control samples Mel 397, 888, and 624, but not in tumor cells harvested from patient PM2-B2 (Fig. 2B). The same was found to be true for mRNA expression of the MART-1/MELAN-A antigen, which is highly expressed in melanoma specimens 397, 888, and 624, but not in Mel PM2-B2 (Fig. 2B). These results could be confirmed in experiments using mRNA and cDNA aliquots prepared from cell lines at different time points in culture. Similar results could be obtained for MART-1/MELAN-A gene products using a different primer set spanning the entire MART-1/MELAN-A gene (data not shown).

TIL Line PM-NH Recognizes T-Cell Epitopes Eluted from HLA-A1+ Allogeneic Tumor Cell Lines as Detected by GM-CSF Release. Since the autologous tumor cell line PM2-B2 initially grew slowly in culture, we used the HLA-A1-matched melanoma cell lines 397 and 888 as the source for class I-associated peptides to be screened for recognition by TIL PM2-B2. Peptides from $5 \times 10^8$ tumor cells were acid eluted and HPLC fractionated as previously described (9, 29). Individual peptide fractions were pulsed on C1R-A1 recipient cells, followed by testing for TIL PM2-B2 recognition, as measured...
Detection of Melanoma T-Cell Epitopes

Tyrosinase-related antigen gp31 (37), and Cw*0601 IMAGE-1 MART-1/MELAN-A mRNA, are expressed in melanoma PM2-B2.

Fig. 2 A RNA was extracted and reverse transcribed into cDNA from melanomas M, molecular weight marker.

Testing of HLA-A1 melanoma 888-derived peptides also resulted in GM-CSF release by TIL PM2-B2 (Fig. 3). Eluted from melanoma 397 exhibited three (fractions 5-7, 35/36, and 52/53) resulting in GM-CSF release (Fig. 3). Melanoma 600-directed T cells to be presented by HLA-A1 (Table 1).

The antigen(s), presumably presented by HLA-A1, appear to be shared by two different (allogeneic) HLA-A1 + melanoma cell lines and the autologous PM2-B2 tumor cells (Table 1). The recently described HLA-A1-presented epitopes provided by the gene products MAGE-1 and MAGE-3 are not recognized by TIL PM2-B2. However, TIL line PM2-B2 recognizes at least two HLA-A1-presented melanoma T-cell epitopes eluting in HPLC fractions 35/36 and 52/53. These HLA-A1-presented peptide species may derive from (a) novel melanoma antigens or (b) already identified melanoma antigens, which thus far have only been evaluated for their ability to provide T-cell epitopes presented by alternate MHC class I molecules. The three bioactive HPLC peaks (of which at least two appear to be shared by Mel 397 and 888, i.e., fractions 35/36 and 52/53) could derive from alternate members of the rapidly growing MAGE-related gene family or from gene products, which have been identified as providing peptides for other MHC class I-restricted T cells. It may well be that these proteins (i.e., MART-1/MELAN-A, tyrosinase, or gp100/Pmel 17) provide peptides for HLA-A1-restricted T-cell recognition, as it has already been described for MAGE-1 [HLA-A1 (23), Cw*1601 (38)], tyrosinase [HLA-A2 (11, 12), MHC class II (36), HLA-A24 (35)], or MAGE-3 [HLA-A1 (24), HLA-A2 (21)]. However, the autologous tumor cells from patient PM2-B2 repeatedly tested negative for mRNA expression of tyrosinase or MART-1/MELAN-A (as detected by PCR, see Fig. 2B), excluding these two possibilities in the resected tumor specimen. We could not use a PCR-based technique to detect gp100 mRNA due to equivocal results obtained using the current primer panel, which is still under investigation.

The absence of the gene products tyrosinase/MART-1/ MELAN-A is of particular interest, since 26/26 freshly collected melanoma samples and 12/21 melanoma cell lines tested positive for MAGE-1/Melan mRNA expression by PCR (18). If tyrosinase stimulates an antitumor response in vivo by providing peptides for MHC class II presentation (36) and subsequent stimulation and expansion of CD4+ T-helper cells, the poor clinical outcome of patient PM2-B2 and the unusual T-cell effector recognition pattern of TIL PM2-B2 (i.e., cytokine release, but not cytolsis) may be due to the lack of appropriate helper T cells required to promote the expansion and cytolytic maturation of MHC class I-restricted cytotoxic T lymphocytes.

The striking observation that the TIL line PM2-B2 does not exhibit any cytotoxic response and that antitumor-directed reactivity results in cytokine release (as measured by GM-CSF) is in concordance with previous reports demonstrating impaired effector T-cell functions, e.g., reduced TCR ζ chain cell surface expression, leading to incomplete T-cell signaling and subsequently to impaired effector T-cell functions (6, 7). Another report, examining impaired effector (melanoma-derived) T-cell

**DISCUSSION**

Several antigens providing "shared" epitopes for anti-melanoma-directed T cells to be presented by HLA-A1 [MAGE-1 (22 and 23), MAGE-3 (24)], HLA-A2 [tyrosinase (11, 12), MART-1/MELAN-A (17-20), gp100/Pmel 17 (13-15), MAGE-3 (21)], HLA-24 [tyrosinase (35)], HLA-A31 [the tyrosinase-related antigen gp31 (37)], and Cw1601 [MAGE-1 (38)] or presented by MHC class II gene products [tyrosinase (36)] have been previously described. Additionally, the fine specificity of TIL lines derived from different patients has revealed the presence of "private" antigens, recognized only by TILs, which are unique to the respective individual (18, 39, 40). We describe here TIL line PM2-B2 derived from a melanoma patient with rapidly progressing, therapy-resistant disease, presenting nodal metastatic disease arising from a primary scalp lesion. The restriction element used for T-cell recognition appears to be HLA-A1, based on blocking studies with a mAb directed against HLA-A1 (Table 1).
functions, identified the lack of the adhesion/costimulatory molecule LFA-3 on melanoma targets as one critical cause for insufficient T-cell functions (8). This appears to be unlikely in our case, since the melanoma cell lines 397 and 888 are excellent targets in cytotoxicity assays (35). Moreover, the “transfer” of class I-presented peptides from presumably poor T-cell targets (i.e., melanoma-lacking adhesion molecules), to good peptide presenting cells (i.e., the surrogate peptide recipient cell line C1R-A1), should be able to overcome this lack of costimulatory function of the target cell. However, it is mechanistically not clear why the TIL line PM2-B2 only responds by cytokine secretion and not by a cytotoxic T-cell response. As already discussed above, this reaction pattern could be attributed to the T-cell compartment, resulting in impaired T-cell effector functions. An alternative explanation would be that the T-cell epitopes, recognized by TIL line PM2-B2, are able to elicit a T-cell response, which is characterized by cytokine release rather than by a cytotoxic T-cell response. Naturally occurring “antagonistic peptides” may account for impaired T-cell immune effector functions. These peptides differ usually only in one amino acid residue, and they can still bind to the proper MHC molecule and engage the appropriate T-cell receptors bearing T lymphocytes (41, 42) and elicit quantitative and qualitative different T-cell responses (43–45).

We demonstrate specific HLA-A1-restricted T-cell recognition using TILs derived from a patient with rapid progressing and apparent therapy-resistant disease and naturally processed and presented peptides eluted from HLA-A1 + melanomas. The value of this T-cell recognition has to be further investigated, since the poor clinical outcome, at least in this particular case, suggests that specific MHC class I-restricted recognition of melanoma may not represent per se an indicator of a more favorable clinical prognosis, particularly if this recognition is only at the level of cytokine release.

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Detection of Melanoma T-Cell Epitopes


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