The Majority of Autologous Cytolytic T-Lymphocyte Clones Derived from Peripheral Blood Lymphocytes of a Melanoma Patient Recognize an Antigenic Peptide Derived from Gene Pmel17/gp100

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Anti-melanoma cytolytic T-lymphocyte (CTL) clones were derived from peripheral blood lymphocytes of HLA-A2 melanoma patient LB265 after stimulation with the autologous tumor cell line LB265-MEL, which showed high expression of melanocyte-lineage specific genes. Of 55 CTL clones, 46 recognized HLA-A2–restricted antigens. These 46 CTL clones were studied for their ability to specifically release tumor necrosis factor in the presence of COS cells cotransfected with the HLA-A2 gene and the cDNA of either tyrosinase, Melan-A/MART1, Pmel17/gp100, gp75/TRP1, or MSH receptor. Six CTL clones recognized the Melan-A/MART1 antigen, whereas the remaining 40 CTL clones recognized a Pmel17/gp100 antigen. These 40 anti-Pmel17/gp100 CTL clones were all able to lyse T2 cells pulsed with the antigenic peptide YLEPGPVTA, as previously reported. The T-cell receptor β chain hypervariable region was sequenced and found to be identical in the 15 CTL clones analyzed. Taken together, these data show a high frequency of Pmel17/gp100-specific T cells in autologous antitumor CTL clones derived from peripheral blood of a melanoma patient. ***Key words: melanoma/CTL/immunotherapy. J Invest Dermatol 107:63–67, 1996***

Cytolytic T lymphocytes (CTL) that recognize tumor cells can often be isolated from melanoma patients (Boon *et al*, 1994). Two main procedures have been successfully followed to determine the nature of the antigens recognized by such antimelanoma CTLs. One utilized a genetic approach based on the transfection of cosmid or cDNA libraries and led to the identification of genes MAGE, BAGE, GAGE, tyrosinase, and Melan-A/MART1 (Van der Bruggen *et al*, 1991; Van Pel *et al*, 1995). Alternatively, tandem mass spectrometry was used to identify HLA-A2–associated nonapeptides recognized by high-affinity CTLs from melanoma patients (Cox *et al*, 1994).

Besides antigens that correspond to point mutations that give rise to unique tumor-specific antigens, two categories of genes coding for antigens recognized on melanomas by CTLs have been so far reported. The first category of genes (including MAGE, BAGE, and GAGE) is strongly expressed not only in melanomas but also in head and neck tumors, non-small–cell lung cancers, bladder carcinoma, and gastric carcinoma. By contrast, these genes are silent in most normal tissues except testis, where they might be exclusively expressed in germ-line cells that lack expression of MHC molecules. The antigens encoded by such genes may therefore be truly tumor-specific (for a review, see Van Pel *et al*, 1995).

A second category of genes corresponds to proteins that are specific to melanocyte-lineage cells. These antigens can therefore be considered as "differentiation antigens." Tyrosinase, Melan-A/MART1, Pmel17/gp100, and gp75/TRP1 belong to this group. Tyrosinase, gp75/TRP1, and Pmel17/gp100 proteins are transmembrane glycoproteins expressed within melanosomes, the melanocyte-specific organelles that are the site of melanin synthesis. Tyrosinase is a key enzyme that catalyzes the initial steps of melanin synthesis (Hearing and Jimenez, 1987). gp75/TRP1 is the most abundant intracellular glycoprotein in melanocytes and displays DHI-2-carboxilic acid oxidase activity in melanogenesis (Jimenez-Cervantes *et al*, 1994). Data support the role of Pmel17/gp100 in melanization as an enzymic or structural component (Kwon *et al*, 1991). The function and localization of the Melan-A/MART1 protein is still unknown.

In our study, we obtained CTL clones from the peripheral blood lymphocytes (PBL) of an HLA-2 melanoma patient whose tumor expressed high levels of melanocyte-lineage–specific genes. We showed that most of these CTL clones recognized two melanocyte-specific antigens in the context of HLA-A2, namely Melan-A/MART1 (6 out of 46 CTL clones) and Pmel17/gp100 (40 out of 46 CTL clones). All the isolated anti-Pmel17/gp100 CTL clones recognized the same antigenic peptide, the nonamer YLEPGPVTA, and the sequences of the T-cell receptor (TCR) hypervariable

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Abbreviations: CTL, cytolytic T lymphocyte; PBL, peripheral blood lymphocytes; TCR, T-cell receptor; MSH-R, melanin-stimulating hormone receptor; PCR, polymerase chain reaction; TIL, tumor infiltrating lymphocyte.

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region were found to be identical for 15 anti-Pmel17/gp100 CTL clones.

MATERIALS AND METHODS

Cell Lines Tumor cell line LB265-MEL was derived from a cervical metastatic lymph node of patient LB265 (HLA-A2, -A3, -B7, -B62, -Cw7, -Cw9). LB43-MEL is a melanoma cell line derived from a primary tumor (kind gift from Dr. D. Vanneste, Institut Médico-Chirurgical, Tournai, Belgium). Melanoma cell lines SK29-MEL and SK23-MEL were a gift from Dr L. Old (Memorial Sloan-Kettering Center, New York). The melanoma cells were cultivated in Iscove's medium supplemented with 10% fetal calf serum (GIBCO BRL, Gaithersburg, MD), L-arginine (116 mg/liter), L-asparagine (36 mg/liter), and L-glutamine (216 mg/liter). COS-7 cells (American Type Culture Collection CRL 1651; Rockville, MD) were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum. WEHI-164 clone 13 (a gift from Dr. T. Espevik, University of Trondheim, Trondheim, Norway) (Espevik and Nissen-Meyer, 1986) and T2 cells (Salter *et al*, 1985) were cultured in RPMI-1640 (GIBCO BRL) supplemented with 10% and 5% fetal calf serum, respectively.

Generation of CTL Clones Autologous mixed lymphocyte-tumor cultures were essentially carried out as previously described (Hérin *et al*, 1987). Briefly, 10^6 FACS-sorted $CD8^+$ T cells were stimulated with 10^5 irradiated (100 Gray from a cesium source) LB265-MEL tumor cells in 2 ml of Iscove's medium supplemented with 10% human serum, L-arginine (116 mg/liter), L-asparagine (36 mg/liter), L-glutamine (216 mg/liter), and 2-mercaptoethanol (0.05 mM), 10 U/ml recombinant human interleukin-2 (IL-2) (donated by Biogen, Geneva, Switzerland) and 5 ng/ml of recombinant human IL-7 (R&D Systems). Derivation and long term culture of CTL clones were carried out as previously described (Hérin *et al*, 1987).

Tumor Necrosis Factor (TNF) Assays Melanoma cell lines were tested for their ability to stimulate the production of TNF by CTL clones as described previously (Traversari *et al*, 1992). Briefly, 3,000 CTLs were added to 100 μl of Iscove medium supplemented with 10% human serum and 25 U/ml recombinant human IL-2 in microwells containing 30,000 target cells. Inhibition of TNF secretion was studied in the presence 3% ascitic fluid containing either anti-HLA-A2 (BB7.2), anti-HLA-B7 (ME1), anti-HLA-B,C (B1.23.2), or anti-HLA class I (W6/32) monoclonal antibodies. After 24 h, the supernatant was collected and its TNF content determined by testing its cytotoxicity for WEHI-164 cells (Espevik and Nissen-Meyer, 1986) in a MTT colorimetric assay as previously described (Traversari *et al*, 1992).

Transient transfections of COS-7 cells were performed by the DEAE-dextran-chloroquine method (Selden, 1995). Briefly, 1.5×10^4 COS-7 cells were cotransfected with 100 ng of plasmid pcDNA3 (Invitrogen, San Diego, CA) containing either Pmel17, gp100, gp75/TRP1, or MSH-R cDNA, and 100 ng of plasmid pcDSRα carrying the HLA-A2.1 gene (Wölfel *et al*, 1993). After 24 h, the transfected cells were tested for expression of the antigen by their ability to induce TNF release by CTL clones.

Assays for Cytolytic Activity Cytolytic activity was measured as described previously (Hérin *et al*, 1987). Target cells were treated for 48 h with 50 U/ml of human recombinant interferon-γ (Boehringer Ingelheim, Germany). CTLs and 1000 ^{51}Cr-labeled targets were incubated at various effector-to-target ratios in 96 conical microplates in a final volume of 200 μl. Chromium release in the supernatant was measured after 4 h of incubation.

Peptides were synthesized on solid phase using Fmoc [*N*-(9-fluorenyl)methoxycarbonyl] for transient NH_2-terminal protection, as described by Atherton *et al* (1981), and were characterized by mass spectrometry. The peptides were >90% pure as determined by analytical high-pressure liquid chromatography. Lyophilized peptides were dissolved at 20 mg/ml in 10 mM acetic acid and stored at -80°C. Cells of the HLA-A2$^+$ cell line, T2, were ^{51}Cr-labeled for 1 h at 37°C, washed extensively, and 1000 of the target cells were then incubated in 96-well microplates in the presence of various concentrations of peptide for 30 min at 37°C before adding CTL clones. After 4 h at 37°C, chromium release was measured.

Isolation of cDNAs Encoding Pmel17 or MSH-R Total RNA was isolated from LB265-MEL cell line using the guanidine isothiocyanate-acid phenol method (Davis *et al*, 1986). For polymerase chain reaction (PCR), first strand cDNA was synthesized from 1 μg of total RNA using oligo(dT)$_{15}$ and Moloney murine leukemia virus reverse transcriptase (GIBCO BRL). Amplification of Pmel17 and MSH-R genes was performed with specific oligonucleotides: 5′-GGA AGA ACA CAA TGG ATC TGG-3′ and 5′-TCC TGC TTC CTG GAC AGG ACT-3′, respectively, as sense primers and 5′-CAC AGC ATC ATA TGA GAG TAC-3′ and 5′-TCC CTC TGC CCA GCA GCA CAC-3′ as antisense primers. The *Pfu* DNA polymerase (Stratagene, La Jolla, CA) was used to avoid PCR-related errors. Twenty-five cycles were performed as follows: 94°C for 1 min; 56°C for 1 min; 72°C for 3 min. The amplification products were cloned into pcDNA3 plasmid (Invitrogen). Nucleotide sequences of the two cDNA clones were identical to Pmel17 and MSH-R sequences available from databases. Independently, a gp100 cDNA, incidentally corresponding to an alternatively spliced mRNA from Pmel17 gene (Adema *et al*, 1994), was obtained from Dr. G. Adema (University of Nijmegen, The Netherlands). The gp75/TRP1 cDNA was kindly provided by Dr. A. Houghton (Memorial Sloan-Kettering Cancer Center, New York) and tyrosinase and Melan-A/MART1 cDNAs were provided by Dr. V. Brichard (Ludwig Institute, Brussels).

Northern Blot Analysis Poly(A)$^+$ RNA was prepared using a microfastrack mRNA extraction kit (Invitrogen). Five micrograms of poly(A)$^+$ RNA, each from LB265-MEL, SK29-MEL, SK23-MEL, LB43-MEL, and NA8-MEL melanoma cell lines, was used for northern blotting. Hybridization was performed with a 2039 bp of ^{32}P-labeled probe corresponding to nucleotides 1–2039 of Pmel17 cDNA. A control hybridization with a β-actin probe was subsequently performed on the same membrane.

TCR Vβ PCR Amplification Single-strand cDNA was prepared from poly(A)$^+$ RNA of anti-Pmel17/gp100 CTL clones with oligo(dT) and Moloney murine leukemia virus reverse transcriptase (Superscript; GIBCO BRL). A combination of TCR Vβ degenerate primers as 5′-primers and TCR Cβ2 primer as 3′-primer were used to amplify the TCR variable region as described previously (Broeren *et al*, 1991). The PCR cycle profile was as follows: 95°C for 1 min; 55°C for 2 min; 72°C for 3 min. Amplification was performed over 40 cycles followed by a final 15-min extension period at 72°C. PCR products were cloned into pBluescript-SK (Stratagene) and sequenced using the T7 sequencing kit (Pharmacia, Uppsala, Sweden) with a Cβ2-specific primer: 5′-CCTTTTGGGTGTGG-GAGATC-3′.

RESULTS

Derivation of Autologous CTL Clones Recognizing Melanoma Cell Line LB265-MEL A 70-year-old caucasian woman, patient LB265, was examined in 1991 for a right axillary mass that, following excision, proved to be a metastatic lymph node of a melanoma. A few months later, the patient presented cervical and axillary metastatic lymph nodes, cutaneous metastatic nodules, as well as vertebral and lung metastases. In April 1993, melanoma cell line LB265-MEL was established from a cervical lymph node metastasis. This cell line expressed a high level of melanocyte-specific genes: tyrosinase, Melan-A/MART1, Pmel17/gp100, gp75/TRP1, and MSH-R but not MAGE, BAGE, or GAGE. Despite surgery, radiotherapy, and chemotherapy, patient LB265 died in November 1993.

Peripheral $CD8^+$ T cells were purified by sorting PBLs collected from patient LB265 in 1993 and were stimulated *in vitro* in the presence of IL-2, IL-7, and irradiated LB265-MEL tumor cells. The responder cells were restimulated every week by addition of irradiated tumor cells. After 3 weeks, we obtained lymphocytes that lysed the autologous melanoma cells but did not lyse the NK-sensitive K562 cell line. The lymphocytes were cloned by limiting dilution, and 55 stable CTL clones were derived from this mixed lymphocyte-tumor cultures. All clones were $CD8^+$ and showed specificity for the tumor cells, because they lysed LB265-MEL cells but not K562 cells. The lytic activity of two representative CTL clones (clones 296/107 and 296/142) is shown in **Fig 1**.

A Pmel17/gp100 Antigen Is Recognized By HLA-A2.1-Restricted CTL Clones Derived from Autologous PBLs The 55 CTL clones released TNF in the presence of LB265-MEL cells. We measured the amount of TNF released by each CTL clone in the presence of antibodies specific either for HLA class I, for HLA-B and -C, for HLA-A2, or for HLA-B7 in order to establish which HLA molecule was involved in the presentation of the respective antigens. In 46 out of 55 CTL clones, TNF production was significantly inhibited by anti-HLA class I and anti-HLA-A2 mAb, indicating that these 46 CTL clones recognized HLA-A2–restricted antigens **(Fig 2*A*)**.

To determine whether these antigens were encoded by melano-

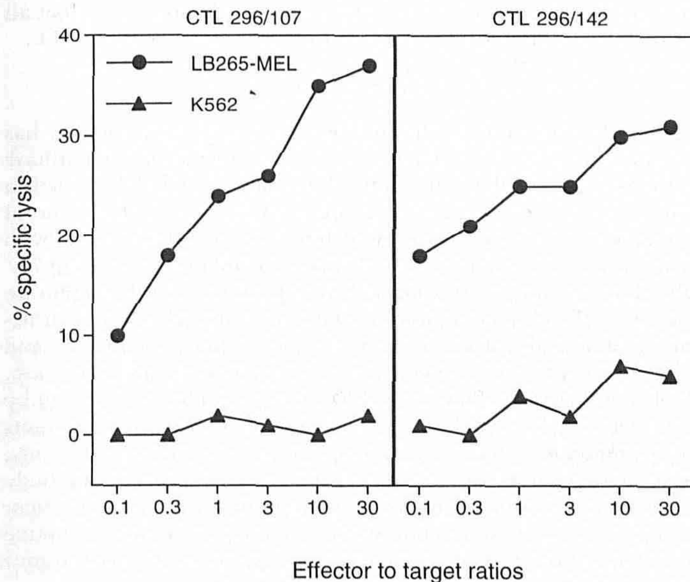


Figure 1. Anti-Pmel17/gp100 CTL clones 296/107 or 296/142 specifically lyse autologous melanoma cell line LB265-MEL. LB265-MEL cells were pretreated with 50 U/ml interferon-γ for 48 h prior to incubation with CTL clones 296/107 or 296/142 in a 4-h chromium release assay.

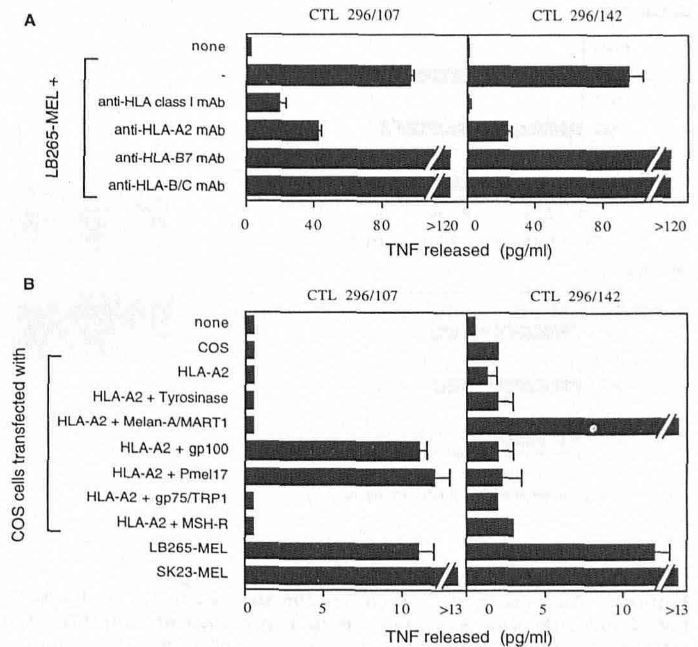


Figure 2. Autologous anti-LB265-MEL CTL clones recognize melanocyte-specific antigens presented by HLA-A2.1. *A*, stimulation of CTL clones 296/107 or 296/142 by the HLA-A2 melanoma cell line LB265-MEL in the presence of anti-HLA antibodies. Three thousand CTLs were co-incubated for 24 h with 30,000 LB265-MEL cells seeded in microwells and in the presence of IL-2 (25 U/ml) and the indicated monoclonal antibodies (ascitic fluid, diluted 1/30). TNF content of the supernatant of this coculture was measured as described under *Materials and Methods*. Shown is the mean and standard deviation calculated from triplicate cultures. CTL clones 296/107 and 296/142 are representative of 40 and 6 different clones, respectively. *B*, stimulation of CTL clone 296/107 or CTL clones 296/142 by COS-7 cells cotransfected with HLA-A2.1 and melanocyte-specific cDNAs. COS-7 transient transfections were performed as described under *Materials and Methods*. After 1 day, CTLs were added to the transfectants, and the TNF content of the supernatant was estimated 24 h later. Shown is the mean and standard deviation calculated from triplicate COS transfections. CTL clones 296/107 and 296/142 are representative of 40 and 6 different clones, respectively.

cyte-specific genes, we cotransfected COS cells with the HLA-A2.1 gene and cDNAs of either tyrosinase, melan-A/MART1, Pmel17, gp100, gp75/TRP1, or MSH-R. Each cDNA was transfected in triplicate COS microcultures. After 48 h, transfected cells were tested for their ability to induce TNF release by each of the 46 CTL clones. Out of 46 CTL clones, 6 produced a large amount of TNF in the presence of Melan-A/MART1–transfected COS cells (as illustrated in **Fig 2B** by CTL clone 296/142). The 40 remaining CTL clones recognized COS cells transfected by the Pmel17 or gp100 cDNAs (as illustrated in **Fig 2B** by CTL clone 296/107), which correspond to mRNA isoforms generated by alternative splicing from a single gene (Adema *et al*, 1994).

In order to confirm that these latter CTL clones recognized an antigen encoded by the Pmel17/gp100 gene, we tested their ability to release TNF specifically in the presence of HLA-A2 melanoma cell lines that do or do not express this gene as determined by northern blot analysis (**Fig 3**). The two HLA-A2 cell lines LB43-MEL and SK23-MEL, which express gene Pmel17/gp100, were recognized by these 40 HLA-A2–restricted CTL clones, while two other melanoma cell lines, SK29-MEL and NA8-MEL, which do not express gene Pmel17/gp100, were not recognized.

YLEPGPVTA Is the Antigenic Peptide Recognized by the Anti-Pmel17/gp100 Autologous CTL Clones Two distinct HLA-A2–restricted antigenic peptides, YLEPGPVTA (amino acids 280–288) (Cox *et al*, 1994) and LLDGTATLRL (amino acids 457–466) (Kawakami *et al*, 1994), encoded by Pmel17/gp100 gene were previously and independently identified as the antigens recognized by tumor infiltrating lymphocytes (TILs) derived from two melanoma patients. To determine whether these HLA-A2–restricted peptides were recognized by the anti-Pmel17/gp100 CTL clones, lysis of T2 cells, preincubated with 25 nM peptide YLEPGPVTA or up to 1 μM peptide LLDGTATLRL, was evaluated with the different CTL clones. The T2 cell line is an HLA-A2 cell line with an antigen-processing defect that results in increased capacity to present exogenous peptide (Cerundolo *et al*, 1990). Only nonamer YLEPGPVTA was able to sensitize pulsed T2 cells to lysis by the anti-Pmel17/gp100 CTL clones (as illustrated in **Fig 4A** for CTL clone 296/107).

The YLEPGPVTA peptide does not possess the dominant HLA-A2–anchoring residue in position nine, even though it is efficiently recognized by anti-Pmel17/gp100 CTL clones. Because it has been reported that the majority of peptides binding to HLA-A2 are nonamers carrying valine, threonine, leucine, or isoleucine in the ninth position (Falk *et al*, 1991), we decided to test peptides with amino acid substitutions in position nine, YLEPGPVTV and YLEPGPVTL, for their ability to sensitize pulsed T2 cells to lysis by the anti-Pmel17/gp100 CTL clones. This experiment, reported in **Fig 4B**, clearly demonstrated that T2 cells incubated with either the original peptide or the substitute peptides were lysed in the same way by CTL clone 296/107.

Identical TCRβ Hypervariable Regions Are Expressed by the Anti-Pmel17/gp100 CTL Clones To determine whether all the anti-Pmel17/gp100 CTL clones were identical, we analyzed the TCRβ hypervariable region. mRNA extraction and PCR using degenerate primers allowed us to amplify the CDR3-like region of 15 anti-Pmel17/gp100 CTL clones including CTL clone 296/107. This region, which corresponds to the hypervariable CDR3-like loops encoded by the VDJ junctions, has been postulated to be directly involved in antigenic peptide binding (Chothia *et al*, 1988; Claverie *et al*, 1989). The PCR products were cloned and sequenced. Each of the 15 anti-Pmel17 CTL clones expressed the same TCRβ chain (**Fig 5**). The sequence of the TCRβ CDR3-like region, as defined by Chothia *et al* (1988), involved the Vβ3.3 and

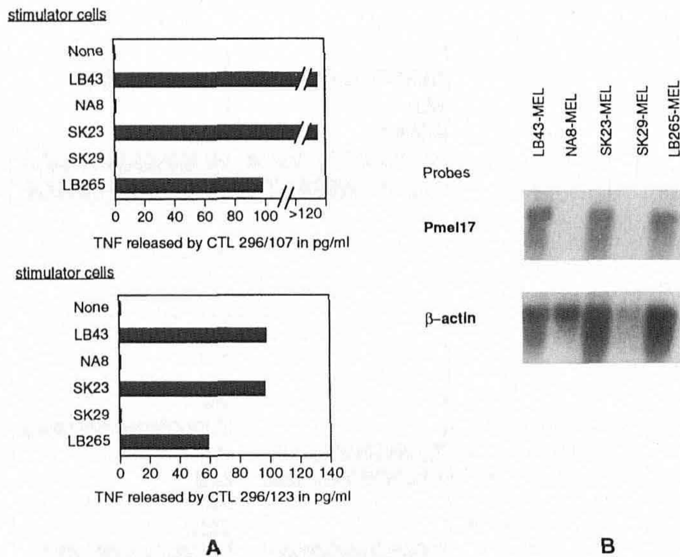


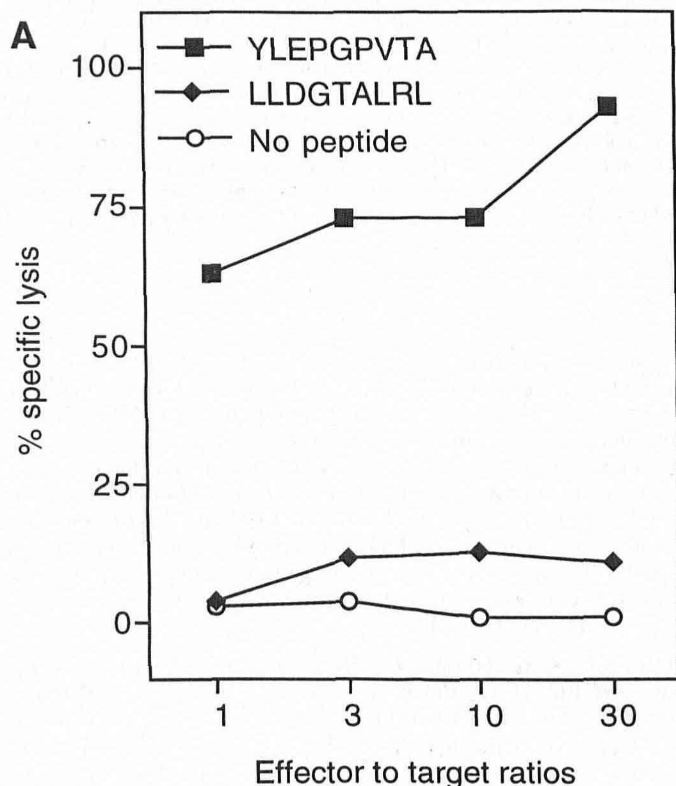
Figure 3. Activation of CTL clones 296/107 or 296/123 by HLA-A2 melanoma cell lines correlates with expression of Pmel17/gp100 mRNA. *A*, TNF production by CTL clones 296/107 or 296/123 stimulated in the presence of five different HLA-A2 melanoma cell lines. Shown is the mean of triplicate cultures that varied from the mean by less than 10%. *B*, northern blot analysis of Pmel17/gp100 in the same melanoma cell lines. Each lane contained 5 μg of poly(A+) RNA. After blotting, the membrane was hybridized with a probe containing the Pmel17/gp100 coding sequence (nucleotides 1–2039) and washed under stringent conditions. Control hybridization was performed on the same membrane with a β-actin probe.

the Jβ2.7 segments. This experiment thereby demonstrated that all of these CTL clones were derived from the same parental CTL.

DISCUSSION

Among human cancers, the immune repertoire to melanoma has been the most widely studied. Analysis of anti-melanoma immune responses provided strong indication that normal differentiation antigens on cancer cells are recognized by the host. Humoral immune responses against these differentiation antigens have been demonstrated by the presence of IgG autoantibodies against gp75/TRP1 antigen (Vijayasaradhi *et al*, 1990). More recently, extensive studies of T-cell recognition of human cancers led to the identification of several antigens expressed in melanoma, melanocytes, and pigmented retinal cells but not in other normal tissues: tyrosinase, Melan-A/MART1, Pmel17/gp100, and gp75/TRP1 (reviewed by Van Pel *et al*, 1995). The role of these antigens as potential targets of the immune system was assessed mainly by *in vitro* experiments, and there is not yet strong evidence that T-cell or antibody responses against these antigens lead to immune rejection of cancer *in vivo*, although some clinical observations made after adoptive transfer of TILs suggest a potential role for these antigens as tumor rejection antigens (Kawakami *et al*, 1994).

The peptide YLEPGPVTA, recognized by the PBL-derived CTL clones of patient LB265, was initially identified using biochemical techniques and nonautologous TILs (Cox *et al*, 1994). Here, by investigating the presence of autologous CTL clones directed against melanocyte-specific antigens in the peripheral blood of the HLA-A2 melanoma patient LB265, we obtained a large number of anti-Pmel17/gp100 CTL clones recognizing this peptide. Our data suggest that a strong T-cell response occurred in the peripheral



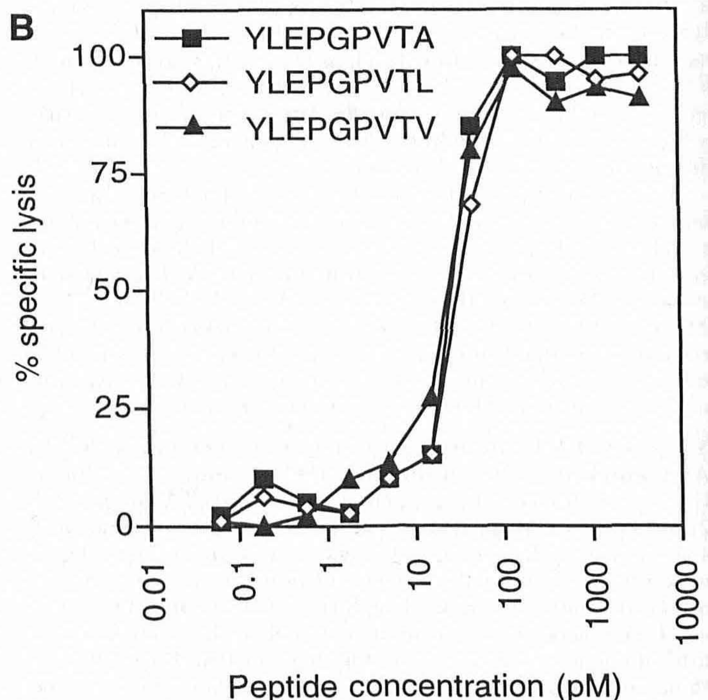


Figure 4. CTL clone 296/107 recognizes peptide YLEPGPVTA from Pmel17/gp100. *A*, lysis by CTL clone 296/107 of T2 target cells incubated with peptides YLEPGPVTA or LLDGTATLRL from Pmel17/gp100. One thousand ^{51}Cr-labeled T2 cells were pulsed with 25 nM peptide YLEPGPVTA or 1 μM peptide LLDGTATLRL and incubated in the presence CTL clones 296/107 at an E/T ratio of 10:1. Chromium release was measured after 4 h. T2 cells alone and LB265-MEL melanoma cells were also tested as controls. Similar results were obtained with 15 other Pmel17/gp100-specific clones. *B*, recognition by CTL clones 296/107 of T2 cells incubated with peptide YLEPGPVTA or two peptides substituted in position nine. One thousand ^{51}Cr-labeled T2 target cells were incubated in 96-well microplates in the presence of various concentrations of peptide for 30 min at 37°C. Each CTL clone was then added at an E/T ratio of 10:1. Chromium release was measured after 4 h at 37°C. Similar results were obtained with three other Pmel17/gp100-specific clones.

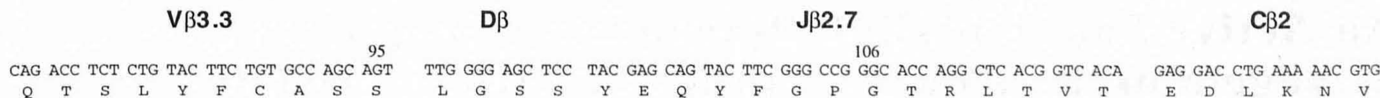


Figure 5. TCRβ junctional nucleotide and amino acid sequences of Pmel17/gp100-specific CTL clones recognizing the nonamer YLEPGPVTA. TCRβ mRNA was reversed transcribed and amplified using TCR Vβ degenerate primers and TCR Cβ2 primers. The indicated sequence (GenBank accession number U46029), involving Vβ3.3 and Jβ2.7 segments, was found in 15 Pmel17/gp100-specific CTL clones tested. The sequence of the CDR3-like region as defined by Chothia *et al* (1988) encompass residues 95 to 106.

blood of the melanoma patient and/or could have been expanded *in vitro*. The determination of the actual intensity of the anti-Pmel17/gp100 T-cell response of patient LB265, however, would require a more careful analysis of the CTL precursor frequency for peptide YLEPGPVTA in the blood of the patient and comparison with other melanoma or normal patients. With regard to this, it should be stressed that, despite the presence of circulating anti-melan-A and anti-Pmel17 CTLs, patient LB265 did not eventually reject her tumor. It is not known, however, whether this failure was due to a subsequent loss of these differentiation antigens by tumor cells, or to a lack of efficacy of the anti-tumor CTL response *in vivo*.

The sequence of the TCRβ CDR3-like region of 15 anti-Pmel17/gp100 CTL clones was determined and found to involve the Vβ3.3 and Jβ2.7 gene segments. This should enable comparison with the TCR region of others' anti-Pmel17 PBL-derived CTL or TILs. This observation might contribute to determine the potential association of certain TCR V regions with recognition of well-defined tumor antigens, especially melanocyte-lineage antigens, as previously suggested (Sensi *et al*, 1995). Such data may be useful in antimelanoma immunotherapy, as it could lead to several clinical applications such as adoptive transfer in melanoma patients of $CD8^+$ T cells selected on the basis of specific TCR V gene expression, or analysis of *in vivo* evolution of the specific T-cell response directed against well-defined tumor antigens.

In conclusion, our results demonstrate the existence of CTL clones recognizing the same Pmel17/gp100 peptide YLEPGPVTA in the peripheral blood of an HLA-A2.1 melanoma patient whose tumor expressed high levels of melanocyte-specific genes. The potential utility of this antigen is emphasized by objective regressions of melanoma metastases after adoptive transfer of TILs populations, which were shown retrospectively to include anti-Pmel17/gp100 CTLs (Kawakami *et al*, 1994). Moreover, association of vitiligo with prolonged survival and spontaneous regression of melanoma, although not observed in patient LB265, supports the role of the differentiation antigens as potentially effective targets for immune responses leading to tumor rejection *in vivo* (Bystryn *et al*, 1987).

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