Identification of a Promiscuous T-Cell Epitope Encoded by Multiple Members of the MAGE Family

Silvia Tanzarella, Vincenzo Russo, Ilaria Lionello, Piero Dalbera, Donata Rigatti, Claudio Bordignon, and Catia Traversari

Telethon Institute of Gene Therapy and Cancer Immunotherapy and Gene Therapy Program, Istituto Scientifico H. S. Raffaele, 20132 Milano, Italy

ABSTRACT

One of the major limitations of tumor-specific vaccination is the generation of antigen-loss variants that are able to escape the immune response elicited by a monoa nogenic peptide epitope. Here, we report the identification of a new HLA-B*3701-restricted epitope shared by four different members of the MAGE family. Peripheral blood lymphocytes isolated from a melanoma patient were stimulated in vitro with the autologous HLA-negative melanoma line transfected with autologous HLA B*3701 molecule. This protocol led to the induction of tumor-specific, B*3701-restricted CTLs specific for a peptide epitope encoded by codons 127–136 of the gene MAGE-1. The same epitope is also encoded by the homologous region of three other members of the MAGE family, MAGE-2, -3, and -6. Consistent with the notion that the peptide encoded by MAGE-1 codons 127–136 is, indeed, processed from the proteins encoded by all four MAGE family members, the CTLs were able to specifically recognize Cos-7 cells cotransfected with HLA-B*3701 and any of these MAGE genes. Moreover, the CTLs also recognized a MAGE-6-positive melanoma line transfected with the B*3701 molecule. These findings allow the inclusion of a new set of tumor patients into clinical cancer vaccination trials. Furthermore, they suggest that some promiscuous peptide epitopes shared by different members of the MAGE family might be less prone to escape the immune response by generation of MAGE antigen loss variants.

INTRODUCTION

A number of studies have demonstrated that CTLs recognizing human tumor cells can be isolated from PBLs and tumor-infiltrating lymphocytes of cancer patients (1). An in vivo role of such tumor-specific effectors is suggested by the results of several immunotherapy trials, mainly performed in melanoma patients (2–5).

Over the last few years, the use of such tumor-specific effectors has allowed the isolation of several genes encoding tumor antigens (6). According to the pattern of expression in neoplastic and normal tissues, these antigens can be classified into four classes, which have different degrees of tumor specificity and clinical relevance. The first class comprises antigens encoded by genes expressed in various tumors of different histotypes but not in normal tissues, other than testis and placenta, such as MAGE, GAGE, and BAGE (7–9).

The second class represents differentiation antigens that are only expressed in melanoma and melanocytomas, such as tyrosinase, Melan-A/MART-1, gp100, TRP-1, and TRP-2 (10–15). The antigens belonging to the third class are generated by point mutations in genes that are ubiquitously expressed (16–18). The fourth class of antigens, which has been defined only recently, is represented by TRP-2-INT2, an antigen shared between melanomas but not expressed in normal cells of the melanocytic lineage (19).

Tumor antigens belonging to the MAGE family have especially elicited considerable interest because six of them, MAGE-1, -2, -3, -4, -6, and -12, are expressed selectively by a significant proportion of primary and metastatic tumors, including melanomas, lung, bladder, ovarian, and breast carcinomas (7, 20–23).

Clinical trials of vaccination, based on the use of antigenic peptides encoded by MAGE-1 and -3, are in progress in patients affected by melanoma and other neoplastic diseases (3). Nevertheless, the use of MAGE-2, -4, and -6 proteins as targets for tumor-specific immunotherapy has been hampered by the uncertainty as to whether these proteins can be recognized by specific CTLs of the immune system.

Potential limitations to the wide application of this therapeutic approach are the limited number of characterized CTL epitopes (i.e., tumor antigen peptides and appropriate HLA class I alleles) and the in vivo generation of antigen loss variants that are able to escape the immune response elicited by a monoa nogenic vaccine (24). Indeed, such clinical protocols apply only to patients carrying a tumor expressing a well-known tumor antigen and a defined HLA allele. Unfortunately, a large majority of cancer patients do not fulfill these including criteria. Therefore, identification of new antigenic determinants is a priority because it would increase the number of patients that could benefit from antitumor vaccination protocols.

Here, we report the identification of a new HLA-B*3701-restricted epitope, encoded by homologous regions of the MAGE-1, -2, -3, and -6 genes. The use of identical antigenic peptides derived from different highly homologous proteins, in protocols of vaccination, may represent a useful tool to avoid the generation of antigen loss variants. Moreover, this study presents, for the first time, evidence for the existence of human CTL recognizing peptides derived from MAGE-2 and -6 proteins, which now can be included in the list of possible antigens for targeted immunotherapy of neoplastic disorders.

MATERIALS AND METHODS

Cell Lines. The melanoma cell line MSR3-mel was established in our laboratory from a metastatic lesion of patient MSR3 and cultured in IMDM supplemented with 10% FCS. PBLs of this patient were serologically typed as: HLA-A1, A11, B37, B5, Cw6. The melanoma line MZ2-MEL.2.2 ET.1 (HLA-A1, B*3701, Cw6), hereafter referred to as ET.1, and the Cos-7 cell line were kindly provided by Prof. T. Boon (Ludwig Institute for Cancer Research, Brussels, Belgium) and maintained in DMEM supplemented with 10% FCS. Me14932 (HLA-A2/A3, B7/Blw50, Cw6/Cw7) was a kind gift of Dr. G. Parmiani (Istituto dei Tumori, Milano, Italy). The B-lymphoblastoid cell line LG2-EBV was kindly provided by Prof. T. Boon, whereas the MSR3-EBV was derived by transformation of peripheral blood B lymphocytes from patient MSR3 with the B95-8 strain of EBV.

Synthetic Peptides. Synthetic peptides were purchased from Primm (Milano, Italy). Peptides were: MAGE127–136 (REPVTKAEML), encoded by codons 127–136 of MAGE-1, -2, -3, and -6 genes; and M4127–136 (KELVTGAEML) and M12127–136 (REPFTKAEML), corresponding to amino acids 127–136 encoded by genes MAGE-4 and -12, respectively. Peptides were dissolved to 10 mM in DMSO and diluted further in 0.9% NaCl.

Subcloning of the HLA-B*3701 Allele. Total RNA was prepared from MSR3 PBLs by the RNeasy® Total RNA Kit (Qiagen, Hilden, Germany), cDNA corresponding to 300 ng of total RNA was amplified by PCR using a primer pair suitable for specific amplification and directional cloning of the full-length

Received 11/30/98; accepted 4/1/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by grants from the Italian Association for Cancer Research (Milan, Italy).

2 To whom requests for reprints should be addressed, at Telethon Institute of Gene Therapy, Istituto Scientifico H. S. Raffaele, Via Olgettina 58, 20132 Milano, Italy. Phone: 39-2-26432357; Fax: 39-2-26434827; E-mail: trava@tigem.it.

3 The abbreviations used are: PBL, peripheral blood lymphocyte; IMDM, Iscove's Modification of Dulbecco's medium; mAbs, monoclonal antibody; IL, interleukin; LNGFR, low-affinity nerve growth factor receptor; β2-m, β2-microglobulin.
coding region of HLA-B alleles (generous gift of Dr. Soo Young Yang, Memorial Sloan-Kettering Cancer Center, New York, NY). The 1.1-kb PCR product was subcloned into the eukaryotic expression vector pcDNA3.1 (Invitrogen Corp., Oxon, United Kingdom). Plasmid clones encoding HLA-B*3701 and B*52011 (the HLA-B*37 and B*5 alleles of patient MSR3) were identified using diagnostic restriction enzymes. The HLA-B*3701 gene was then sequenced to verify the correspondence to the published DNA sequence. This plasmid was called pcDNA3.1/B*3701.

**Transfection of Melanoma Cell Lines.** Melanoma cell lines were transfected by the calcium phosphate precipitation technique with pcDNA3.1/HLA-B*3701 and selected in G418. Expression of the transfected HLA-B*3701 molecule in stable transfectants was verified by flow cytometry with the HLA-A-, HLA-B-, and HLA-C-specific mAb W6/32.

**In Vitro Induction of CTL Line 337.** CTL line 337 was obtained using a protocol previously described by others (25), with slight modifications. Briefly, PBLs from patient MSR3 were separated by Ficoll gradient and cultivated (1 × 10^5–2 × 10^6 cells/well) with the autologous, irradiated MSR3-B37 melanoma cells (0.5 × 10^5–1 × 10^6 cells/well) in 2 ml of IMDM supplemented with 10% human serum, glutamine, and antibiotics. After 3 days of culture, 10 units/ml IL-2 (Chiron, Milan, Italy) and 5 ng/ml IL-7 (Genzyme Corp., Cambridge, MA) were added. Lymphocytes were restimulated weekly with 0.5 × 10^5 irradiated MSR3-B17 cells and tested in a cytotoxicity assay after three stimulations. After the fifth restimulation, 2 × 10^5 irradiated LG2-EBV cells were added as feeder cells, and IL-2 was increased to 50 units/ml.

**Assay for Cytolytic Activity and Peptide Binding Studies.** Lytic activity of the cytotoxic T-cell lines was tested in a chromium release assay as described previously (26). Peptides were tested in chromium release assays: ^3H-Cr-labeled target cells were incubated for 1 h at room temperature in 96-well microplates with various concentrations of the peptide before addition of effector cells at a fixed E:T ratio. Binding of peptides M4127–136 and M1212–136 to the HLA-B*3701 molecule was studied in a competition assay, as described previously (27). As standard peptide, we used peptide MAGE127–136 (300 nm), recognized by CTL 337. CTLs were used at an E:T ratio of 30:1.

**Production of Subfragments of MAGE-1.** Subfragments of MAGE-1 gene (495- and 1072-bp fragments) were obtained by digestion of MAGE-1 cDNA with BglII and EcoRI. After purification on agarose gel, the fragments were cloned into the pcDNA3.1 plasmid. Clones were isolated, plasmid DNA was extracted and transfected into Cos-7 cells along with the HLA-A*0201 gene.

**Transfection of Cos-7 Cells and IFN-γ Release Assay.** Transfection of Cos-7 cells was performed by the DEAE-dextran-chloroquine method (12). Briefly, 1.5 × 10^5 Cos-7 cells were transfected with 100 ng of plasmid pcDNA3.1/B*3701 and 100 ng of expression vectors containing the cDNA of one of the following genes: MAGE-1, -2, -3, -4, -6, and -12. Transfected Cos-7 cells were tested in an IFN-γ assay after 48 h: 5000 responder CTLs, at day 5 after stimulation, were added in 150 μl of IMDM-10% human serum supplemented with 25 units/ml IL-2. After 24 h at 37°C, 100 μl of supernatant were harvested, and the IFN-γ concentration was measured using a IFN-γ release kit (Genzyme Corp.) according to the manufacturer’s recommendations.

**Retroviral Vector-mediated Gene Transfer of HLA-B*3701 into Me14932.** The retroviral vector B37-CSM, coding for the HLA-B*3701 molecule of patient MSR3, was constructed as described previously (28). Briefly, PBLs from patient MSR3 were separated by Ficoll gradient and cultivated (1 × 10^5–2 × 10^6 cells/well) with the autologous, irradiated MSR3-B37 melanoma cells (0.5 × 10^5–1 × 10^6 cells/well) in 2 ml of IMDM supplemented with 10% human serum, glutamine, and antibiotics. After 3 days of culture, 10 units/ml IL-2 (Chiron, Milan, Italy) and 5 ng/ml IL-7 (Genzyme Corp., Cambridge, MA) were added. Lymphocytes were restimulated weekly with 0.5 × 10^5 irradiated MSR3-B17 cells and tested in a cytotoxicity assay after three stimulations. After the fifth restimulation, 2 × 10^5 irradiated LG2-EBV cells were added as feeder cells, and IL-2 was increased to 50 units/ml.

**RT-PCR Assays.** MAGE-1, -2, -3, -4, -6, and -12 and β2-m cDNAs were detected by PCR amplification. Reaction mixture contained 5 μl of cDNA suspension, 4 μl of a 10 mM dNTPs mixture (containing each dNTP at 2.5 mM), 5 μl of 10× DNA polymerase buffer (Finnzymes Oy, Espoo, Finland), 2 units of DynaZyme DNA polymerase (Finnzymes Oy), and sterile distilled water up to a 50-μl total reaction volume. For oligonucleotide primer sequences and PCR amplification programs, see Weynants et al. (Ref. 20; MAGE-1, -2, -3, and -6) and De Plaen et al. (Ref. 29; MAGE-4, -6, and -12). β2m cDNA was amplified using the sense primer β 5′ (5′-AAC CAC GTG ACT TTG TCA CAG C-3′) and antisense primer β 5′ (5′-CTG CTC AGA TAC TAT AAC CAT G-3′). PCR amplification was performed for 30 cycles (1 min at 94°C, 30 s at 56°C, and 2 min at 72°C); the expected length of β2m amplification product was 230 bp. RNA integrity was tested by PCR with β-actin-specific oligonucleotide primers (30). Samples scored positive when a band of the appropriate size was visible on a agarose gel in the presence of ethidium bromide.

**RESULTS**

**MRS3-B37 Induces an Antigen-specific Immune Response.** The melanoma line MSR3 was established from a cutaneous metastasis resected from patient MSR3. Expression of HLA class I alleles by the tumor cells was barely detectable (Fig. 1) and appeared to be inadequate to allow antigen presentation to immune effectors. Indeed, the MSR3 melanoma line failed to induce a cytotoxic response from autologous PBLs (data not shown). The lack of class I cell surface expression by MSR3-mel was not caused by impaired β2m synthesis because a β2m-specific mRNA was detected by RT-PCR analysis (data not shown).

To determine whether HLA class I antigen expression could be restored, MSR3-mel cells were stably transfected with cDNA encoding the autologous HLA-B*3701 molecule. After G418 selection flow cytometric analysis showed staining of the transfected MSR3-B37 cell line by the W6/32 mAb (Fig. 1).

To evaluate the presence on the surface of MSR3-B37 line of tumor-specific antigens, the melanoma cells were tested for their ability to induce tumor-specific cytotoxic effectors and for their susceptibility to lysis by these CTLs. Patient’s PBLs were in vitro stimulated by MSR3-B37 as described in “Materials and Methods.” After three rounds of stimulation, the polyclonal cytotoxic T cell line 337 (CTL 337) specifically lysed the MSR3-B37 cell line but not the untransfected MSR3-mel (Fig. 2). Autologous MSR3-EBV cells and PHA-activated T blasts were not recognized (data not shown), suggesting that the epitopes recognized by these CTLs are melanoma-/melanocyte-specific. Indeed, in addition to the autologous melanoma cells, CTL 337 also lysed the HLA-B*3701-positive melanoma line ET1 (Fig. 2), suggesting that one or more shared melanoma antigens are recognized.

These data indicate that HLA class I expression can be restored by transfection of MSR3 melanoma cells and that the melanoma line transfected with the HLA-B*3701 molecule is able to induce a tumor-specific cytotoxic T-cell response.

**Identification of the Antigenic Epitope Recognized by CTL 337.** To identify the antigen recognized by CTL 337, we evaluated the IFN-γ release of CTL 337 in the presence of Cos-7 cells transfected with plasmid pcDNA3.1/B*3701, along with cDNA encoding six members of the MAGE family (i.e., MAGE-1, -2, -3, -4, -6, and -12), some of which are expressed by both MSR3-mel and ET1. CTL 337 specifically recognized Cos-7 cells transfected with MAGE-1, -2, -3, and -6 (Fig. 3), suggesting that the epitope target of CTL 337 was shared among the four different antigens or that distinct components of the oligoclonal T-cell line were recognizing peptides derived from the four MAGE gene products. A low level of IFN-γ was detected in
peptide, denominated MAGE-127–136, was used to sensitize the MSR3-EBV line to lysis by CTL 337 cells in a titration assay (Fig. 6A). The half-maximal lysis was reached with 90 nm peptide. No lysis of MSR3-EBV pulsed with an unrelated peptide that was able to bind to HLA-B*3701 was observed (Fig. 6B and data not shown).

Low levels of IFN-γ were released by the CTL 337 cells in the presence of Cos-7 cells expressing MAGE-4 and -12 (Fig. 3). To verify whether this release could be ascribed to recognition of peptides encoded by codons 127–136 within MAGE-4 and MAGE-12, a peptide-binding study was performed, using MSR3-EBV cells pulsed with the two peptides as targets. Peptide M4-127–136, KELVTKAEML, differs by two amino acids (lysine versus arginine in position 1 and leucine versus proline in position 3) from peptide REPVTKAEML, whereas peptide M12-127–136, REPFTKAEML, differs by only one amino acid (phenyalanine versus valine in position 4). The results revealed that the two peptides can bind to HLA-B*3701 because increasing amounts of both were able to inhibit the lysis of MSR3-EBV pulsed with peptide REPVTKAEML but not with an unrelated HLA-A1-binding peptide (i.e., M3.271–279; Fig. 6B). However, no recognition of EBV cells pulsed with peptides M4-127–136 and M12-127–136 was observed (data not shown).

Taken together, these data indicate that CTL 337 cells are able to recognize a peptide endogenously processed from MAGE-1, -2, -3, and -6 products. The two peptides, encoded by the same region of MAGE-4 and -12, respectively, are able to bind to HLA-B*3701, but they are not recognized by CTL 337 cells.

**CTL 337 Cells Specifically Recognize MAGE-2 and -6 Gene Products.** Until now, there was no evidence of the immunogenicity of MAGE-2 and MAGE-6-encoded proteins in humans. Indeed, peptides encoded by MAGE-1, -3, -4, and -12 have been found to bind to various class I molecules to form antigens recognized by different CTLs, whereas no peptides encoded by the genes MAGE-2 or MAGE-6 have thus far been identified.

To demonstrate that peptide REPVTKAEML could also be processed from MAGE-2 and -6 and presented to CTL 337 cells, we attempted to look for melanoma cell lines expressing MAGE-2 or -6 but none of the other MAGE genes. Unfortunately, expression of the MAGE genes in melanomas is strictly correlated: most of the melanomas that express one member of the MAGE gene family also express the others. Indeed, we were unable to find a melanoma line

---

**Fig. 1.** Expression of HLA-class I molecules by MSR3-mel and MSR3-B37. Tumor cells were incubated with mAb W6/32 (anti-HLA-class I) or with an isotype control, washed, and labeled with goat antimouse immunoglobulin antibodies coupled to fluorescein. The analysis was performed before and after HLA-B*3701 transfection of MSR3-mel.

**Fig. 2.** Recognition of a shared HLA-B*3701-restricted antigen by CTL 337. The cytotoxic activity of CTL 337 cells was assessed against the autologous melanomas MSR3-mel and MSR3-B37 and against the allogenic melanoma ET1 at various E/T ratios.

**Fig. 3.** Identification of the tumor-antigens recognized by CTL 337. Cos-7 cells were cotransfected with HLA-B*3701, alone or together with cDNAs encoding genes MAGE-1, -2, -3, -6, and -12. After 48 h, CTL 337 cells were added, and the IFN-γ release was measured 24 h later, as described in “Materials and Methods.” MSR3-B37 was included as positive control.

---

SPECIFIC CTL RECOGNITION OF A SHARED MAGE-ENCODED PEPTIDE
that selectively expresses MAGE-2, but we succeeded in finding a single melanoma line, Me14932, that selectively expresses MAGE-6 at low level (data not shown).

To verify whether peptide REPVTKAEML is endogenously processed from MAGE-6 products and presented by HLA-B*3701, Me14932 was transduced by a retroviral vector encoding the HLA-B*3701 molecule. As indicated by immunofluorescence staining with a HLA-Bw4-specific mAb, cell surface expression of HLA-B*3701 on a pure population of transduced Me14932 cells was at least 2-fold lower than that of MSR3-B37 melanoma cells (data not shown). CTL 337 cells were able to recognize the Me14932-LB37 line in a cytotoxicity assay, and the level of lysis was increased by the exogenous addition of peptide REPVTKAEML, whereas there was no recognition of the pulsed and unpulsed Me14932 lines (Fig. 7). The low levels of lysis of the melanoma Me14932-LB37 might be explained either by weak expression of gene MAGE-6 and by the weak surface expression of HLA-B*3701 molecules.

To evaluate whether the inclusion of MAGE-2 and -6 in the list of possible target antigens for specific immunotherapy could increase the proportion of eligible patients, we analyzed the expression of MAGE-1, -2, -3, and -6 in fresh tumor samples of various histotypes. Melanomas were not analyzed because expression of the different MAGE genes was clearly correlated (32). The results indicate that 12% of the ovarian carcinomas and 5% of colon and breast carcinomas express MAGE-2 and/or -6 in the absence of MAGE-1 and -3 (Table 1). On the other hand, in all bladder and lung carcinomas studied the four genes were always coexpressed.

In conclusion, the data reported in this study indicate that MAGE-2 and -6 can be included in the list of possible target antigens for
tumor-specific immunotherapy, increasing the number of patients that could benefit from this therapy.

**DISCUSSION**

In the last few years, there has been a considerable effort to characterize antigenic peptides encoded by tumor-associated antigens and the HLA molecules responsible for their presentation (6). Several immunotherapy clinical trials of cancer vaccinations based on the use of these peptides are in progress, with quite positive preliminary results. Indeed, some objective cancer responses have been observed, consisting of both tumor regression (3, 5) and a few long-term complete responses (4).

One major limitation outlined by those studies is the development, in a significant proportion of the treated patients, of tumor variants that fail to express the antigen recognized by tumor-reactive lymphocytes (24). Those variants can be generated either by loss of the nominal antigen (referred to as antigen loss variants; Refs. 33 and 34) or by molecular defects affecting different steps of the antigen presentation pathway (referred to as presentation loss variants; Refs. 35–37). An active intervention of the immune system in the selection of antigen loss and presentation loss variants have been observed in both treated (24) and untreated patients (33, 38). However, escape from classical tumor-specific CTLs may be counteracted in vivo by the intervention of different immune effectors. Indeed, tumor cells that have lost expression of some but not all HLA class I molecules can be recognized by a new category of antitumor lymphocytes expressing killer-cell inhibitory receptors (39), whereas HLA-negative tumor cells can be targeted by NK cells.

The melanoma cell line used in this study belongs to the presentation loss variant class of HLA-negative tumor cells. The molecular defect responsible for the HLA class I phenotypes of MSR3-mel has not yet been identified; however, our melanoma line exhibits barely detectable levels of HLA class I expression by immunofluorescence analysis, which are not sufficient for stimulation of a tumor-specific T-cell response (data not shown). This altered phenotype does not seem to be due to β2m or TAP alterations or to deletions of MHC genes but rather to a defect in the transcriptional machinery. Indeed, HLA class I expression in MSR3-mel can be restored by transfection of cDNAs encoding autologous HLA class I alleles.

The HLA-B*3701-transfected cell line (i.e., MSR3-B37) allowed the isolation of HLA-B*3701-restricted and tumor-specific CTLs that recognized a nonapeptide encoded by the same region (i.e., residues 127–136) of MAGE-1, -2, -3, and -6 proteins.

---

**Table 1** Expression of MAGE genes by fresh tumor samples

<table>
<thead>
<tr>
<th>Histological type</th>
<th>MAGE-1</th>
<th>MAGE-3</th>
<th>MAGE-2</th>
<th>MAGE-6</th>
<th>MAGE-2 or MAGE-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung carcinoma (28)</td>
<td>35</td>
<td>39</td>
<td>32</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Breast carcinoma (20)</td>
<td>30</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Ovary carcinoma (25)</td>
<td>24</td>
<td>20</td>
<td>32</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Bladder carcinoma (25)</td>
<td>28</td>
<td>28</td>
<td>20</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Colon carcinoma (17)</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

- **MAGE-1** 35%
- **MAGE-2** 39%
- **MAGE-3** 32%
- **MAGE-6** 29%
- **MAGE-2 or MAGE-6** 0%

a As determined by reverse transcription-PCR (RT-PCR) analysis.

b Numbers in parentheses represent numbers of fresh tumor samples analyzed.

---

**Fig. 6.** A, recognition of the peptide MAGE127–136 by CTL 337 cells. MSR3-EBV cells were incubated with 3-fold dilutions of peptide MAGE127–136 starting from 10 μM and used as target cells in a standard cytotoxicity assay. The E:T ratio was fixed at 10:1. B, binding of peptides M4127–136 and M12127–136 to HLA-B*3701, evaluated in a competition assay. Competitor peptides included the M4127–136 peptide KELVTKAEM and the M12127–136 peptide REPFTKAEM. The M3.A1 (i.e., M3271–279) peptide, which was unable to bind to the HLA-B*3701 molecule, was used as negative control. Percentage lysis without competitor peptides was 52%.

**Fig. 7.** Recognition of a MAGE-6-positive melanoma cell line by CTL 337. The HLA-B*3701-negative line Me14932 and the HLA-B*3701-positive line Me14932-LB37 were pulsed or not with 16 μM peptide MAGE127–136 and used as target cells in a standard cytotoxicity assay at the indicated E:T ratios.

---

**Fig. 6 A** - Recognition of the peptide MAGE127–136 by CTL 337 cells. MSR3-EBV cells were incubated with 3-fold dilutions of peptide MAGE127–136 starting from 10 μM and used as target cells in a standard cytotoxicity assay. The E:T ratio was fixed at 10:1. **B** - Binding of peptides M4127–136 and M12127–136 to HLA-B*3701, evaluated in a competition assay. Competitor peptides included the M4127–136 peptide KELVTKAEM and the M12127–136 Peptide REPFTKAEM. The M3.A1 (i.e., M3271–279) peptide, which was unable to bind to the HLA-B*3701 molecule, was used as negative control. Percentage lysis without competitor peptides was 52%.

---

**Fig. 7** - Recognition of a MAGE-6-positive melanoma cell line by CTL 337. The HLA-B*3701-negative line Me14932 and the HLA-B*3701-positive line Me14932-LB37 were pulsed or not with 16 μM peptide MAGE127–136 and used as target cells in a standard cytotoxicity assay at the indicated E:T ratios.
To our knowledge, this is the first B*3701-restricted tumor-specific epitope that has been identified thus far. Note that the HLA-B*3701 molecule is present on both lymphocytes and tumor cells of patient MZ2 (7), from which a large variety of MAGE-specific CTL clones restricted by different HLA class I molecules were isolated (8–9, 40–42). Those results suggest a subdominant role of HLA-B*3701 in the MSR3 system by the absence on the stimulating cells of a HLA class I molecule other than HLA-B*3701. Indeed, dominance of a specific epitope not only increases the number of patients eligible for immunization but also may prove highly efficient for immunotherapy because of reduced risk of tumor escape due to the occurrence of antigen loss variants.

ACKNOWLEDGMENTS

We are grateful to Dr. Katharina Fleischhauer for useful discussions.

REFERENCES

SPECIFIC CTL RECOGNITION OF A SHARED MAGE-ENCODED PEPTIDE


Identification of a Promiscuous T-Cell Epitope Encoded by Multiple Members of the MAGE Family

Silvia Tanzarella, Vincenzo Russo, Ilaria Lionello, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/11/2668

Cited articles
This article cites 47 articles, 18 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/11/2668.full#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/11/2668.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link:
http://cancerres.aacrjournals.org/content/59/11/2668.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.