

A HLA-Cw*0701 restricted Melan-A/MART1 epitope presented by melanoma tumor cells to CD8+ tumor infiltrating lymphocytes

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Abstract Melan-A/MART1 is a melanocytic differentiation antigen recognized on melanoma tumor cells by CD8+ and CD4+ T cells. In this study, we describe a new epitope of this protein recognized in the context of HLA-Cw*0701 molecules by a CD8+ tumor infiltrating lymphocyte (TIL) clone. This CD8+ TIL clone specifically recognized and killed a fraction of melanoma cells lines expressing Melan-A/MART1 and HLA-Cw*0701. We further show that the Melan-A/MART1_{51–61} peptide is the optimal peptide recognized by this clone. Together, these data significantly enlarge the fraction of melanoma patients susceptible to benefit from a Melan-A/MART1 vaccine approach.

Keywords CD8+ T cells · Cancer · Melanoma · Melan-A/MART1 · HLA-Cw*0701

Abbreviations

B-LCL B lymphocyte cell line
PHS Pooled human serum
TIL Tumor infiltrating lymphocytes

Introduction

Characterization of tumor-associated antigens (TAA)-derived peptides efficiently presented by tumor cells and recognized by tumor reactive TIL is critical for the devel-

opment of TAA-specific immunotherapies. TAA identification has been especially effective for melanoma tumors, evidencing two major classes of melanoma-associated Ags (MAA): tumor-specific proteins unexpressed in normal tissues and melanocytic differentiation Ags, like Melan-A/MART1, which are also expressed in normal melanocytes [27].

Among melanoma-associated antigens recognized by CD8+ and CD4+ T cells, Melan-A/MART-1 is one of the most consistently expressed by both a high fraction of melanoma tumor cells and melanoma tumor samples [8, 21]. Melan-A/MART1 epitopes recognized by CD8+ T cells and CD4+ T cells have been described [2, 4, 8, 13, 20, 23, 35]. Furthermore, others and we found that the Melan-A/MART1_{26–35} peptide is the epitope most frequently recognized by CD8+ TIL from HLA-A2+ melanoma patients [1, 19, 22, 31]. Since HLA-A2 is the prevalent HLA class I allele within the Caucasian population, vaccines, and adoptive therapy trials targeting this Melan-A/MART1 epitope have been performed [3, 9, 24, 26, 32, 33]. However, despite the induction of specific T cells responses, clinical efficacy of these treatments remained disappointing. One possible explanation is that these treatments might have induced a tumor escape by selecting antigen- or HLA-loss variants [17, 34]. The simultaneous targeting of multiple epitopes presented by diverse HLA molecules and capable of stimulating CD8+ and CD4+ T cells may at least partially counteract this tumor escape mechanism. Hence, the identification of new epitopes from commonly expressed Ags restricted by various frequently expressed HLA alleles is necessary.

In this study, we identified a HLA-Cw*0701 restricted Melan-A/MART1 epitope presented by HLA-Cw*0701+ melanoma tumor cells and recognized by a CD8+ TIL. This epitope is located in the region 51–73 of the protein,

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already shown to contain HLA-DR4 [35] and HLA-DQ5 [23] restricted epitopes. These data widen the fraction of melanoma patients susceptible to benefit from a Melan-A/MART1 specific vaccine treatment.

Materials and methods

Culture medium

Culture medium RPMI1640 (Gibco BLR, Gaithersburg, MD, USA) was supplemented with penicillin–streptomycin (10 µg/ml) and L-Glutamine (2 mM) (Life Technologies, Cergy-Pontoise, France) and 8% pooled human serum (pHS), or 10% fetal calf serum (FCS, Eurobio, Les Ulis, France).

Cell lines

B lymphocyte cell lines (BLCLs): Crep, Hob, Dab, Do 45, Hen, Hour, M.H, and UPN221 were a kind gift from Dr H. Vié (INSERM U601, Nantes). Melanoma cell lines M6, M67, M74, M113, M125, M134, M153, M171, M199, and M200 were established in our laboratory. All these cell lines were maintained with RPMI 1640 containing 10% FCS.

Peptides

Melan-A/MART1_{51–73} (RNGYRALMDKSLHVGTCAL-TRR) was purchased from Epytop (Nimes, France). Melan-A/MART1_{52–73,51–62,51–61} were purchased from Genepep (Montpellier, France). Melan-A/MART1_{51–60,51–59} were purchased from NeoMPS (Strasbourg, France). All peptides were at least 80% pure. Lyophilized peptides were diluted in DMSO and stored at –80°C.

Detection of Melan-A/MART1_{51–63} and Melan-A/MART1_{51–73} CD8+ T cell responses among TIL of melanoma patients

TIL from melanoma patients were obtained as previously described [1]. TIL were cultured with or without 10 µM peptide in presence of 10 µg/ml of brefeldin-A. After 6 h, cells were fixed and IFN-γ production by CD8+ T cells was assessed by IFN-γ intracytoplasmic and CD8 surface staining.

IFN-γ cells sorting

Specific T cells were isolated using an IFN-γ catching assay [25], according to the manufacturer's instructions (Miltenyi Biotech, Hamburg, Germany). Briefly, 3×10^6 TIL were incubated in 200 µl of RPMI1640 8% pHS with 10 µM of

Melan-A/MART1_{51–63} peptide at 37°C, 5% CO₂, for 3 h. Cells were subsequently labeled with a bispecific CD45/IFN-γ catching Ab and incubated for 45 min at 37°C. After several washes, the IFN-γ producing cells were stained with a second IFN-γ-PE detection mAb and separated by anti-PE mAb conjugated with magnetic beads. The isolated cells were then amplified and cloned.

Cloning of T cells

Cells from polyclonal cultures containing specific T cells were cloned by limiting dilution as previously described [11]. Briefly, T cells were plated in U-bottom 96-well plates with irradiated feeder cells, at concentrations of 1, 0.6 or 0.3 T cells/well. Irradiated (35 grey) feeder cells consisted of 1×10^5 allogenic PBMCs and 1×10^4 BLCL cells/well. The stimulatory medium consisted of RPMI1640 8% pHS containing 150 U/ml IL-2 and 1 µg/ml Phytohemagglutinin-L (PHA-L, Sigma). After 5 days, half of the volume of medium (75 µl/well) was replaced with culture medium containing 8% pHS and 150 U/ml IL-2 without PHA-L. After 8 or 9 days, proliferating clones could be seen by microscopy and were maintained with less than 1.5×10^6 T cells/ml. After 2 weeks, aliquots of each clone (60 µl) were tested for specificity by TNF production assay. Specific clones were restimulated as above beginning with 10,000 T cell clone/wells. After 2 weeks, clones reached a resting state and were frozen for further expansion. For all experiments, clones were used at least 14 days after the start of expansion.

IFN-γ intracellular staining

T cell clones or polyclonal TIL populations (1×10^5) were cultured with or without Melan-A/MART1_{51–63} peptide in the presence of 10 µg/ml of Brefeldin A (Sigma) for 6 h at 37°C. In some experiments mAb against HLA class I (clone W6.32), HLA-B/C (clone B1.23.2), HLA-A2 (clone BB7.2) produced in our laboratory or IgG2A control mAb (Immunotech) were added to cultures. Finally, cells were fixed for 10 min at room temperature with PBS containing 4% paraformaldehyde (Sigma). In some experiments, cells were stained with PE conjugated mAb specific for CD8 molecule (Beckman Coulter) before fixation. IFN-γ intracellular staining of fixed cells was performed as described by Jung et al. [18]. Briefly, cells were stained with 5 µg/ml of FITC conjugated mAb specific for IFN-γ (clone 4S.B3, BD Pharmingen) and then washed. Antibody dilutions and washes were made with PBS containing 0.1% of bovine serum albumin and 0.1% of saponin at room temperature. After staining, cells were resuspended in PBS and analyzed on a FACScan (Beckton & Dickinson, Franklin Lake, NJ, USA) with a gate set on T cells on the FSC-SSC dot plot.

TNF production assay

T cell clones (1×10^4) were cultured for 6 h at 37°C with 3×10^4 unpulsed or Melan-A/MART1_{51–63} peptide pulsed B-LCL, with 3×10^4 unpulsed or Melan-A/MART1_{51–63} peptide pulsed transfected COS-7 cells or with 3×10^4 untreated or IFN- γ treated (48 h, 500 IU/ml) melanoma cell lines unpulsed or pulsed with Melan-A/MART1_{51–63} peptide. Then cultures supernatants were collected and TNF released by T cells was measured using a colorimetric assay based on cytotoxicity to WEHI 164 clone 13 [14]. TNF production is considered positive when more than 10 pg/ml of TNF was released.

Transfection of COS-7 cells

The COS cells were transfected with plasmids containing the appropriate HLA-B or HLA-C allele cDNA (corresponding to each patient's alleles) and, in some conditions, cotransfected with plasmid containing the cDNA coding for Melan-A/MART1. cDNA were kindly provided by T. Boon (LICR, Brussels, Belgium) or cloned in our laboratory. COS-7 cells transfection was performed by the DEAE-dextran-chloroquine method [6, 8]. In brief, 1.5×10^4 /well COS-7 cells were plated in flat-bottomed 96-wells plates 24 h before transfection. Cells were transfected with 100 ng of plasmid coding for an HLA alone or cotransfected with 100 ng of plasmid coding for Melan-A/MART1. Each transfection condition was performed in duplicate. After 24 h, transfected COS cells were unpulsed or pulsed with Melan-A/MART1_{51–63} peptide for 1 h and then washed twice. The transfected cells were then used for experiments.

Melanoma cell lines phenotype

Untreated or IFN- γ treated (48 h, 500 IU/ml) melanoma cell lines (1×10^5) were stained with mAb specific for HLA class I (clone W6.32) or HLA B/C molecules (clone B1.23.2). For Melan-A/MART1 intracellular staining, cells were fixed with PBS containing 4% paraformaldehyde 10 min at room temperature and stained with Melan-A/MART1 specific mAb (clone A103, Dakocytomation, Denmark). Melanoma cell lines were then washed and incubated with PE conjugated goat anti-mouse mAb (Beckman Coulter). After staining, cells were resuspended in PBS and analyzed on a FACScan (Beckton & Dickinson, Franklin Lake, NJ, USA) with a gate set on melanoma cells on the FSC-SSC dot plot.

⁵¹Cr release assay

Target cells (melanoma cell lines) were pulsed for 1 h with Na₂⁵¹CrO₄ (NEN life science, Paris, France) and then

washed. 1×10^3 target cells were mixed with effector T cells at effector to target ratio 20/1. After 4 h of incubation at 37°C, 25 μ l of supernatant was harvested, and added to 100 μ l scintillation cocktail (optiphase supermix, Wallac, UK) before liquid scintillation counting. The % specific lysis was calculated as follows: (sample release – spontaneous release/maximum release – spontaneous release) \times 100. The spontaneous release was calculated from targets incubated with culture medium, and the maximum release from targets lysed with RPMI containing 2.5% Triton X-100. Lysis was considered positive when more than 10% specific lysis was observed.

Results

Presence of spontaneous CD8+ T cell responses to Melan-A/MART1_{51–63} and Melan-A/MART1_{51–73} among the TIL of a melanoma patient

Based on the work of Storkus' group [35] and on our previous study [23], we looked for the presence of CD4+ and CD8+ T cells specific for Melan-A/MART1_{51–63}, and Melan-A/MART1_{51–73} peptide among tumor-infiltrating lymphocytes (TIL) populations of six patients. We found that 0.5 and 0.3% of CD8 positive TIL from one patient, M74, responded to the Melan-A/MART1_{51–63}, and to the Melan-A/MART1_{51–73} peptide, respectively (Fig. 1a). So, we performed an IFN- γ positive cell sorting of TIL after stimulation by Melan-A/MART1_{51–63}. The sorted TIL were expanded and tested for their response to specific peptides by CD8 surface and IFN- γ intracellular staining after an "autopresentation" assay. We observed an enrichment of CD8+ specific T cells with 14% of CD8+ TIL responding to the Melan-A/MART1_{51–63} peptide and 5% to Melan-A/MART1_{51–73} peptide (Fig. 1b).

HLA-Cw*0701 molecules present Melan-A/MART1_{51–63} peptide to specific CD8+ T cells

To determine the HLA class I restriction of Melan-A/MART1_{51–63} reactive CD8+ T cells, we cloned under limiting dilution the enriched population as previously described [11]. We obtained ten clones, which produced TNF in response to Melan-A/MART1_{51–63} peptide. Three out of these ten clones could be expanded and cryopreserved. These three TIL clones were tested for V β expression and we found that 100% of cells of the three clones expressed V β 13.1 (data not shown). The clone M74.19 was further expanded for additional experiments. We confirmed that this clone was CD4–CD8+ (data not shown), and by auto-presentation followed by IFN- γ intracytoplasmic staining, that it was able to respond to Melan-A/MART1_{51–63} peptide

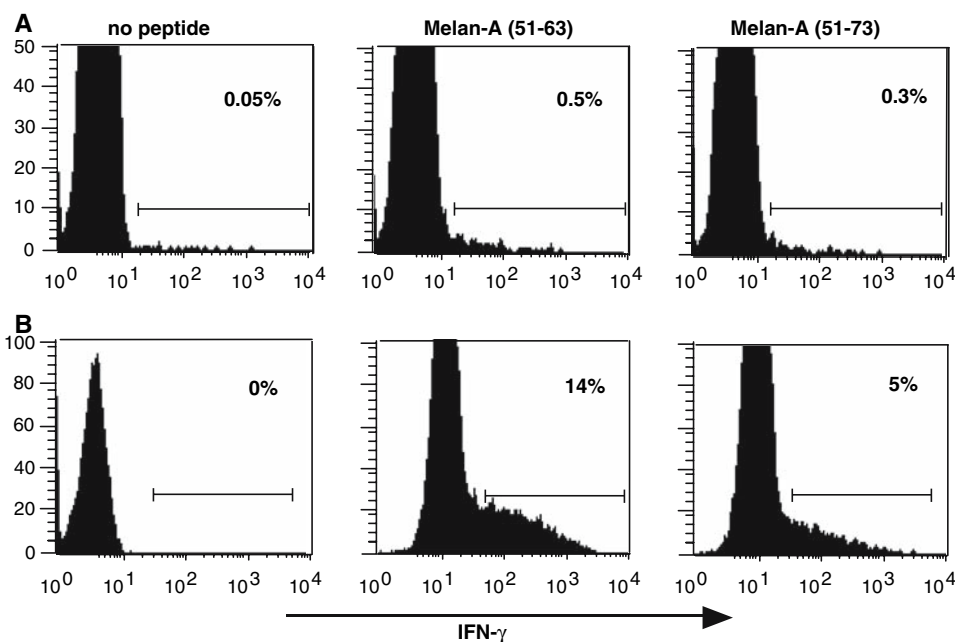


Fig. 1 Enrichment of CD8⁺ T cells specific for Melan-A/MART1_{51–63} and Melan-A/MART1_{51–73} peptide from a polyclonal TIL population. **a** T cells were obtained from TIL of a melanoma patient. T cells were cultured with or without Melan-A/MART1_{51–63} or Melan-A/MART1_{51–73} peptide in presence of Brefeldin A at 37°C. After 6 h, cells were stained for surface CD8, fixed, and then stained for intracytoplasmic IFN- γ . Fluorescence was analyzed by flow cytometry with a gate set on CD8⁺ T cells. **b** CD8⁺ T cells specific for Melan-A/

MART1_{51–63} peptide were enriched by IFN- γ magnetic beads cell sorting and were expanded during 2 weeks. The sorted TIL were then cultured with or without Melan-A/MART1_{51–63} and Melan-A/MART1_{51–73} peptide in presence of Brefeldin A at 37°C during 6 h. Cells were stained for surface CD8, fixed, and then stained for intracytoplasmic IFN- γ . Fluorescence was analyzed by flow cytometry with a gate set on CD8⁺ T cells

(Fig. 2a). Anti-HLA class I and anti-HLA-B/C, but not anti HLA-A2 and IgG2A control specific mAb, inhibited the response of clone M74.19 in a concentration-dependent manner (Fig. 2a). Therefore, this clone recognizes a Melan-A/MART1 peptide in the HLA-B (HLA-B*1402 and HLA-B*0801) or HLA-C context (HLA-Cw*0701 and HLA-Cw*0801).

To identify the restricting HLA allele, we first used B lymphocytes cell lines (B-LCL) expressing one or several donor HLA alleles to present the peptide to the clone M74.19: Crep, Hob, Do 45, Hen (HLA-B*0801+ and HLA-Cw*0701+), Dab (HLA-Cw7+), Hour (HLA-Cw*0702+), M.H (HLA-Cw*0801+), and UPN221 (HLA-Cw*0704+). All, and only, HLA-Cw*0701 expressing B-LCL were able to stimulate TNF production from the clone M74.19, suggesting that this allele is the restriction element (Fig. 2b). To confirm this result, we tested the capacity of COS-7 cells transfected with plasmids encoding donor HLA-B or HLA-C molecules with, or without plasmids encoding Melan-A/MART1, to stimulate the clone M74.19. Transfected cells were unpulsed or pulsed with 10 μ M of Melan-A/MART1_{51–63} peptide. Only COS-7 cells co-transfected with the HLA-Cw*0701 and Melan-A/MART1 plasmids were able to induce a response of the clone in the absence of peptide addition (Fig. 2c). That was strongly increased by

peptide addition. Therefore, the Melan-A/MART1 specific TIL clone is restricted by HLA-Cw*0701 and the recognized peptide can be presented by the endogenous pathway of COS-7 cells transfected with the Melan-A/MART1 cDNA. Furthermore, clone M74.19 is unable to recognize the peptide in the HLA-Cw*0702 context, despite minor differences of sequence in the α 2 and α 3 region of this molecule as compared to HLA-Cw*0701 molecule.

Mapping of the minimal Melan-A/MART1 peptide presented by HLA-Cw*0701 molecules

To determine the minimal peptide sequence able to stimulate the M74.19 clone, we tested a panel of overlapping synthetic peptides located in the Melan-A/MART1_{51–73} region for their ability to induce TNF secretion by this clone in an auto-presentation assay. Removal of the N-terminal arginine 51 abrogated the clone response (Fig. 3). In contrast, removal of C-terminal residues localized between position 73 and 61, increased response of the clone. This response decreased with Melan-A/MART1_{51–60} peptide and was suppressed by the removal of lysine 60. Therefore, Melan-A/MART1_{51–61} peptide is the peptide optimally recognized by this clone. However, other peptides contained within the region 51–73 represent potential epitopes for this clone.

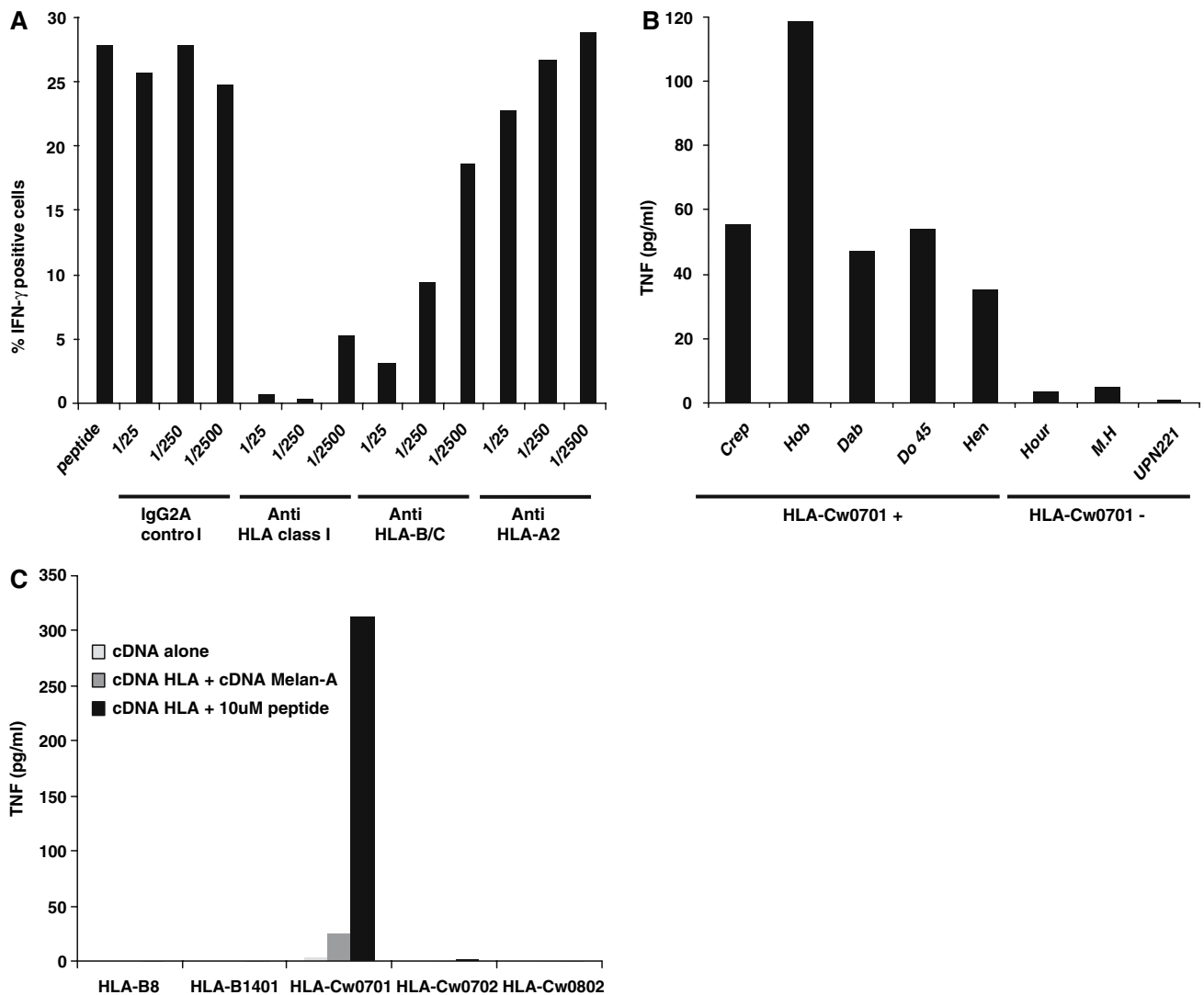


Fig. 2 The Melan-A/MART1_{51–63} specific CD8⁺ T cell clone, M74.19, is restricted by the HLA-Cw*0701 molecules. **a** M74.19 CD8⁺ T cell clone was cultured alone or with 10 μ M of Melan-A/MART1_{51–63} peptide in presence of Brefeldin A at 37°C. Inhibitory mAb against HLA class I, HLA-B/C or HLA-A2 molecules or isotype control mAb were added to cultures at different concentrations. After 6 h, cells were fixed, permeabilized and stained for intracytoplasmic IFN- γ . Fluorescence was analyzed by flow cytometry. **b** M74.19 T cell clone was cultured alone or with 10 μ M Melan-A/MART1_{51–63} peptide

pulsed HLA-Cw*0701+ or HLA-Cw*0701– B-LCL. After 6 h, TNF was measured in co-culture supernatants. **c** COS-7 cells were co-transfected with plasmid encoding the HLA-B8, HLA-B*1401, HLA-Cw*0701, HLA-Cw*0702 or HLA-Cw*0802 and with or without plasmid encoding Melan-A/MART1. 24 h after transfection, COS-7 cells were either unpulsed or pulsed with 10 μ M of Melan-A/MART1_{51–63} peptide and washed. Then, they were co-cultured with M74.19 CD8⁺ T cell clone. After 6 h, TNF was measured in co-culture supernatants

The Melan-A/MART1 epitope recognized by M74.19 CD8⁺ T cell clone is presented by Melan-A/MART1+ HLA-Cw*0701+ melanoma tumor cells

To address the presentation of the M74.19 clone epitope by melanoma tumor cells, we then tested a panel of melanoma cell lines for their ability to stimulate the clone. Because HLA-Cw expression is spontaneously low and is increased by IFN- γ [16, 36], we also tested the same cell lines pre-treated by IFN- γ for 48 h. All these cell lines were first analyzed for HLA-B/C and for Melan-A/MART1 expression.

As shown for the autologous M74 cell line, as a representative result (Fig. 4a), all melanoma cell lines expressed HLA-B/C molecules and this expression was increased by IFN- γ treatment. All cell lines also expressed the Melan-A/MART1 protein, but IFN- γ decreased this expression as reported previously [16].

Two out of five HLA-Cw*0701+ melanoma cell lines, including the autologous cell line M74, induced TNF secretion by the clone and this stimulation was stronger upon pre-treatment by IFN- γ (Fig. 4b). Another HLA-Cw*0701+ cell line, M134, could stimulate the clone, but only after

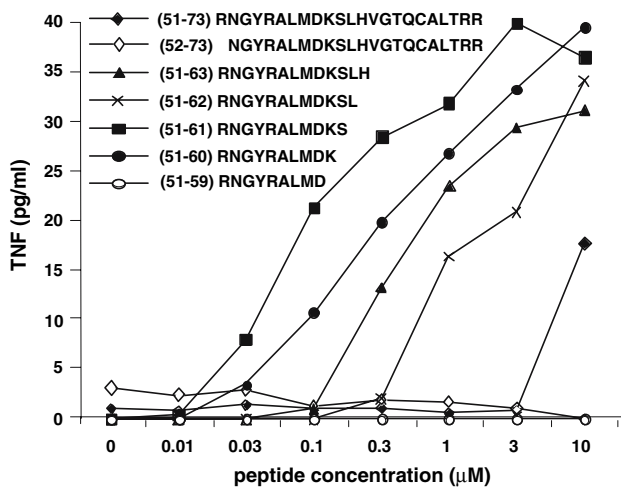
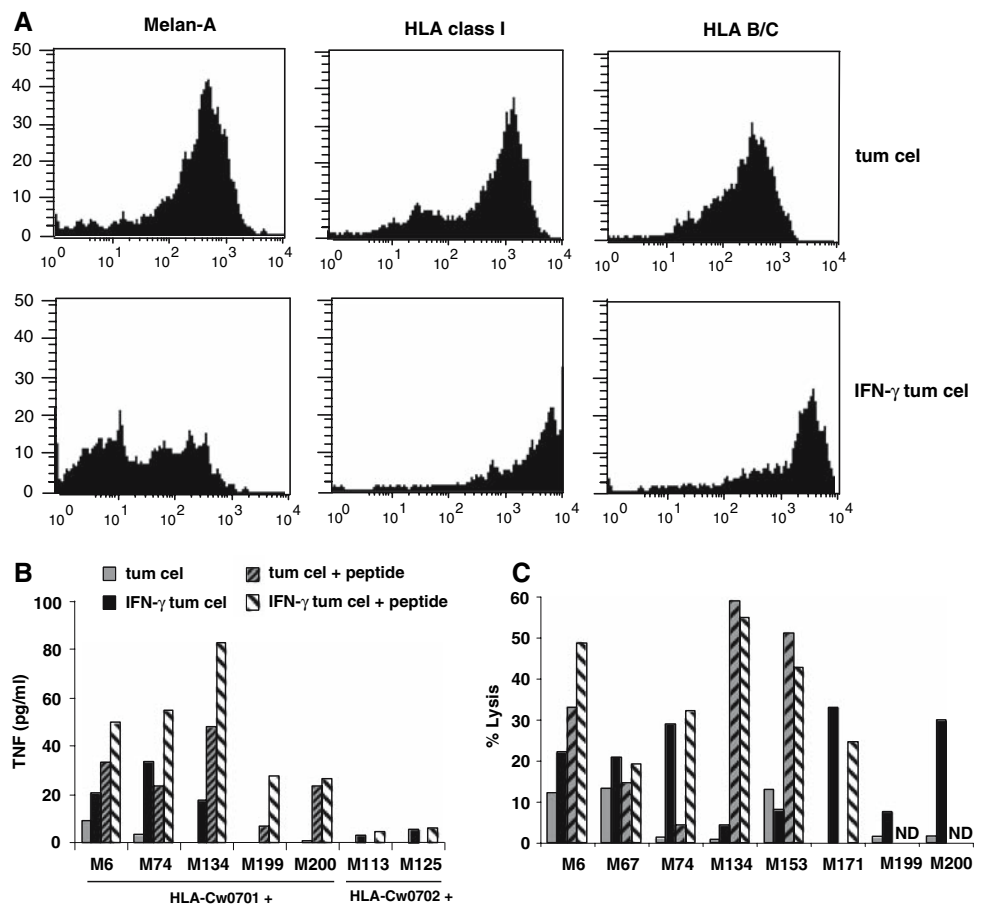


Fig. 3 Melan-A/MART1₅₁₋₆₁ peptide is the optimal epitope recognized by M74.19 CD8+ T cell clone. M74.19 CD8+ T cell clone was cultured with different concentrations of a panel of truncating peptides located in Melan-A/MART1₅₁₋₇₃ region. After 6 h, TNF was measured in co-culture supernatants

IFN- γ treatment. In contrast, two out of these five cell lines, M199 and M200, remain unable to stimulate the clone even after IFN- γ treatment. However, as expected, upon peptide

Fig. 4 The Melan-A/MART1 epitope recognized by M74.19 CD8+ T cell clone is presented by Melan-A/MART1+ HLA-Cw*0701+ melanoma tumor cells. **a** Untreated, or IFN- γ treated melanoma tumor cell line M74 was stained with unlabeled primary mAb produced in mouse, specific for HLA class I or HLA B/C molecule or Melan-A/MART1 protein. Then melanoma tumor cell lines were stained with PE conjugated goat anti-mouse mAb. Fluorescence was analyzed by flow cytometry, **b** M74.19 CD8+ T cell clone was cultured with untreated, or IFN- γ treated melanoma tumor cell lines unpulsed or pulsed with Melan-A/MART1₅₁₋₆₃ peptide. After 6h, TNF was measured in co-culture supernatants. **c** Cytolytic activity of M74.19 CD8+ T cell clone against untreated or IFN- γ treated melanoma tumor cell lines unpulsed or pulsed with Melan-A₅₁₋₆₁ peptide, was assessed by 4 h ⁵¹Cr release assay. ND not done



loading, the five cell lines, but not HLA-Cw*0702+ cell lines, could induce TNF secretion by the clone.

Finally, we asked whether the clone was able to lyse HLA-Cw*0701+ melanoma cell lines. Three melanoma cell lines: M6, M67, and M153 out of eight were killed at a significant, but rather low level (Fig. 4c). IFN- γ pre-treatment significantly increased the lysis of two of these cell lines: M6 and M67, whereas it decreased the lysis of M153 cell line. Furthermore, IFN- γ pre-treatment induced the lysis of three cell lines, which were not killed without IFN- γ pre-treatment: M74, M171, and M200. Lysis of melanoma cell lines can be further increased by the addition of Melan-A₅₁₋₆₁ peptide. Altogether, the cytotoxic and TNF responses of the clone to melanoma cell lines were well correlated. This strongly suggests that a fraction of HLA-Cw*0701+ melanoma cell lines spontaneously present the clone epitope and that this presentation is increased by IFN- γ .

Discussion

During the past years, the majority of identified Melan-A/MART1 epitopes presented on melanoma tumor cells in association with HLA class I or class II molecules were

located in the region 25–40 of the protein [2, 4, 7, 8, 30]. This study and others show that region 51–73 of Melan-A/MART1 also contains several tumor epitopes recognized by CD4+ and CD8+ T cells [23, 35]. In this study, while trying to detect CD4+ and CD8+ T cells specific for Melan-A/MART1_{51–73} among TIL of melanoma patients, we obtained a HLA-Cw*0701 restricted specific CD8+ T cell clone from one patient. Using this clone, we demonstrated the existence of a HLA-Cw*0701 restricted Melan-A epitope in this region. Although, we did not formally identify the natural epitope presented by melanoma cells, we showed that it starts with arginine residue 51 and ends between residue 60 and 73. One could hypothesize that the response of TIL to the long Melan-A/MART1_{51–73} peptide could be due to the processing of this peptide into smaller peptides like Melan-A/MART1_{51–63} or Melan-A/MART1_{51–61} (Fig. 1b). We rather think that this response resulted from the direct presentation of the long peptide, since the presence of brefeldin A in this assay prevents transport of HLA-Cw*0701/peptide complexes to the cell surface. Other unidentified epitopes may be present in this region, like the Melan-A/MART1_{53–62} peptide recognized by T cell in an unknown HLA context after vaccination of melanoma patients with killed allogenic melanoma cells loaded dendritic cells [28]. Characterization of other epitopes from the Melan-A/MART1_{51–73} region would be useful for development of Melan-A/MART1-based immunotherapy.

HLA-Cw*0701 allele is expressed by about 17% of the European population (<http://www.ncbi.nlm.nih.gov/mhc/ihwg.cgi?ID=9&cmd=PRJOV>). Therefore, epitopes from Melan-A_{51–73} could be used to stimulate CD8+ tumor reactive T cell responses from a significant fraction of melanoma patients. Since two other HLA-Cw7 restricted epitopes have been identified from MAGE-A2, -A3, -A6, and -A12 protein expressed by a significant fraction of melanoma tumors [5, 15, 29], multipitopic vaccination with peptide from Melan-A/MART1 and MAGE proteins might be performed in HLA-Cw7+ melanoma patients to decrease the risk of antigen loss variant selection.

The recognition of HLA-Cw*0701+, Melan-A/MART1+ melanoma cells by the CD8+ T cell clone M74.19 was rather weak. Furthermore, we detected a CD8+ T cell response against this epitope only in 1 TIL population (M74) out of 13 HLA-Cw*0701+ melanoma patients tested (data not shown). Recognition of this epitope is largely increased by IFN- γ treatment of melanoma tumor cells, which induces an increase of HLA class I molecules expression. This result suggests that an in vivo recognition of this epitope on the surface of melanoma tumor cells may depend on the presence of IFN- γ in the tumor microenvironment that could be produced by other tumor reactive TIL. However, we also show that IFN- γ decreases Melan-/MART-1 expression by melanoma tumor cells. Other cyto-

kines like IFN- β may be better candidates to increase tumor cell recognition by increasing not only HLA class I expression, but also melanoma antigen expression [10]. Other factors such as the weak expression on cell surface of HLA-C molecules compared to HLA-A or HLA-B expression [36], or the decrease or loss of HLA class I expression during cancer progression [12] may explain the absence of response in 12 out of 13 HLA-Cw*0701+ melanoma patients tested.

In conclusion, we reported here that Melan-A/MART1 contains a CD8+ T cell epitope within the region 51–73 efficiently presented by HLA-Cw*0701+ melanoma tumor cells. Such epitope may be helpful to design and to evaluate Melan-A/MART1 based anti-tumoral vaccination strategies.

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