Diversity of the Fine Specificity Displayed by HLA-A*0201-Restricted CTL Specific for the Immunodominant Melan-A/MART-1 Antigenic Peptide

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Diversity of the Fine Specificity Displayed by HLA-A*0201-Restricted CTL Specific for the Immunodominant Melan-A/MART-1 Antigenic Peptide

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HLA-A*0201 melanoma patients often develop a CTL response to an immunodominant peptide derived from the melanocyte lineage-specific protein Melan-A/MART-1. We have shown previously that the antigenic peptide most often involved is the decapeptide Melan-A26–35 (EAAGIGILTV). We also observed some clonal diversity in the fine specificity of Melan-A-specific CTL. To substantiate this observation, we have now tested a series of Melan-A26–35 variant peptides containing single alanine substitutions for binding to HLA-A*0201 and recognition by polyclonal and monoclonal Melan-A-specific CTL. Substitution of several residues with alanine reduced peptide binding activity by >10-fold. In contrast, substitution of E26 with alanine (AAAGIGILTV) resulted in a 5-fold higher binding activity as well as in stronger stability of the corresponding HLA-A*0201/peptide complexes. Interestingly, the peptide variant AAAGIGILTV was recognized more efficiently than the natural decapeptide by short term cultured, tumor-infiltrated lymph node cell cultures and a number of Melan-A-specific CTL clones derived from different individuals. Moreover, this analysis revealed that the fine specificity of the CTL response to the Melan-A immunodominant epitope is quite diverse at the clonal level. At least three distinct patterns of fine specificity were identified. This diversity appears to reflect the diversity of the TCR repertoire available for this Ag, since similar results were obtained with a panel of Melan-A-specific CTL clones derived from a single melanoma patient. These findings have important implications for the formulation of Melan-A peptide-based vaccines as well as for the monitoring of Melan-A-specific CTL responses in melanoma patients. The Journal of Immunology, 1998, 161: 6956–6962.

The recent identification of CTL-defined melanoma tumor-associated Ags (for review, see Refs. 1 and 2) has opened the possibility of developing Ag-specific immunotherapy for patients with melanoma. Melan-A/MART-1 protein (referred to as Melan-A hereafter) is the melanoma-associated Ag most frequently recognized by tumor-reactive CTL derived from HLA-A*0201 melanoma patients (3–8). The antigenic peptide recognized by HLA-A*0201-restricted Melan-A-specific CTL has been initially mapped to amino acid residues 27–35 corresponding to the nonapeptide AAGIGILTV (5). In addition, several overlapping antigenic peptide sequences have been identified in the same Melan-A protein region (5, 9). More recently, we have reported that the decapeptide Melan-A26–35 (EAAGIGILTV) is better recognized than the other antigenic peptides by several tumor-reactive CTL lines and clones (10). In addition, the use of Melan-A26–35 peptide analogues revealed some clonal diversity in the fine specificity displayed by these CTL (10, 11).

To document further such a diversity we have now tested single alanine-substituted Melan-A26–35 peptide variants for binding to HLA-A*0201 and recognition by Melan-A-specific CTL. This analysis allowed the identification of a variant peptide (AAA GIGILTV) with increased antigenicity compared with the natural peptide(s). Moreover, comparative analysis of the recognition pattern displayed by 10 independently derived CTL clones known to react with HLA-A*02011 Melan-A2 tumor cells revealed a striking diversity in terms of both affinity and fine specificity. In addition, a similar degree of diversity was found by analyzing a panel of Melan-A-specific CTL clones derived from the tumor-infiltrated lymph node (TILN)3 of a single melanoma patient.

Materials and Methods

Synthetic peptides

Peptides were synthesized by standard solid phase chemistry on a multiple peptide synthesizer (Applied Biosystems, Foster City, CA) using F-moc for transient NH2-terminal protection and were analyzed by mass spectrometry. All peptides were >90% pure as indicated by analytical HPLC. Lyophilized peptides were diluted in DMSO and stored at −20°C.

3 Abbreviations used in this paper: TILN, tumor-infiltrated lymph node; nM 50%, concentration of each competitor peptide required to achieve 50% inhibition of target cell lysis.
Tumor cell lines and clones

Tumor cell lines and EBV-transformed B cell lines were maintained in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS, 0.55 mM Arg, 0.24 mM Asn, and 1.5 mM Gln. Melanoma cell line Me 290 was established at the Ludwig Institute for Cancer Research, Lausanne Branch, from surgically excised melanona metastases from patient LAU 203. CTL clone LAU 132/2 specific for peptide 368–376 from tyrosinase (12) was derived from TILN of melanoma patient LAU 132 by cloning after in vitro stimulation with the corresponding synthetic peptide. Melan-A-specific CTL clones M77.80 (Vβ3) and M77.84 (Vβ14) were derived from TILN of melanoma patient M77 from limiting dilution cultures in the presence of irradiated autologous tumor cells, EBV-transformed B lymphocytes, PHA, and rIL-2 as previously described (13). CTL clones LAU 132/6 and LAU 132/14 (Vβ7 and Vβ13, respectively) (P. Y. Dietrich, unpublished observation) were similarly derived, with minor modifications, from TILN of patient LAU 132 after in vitro restimulation with peptide Melan-A26–35. CTL clone A2 (Vβ7) was established by limiting dilution from a TIL culture, as previously described (5). CTL clones M1.13 (Vβ14), 7.10, 5.3.9, 7.23, and Mel 1.33 (Vβ9) were derived from PBMC of normal HLA-A2 donors after in vitro restimulation with peptide Melan-A27–34 (N. Gervois et al., manuscript in preparation). CTL clones 1.29, 1.36, 1.37, 1.40, 1.48, 1.59, 2.14, 2.30, 2.34, 2.36, and 2.47 were derived from TILN of melanoma patient LAU 203 from limiting dilution cultures in the presence of irradiated autologous PBMC, EBV-transformed B lymphocytes, PHA, and rIL-2. Clones were derived from wells with a probability of clonality >90% according to single hit Poisson distribution. They were subsequently expanded by plating 5 × 10^5 cells every 3–4 wk into microtiter plates together with irradiated feeder cells (5 × 10^5 autologous PBMC and 2 × 10^6 EBV-transformed B cells) in the presence of PHA and rIL-2.

**HLA-A*0201 binding assay**

The peptide binding capacity to HLA-A*0201 was assessed in a functional competition assay based on inhibition of recognition of the antigenic peptide tyrosinase 368–376 (YMDGTMSQV) (14) by HLA-A*0201-restricted CTL clone LAU 132/2. Briefly, T2 cells were ¹¹¹Cr labeled in the presence of anti-class I mAb W6/32. Various concentrations of competitor peptides (50 μM) were incubated with ¹¹¹Cr-labeled T2 cells (50 μL; 1000 cells/μL for 15 min at room temperature; A suboptimal dose (1 nM) of the antigenic peptide (50 μM) was then added together with specific CTL (5000 cells/well; 50 μL). Chromium release was measured after 4-h incubation at 37°C. The concentration of each competitor peptide required to achieve 50% inhibition of target cell lysis was then determined and is indicated as [nM] 50%. To facilitate comparison, the relative competitor activity was calculated as the [nM] 50% of the unmodified Melan-A decapeptide EAAGIGILTV divided by the [nM] 50% of the competitor peptide.

**Assessment of the stability of single A-substituted Melan-A 26–35 peptide analogues/HLA-A*0201 complexes**

The stability of peptide/HLA-A*0201 complexes was assessed as previously described (11). Briefly, T2 cells were loaded with saturating concentrations (10 μM) of the different peptide analogues by overnight incubation at room temperature in the presence of βm (3 μg/mL; Sigma, Buchs, Switzerland) in serum-free medium (X-VIVO 10, BioWhittaker, Walkersville, MD). After peptide removal and addition of emetine (10 μg/mL; Sigma) to block protein synthesis, cells were incubated at 37°C for the indicated time periods. For each time point, an aliquot of cells was stained with mAb BB7.2 (HLA-A2 specific) to measure HLA-A2 Ag expression. Influenza matrix 58–66 peptide, which has been shown to bind to HLA-A*0201 with high affinity and form stable peptide/HLA-A*0201 complexes (15), was used as an internal standard. Results are expressed as relative complex stability = 100 × [(mean fluorescence with Melan-A analogue – background mean fluorescence)/(mean fluorescence with fluorescence matrix peptide – background mean fluorescence)]. Where background mean fluorescence represents the fluorescence value obtained on a background mean fluorescence, the stability of each peptide variant relative to that observed for influenza A matrix 58–66 peptide/HLA-A*0201 complexes as shown previously (11) HLA-A*0201 complexes formed with peptide Melan-A26–35 were unstable, i.e., they completely dissociated in <1 h of incubation at 37°C (Fig. 2). In contrast, complexes formed with the peptide variant AEAAGIGILTV were relatively stable over a 6-h

**Generation of TILN**

TILN were generated from tumor-invaded lymph nodes of HLA-A*0201 melanoma patients obtained by surgery at the Centre Pluridisciplinaire d’Oncologie, Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland). Tumoral lymph node fragments were minced to single cell suspensions (10, 11) and were cultured in 24-well tissue culture plates (Costar, Cambridge, MA) in 2 ml of Iscove’s modified Dulbecco’s medium supplemented with Asn, Arg, Gln, and 10% pooled human A+ serum in the presence of IL-2 and IL-7 (100 U/ml and 10 ng/ml, respectively). After 2–3 wk of cell culture, the TILN were tested for cytolitic activity, and their cell surface phenotypes were determined by flow cytometry. The TILN chosen for this study were >90% CD3+ CD8+ cells that had cytotilic activity against autologous or HLA-A*0201+ tumor cell lines and recognized peptides Melan-A26–35 and Melan-A27–35 presented by HLA-A*0201.

**Assessment of Ag recognition by TILN and Melan-A-specific CTL clones**

Ag recognition was assessed using chromium release assays. Target cells were labeled with ¹¹¹Cr for 1 h at 37°C and washed twice. Labeled target cells (1,000 cells in 50 μL) were then added to varying numbers of effector cells (50 μL) in V-bottom microwells in the presence or the absence of 1 μg ml of the antigenic peptide (50 μM). In the peptide titration experiments target cells (1,000 cells in 50 μL) were incubated in the presence of various concentrations of peptide (50 μM) for 15 min at room temperature before the addition of effector cells. In each case the effector cells were preincubated for at least 20 min at 37°C in the presence of unlabeled K562 cells (50,000/well) to eliminate nonspecific lysis due to NK-like effectors present in stimulated T cell populations. Chromium release was measured after incubation for 4 h at 37°C. The percent specific lysis was calculated as: 100 × [(experimental – spontaneous release)/(total – spontaneous release)].

**Results**

**Binding of single A-substituted Melan-A peptide variants to HLA-A*0201**

To determine the contributions of single amino acid side chains to the interaction between peptide Melan-A26–35 and the HLA-A*0201 molecule, single alanine (A)-substituted variants of the peptide were synthesized and tested for binding to HLA-A*0201 in a functional peptide competition assay. As previously reported, the nonapeptide Melan-A27–35 was 5-fold less efficient than the decapeptide Melan-A26–35 in this assay (10). As illustrated in Fig. 1, substitution of A for E at position 1 of the decapeptide (peptide variant AEAAGIGILTV) resulted in a 5-fold increase in competitor activity. Substitution of A for G at position 4 or 6 resulted in a decreased competitor activity (20- and 10-fold, respectively). Substitution of A for G at position 1 also resulted in a drastic reduction in competitor activity (25-fold). In contrast, single substitution of A for the amino acids at the remaining positions did not result in a significant change in competitor activity.

**Assessment of the dissociation rate of single A-substituted Melan-A26–35 peptide variants from HLA-A*0201**

The stability of complexes formed between HLA-A*0201 and single A-substituted Melan-A26–35 peptide variants was assayed on T2 cells as described previously (11). Briefly, T2 cells were incubated overnight with saturating amounts of each peptide variant in the presence of human βm. After peptide removal and addition of emetine to inhibit protein synthesis, T2 cells were incubated at 37°C, and the relative amount of HLA-A*0201 molecules remaining at the cell surface was determined after various incubation times. The stabilities of the various peptide/HLA-A*0201 complexes were then normalized relative to that observed for influenza A matrix 58–66 peptide/HLA-A*0201 complexes. As shown previously (11) HLA-A*0201 complexes formed with peptide Melan-A26–35 were unstable, i.e., they completely dissociated in <1 h of incubation at 37°C (Fig. 2). In contrast, complexes formed with the peptide variant AEAAGIGILTV were relatively stable over a 6-h
period. Complexes of low stability were observed with all the remaining peptide variants tested.

Recognition of single A-substituted Melan-A peptide variants by Melan-A-specific TILN and CTL clones

The relative antigenic activities of single A-substituted Melan-A26–35 peptide variants were quantitated in a standard CTL assay using TILN populations obtained from two patients and a panel of 10 independent, Melan-A-specific CTL clones. Titration curves over a wide range of concentrations (10^{-5}-10^{-13} M) were generated for each peptide variant. For a quantitative comparison, the concentration required for 50% maximal activity was determined from individual titration curves and was normalized to the 50% concentration value of peptide Melan-A26–35 (Table I). As shown in a previous study (11), TILN LAU 132 and LAU 203 recognized peptide Melan-A26–35 at least 1 order of magnitude more efficiently than the nonapeptide Melan-A27–35. Interestingly, the peptide variant AAAGIGILTV was recognized 20- to 60-fold better than the peptide Melan-A26–35 by the two TILN populations. In contrast, the other peptide variants were recognized less efficiently.

In agreement with our previous findings (10), marked differences were observed among the 10 CTL clones tested in terms of recognition of the two natural peptides Melan-A26–35 and Melan-A27–35 (Table II). Thus, five clones recognized the decapeptide better than the nonapeptide (relative antigenic activity, >10-fold higher). For one of these clones (LAU 132/6), there was poor recognition of peptide Melan-A27–35 despite a very efficient recognition of peptide Melan-A26–35 (50% maximal lysis at 0.04 nM).

![FIGURE 1. A. Relative competitor activities of single A substituted Melan-A26–35 peptide variants. Data are from one of two independent experiments that gave comparable results. Competitor activity was measured on the basis of inhibition of recognition of the tyrosinase 368–376 antigenic peptide by the HLA-A*0201-restricted peptide-specific CTL clone LAU 132/2. The relative competitor activity represents the improved or decreased capacity of peptide variants to inhibit recognition. It was calculated as described in Materials and Methods, using peptide Melan-A26–35 as the reference peptide with an arbitrary competitor activity of 1. B. Amino acid residues strongly associated with good or poor binding to HLA-A*0201. Modified from Ref. 17.]

![FIGURE 2. Dissociation rates of single Ala-substituted Melan-A26–35 peptide variants from HLA-A*0201. After overnight incubation with saturating amounts of peptide, T2 cells were treated with emetine (to inhibit protein synthesis) and were incubated at 37°C. At the indicated time points, cells were stained with mAb to HLA-A2 and were analyzed by flow cytometry. The mean fluorescence intensity was determined for each histogram. The stability of each peptide/HLA-A*0201 complex was normalized relative to that observed for the influenza A matrix 58–66 peptide/HLA-A*0201 complex.]

<table>
<thead>
<tr>
<th>Peptide Sequence</th>
<th>Peptide LAU 132</th>
<th>Relative antigenic activity</th>
<th>Peptide LAU 203</th>
<th>Relative antigenic activity</th>
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<tr>
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<td>2</td>
<td>1</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>AAAGIGILTV</td>
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<td>60</td>
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<td>0.004</td>
</tr>
</tbody>
</table>

Letters in bold indicate amino acids substituted by alanine.

T2 cells were incubated with various concentrations of the parental peptide Melan-A26–35 or with the different peptide variants. Lysis by TILN from patients LAU 132 and LAU 203 was measured in a 4-h 51Cr release assay at a lymphocyte-to-target cell ratio of 30:1. The peptide nanomolar concentration giving 50% of maximal activity, [nM] 50%, was then determined from the titration curve for each peptide.

The relative antigenic activity of each peptide, normalized to that of the parental peptide Melan-A26–35, was calculated as described in Materials and Methods.
nM). In contrast, three clones recognized the nonapeptide as efficiently as the decapeptide, and two clones recognized the nonapeptide more efficiently than the decapeptide (Table II). In general, single A-substituted peptide variants were recognized less efficiently than peptides Melan-A27–35 or Melan-A26–35, with one striking exception, peptide AAAGIGILTV. Indeed, this peptide variant exhibited enhanced recognition by eight of the 10 clones tested. As shown in Fig. 3A, recognition of the peptide variant AAAGIGILTV and the natural peptide Melan-A26–35 was sometimes mutually exclusive (for example, compare CTL clones LAU 132/6 and 7.23), whereas the difference in the efficiency of recognition of either peptide by another clone (M77.84) was small and presumably reflected the difference in their binding activity to HLA-A*0201 (Fig. 1). Together, the results obtained with Melan-A26–35, Melan-A27–35, and AAAGIGILTV peptides revealed a striking diversity in the fine specificity of tumor-reactive CTL clones. The distinct patterns of specificity identified in this analysis are illustrated in Fig. 3. Despite their different fine specificities, all CTL clones were able to efficiently kill Melan-A-expressing melanoma cells (Fig. 3B).

**Fine specificity of Melan-A-specific CTL clones derived from a single melanoma patient**

To determine whether the diversity of antigenic specificity displayed by the CTL clones tested above was a general characteristic of the TCR available against this epitope or could be attributed to the different protocols used to derive these clones, we assessed the fine specificity of Melan-A-specific CTL clones that were derived from a single melanoma patient. To avoid any Ag-driven selection in relation with in vitro stimulation with synthetic peptides, we used as a source of specific CTL clones the TILN population from patient LAU 203, which exhibits a relatively high CTL activity against Melan-A*0201, as determined by comparing the patterns of recognition of the two parental peptides and the peptide variant AAAGIGILTV. As illustrated in Table III, the results of this analysis were very similar to those described above. For example, four CTL clones recognized peptide Melan-A26–35 better than Melan-A27–35 (relative antigenic activity, >10-fold higher), whereas the opposite reactivity was observed with one CTL clone. Moreover, peptide variant AAAGIGILTV was recognized more efficiently than peptide Melan-A26–35 by eight of the 11 CTL clones. Strikingly, one of the CTL clones (2.36) was found to recognize AAAGIGILTV at nanomolar concentrations, whereas recognition of either of the parental peptides was observed only at micromolar concentrations.

**Discussion**

Among the melanoma-associated Ags defined by MHC class I-restricted CTL that have been identified to date, Melan-A is the most frequently recognized in HLA-A2+ melanoma patients (3–8). Although the nonapeptide Melan-A27–35 was originally described as the immunodominant antigenic peptide (5), we have recently shown (and confirmed in the present study) that the decapetide Melan-A26–35 is the natural peptide that is recognized most efficiently by a number of HLA-A*0201-restricted CTL clones as well as by polyclonal TILN populations (10, 11). In this report we demonstrate that the dominant Melan-A-specific CTL response in HLA-A*0201 melanoma patients is quite diverse in terms of fine specificity and includes CTL that recognize a defined peptide variant much better than the above-mentioned natural peptides.

As we found that both peptides Melan-A27–35 and Melan-A26–35 form unstable complexes with HLA-A*0201 (11), we first performed an analysis of the individual amino acid side chains within the Melan-A26–35 sequence that contribute to peptide binding to HLA-A*0201 and stability of HLA-A*0201/peptide complexes. The results of this analysis were largely in agreement with the findings of studies dealing with other HLA-A2 binding peptides (16, 17). In addition to substitution of A for the anchor residue V at position 10 of peptide Melan-A26–35, substitution of G29 and G31 with A also resulted in a significant decrease in HLA-A*0201 binding activity. Indeed, the presence of G at positions 4 and 6 of the sequence of decapeptides has been shown to correlate with good binding to HLA-A*0201 (17). Our analysis also revealed the
contribution of another residue, I at position 7, to the binding of peptide Melan-A 26–35 to HLA-A*0201.

Structural data obtained for the decapeptide HBV nucleopeptide 18–27/A*0201 complex provide a structural basis for our results (16). In particular, to be accommodated in the binding cleft, the backbone of the decapeptide was found to zigzag twice, first horizontally between residues at positions 5 and 6 and then vertically between residues at positions 6 and 8. It is conceivable that the presence of G at positions 4 and 6 of the decapeptide sequence may favor this conformation by conferring the required flexibility to the peptide backbone. In addition, the side chain of the residue at position 7, located at the level of the second vertical zigzag, was
found to penetrate deeply into the binding cleft and thus might be involved in interactions with the HLA-A*0201 region corresponding to the C and E pockets (16).

It has been reported that an E residue at position 1 of decapeptides is associated with poor binding to HLA-A2 (17). Indeed, we have recently shown that substitution of E at position 1 of the decapeptide Melan-A26-35 with amino acids reported to have a positive effect on peptide binding, such as F and Y, significantly improved binding of the decapeptide to HLA-A*0201 (10). In the current study we found that substitution of A for E at position 1 of peptide Melan-A26-35 also resulted in a significant increase in HLA-A*0201-binding activity as well as in the stabilization of peptide/HLA-A*0201 complexes (Fig. 2). It is thus conceivable that the presence of a negatively charged side chain at position 1 of the decapeptide is involved in the lack of stability of peptide Melan-A26-35/HLA-A*0201 complexes. It is noteworthy that this negative effect can also be counteracted by introduction of an appropriate anchor residue at position 2 of peptide Melan-A26-35. Indeed, the peptide analogue ELAGIGILTV is able to form stable complexes with HLA-A*0201 (11).

Although the majority of Melan-A-specific CTL clones tested recognized the decapeptide Melan-A26-35 more efficiently than the nonapeptide Melan-A27-35, few clones recognized the two peptides equally well (10) or recognized peptide Melan-A27-35 better than peptide Melan-A26-35, thus suggesting a clonal diversity in the efficiency of recognition of these two peptides. Interestingly, all the CTL clones, except one, that efficiently recognized peptide Melan-A26-35 recognized the peptide variant AAAGIGILTV even more efficiently, thus indicating that E at position 1 was not a crucial residue involved in the peptide contact site with the TCR. Importantly, the different patterns of fine specificity were also observed in the analysis of 11 Melan-A-specific CTL clones derived from TILN cultured in the absence of any exogenous peptide. It thus appears that the clonal diversity we observed in the fine specificity of Melan-A-specific CTL reflects a general diversity in the TCR repertoire of CD8 T cells that react with this epitope.

As shown in this study, clones of different fine specificity were able to efficiently kill a HLA-A*0201 +, Melan-A-expressing melanoma cell line, thus suggesting that both nona- and decapeptides are presented at the surface of melanoma cells. However, to date, no conclusive data are available concerning the precise nature of the naturally processed Melan-A peptides expressed at the surface of HLA-A2 + and Melan-A/MART-1-expressing melanoma cells (9, 10, 18). The definitive characterization of the Melan-A peptide species expressed by HLA-A2 + melanoma cells may require a direct approach, such as mass spectrometry sequencing of the active peptide species eluted from melanoma cells (14).

A large fraction of the Melan-A-specific CTL clones derived from TIL LAU 203 required a high concentration of natural peptides to efficiently lyse T2 cells. Indeed, studies in progress in our laboratory indicate that the two natural peptides behave as weak, rather than full, agonists for these CTL cells clones (D. Valmori et al., manuscript in preparation). We have previously reported that most TILN stimulated by melanoma cells efficiently killed the tumor cells and secreted TNF-α, but few of them produced detectable levels of IL-2 and IFN-γ (19). This finding could at least partially be explained by the low levels of Ag expressed by some tumor cells (20). However, the high proportion of low affinity CTL clones found in the present study together with the relative inefficiency of natural peptides at inducing full CTL activation may also explain the functional dissociation mentioned above.

Interestingly, the Melan-A26-35 peptide variant AAAGIGILTV was not only able to form stable peptide/HLA-A*0201 complexes, but was also recognized more efficiently than the natural decapeptide by the two TILN as well as by the majority of CTL clones tested. We have recently reported that a Melan-A26-35 peptide analogue with similar characteristics can be generated by substitution of A at position 2 of the decapetide (i.e., a tolerated, but not a major, anchor residue) with a major anchor residue such as L (leucine) (11). This apparent degeneracy in CTL recognition of the antigenic peptide may seem surprising. However, it has been proposed that degeneracy in Ag recognition by T cells may be the rule rather than the exception. This conclusion was based on the results of an extensive analysis of Ag recognition by T cells using combinatorial peptide libraries (21). Moreover, epitope mimics of the Melan-A27-35 nonapeptide have been reported (22). Direct analysis ex vivo of Melan-A-specific CTL using tetramerized HLA-A2 molecules complexed with the various antigenic peptides described here is in progress in our laboratory to further evaluate the extent of degeneracy in T cell recognition of Melan-A.

In agreement with the strong correlation found between the ability of a peptide to form stable HLA-A2 complexes and its immunogenicity (23), we found that Melan-A26-35 peptide analogue ELAGIGILTV is much more efficient than both natural peptides for in vitro generation of tumor-reactive CTL by stimulation of PBMC from HLA-A*0202 melanoma patients (11). Experiments in progress in our laboratory suggest that the Melan-A26-35 peptide variant AAAGIGILTV also displays enhanced stimulatory capacities compared with those of natural sequences. Such peptide analogues may thus represent useful tools for in vitro monitoring of Melan-A-specific CTL responses as well as for improving the efficacy of in vitro procedures required to obtain specific CTL populations for adoptive transfer.
step toward possible clinical applications we are currently assessing the extent of cross-reactivity exhibited by CTL elicited in vitro by stimulation with peptide analogues with the natural sequences expressed by melanomas.

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