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Recognition of an Antigenic Peptide Derived from Tyrosinase-Related Protein-2 by CTL in the Context of HLA-A31 and -A33

Rong-Fu Wang,* Samuel L. Johnston,* Scott Southwood,† Alessandro Sette,‡ and Steven A. Rosenberg*

Tumor-infiltrating lymphocytes (TILs) derived from tumor-bearing patients recognize tumor-associated Ags presented by MHC class I molecules. The infusion of TIL586 along with IL-2 into the autologous patient with metastatic melanoma resulted in the objective regression of tumor. Two T cell epitopes derived from tumor Ags, tyrosinase-related protein (TRP)-1 and TRP-2, were shown to be recognized by HLA-A31 restricted TIL586 and its T cell clones. In this study we tested the hypothesis that these two peptides can be recognized by CTL from non-HLA-A31 patients with melanoma. It was found that both peptides were capable of binding to HLA-A3, -A11, -A31, -A33, and -A68 of the HLA-A3 supertype. Importantly, we found that HLA-A33-positive TIL1244 and its T cell clones can recognize TRP197–205 presented by both HLA A31 and A33 molecules, suggesting that a single TCR can recognize peptide/A31 and peptide/A33 complexes. However, peptide titration experiments showed that the affinity of TCR receptor to peptide/A33 could be higher than that to the peptide/A31. These studies have important implications for the development of peptide-based cancer vaccines. The Journal of Immunology, 1998, 160: 890–897.

In the last few years, significant progress has been made toward understanding the rules governing peptide binding to MHC class I molecules, the so-called peptide binding motifs (21). Based on the structural similarities of a group of HLA alleles, peptide binding motifs, sequencing analysis of pools of naturally processed and endogenously bound peptides eluted from MHC class I molecules, and peptide binding assays, several supertypes were proposed: the HLA-A2-like, -A3-like, and -B7-like (22). The A3-like supertype includes the allelic products of at least five of the most common HLA-A alleles: A3, A11, A31, A33, and A68.

In the present study we sought to expand the potential population coverage of TRP-1 and TRP-2-derived epitopes by testing the hypothesis that a particular peptide not only can bind to more than one type of HLA allele, but can still be recognized by CTL. As the first step, we demonstrate here that the antigenic peptides of TRP-1 and TRP-2 can bind to HLA-A3, -A11, -A31, -A33, and -A68. After screening 13 TILs (possibly restricted by HLA-A3, -A11 and -A33), one HLA-A33-restricted TIL1244 was identified that recognized the TRP197–205 in the context of both HLA-A31 and A33, suggesting that a single TCR can recognize a tumor-specific self-peptide presented by two different HLA-A alleles. These findings indicate that the TRP-1 and TRP-2 antigenic peptides can be used for the development of peptide-based vaccines for the treatment of melanoma patients expressing not only HLA-A31, but also HLA-A33. Based on their good peptide binding affinities, it is also possible to use TRP197–205 or ORF3P to raise CTL from patients expressing HLA-A3, -A11, and -A68.

Materials and Methods

Chemicals and reagents

The following chemicals and reagents were purchased from the sources indicated: RPMI 1640 medium, AIM-V medium, Lipofectamine, and G418 from Life Technologies (Gaithersburg, MD); the eukaryotic expression vector pCR3 from Invitrogen (San Diego, CA); anti-HLA-A31 and anti-HLA-A33 mAbs from One Lambda (Canoga Park, CA); and anti-IgM Ab conjugated with FITC from Vector Laboratories, Inc. (Burlingame, CA).

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3 Abbreviations used in this paper: TRP, tyrosinase-related protein; TIL, tumor-infiltrating lymphocyte; ORF3P, open reading frame peptide-3; GM-CSF, granulocyte-macrophage colony-stimulating factor.
T cell clones and lines

TIL586 and TIL1244 were isolated separately from tumor specimens of patients with metastatic melanoma and grown in medium containing IL-2 (6000 IU/ml; Chiron, Emeryville, CA) for 32 to 60 days as previously described (23). TIL586 and TIL1244 were predominantly CD8+ T cells. The T cell clones were generated by limiting dilution methods (at one cell per well) from the TIL1244 cell line using irradiated allogeneic PBL (1 × 10^7 cells/well) as feeder cells in RPMI 1640 medium containing 10% human AB serum and 500 IU of IL-2. After 12 days, T cell clones were expanded in AIM-V medium containing 6000 IU/ml IL-2. To obtain optimal expansion, we used the OKT3 expansion method described by S. Riddell (24). Briefly, on day 0, 5 × 10^5 T cells were cocultured with HLA-A*33 PB1 (500.1: PBL:T cell ratio) and 1500EBV B cells (100:1; EBV:T cell ratio) in 25 ml of RPMI 1640 medium containing 11% human AB serum, 30 ng/ml OKT3 Ab, and antibiotics. On day 1, IL-2 was added to a final concentration of 180 IU/ml. On day 5, the cell culture was changed to fresh medium containing 11% human AB serum and 180 IU/ml of IL-2. The medium was then changed every 3 days. On days 12 to 14, T cells were harvested, counted, and cryopreserved.

Melanoma cell lines 397mel, 397mel/A31, 586mel, 624mel, and 642mel/A31; EBV-transformed B cell lines 586EBV and 1500EBV; and T2 cells were established in our laboratory and cultured in RPMI 1640 medium containing 10% human serum and 400 IU/ml of IL-2. All cell lines are HLA-A33-positive cell lines. The COS-7 cell line was provided by Dr. W. Leonard (National Institutes of Health). The following EBV-transformed cell lines were used as sources of class I molecules: GM3107 (A*0301), BVR (A*1101), SPACH (A*3101), and LWAQIS (A*3301). A CIR transfectant, characterized by Dr. Walter Stojdl, was used as a source of A*6801. Cells were maintained in vitro by culture in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% heat-inactivated FCS. Cell cultures were also supplemented with 100 µg/ml of streptomycin (Irvine Scientific, Santa Ana, CA) and 100 U/ml of penicillin (Life Technologies, Grand Island, NY). Large quantities of cells were grown in spinner cultures.

GM-CSF secretion assay

DNA transfection and GM-CSF assays were performed as previously described (15). Briefly, 200 ng of DNA encoding Ags and 50 ng of HLA-A31 DNA were mixed with 2 µl of Lipofectamine in 100 µl of DMEM and incubated at room temperature for 15 to 45 min. The DNA/Lipofectamine mixture was then added to the COS-7 cells (5 × 10^4) and incubated overnight. The following day, cells were washed twice with DMEM medium. TIL586 was added at a concentration of 1 × 10^5 cells/well in AIM-V medium containing 120 IU/ml of IL-2. For T cell clones, only 1 to 2 × 10^5 cells/well were added. After an 18- to 24-h incubation, 100 µl of supernatant was collected, and GM-CSF was measured in a standard ELISA assay (R&D Systems, Minneapolis, MN). For peptide recognition, 586EBV or T2 cells were incubated with peptides at 37°C for 90 min and then washed three times with AIM-V medium containing 120 IU/ml of IL-2. T cells were added and incubated for an additional 18 to 24 h; 100 µl of supernatant was collected for the GM-CSF assay.

Cytotoxicity assays

The cytolytic assay was performed as previously described (7). Briefly, the target cells were labeled with chromium for 90 min. After washing three times, the cells were incubated with peptides at a concentration of 1 µg/ml for 90 min. The cells were washed again, counted, and then mixed with TIL1244, T cell clones, or CTL clone 4 at the indicated E:T cell ratio. Chromium release was measured as described (15). For peptide incubation, 4°C peptide-DNA complexes were mixed with a CIR transfectant for 18 h of ORF3P and TRP197–205 peptides, 586EBV B cells and 1500EBV B cells were incubated with various concentrations of the purified peptide. The DNA/Lipofectamine complexes were mixed with 2 µl of Lipofectamine in 100 µl of super- natant was collected, and GM-CSF was measured in a standard ELISA assay. For the A3, A11, and A*6801 clones, a T7Y analogue of HBVC41-51 (sequence STLPETYVVR) was used as the radiolabeled probe for the A*3301 assay.

The average IC_{50} values of A3CON1 for the A3, A11, A31, and A*6801 assays were 11, 6, 18, and 8 nM, respectively. The average IC_{50} of the HBVC41-51 peptide in the A*3301 assay was 29 nM.

RT-PCR analysis

RNA was extracted from T cells using the Trizol reagent according to manufacturer’s procedure (Life Technologies). T cells (1 × 10^7) of CTL clones 35 and 38 were used to isolate total RNA. RT-PCR was performed using the One-Step RT-PCR kit from Life Technologies and 6V subfamily-specific primer combined with a CβR primer from the constant region of a TCR as previously described (31). Two hundred nanograms of total RNA was used in a 50-µl RT-PCR reaction. RT-PCR was performed in one cycle of 94°C for 2 min and 50°C for 30 min, followed by 40 cycles of 94°C for 30 s for denaturation, 62°C for 20 s for annealing, and 72°C for 1 min for extension. The positive control used the constant region primers CβF and CβR. Negative controls used RNA or water instead of cDNA in the PCR reactions. RT-PCR products were resolved on a 1% agarose gel.

Results

Peptide binding to HLA class I molecules of the HLA-A3 superfamily

Based on the structural similarities and sequencing of pools of naturally processed peptides bound on MHC class I molecules, it was recently proposed that a set of HLA-A alleles, including HLA-A3, -A11, -A31, -A33, and -A68, could be grouped into a superfamily or designated the A3-like supertype (28). These HLA class
I molecules bind to peptide ligands with hydrophobic anchor residues at position 2 and positively charged residues at the COOH-termini (21, 28) (Table I). Two tumor Ag peptides, ORF3P, derived from the gene product of alternative open reading frame of TRP-1, and TRP197–205, derived from the TRP-2 normal coding sequence, were recently identified. Both of these epitopes conformed to the previously described canonical HLA-A31 binding motif (Table I). A modified peptide of TRP197–205, TRP197–205K, was also included in Table I, as this peptide was also recognized by the HLA-A31-restricted CTL clone 4 when pulsed onto HLA-A31 EBV B cells. Since these tumor Ag peptides were recognized by HLA-A31-restricted CTLs (16, 20), it was of interest to test whether TRP-1 and TRP-2 could be recognized by CTLs in the context of other HLA alleles, such as HLA-A3, -A11, -A33, and -A68. As the first step, we performed peptide binding assays to determine whether ORF3P, TRP197–205 and TRP197–205K could bind to members of the A3-like family. As shown in Table II, these three peptides were indeed capable of binding to A3, A11, A31, A33, and A68 molecules. The peptide ORF3P bound to all five MHIC class I members of the A3-like superfamily with relatively high binding affinity. The peptide TRP197–205 exhibited a relatively high binding affinity to HLA-A31, intermediate binding affinity to HLA-A33, and relatively low binding affinity to HLA-A3, -A11, and -A68. Interestingly, the binding affinity of TRP197–205 to HLA-A33 and -A11 could be improved significantly by the substitution of Arg with Lys at the C-terminus of the peptide. As shown in Table II, TRP197–205K exhibits a high binding affinity to both HLA-A3 and -A11, approximately 100-fold higher than the parental peptide TRP197–205. Furthermore, this substitution had little or no effect on the binding affinity of the peptide to HLA-A31 and -A33, and only a marginally negative effect on the binding affinity of the peptide to HLA-A68.

Identification of TILs with reactivity to the TRP-2 peptide

Although many tumor Ags have been identified and their T cell epitopes have been determined (7), it has not been reported that the same T cell epitope peptide can bind to different MHC alleles in the superfamily and still be recognized by the corresponding CTL. To test this possibility, we collected five TILs isolated from patients expressing HLA-A3, six TILs from patients expressing HLA-A11, and two TILs from patients expressing HLA-A33 and grew them in RPMI 1640 medium with 10% human AB serum and 6000 IU/ml of IL-2. After 1 wk, these TILs were tested for recognition of TRP197–205 or TRP197–205K pulsed on HLA-A3+, -A11+, and -A33+ EBV B cells, respectively. No T cell recognition was found from TILs isolated from patients expressing either HLA-A3 or -A11. Failure to identify HLA-A3- and HLA-A11-restricted TILs that recognize ORF3P or TRP197–205 may be due to lack of true HLA-A3- and HLA-A11-restricted CTL in the bulk TIL populations. However, one of two HLA-A33-restricted TILs, TIL224, was found to recognize the same TRP197–205 peptide in the context of HLA-A33 (Table III). The phenotype of HLA-A33+ 1500EBV was confirmed by FACS analysis. 1500EBV stained positive with anti-HLA-A33 Ab, but negative with anti-HLA-A31 Ab. By contrast, 586mel and 586EBV were positive for HLA-A31 and negative for HLA-A33 (data not shown), suggesting that HLA-A31 and -A33 molecules can be distinguished by mAbs.

Table I. Peptide binding motifs of the A3-like superfamily and peptides used in this study

<table>
<thead>
<tr>
<th>HLA-A Alleles</th>
<th>Peptide Binding Motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>KVM FY IMFVL ILMF K</td>
</tr>
<tr>
<td>A11</td>
<td>VIFY MLIY ILVVF K</td>
</tr>
<tr>
<td>A31</td>
<td>LVST FLYW LIVF YFA</td>
</tr>
<tr>
<td>A33</td>
<td>ALIF YVS R</td>
</tr>
<tr>
<td>A68</td>
<td>VT R/K</td>
</tr>
</tbody>
</table>

Peptides used in this study

- ORF3P
- TRP197–205
- TRP197–205K

Table II. Binding affinity of antigenic peptides to the A3-like superfamily

<table>
<thead>
<tr>
<th>Peptides</th>
<th>A3</th>
<th>A11</th>
<th>A31</th>
<th>A33</th>
<th>A68</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF3P</td>
<td>54</td>
<td>8.3</td>
<td>8.3</td>
<td>14</td>
<td>8.5</td>
</tr>
<tr>
<td>TRP197–205</td>
<td>570</td>
<td>656</td>
<td>11</td>
<td>106</td>
<td>1086</td>
</tr>
<tr>
<td>TRP197–205K</td>
<td>5.0</td>
<td>8.8</td>
<td>11</td>
<td>60</td>
<td>1569</td>
</tr>
</tbody>
</table>

*Peptide binding affinity was presented as peptide concentrations (nmol) required for 50% inhibition of the standard peptide (IC50) in the competitive inhibition assay. IC50 values for a high affinity binder, an intermediate affinity binder, and a low affinity binder are defined as 1 to 50 nM, 51 to 500 nM, and >501 nM, respectively.

Table III. Screening of TILs with reactivity to TRP-1 or TRP-2 peptides

<table>
<thead>
<tr>
<th>GM-CSF Secretion (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines</td>
</tr>
<tr>
<td>A3-TIL</td>
</tr>
<tr>
<td>A11-TIL</td>
</tr>
<tr>
<td>A33-TIL</td>
</tr>
<tr>
<td>907EBV A3 None</td>
</tr>
<tr>
<td>907EBV A3 ORF3P</td>
</tr>
<tr>
<td>907EBV A3 TRP197–205</td>
</tr>
<tr>
<td>1485EBV A11 None</td>
</tr>
<tr>
<td>1485EBV A11 ORF3P</td>
</tr>
<tr>
<td>1485EBV A11 TRP197–205</td>
</tr>
<tr>
<td>1500EBV A33 None</td>
</tr>
<tr>
<td>1500EBV A33 ORF3P</td>
</tr>
<tr>
<td>1500EBV A33 TRP197–205</td>
</tr>
</tbody>
</table>

*907EBV, 1485EBV, and 1500EBV were incubated with individual peptides at a concentration of 1 mg/ml for 90 min. GM-CSF release was measured after coincubation of peptide-loaded EBV cells with TILs. GM-CSF secretion by TIL alone without stimulators was subtracted. 907EBV, 1485EBV, and 1500EBV were EBV-transformed B cell lines expressing HLA-A3, -A11, and -A33, respectively.

T cell recognition of the TRP-2 peptide in the context of HLA-A31 and -A33

Since TRP197–205 is a good HLA-A31 binder (Table II), we next tested whether the HLA-A33-restricted TIL224 was also capable of recognizing the A31/peptide complex. To this end, ORF3P and TRP197–205 were pulsed onto HLA-A31+ 586EBV, HLA-A33+ 1500EBV, and T2 cells, respectively, and evaluated for T cell recognition by measuring GM-CSF release. TIL224 recognized both HLA-A31+ and HLA-A33+ EBV B cells pulsed with the TRP197–205 peptide, but not T2 cells pulsed with the TRP197–205 peptide or HLA-A31+ or HLA-A33+ EBV B cells pulsed with the ORF3P peptide (Fig. 1). TIL224 also recognized the modified peptide TRP197–205K.
peptide TRP$_{197-205}$K as effectively as the parental peptide. In contrast, CTL clone 4, which recognized the TRP$_{197-205}$ peptide pulsed onto HLA-A311 EBV B cells, did not respond to the TRP$_{197-205}$ and TRP$_{197-205}$K peptides presented by HLA-A331 EBV B cells (Fig. 1).

To confirm this observation, we transfected COS-7 with either HLA-A31 or HLA-A33 combined with either TRP-1 or TRP-2 cDNA. As expected, TIL1244 recognized COS-7 transfected with HLA-A33 and TRP-2 cDNA, but not COS-7 transfected with HLA-A33 and TRP-1 cDNA (Fig. 2A). Interestingly, TIL1244 also recognized COS-7 transfected with HLA-A31 plus TRP-2. However, TIL1244 did not recognize COS-7 transfected with HLA-A31 plus TRP-1 or with HLA-A31 alone (Fig. 2A). These results demonstrated that TIL1244 were capable of recognizing the TRP$_{197-205}$ peptide presented by either HLA-A31 or HLA-A33 molecules.

CTLs generated in vitro in many cases are capable of recognizing a peptide pulsed onto EBV B cells, but fail to recognize tumor cells (7). Therefore, it was of interest to test whether TIL1244 was capable of recognizing HLA-A31$^+$ tumor lines. The experimental results in Figure 2B indicated that 586mel (A31$^+$ TRP-2$^+$), 397mel/A31 (A31$^+$, TRP-2$^+$), and 624mel/A31 (A31$^+$, TRP-2$^+$) all stimulated GM-CSF release from TIL1244, while 397mel (A31$^+$, TRP-2$^+$) and 624mel (A31$^+$, TRP-2$^+$) failed to stimulate cytokine release from TIL1244. In addition, 624mel transfected with HLA-A33 was shown to confer recognition by TIL1244. Taken together, these results indicated that TIL1244 is capable of recognizing tumor cells expressing TRP-2 and either HLA-A31 or HLA-A33 molecules.

T cell clones derived from TIL1244

Since TIL1244 is a bulk T cell line, recognition of TRP$_{197-205}$ by TIL1244 in the context of HLA-A31 and -A33 could be due to the coexistence of different subsets of T cell populations: one recognizing TRP-2 in the context of HLA-A31 and the other recognizing TRP-2 in the context of HLA-A33. To test this possibility, T cell clones were generated by limiting dilution. Of 136 clones tested, 50 clones (36%) were found to recognize 586EBV B cells and 1500EBV pulsed with TRP$_{197-205}$, but not EBV B cells alone. Four CTL clones were further expanded using the anti-OKT3 rapid expansion method and tested for their recognition of either HLA-A31$^+$ or HLA-A33$^+$ tumor cells. All four CTL clones recognized 586mel, 397mel/A31, 624mel/A31, and 624/A33 (data not shown). The cytolytic activity of TIL1244 and its derived CTL clones against different targets was also tested at different E:T ratios. As shown in Figure 3, A and B, TIL1244 efficiently lysed 586mel, TRP$_{197-205}$-pulsed 586EBV, and 1500EBV B cells, while no lysis was observed in the case of 397mel or T2 cells pulsed with TRP$_{197-205}$. Similar results were obtained when the CTL clones 35 and 38 were used as effector cells (Fig. 3, C and D). The clonality of CTL clones 35 and 38 was confirmed by RT-PCR analysis using TCR $\beta$ subfamily-specific primers (31). Only one DNA band of about 600 bp was detected from RT-PCR products amplified with $\beta$V16-specific primer combined with a C$\beta$R primer from the constant region of TCR, while no DNA band was observed from the RT-PCR reactions using other $\beta$V-specific primers combined with the C$\beta$R primer (Fig. 4). These results suggested that a single TCR receptor can recognize the same peptide presented by either HLA-A31 or A33 molecules.

Characterization of T cell recognition

From the experiments presented in Figure 3, it was evident that lysis of 1500EBV pulsed with TRP$_{197-205}$ peptide by TIL1244 and CTL clones 35 and 38 was higher than that of 586EBV pulsed with the same peptide. To further analyze the differential recognition of target cells by both TIL1244 and its CTL clones, peptide titration experiments were performed. As shown in Figure 5A, there was a significant difference in the peptide concentrations required to obtain similar cytokine release from the TIL1244 stimulated by different APCs. The T cell response could be detected down to 1 ng/ml of TRP$_{197-205}$ and plateaued at 1 $\mu$g/ml in the case of
However, at least a 10-fold higher peptide concentration was required for detectable responses when the same peptide was pulsed onto 586EBV B cells. The T cell response in this case did not reach a plateau at a concentration of 1 μg/ml of the peptide. Although the poor response of TIL1244 to the TRP197–205 peptide when loaded on HLA-A31 cells could result from the difference in peptide binding affinity to HLA-A31 molecules on 586EBV vs that to HLA-A33 molecules on 1500EBV, the peptide binding assay in Table II ruled out this possibility. In contrast, the response of HLA-A31-restricted CTL clone 4 to the TRP197–205 peptide reached a plateau at 1 ng/ml when pulsed onto HLA-A31 cells, and no recognition was observed when the same peptide was pulsed onto HLA-A33 cells (Fig. 5B). Furthermore, both 586EBV and 1500EBV B cells expressed a comparable level of MHC class I molecules (HLA-A31 or -A33) on the cell surface (data not shown). These results indicated that the differential recognition of the same peptide on either HLA-A31 or -A33 by TIL1244 might be due to the difference in the TCR affinity of TIL1244 to A31/peptide compared with that to A33/peptide. Consistent with this hypothesis were results obtained in experiments in which various peptide concentrations were evaluated (Fig. 6), strongly suggesting that TCR affinity rather than MHC/peptide affinity is responsible for the observed patterns of T cell recognition and target lysis.
Discussion

In this study we demonstrate that the antigenic peptides derived from TRP-1 and TRP-2 are capable of binding to all members of the A3-like supertype. Although the majority of antigenic peptides identified from melanoma Ags to date have intermediate or relatively low binding affinity to MHC molecules (6), the ORF3P peptide has a relatively high binding affinity to all alleles tested. By contrast, the TRP197–205 peptide has different affinities, ranging from high to low, for the five alleles. Interestingly, ORF3P and TRP197–205 display identical or similar binding to HLA-A31 and -A33, but their binding affinities to HLA-A3,-A11, and -A68 are very different, suggesting that secondary anchor residues play an important role in determining the binding affinity of this particular peptide to A3 supertype MHC molecules.

The binding affinity of TRP197–205 to HLA-A3 and -A11 can be improved by substituting Arg with Lys. It was reported that the improved binding affinity of the modified peptides by substitution of anchor residues with optimal or preferred residues enhanced the immunogenicity of peptides in vitro (32). However, the correlation between the peptide binding affinity and immunogenicity in vivo
remains to be established. Recent reports suggest that peptide binding affinity for MHC class I molecules is a major factor, but not the sole factor, in determining immunogenicity (32, 33). Other factors, such as peptide liberation, TAP transport, and TCR repertoire for a particular peptide, also affect the immunogenicity of peptide in vivo (33). The existence of immunodominant peptides may affect the immunogenicity of the other peptides, such as a cryptic peptide (33, 34). For this reason, peptide-based vaccines may have advantages over other types of vaccines because one should be able to direct the immune response toward cryptic peptides in cases where an immunodominant epitope suppresses cryptic peptides to elicit an immune response (34).

Direct peptide binding assay has allowed the definition of several HLA supertypes initially suggested by HLA structural similarity and peptide binding motifs (22, 28). However, these studies did not demonstrate that these MHC/peptide complexes can be recognized by CTL. It was previously reported that a viral peptide of HBCAg from hepatitis B virus was capable of binding to HLA-A31 and -A68 and was recognized by their corresponding CTLs isolated from a single patient (35). To our knowledge, this is the first demonstration that a self cancer peptide, TRP$_{197–205}$, not only binds to all members of the HLA-A3 superfamily, as discussed above, but is also recognized by the HLA-A31-restricted CTL clone 4 and the HLA-A33-restricted CTL derived from different patients. Furthermore, it was found that TIL1244 was capable of recognizing the TRP$_{197–205}$ peptide presented by either HLA-A31 or -A33 molecules. These studies suggest that self Ags may be used to treat patients expressing HLA-A31 or HLA-A33 and perhaps other members of the HLA-A3-like supertype. Since the modified peptide TRP$_{197–205K}$ exhibits better binding to HLA-A3 and -A11, and still can be recognized by the HLA-A31-restricted CTL clone when pulsed onto A31 EBV B cells as well as by TIL1244 when presented by both HLA-A31 and -A33 EBV B cells, this peptide may be a good candidate for a cancer vaccine for the treatment of patients expressing HLA-A3. -A11, -A31, and -A33. Generation of HLA-A3- or HLA-A11-restricted CTLs that are capable of recognizing these peptides derived from TRP-1 and TRP-2 is important for our understanding of T cell recognition of peptides presented by members of the A3 superfamily. These studies are currently under investigation.

Although the TRP$_{197–205}$ peptide bound to HLA-A31 10 times better than to HLA-A33 (Table II), recognition of the peptide/HLA-A31 complex by TIL1244 and its T cell clones was 10 times worse than that of the peptide/HLA-A33 complex (Figs. 5 and 6), strongly suggesting that the TCR receptor of TIL1244 or its T cell clones has a low avidity for peptide/HLA-A31 complexes. Nonetheless, TIL1244 strongly recognized both tumor cells expressing TRP-2 and HLA-A31 and tumor cells expressing TRP-2 and HLA-A33 (Fig. 2B). This may be due to high expression levels of TRP-2 in tumor cells (16), leading to a high density of the TRP-2 peptide/HLA-A31 or HLA-A33 complexes on the surface of tumor cells. T cell clones with differential TCR avidity for antigenic peptide/MHC complexes have indeed recently been described (36). Fully understanding the relationship between TCR avidity and the number of MHC/peptides required for optimal TCR engagement will have important implications for the treatment of patients with cancer or autoimmune disease.

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References


