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

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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 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).
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 x 10^6 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 x 10^5 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 x 10^5

Class I peptide binding assays

Quantitative assays for the binding of three peptides to soluble MHC class I molecules on the basis of the inhibition of binding of a radiolabeled standard probe peptide to detergent solubilized MHC molecules were conducted as previously described (28). Briefly, purified human class I molecules (5-500 nM) were incubated with 1 to 10 nM [3H]-radiolabeled probe peptide, iodinated by the chloramine-T method (29), for 48 h at room temperature in the presence of 1 μM human β2m (Scripps Laboratories, San Diego, CA) and a mixture of protease inhibitors. The final concentrations of protease inhibitors were 1 mM PMSF, 1.3 nM 1.10 phenanthroline, 73 μM pepstatin A, 8 μM EDTA, and 200 μM Nα-p-tosyl-L-lysine chloromethyl ketone (TLCK). Class I peptide complexes were separated from free peptide by gel filtration on TSK200 columns, and the fraction of bound peptide was calculated as previously described (25). In preliminary experiments, the HLA class I prep was titrated in the presence of fixed amounts of radiolabeled peptides to determine the concentration of class I molecules necessary to bind 10 to 20% of the total radioactivity. All subsequent inhibition and direct binding assays were performed using these class I concentrations. In the inhibition assays, peptide inhibitors were typically tested at concentrations ranging from 120 μg/ml to 1.2 ng/ml. The data were then plotted, and the dose yielding 50% inhibition was measured. Peptides were tested in two to four completely independent experiments. Since under these conditions concentration of label < concentration of MHC and IC50 conjugation of MHC, the measured IC50 values are reasonable approximations of the true kilodalton values. The radiolabeled probe and standard control peptides used are as follows. The A*0101 peptide sequence KYVPYPALINK (30) was used as the radiolabeled probe for the A3, A11, A31, and A*6801 alleles. A T7Y analogue of HBVpC141-151 (sequence STLPETYVVVR) was used as the radiolabeled probe for the A*3301 allele. The average IC50 values of A301 for the A3, A11, A31, and A*6801 alleles were 11, 6, 18, and 8 nM, respectively. The average IC50 of the HBVpC141-151 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 x 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 5′ 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 superfamily members present a diverse range of naturally processed peptides, and this ability may be characteristic of HLA-A3 superfamily members.
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, TRP 197–205K, 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 MHC 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-A3 and -A11 could be improved significantly by the substitution of Arg with Lys at the COOH-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 ORF3P and TRP197–205 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, TIL244, 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.

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 TIL244 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. TIL244 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). TIL244 also recognized the modified peptide TRP197–205K.

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>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>KVM FY IMFLV ILMF K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11</td>
<td>VIFY MLIVY ILVYF K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A31</td>
<td>LVST FLYW LIVF YF</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A33</td>
<td>AILF YVS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>VT</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRP197–205</td>
<td>L L G P G P Y R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRP197–205K</td>
<td>L L G P G P Y K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></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>
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<tr>
<td>ORF3P</td>
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<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>
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<tr>
<td>TRP197–205K</td>
<td>5.0</td>
<td>8.8</td>
<td>8.8</td>
<td>160</td>
<td>1569</td>
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</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>Cell lines</th>
<th>MHC</th>
<th>Peptides</th>
<th>(5 TILs tested)</th>
<th>(6 TILs tested)</th>
<th>TIL1138</th>
<th>TIL1244</th>
</tr>
</thead>
<tbody>
<tr>
<td>907EBV</td>
<td>A3</td>
<td>None</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>907EBV</td>
<td>A3</td>
<td>ORF3P</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>907EBV</td>
<td>A3</td>
<td>TRP197–205</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>1485EBV</td>
<td>A11</td>
<td>None</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>1485EBV</td>
<td>A11</td>
<td>ORF3P</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>1485EBV</td>
<td>A11</td>
<td>TRP197–205</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>1500EBV</td>
<td>A33</td>
<td>None</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>1500EBV</td>
<td>A33</td>
<td>ORF3P</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>1500EBV</td>
<td>A33</td>
<td>TRP197–205</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

*907EBV, 1485EBV, and 1500 EBV 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.
peptide TRP 197–205K as effectively as the parental peptide. In contrast, CTL clone 4, which recognized the TRP 197–205 peptide pulsed onto HLA-A31 B cells, did not respond to the TRP 197–205 and TRP 197–205K peptides presented by HLA-A33 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 plus TRP-2. However, TIL1244 did not recognize COS-7 transfected with HLA-A31 plus TRP-2. 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 also 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 cytolylactic 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 βV subfamily-specific primers (31). Only one DNA band of about 600 bp was detected from RT-PCR products amplified with a βV16-specific primer combined with a CβR primer from the constant region of TCR, while no DNA band was observed from the RT-PCR reactions using other βV-specific primers combined with the CβR 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 µg/ml in the case of
1500EBVB cells. 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

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

B cells, and no recognition was observed when the same peptide was pulsed onto HLA-A33

B 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 TRP_{197-205} peptide has different affinities, ranging from high to low, for the five alleles. Interestingly, ORF3P and TRP_{197-205} 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 TRP_{197-205} 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. 28). 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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