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*J Immunol* 2000; 164:5192-5198; doi: 10.4049/jimmunol.164.10.5192

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An Altered Peptide Ligand Antagonizes Antigen-Specific T Cells of Patients with Human T Lymphotropic Virus Type I-Affiliated Neurological Disease

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Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is an inflammatory neurologic disease associated with HTLV-I infection, in which chronically activated, HTLV-I-specific CD8⁺ T cells have been suggested to be immunopathogenic. In HLA-A2 HAM/TSP patients, CD8⁺ HTLV-I-specific CTLs recognize an immunodominant peptide of the HTLV-I Tax protein, Tax₁₁₋₁₉. We examined the functional outcome on activation of both cloned peripheral blood and cerebrospinal fluid-derived CTL and bulk PBMC from HAM/TSP patients by altered peptide ligands (APL) derived from HTLV-I Tax₁₁₋₁₉. In CTL clones generated from PBMC and CSF of HLA-A2 HAM/TSP patients, an APL substituted at position 5 significantly decreased CTL responses when compared with the native peptide. Moreover, these ligands were also shown to inhibit CTL responses to the native peptide in bulk PBMC of HLA-A2 HAM/TSP patients. These data suggest that a modification of an antigenic peptide at the central position can manipulate the T cell responses in bulk PBMC from different individuals with an inflammatory disease. Additionally, these results have implications for the potential use of APL-based immunotherapy in this T cell-mediated CNS disease. The Journal of Immunology, 2000, 164: 5192–5198.

T cells specifically recognize antigenic peptide bound to self MHC molecules on APC. However, recent studies have clearly demonstrated that the TCR can interact with a spectrum of ligands, suggesting that TCR recognition of peptide-MHC complexes is more degenerate than previously considered (1–6). Moreover, modifications of agonist ligands in TCR contact positions by altered peptide ligands (APL) may result in a broad spectrum of functional outcomes, ranging from TCR agonism to partial agonism or antagonism (1, 7–11). The latter APL can antagonize T cell functions through direct interaction with the TCR, rather than competing for binding to the restricting MHC molecules with the native peptide (2, 7, 11). APLs are biologically relevant during thymic positive selection (12), and during chronic viral infections, e.g., via escape mutants of important viral epitopes that inhibit protective immune responses (13, 14). Furthermore, APL variants of Plasmodium falciaparam circumsporozoite epitopes have been shown to interfere with Ag priming (15). Consequently, APLs have also been considered for immunotherapeutic strategies to manipulate T cell functions in vivo. In experimental allergic encephalomyelitis (EAE), an animal model for autoimmune disease of CNS, it has been reported that APL can ameliorate or prevent disease (16–18). In humans, functions of Ag-specific T cell clones have been demonstrated to be altered by appropriate APL (19, 20). However, in human diseases caused by Ag-specific T cells that often display a broad range of fine specificity patterns (6), it remains uncertain whether an APL can modulate functions of immunopathogenic T cells or alter the course of a given disease.

Human T lymphotropic virus type I (HTLV-I) is a human retrovirus well known as the causative agent of adult T cell leukemia/lymphoma and a slowly progressive neurological disorder, termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (21–23). The disease is characterized by perivascular inflammatory cell infiltration with a predominance of CD8⁺ lymphocytes in the spinal cord (24, 25). In such lesions, the existence of HTLV-I-specific CD8⁺ CTL as well as expression of HTLV-I gene products has also been shown (26–29). HAM/TSP patients show extraordinarily high levels of circulating CD8⁺ CTL specific for HTLV-I pX products, which predominantly recognize the HTLV-I Tax₁₁₋₁₉ peptide (LLFGYPVYYV) in human leukocyte Ag (HLA)-A2 HAM/TSP patients (30–32). Collectively, these studies support the view that the virus-specific CD8⁺ CTL may be immunopathogenic for HAM/TSP (33).

Recently, a crystal structural analysis of two different human TCR/HTLV-I Tax₁₁₋₁₉/HLA-A2 complexes showed that these TCR Vα and Vβ bind diagonally to the Tax/HLA-A2 complexes and form central pockets by the complementarity-determining region (CDR) 3α and CDR3β (34, 35). The tyrosine at position 5 of the Tax₁₁₋₁₉ peptide fits into these pockets and makes numerous contacts with the TCR. Furthermore, glycine at position 4 and tyrosine at position 8 bind to the CDR3α and the CDR3β and CDR1β, respectively, which are associated with fewer TCR contacts than the tyrosine at position 5. This suggests that the tyrosine at the central position of the Tax peptide may be a significant residue for TCR recognition. Based on this information, we questioned whether or not the tyrosine at position 5 of the Tax₁₁₋₁₉ peptide plays an important role in TCR recognition of HLA-A2-restricted, HTLV-I Tax₁₁₋₁₉-specific CTLs and whether APLs substituted at TCR contact residues antagonize HTLV-I-specific CTLs.
from HAM/TSP patients. The high frequency of circulating CTL specific for HTLV-I Tax peptide in patients with HAM/TSP and the defined TCR/HTLV-I Tax structure-function relationship afford the unique opportunity to test whether APLs can alter T cell function in bulk PBMC. Thus, this system is uniquely suited to address whether APL-based therapies targeting a disease-relevant part of the T cell immune response can be employed in vivo in humans.

In the present study, we investigated HTLV-I Tax_{11–19}-specific CTL responses both in CD8\(^+\) T cell clones and bulk PBMC from HLA-A2 HAM/TSP patients using APL derived from Tax_{11–19}. Our data suggest that tyrosine at position 5 is a primary TCR contact residue in the majority of these T cells, although CTL responses are heterogeneous to each APL. Moreover, CTL responses of both CD8\(^+\) T cell clones and bulk PBMC were significantly antagonized by the APL, implying that this APL may potentially be beneficial as an immunotherapy for HAM/TSP patients.

Materials and Methods

Subjects

Four HLA-A2 HAM/TSP patients (1–4) were examined under a National Institute of Neurological Disorders and Stroke (NINDS) Institutional Review Board-approved protocol. HTLV-I infection was confirmed by Western blot of these patients’ sera (HTLV Blot 2.4; Genelabs Technologies, Singapore). The diagnosis of HAM/TSP was made using the World Health Organization criteria and is based on the patients’ neurological symptoms and a serological test for HTLV-I (36). PBMC were isolated by Ficoll-Hypaque centrifugation and stored in liquid nitrogen until use.

Peptides

The amino acid sequence of HTLV-I Tax_{11–19} was LLFGYPVVYV (31). According to the crystal structure of two TCR/Tax_{11–19}/HLA-A2 complexes, glycine at position 4, tyrosine at position 5, and tyrosine at position 8 of the Tax peptide are major TCR contact residues (34, 35, 37), while leucine in position 2 and valine in position 9 are crucial for MHC binding to HLA-A2 (38). Therefore, we synthesized 1-alanine-substituted peptides at positions 1, 4, 5, 6, 8, and 9 and designated them L1A, G4A, Y5A, P6A, Y8A, and V9A, respectively. Influenza virus M1 peptide (GILGFVFTL) was used as a control peptide, which binds to HLA-A2 (39). Peptides were synthesized with the Multisynthesizer Synthesis System (Chiron Mimotopes, Clayton, Victoria, Australia) at greater than 93% purity, as determined by HPLC analysis. Peptides were dissolved in PBS containing 50% DMSO at a concentration of 1 mM. When used, peptides were diluted with indicated medium. HLA-A*0201-binding affinities of the peptides can be predicted via the Internet (http://bimas.dcrt.nih.gov/molbio/hla_bind/). The estimated 1/2 (min) was 2406 by Tax_{11–19}, 1415 by L1A, 2406 by G4A, 2406 by Y5A, 2406 by P6A, 437 by Y8A, 171 by V9A, and 550 by M1 peptide.

Generation of HTLV-I Tax_{11–19}-specific CD8\(^+\) CLT clones

HTLV-I Tax_{11–19}-specific CTLs were derived from PBMC of three HLA-A2 HAM/TSP patients, 1, 2, and 3, and CSF cells of a HAM/TSP patient 1. Peripheral blood CD8\(^+\) cells from patient 1 were positively purified by anti-CD8 Ab-coupled immunomagnetic beads (Dynal, Lake Success, NY). After detachment of bound beads, cells were seeded at limiting dilution (0.3–1 cell/well) in a 96-well U-bottom plate with 200 μL of IMDM (Life Technologies, Gaithersburg, MD) containing 20% human serum, 2.8 mM L-glutamine, 40 μU/ml streptomycin, and 100 μU/ml penicillin. After 3-h incubation at 37°C, the supernatant was harvested and the fluorescence intensity was measured as described above. The percent inhibition was calculated using the following formula: [(the percent specific lysis for APL)/(the percent specific lysis for Tax_{11–19})] × 100. The assay was conducted in triplicate.

**CTL antagonist assay**

The CTL antagonist assay was performed according to the protocol previously described, with minor modifications (11, 13, 14). Briefly, a suboptimal concentration of native Tax peptide was first determined by titration assays with the peptide. The concentration was determined as that which MHCs on the target cells may not be saturated with the peptide, but CTL response can be induced. Hmy-A2 target cells were prepulsed with a suboptimal concentration of Tax_{11–19} peptide (0.1 nM for both T cell clones and bulk PBMC) for 1 h, and washed three times. Three thousand target cells were transferred to 96-well plates and incubated with serially diluted APL for 30 min. Then, effector cells were added to reach an E:T ratio of 1:1 for T cell clones or of 100:1 for PBMC. After 3-h incubation at 37°C, the supernatant was harvested and the fluorescence intensity was measured as described above. The percent inhibition was calculated using the following formula: [(1 – (the lysis with APL – background lysis)/(the lysis without APL − background lysis))] × 100. The assay was conducted in triplicate.

**Statistical analysis**

Specific lysis to each target was compared by using Student’s t test. The χ² test was used to determine what APL predominantly acts as an agonist or an antagonist for TCR in the T cell clones. A p value <0.05 was considered statistically significant.

**Results**

**TCR agonism of HTLV-I Tax_{11–19}-specific CTL clones**

To determine which concentration of HTLV-I Tax_{11–19} induces cytolyis by the HTLV-I Tax_{11–19}-specific CTL clones, a dose titration assay was performed using 13 CTL clones from two HLA-A2 HAM/TSP patients. As shown in Fig. 1A, cytolytic activity of targets prepulsed with Tax_{11–19} appeared at 0.01–0.1 nM and reached a plateau at 1 nM in the CTL clone N1104, whereas cytolytic activity by APLs was observed only in P6A at 1 μM. In the other CTL clones tested, the titration curves of Tax_{11–19} were similar to that in clone N1104, whereas cytolytic activities to APLs at 1 μM varied in each clone (data not shown). Therefore, we chose 1 μM peptide to assess the magnitude of TCR recognition in further studies. Fig. 2 indicates the TCR recognition profile of representative HTLV-I Tax_{11–19}-specific clones. CTL clone N1106 from PBMC of HAM/TSP patient 1 was tolerant to the alanine substitution at position 4, but not at position 5. However, none of the APLs sensitized targets for lysis by CTL clone N1216.
from the same patient’s PBMC. In CTL clone 2208 from PBMC of HAM/TSP patient 2, G4A, P6A, and Y8A were well tolerated by the TCR. However, Y5A abrogated recognition by this clone. In CTL clone 3204 from PBMC of HAM/TSP patient 3, no APL was recognized. The relative cytolysis for each APL to the native peptide in 36 HTLV-I Tax 11–19-specific CTL clones is summarized in Fig. 3 (which is shown as a figure in Ref. 6). Almost all clones tolerated V9A. In 23 CTL clones from PBMC of three HAM/TSP patients, over 60% of relative cytolysis to the native Tax peptide was observed in 18, 6, 11, and 11 clones in response to G4A, Y5A, P6A, and Y8A, respectively. Alternatively, less than 30% of relative cytolysis was shown in 5, 10, 7, and 6 clones, respectively. T cell clones derived from the CSF of patient 1 showed a more restricted recognition repertoire to these APLs compared with the PBMC-derived clones from the same patient. Over 60% of relative cytolysis was observed in 6, 1, 1, and 1 CSF clones in response to the G4A, Y5A, P6A, and Y8A ligand, whereas less than 30% of the cytolysis was observed in 5, 8, 8, and 10 clones, respectively. In patient 1, the average relative cytolysis of P6A and Y8A significantly decreased in CSF-derived clones when compared with that in PBMC-derived clones from the same patient. Over 60% of relative cytolysis was observed in 6, 1, 1, and 1 CSF clones in response to the G4A, Y5A, P6A, and Y8A ligand, whereas less than 30% of the cytolysis was observed in 5, 8, 8, and 10 clones, respectively.

**FIGURE 1.** Cytolysis with titrated HTLV-I Tax 11–19 peptide and APLs in cloned CTL and bulk PBMC. HLA-A2-transfected targets were pulsed with different concentrations of peptides. The CTL assay was conducted in triplicate. The data point indicates mean ± SD. A. Shows the cytolytic activity of HTLV-I Tax 11–19-specific CD8⁺ CTL clone N1104 against either Tax-pulsed targets (lysis started at 0.01–0.1 nM and reached a plateau above 1 nM), or targets pulsed with various APL (lysis of P6A-pulsed targets appeared at concentration of 1 μM and was very low). E:T ratio was 1:1. B. Demonstrates a representative CTL experiment of bulk PBMC from two HAM/TSP patients. Lysis appeared at approximately the same concentration as that observed in T cell clones and plateaued at 10 nM. The E:T ratio was 100:1.

**FIGURE 2.** Representative TCR agonist assay for HTLV-I Tax 11–19-specific CTL clones. HLA-A2-transfected target cells were pulsed with 1 μM of Tax peptide, APLs, or influenza virus M1 peptide (control). NP indicates the lysis of unpulsed targets, i.e., no peptide. The specific lysis is presented by mean ± SD. The specific lysis for APL or M1 peptide was compared with that for Tax 11–19 by using Student’s t test (*, p < 0.05). APL Y5A was not recognized by any of the clones. Overall, there was a differential response to the various APL by the four clones. The CTL assay was conducted in triplicate. The E:T ratio was 1:1.

**FIGURE 3.** Summary of TCR agonist assays of 36 HTLV-I Tax 11–19-specific CTL clones from three HLA-A2 HAM/TSP patients. Target cells were pulsed with 1 μM of each peptide. Specific lysis was standardized to relative cytolysis by dividing the percent specific lysis for each APL by the percent specific lysis for Tax peptide, and ranked according to the relative cytolysis to the native peptide. Asterisks indicate the minimal lysis to the respective APL for each clone. The clone number showing the minimal recognition was 8 by G4A, 12 by Y5A, 4 by P6A, and 12 by Y8A. The CTL assay was conducted in triplicate. The E:T ratio was 1:1.
TCR antagonism of HTLV-I Tax11–19-specific CTL clones

For the TCR antagonist assay, a suboptimal concentration of the APL by using Student’s t test (*, p < 0.05). The x-axis indicates the concentration of APL added. Zero on the x-axis indicates the extent of cytolysis of targets prepulsed with Tax peptide in the absence of APL. NP indicates cytolysis of targets not prepulsed with Tax peptide. The CTL assay was conducted in triplicate. The E:T ratio was 1–2:1.

tested in cytolytic assays with this large sample of PBL-derived T cell clones from HAM/TSP patients. In the CSF-derived clones, Y5A, P6A, and Y8A were less potent ligands than G4A.

TCR antagonism of HTLV-I Tax11–19-specific CD8$^+$ CTL clones

For the TCR antagonist assay, a suboptimal concentration of the native Tax11–19 peptide (0.1 nM) was chosen to prepulse targets based on the titration assay in Fig. 1A. As shown in Fig. 4, a control influenza virus M1 peptide, known to bind to HLA-A2, did not inhibit the cytolysis by the HTLV-I Tax11–19-specific CTL clones in this antagonist assay. As expected, some APLs that were not active in the agonist assay inhibited the cytolysis of the targets pulsed with the native peptide (e.g., Y5A and Y8A peptide in CTL clone N1106, and, to a lesser extent, Y5A and G4A for CTL clone N1216 in Fig. 4). Inhibition appeared at a concentration of 0.1 nM, and in some CTL clones was greatest at a concentration of 10 nM (e.g., CTL clone N1216 in Fig. 4). In CTL clone N1106, G4A, which was a weak agonist in the agonist assay (Figs. 2 and 3), inhibited cytolysis over 50% at 1 nM, and, at higher concentrations, returned to baseline cytolysis, i.e., displayed mixed weak agonism/antagonism properties (Fig. 4). Similar dose–titration curves were observed with G4A and Y5A in CTL clone 3204 (Fig. 4). The maximum inhibition by these APLs in all 17 CTL clones tested is summarized in Table I. All APLs inhibited the cytolysis to some degree with exception of four clones (Table I). The total number of clones that were maximally inhibited was three by G4A, seven by Y5A, zero by P6A, and three by Y8A.

TCR agonism of bulk PBMC from HAM/TSP patients

We next wanted to assess whether HTLV-I Tax-derived APLs are able to affect the bulk PBMC response. For this purpose, we first determined the concentration of the HTLV-I Tax11–19 peptide able to induce cytolysis by HTLV-I Tax11–19-specific CTL in bulk PBMC of HAM/TSP patients. Peptide titration assays were performed using PBMC from HAM/TSP patients 1 and 4. As shown in Fig. 1B, the profile of the cytolysis in the PBMC was similar to that in the Tax-specific CTL clones (Fig. 1A). The cytolysis appeared at concentration of 0.01–0.1 nM and reached a plateau at 1 nM. Therefore, 1 μM of peptide was used to determine the magnitude of TCR recognition for the native HTLV-I Tax peptide and APLs. At 1 μM of HTLV-I Tax peptide, the specific lysis ranged from 10 to 28% in PBMC from four HAM/TSP patients and was higher than that of any APL-pulsed targets (Fig. 5A). Y5A was not or barely recognized in cytolysis assays with PBMC of all four

Table I. Summary of TCR antagonist assay of HTLV-I Tax11–19-specific CD8$^+$ CTL clones using APLs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clone</th>
<th>% Inhibition of Specific Lysis to Tax11–19-Pulsed APCs by APLs$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G4A</td>
</tr>
<tr>
<td>#1/PBMC</td>
<td>N1104</td>
<td>12.2 ± 10.6</td>
</tr>
<tr>
<td></td>
<td>N1106</td>
<td>55.5 ± 6.6$^*$</td>
</tr>
<tr>
<td></td>
<td>N1201</td>
<td>55.6 ± 6.6$^*$</td>
</tr>
<tr>
<td></td>
<td>N1207</td>
<td>3.3 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>N1208</td>
<td>4.7 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>N1216</td>
<td>33.4 ± 3.1$^*$</td>
</tr>
<tr>
<td></td>
<td>N1217</td>
<td>78.5 ± 5.2$^*$</td>
</tr>
<tr>
<td></td>
<td>N1218</td>
<td>78.5 ± 5.2$^*$</td>
</tr>
<tr>
<td>#1/CSF</td>
<td>N706$^b$</td>
<td>7.4 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>N710$^a$</td>
<td>12.8 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>N715</td>
<td>46.8 ± 12.1$^*$</td>
</tr>
<tr>
<td></td>
<td>N722</td>
<td>35.9 ± 9.8$^*$</td>
</tr>
<tr>
<td>#2/PBMC</td>
<td>2210</td>
<td>0 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>2213$^b$</td>
<td>0 ± 10.8</td>
</tr>
<tr>
<td>#3/PBMC</td>
<td>3204</td>
<td>83.6 ± 10.6$^*$</td>
</tr>
<tr>
<td></td>
<td>3206</td>
<td>11.1 ± 13.9</td>
</tr>
<tr>
<td></td>
<td>3207</td>
<td>34.4 ± 3.9$^*$</td>
</tr>
</tbody>
</table>

$^a$ The percent inhibition of specific lysis to Tax11–19-pulsed APCs by the APL was calculated by the formula described in Materials and Methods (mean ± SD). The specific lysis to APL-added APCs prepulsed with Tax11–19 was compared with that to Tax11–19-prepulsed APCs without APL by using the Student t test (*, p < 0.05). Bold numbers indicate the largest degree of inhibition for each clone.

$^b$ We could not determine what APL maximally inhibits the CTL response for Tax, since there is no significant inhibition.

$^c$ The frequency is significantly increased when compared to that of P6A and M1 by χ² test (p < 0.01). The CTL assay was carried out in triplicate.
patients. APLs G4A and P6A induced cytolysis, but to a lesser degree than the native Tax peptide. The cytolysis against Y8A-pulsed targets was variable. In HAM/TSP patients 1, 2, and 4, it was approximately the same as Y5A, while it was recognized similar to the native HTLV-I Tax peptide in patient 3.

TCR antagonism of bulk PBMC from HAM/TSP patients

APL Y5A was among the most potent with respect to TCR antagonism in the tested HTLV-I Tax-specific clones; however, there was overall significant heterogeneity in terms of fine specificity, suggesting that it may be difficult to exert TCR antagonism in bulk T cell populations. Surprisingly, the inhibition of CTL activity against targets sensitized with the native Tax peptide could be shown also with bulk PBMC and was strongest with the Y5A APL in all HAM/TSP patients (Fig. 5B). In patients 1, 3, and 4, the inhibition by Y5A appeared at 0.1 nM, in which the agonist to antagonist ratio was 1:1, and ranged from 36 to 46%. The maximum inhibition was shown over 80% at 1–10 nM. In patient 2, the inhibition by Y5A was similar to Y8A, consistent with the result from the agonist assay in which Y8A strongly abrogated the cytolysis (Fig. 5, A and B). In patient 3, the inhibition by Y8A was similar to the control peptide M1, which again paralleled the data from the agonist assay in which Y8A was well recognized by PBMC from HAM/TSP patient 3 (Fig. 5, A and B). The degree of the inhibition by P6A and G4A was moderate in all HAM/TSP patients tested.

Discussion

The data presented in this study indicate that CTL responses in the majority of Ag-specific CTL clones and Ag-specific CTL responses in bulk PBMC from HAM/TSP patients can be abolished by an alanine substitution of the antigenic HTLV-I Tax11–19 peptide at position 5. These data suggest that tyrosine at position 5 is an important TCR contact residue for the majority of HTLV-I Tax-specific CTL in vivo. This is consistent with recent reports, which demonstrated that this amino acid fits into the central pocket formed by TCR CDR3α and CDR3β for which crystal structures have been obtained (34, 35, 37). APL Y5A can significantly antagonize CTL responses to the native peptide not only in Ag-specific CD8+ T cell clones, but also in bulk PBMC of HLA-A2 HAM/TSP patients. The inhibition is not solely accounted for by a simple competition for binding between the native peptide and APLs for MHC class I molecules. This is based on the following evidence: 1) T cell recognition of Tax11–19 appeared at 0.01–0.1 nM and reached a plateau at 1 nM (Fig. 1). Therefore, antagonist assays were conducted using prepulsed targets with a suboptimal concentration (0.1 nM) of the native peptide. The inhibition of cytolysis by APLs appeared at 0.1–10 nM (Figs. 4 and 5). 2) Profiles of the inhibition by each APL were not the same in different CTL clones, although the predicted HLA-binding affinity of G4A, Y5A, and P6A is the same to that of Tax11–19, with a lesser extent for Y8A (e.g., in Fig. 4, Y8A maximally inhibited in CTL clone N1106, but in CTL clone N1216, Y8A inhibited the cytolysis stronger than Y8A), and the known HLA-A2 binder influenza virus M1 peptide only moderately competed for CTL activity (Figs. 4 and 5). 3) Y5A inhibited the cytolysis up to 46% in bulk PBMC at the low agonist to antagonist ratio of 1:1 (Fig. 5B, in patient 1, at 0.1 nM). Therefore, it appears likely that the inhibition of CTL responses both in the CTL clones and bulk PBMC occurs at the TCR level.

It is of interest that some APLs have mixed weak agonist/antagonist properties for some T cells, by which they act as a TCR antagonist at low concentration (0.1–10 nM) and as a TCR agonist at high concentration (over 10 nM); e.g., G4A in clone N1106, and G4A and Y5A in clone N3204 in Fig. 4). It has been suggested that TCR antagonism by APL could occur by inhibition of TCR signal for T cell activation. However, it has recently been shown that an antagonistic peptide can induce a negative signal to inactivate T cells, which is independent of the positive signal from TCR bound to agonist/MHC complex (42, 43). In the present study, the weak agonist/antagonist peptides act as antagonists at increasing concentrations. This suggests that antagonistic effect of the APLs may not result from the induction of such negative signals. It would be likely that the APLs inhibit the positive signal induction, resulting...
in inhibition of T cell activation in our T cell clones (44). With the increase of peptide concentration, serial TCR triggering by the weak agonist/antagonist peptide bound to MHC molecule may increase, which may result in the APL acting as an agonist. In our study, the cytolytic to the native peptide of the majority of T cells can be inhibited by the YSA APL, suggesting that this peptide is a candidate for a potential immunotherapy. However, in small numbers of T cells, antagonism is lost at a high concentration of YSA (Fig. 4, clone 3204). Therefore, caution must be exercised in the application of APL for therapeutic use in human disease. APL concentration as well as TCR heterogeneity of pathogenic T cells must be considered when developing APL-based immunotherapies.

In general, the CSF-derived T cell clones from patient 1 did not respond as well to this panel of APLs in comparison with the PBMC derived clones (Fig. 3). This suggests that these Ag-specific T cells in the CSF have functionally more limited recognition for the native peptide than the PBMC-derived T cells (manuscript in preparation). HAM/TSP is an inflammatory disease of the CNS, in which HTLV-I-infected CD4⁺ cells and HTLV-I-specific CD8⁺ CTL are accumulated (25, 28, 29). Increase in CTL with highly focused specificity for HTLV-I in the CNS, together with the recent data that the frequency of the HTLV-I-specific CTL is much higher in CSF than in peripheral blood of HAM/TSP patient (45), further support the view that HTLV-I-specific CTL may be immunopathogenic in HAM/TSP. The TCR sequence analysis using HTLV-I-specific T cells established from PBMC has revealed structural TCR diversity in HAM/TSP patients (46). In fact, recognition profile of APLs varied between PBMC-derived T cells in the present study (Figs. 2 and 3). However, in the CNS, the limited diversity of functional TCR of potential pathogenic T cells may facilitate the use of APL for a TCR-targeted immunotherapy for HAM/TSP.

Recent studies of chronic retroviral infection such as HIV-1 have indicated that some naturally occurring mutations of viral Ags in vivo can act as antagonists to the original peptide, suggesting a mechanism by which this virus may escape host immune surveillance (14, 47). In HTLV-I infection, it has been reported that naturally occurring variants of the HTLV-I Tax₁₁₁₋₁₉ peptide at positions 3 and 5 can abolish CTL recognition (48). In HLA-A₂/HAM/TSP patients, antiviral CTL response is primarily directed against the HTLV-I Tax₁₁₁₋₁₉ peptide. Therefore, the present study suggests that, if natural mutations occurred at TCR contact residue at position 4, 5, or 8 of the HTLV-I Tax peptide, then these natural mutations of viral peptides may efficiently facilitate the escape of HTLV-I from HAM/TSP.

In multiple sclerosis (MS), a demyelinating disease of the CNS that is clinically similar to HAM/TSP and thought to be mediated by autoreactive myelin-specific CD4⁺ T cells, extensive efforts have characterized the TCR repertoire of these autoreactive clones. In EAE, an animal model for MS, TCR analysis has shown an oligoclonal expansion of T cells specific for self Ags (49, 50) and treatment with an APL improved disease symptoms and prevented EAE induction by adoptive transfer of the encephalitogenic T cells (16–18). Furthermore, it has been shown that a single APL can inhibit T cell responses to a native peptide in six proteolipid protein–specific T cell clones despite diverse TCR usage, and can prevent EAE induction (16). However, in the human disease MS, TCR analysis of myelin basic protein (MBP)-specific T cells shows a heterogeneity in the TCR sequences within individual patients with advanced diseases as well as extensive diversity in TCR sequence from patient to patient, even if the T cells are restricted to the same MHC class II molecules (51). Moreover, recent intensive functional studies of 41 HLA-DR₂-, DR₄-, and DR6-restricted autoreactive CD4⁺ T cell clones specific for the MBP₈₃₋₉₉ peptide revealed few similarities of the overall fine specificity patterns to a series of alanine substitutions of the native MBP peptide, even among T cell clones derived from the same patient and restricted by the same MHC class II (6). This is consistent with the notion that the MBP-specific TCR repertoire of these T cell clones is diverse. However, an alanine-substituted peptide at position 91 (K91-A) was not recognized by 36 of 41 T cell clones despite heterogeneous TCR usage, indicating that the amino acid at position 91 is critical for most T cell clones specific for MBP₈₃₋₉₉ restricted by HLA-DR₂, -DR₄, or -DR₆ (6).

In HAM/TSP patients, TCR sequence analysis has revealed that some TCR sequences of HLA-A₂-restricted, HTLV-I Tax₁₁₁₋₁₉-specific CTL clones are the same within individuals, although sequences vary between patients (47, 52). In the agonist assay of bulk PBMC (Fig. 5A), the functional TCR recognition profiles of HLA-A₂ HAM/TSP patients were not the same, especially the recognition of Y8A in PBMC of patients 2 and 3 (Fig. 5A). This suggests that the TCR repertoire may be different in these patients. However, the Y5A APL significantly antagonized the CTL responses to the HTLV-I Tax peptide in PBMC of these patients (Fig. 5B), indicating that a modification of the antigenic peptide can manipulate T cell responses in the majority of the Ag-specific T cells, despite differences in fine specificity repertoires at the clonal level. These results imply that an HTLV-I Tax-derived APL with a modification of tyrosine at position 5 could be used for a peptide-specific immunotherapy in HAM/TSP. The unique expansion of HTLV-I-specific, MHC class I-restricted CD8⁺ T cells in these patients both in the periphery and the target organ as well as the wealth of data available on structural aspects of interaction between Tax-specific TCR and HLA-A₂/Tax complexes and the phenotype and fine specificity of these T cells renders HAM/TSP a unique model to address the use of APL-based immunotherapies in humans.

Acknowledgments

We thank Allen Waziri and Dr. Masahiro Nagai for critical reading of the manuscript.

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