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

Ryuji Kubota, Samantha S. Soldan, Roland Martin and Steven Jacobson

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

http://www.jimmunol.org/content/164/10/5192

---

**References**

This article cites 52 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/164/10/5192.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

---

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

Copyright © 2000 by The American Association of Immunologists All rights reserved.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.
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^+ CTL 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, Tax_{11-19}. We examined the functional outcome on activation of both cloned peripheral blood and cerebrospinal spinal fluid-derived CTL and bulk PBMC from HAM/TSP patients by altered peptide ligands (APL) derived from HTLV-I Tax_{11-19}. 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 falciparum 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 Tax_{11-19} peptide (LLFGYPVYV) 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 Tax_{11-19}/HLA-A2 complexes showed that these TCR V_{α} and V_{β} bind diagonally to the Tax/HLA-A2 complexes and form central pockets by the complementarity-determining region (CDR) 3{α} and CDR3{β} (34, 35). The tyrosine at position 5 of the Tax_{11-19} 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 CDR3{α} and CDR3{β} and CDR1{β}, 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 Tax_{11-19} peptide plays an important role in TCR recognition of HLA-A2-restricted, HTLV-I Tax_{11-19}-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 Tax11-19-specific CTL responses both in CD8+ T cells and bulk PBMC from HLA-A2 HAM/TSP patients using APL derived from Tax11-19. 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 cells 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 Tax11-19 was LLFGYPVYV (31). According to the crystal structure of two TCR/Tax11-19/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 (GILGFVFLT) was used as a control peptide, which binds to HLA-A2 (39). Peptides were synthesized with the Multispec 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 affinity of the peptides can be predicted via the Internet (http://bimas.dccr.nih.gov/molsoft/hla_bind/). The estimated 1/2 (min) is 2406 by Tax11-19, 1415 by L1A, 2046 by G4A, 2406 by Y5A, 2046 by P6A, 437 by Y8A, 171 by V9A, and 550 by M1 peptide.

Generation of HTLV-I Tax11-19-specific CD8+ CTL clones

HTLV-I Tax11-19-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% fetal calf serum, 2.8 mM l-glutamine, 40 μM penicillin, 40 μg/ml streptomycin, and 50 μM 2-mercaptoethanol (Boehringer Mannheim, Mannheim, Germany), and HTLV-I Tax11-19 (1 μM), irradiated 106 PBMC from HLA-A2-matched, non-HTLV-I-infected individuals. CSF cells from patient 1 were directly seeded to the wells at 0.3–1 cell/well under the same conditions. Alternatively, 105 × 106 CD8+ CD69+ cells separated by immunomagnetic beads from PBMC of patients 2 and 3 were stimulated three to four times with 5 × 106 HLA-A2-matched allogeneic PBMC pulsed with 1 μM Tax11-19 in 10 μl medium. CD8+ cells were separated from the cultured cells, and a limiting dilution was conducted as described above. Three to four weeks later, HTLV-I Tax11-19-specific CTLs were screened by CTL assay with targets pulsed with these Tax11-19 or influenza M1 peptide. HTLV-I Tax11-19-specific CTL clones were maintained by restimulation with 5 × 106 HLA-A2-matched allogeneic PBMC pulsed with 1 μM Tax peptide and 30 μM IL-2 every 8–12 days. All of these CTL clones were restricted to HLA-A2, and expressed CD3, CD8, and TCR αβ molecules, as confirmed by flow cytometry.

 CTL agonist assay

For the CTL assay, CTL clones were used 9–11 days after restimulation. The CTL assay was performed using Euromax, as previously described (40). HLA-A2-transfected Hmy-A2 cells served as targets (41). Two million Hmy-A2 cells were labeled with 50 μM fluorescence-enhancing ligand, bis (acetoxyethyl) 2.2’-6’-2’-terpyridine-6,6’-dicarboxylase (BATD; Wallac Instruments, Turku, Finland) for 30 min. After washing with IMDM containing 5% FCS, 2.8 mM l-glutamine, 40 μM penicillin, 40 μg/ml streptomycin, and 125 μM sulfisoxypyrazine (Sigma, St. Louis, MO), target cells were mixed with the indicated concentration of Tax peptide or APL for 1 h and washed twice. Three thousand target cells were transferred to wells in a 96-well U-bottom plate. Effector cells were transferred to the well at an E:T ratio of 1:1 for T cell clones and 100:1 for PBMC. Cells were incubated for 3 h at 37°C, and 40 μl of supernatant was transferred to wells containing 160 μl of 50 μM europium solution (Aldrich Chemical, Milwaukee, WI). After mixing for 5 min at room temperature, the fluorescence of the chelates formed by Europium and fluorescence-enhancing ligand was measured by a fluorometer (DELFIA 1234; Wallac). Maximal release was produced by incubation of target cells in 1% Triton X-100, and spontaneous release was produced by incubation in medium alone. The specific lysis was calculated with the following formula: [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100. The specific lysis in the CTL agonist assay was standardized to relative cytolysis using the following formula: [(the percent specific lysis for APL)/(the percent specific lysis for Tax11-19)] × 100. The assay was conducted in triplicate.

 CTL antagonist assay

The CTL agonist 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 at which MHCs on the target cells may not be saturated with the peptide, but CTL response can be induced. Hmy-A2 target cells were pulsed with a suboptimal concentration of Tax11-19 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 1:2–1 for T cell clones or of 100:1 for PBMC. After 3–4 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 Tax11-19-specific CTL clones

To determine which concentration of HTLV-I Tax11-19 induces cytolysis by the HTLV-I Tax11-19-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 pulsed with Tax11-19 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 Tax11-19 were similar to that in clone N1104, although 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 Tax11-19-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 shown 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 shown 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 shown 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 shown 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 shown 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 shown 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 shown in 5, 8, 8, and 10 clones, respectively. APL Y5A followed by P6A was the least potent when
Based on the titration assay in Fig. 1A. As shown in Fig. 4, a summary of TCR antagonist assay of HTLV-I Tax 11–19-specific CD8+ assay was conducted in triplicate. For the TCR antagonist assay, a suboptimal concentration of the effector cells and the APL peptides were added. The specific lysis is presented by mean ± SD. The specific lysis to APL-added APCs prepulsed with Tax 11–19 was compared with that to Tax 11–19-prepulsed APCs with ± 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 cytolytic targets prepulsed with Tax peptide in the absence of APL. NP indicates cytolyis 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 Tax 11–19-specific CTL clones**

For the TCR antagonist assay, a suboptimal concentration of the native Tax 11–19 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 cytolyis 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 cytolyis of the targets pulsed with the native peptide (e.g., Y5A and Y8A peptide in CTL clone N1216, 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 cytolyis over 50% at 1 nM, and, at higher concentrations, returned to baseline cytolyis, 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 cytolyis 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 cytolyis 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 cytolyis in the PBMC was similar to that in the Tax-specific CTL clones (Fig. 1A). The cytolyis 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 cytolyis assays with PBMC of all four patients.

---

**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&lt;sup&gt;a&lt;/sup&gt;</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>N1016</td>
<td>55.5 ± 6.6*</td>
</tr>
<tr>
<td></td>
<td>N1120</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&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 10</td>
</tr>
<tr>
<td>#1/CSF</td>
<td>N700&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>N710&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.8 ± 11.4</td>
</tr>
<tr>
<td></td>
<td>N715&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.8 ± 12.1*</td>
</tr>
<tr>
<td></td>
<td>N722&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.9 ± 9.8*</td>
</tr>
<tr>
<td>#2/PBMC</td>
<td>2210&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>2213&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 ± 10.8</td>
</tr>
<tr>
<td>#3/PBMC</td>
<td>3204&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.6 ± 10.6*</td>
</tr>
<tr>
<td></td>
<td>3206&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3 ± 13.9</td>
</tr>
<tr>
<td></td>
<td>3207&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.4 ± 3.9*</td>
</tr>
<tr>
<td>No. of clones maximally inhibited&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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's t test (*, p < 0.05). Bold numbers indicate the largest degree of inhibition for each clone.

<sup>b</sup> We could not determine what APL maximally inhibits the CTL response for Tax, since there is no significant inhibition.

<sup>c</sup> 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, Y5A 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 3204 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 agonistic 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 agonists 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 immunopathogen 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 Tax11–19 peptide at position 4, 5, or 8 of the HTLV-I Tax peptide, then these natural mutations occurred at TCR contact residue (14, 47). In HTLV-I infection, it has been reported that the TCR repertoire of HTLV-I-specific, MHC class I-restricted CD8+ 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.

References


