

# Direct Analysis of Viral-Specific CD8<sup>+</sup> T Cells with Soluble HLA-A2/Tax11-19 Tetramer Complexes in Patients with Human T Cell Lymphotropic Virus-Associated Myelopathy<sup>1</sup>

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Human T cell lymphotropic virus-I (HTLV-I)-associated myelopathy is a slowly progressive neurologic disease characterized by inflammatory infiltrates in the central nervous system accompanied by clonal expansion of HTLV-I-reactive CD8<sup>+</sup> T-cells. In patients carrying the HLA-A2 allele, the immune response is primarily directed to the Tax11-19 peptide. The frequency, activation state, and TCR usage of HLA-A2/Tax11-19 binding T cells in patients with HTLV-I-associated myelopathy was determined using MHC class I tetramers loaded with the Tax11-19 peptide. Circulating Tax11-19-reactive T cells were found at very high frequencies, approaching 1:10 circulating CD8<sup>+</sup> T cells. T cells binding HLA-A2/Tax11-19 consisted of heterogeneous populations expressing different chemokine receptors and the IL-2R  $\beta$ -chain but not the IL-2R  $\alpha$ -chain. Additionally, Tax11-19-reactive CD8<sup>+</sup> T cells used one predominant TCR V $\beta$ -chain for the recognition of the HLA-A2/Tax11-19 complex. These data provide direct evidence for high frequencies of circulating Tax11-19-reactive CD8<sup>+</sup> T cells in patients with HTLV-I-associated myelopathy. *The Journal of Immunology*, 1999, 162: 1765–1771.

The human T cell lymphotropic virus type I (HTLV-I)<sup>3</sup> was the first human retrovirus identified (1). It can persistently infect CD4<sup>+</sup> T cells, and, in a subset of infected individuals, chronic inflammatory organ-specific diseases can occur (2, 3). HTLV-I-associated myelopathy (HAM) is a slowly progressive neurologic disease characterized by inflammatory infiltrates in the central nervous system that consist predominantly of monocytes and CD8<sup>+</sup> T cells (4). Systemically, there is an increase in viral load associated with clonal expansion of HTLV-I-reactive CD8<sup>+</sup> T cells, which are primarily reactive with the Tax protein. In patients carrying the HLA-A2 allele, the immune response is dominated by CD8<sup>+</sup> T cells that recognize the Tax11-19 peptide. Based on limiting dilution analysis (LDA), estimation of the frequencies of CTLs specific for HTLV-I revealed precursor frequencies from 1 in 75 cells to 1 in 320 T cells in the circulation of patients with HAM (5). In asymptomatic patients, the magnitude of CTL activity was significantly lower or absent (5). High fre-

quencies of HTLV-I-reactive CD8<sup>+</sup> T cells in the peripheral blood and the accumulation of CD8<sup>+</sup> T cells in central nervous system plaques might suggest the involvement of virus-specific CD8<sup>+</sup> T cells in the disease's pathogenesis.

The characterization of T cell responses to Ag is critical in understanding the pathophysiology of human diseases induced by chronic viral infections. In humans, this has been hampered by the need to expand Ag-specific T cells in vitro to large numbers to examine their function. However, the process of cloning T cells may irreversibly alter their function (6). For example, activated T cells may selectively undergo activation-induced cell death by in vitro Ag stimulation (7). A recent major advance has been the generation of MHC/peptide tetramers or MHC/Ig fusion proteins that can directly bind the TCR (8, 9). The new technology allows direct analysis of Ag-specific T cells without in vitro manipulation. Direct visualization of Ag-specific CD8<sup>+</sup> cells in HIV and EBV infection revealed a high frequency of activated virus-specific CD8<sup>+</sup> T cells, a frequency at least two orders of magnitude higher than that expected by LDA (10). Similarly, staining with soluble MHC class I complexes revealed high frequencies of Ag-specific CD8<sup>+</sup> cells in animal models of viral infection (10, 11). The use of a soluble MHC/Ig fusion protein for visualization of virus-specific CD8<sup>+</sup> T cells similarly revealed high frequencies of cells in HAM/tropical spastic paraparesis patients (12).

Here, we analyzed in detail the activation state, chemokine receptor expression and TCR usage of HLA-A2/Tax11-19-binding T cells in patients with HAM. Circulating Tax11-19-reactive T cells were high in frequency, approaching 1:10 circulating CD8<sup>+</sup> T cells, and were expressing chemokine receptors and the IL-2R  $\beta$ -chain but not the IL-2R  $\alpha$ -chain. Nevertheless, the phenotype of Tax11-19-reactive T cells was surprisingly heterogeneous, being found equally in both CD28-positive or -negative and CD45RO or CD45RA populations. Moreover, Tax11-19-reactive CD8<sup>+</sup> T cells were shown to use one predominant TCR V $\beta$ -chain in the recognition of Tax11-19 peptide. These data provide direct evidence for

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<sup>3</sup>Abbreviations used in this paper: HTLV-I, human T cell lymphotropic virus type I; HAM, HTLV-I-associated myelopathy; LDA, limiting dilution analysis; PE, phycoerythrin.

Table I. Clinical summary of HAM patients studied

Patient	Age/Sex	Disease Duration	AI <sup>a</sup>	EDSS <sup>b</sup>	Anti-HTLV-I Ab <sup>c</sup>
HAM 1	50 M	3 mo	2	3–4	+
HAM 10	42 F	14 yr	5	6	+
HAM 23	62 F	23 yr	5	6	+
HAM 28	44 F	10 yr	6	6.5	+

<sup>a</sup> AI, ambulatory index.

<sup>b</sup> EDSS, extended disability status score (Kurtzke score).

<sup>c</sup> Anti-HTLV-I Ab for viral envelope protein (gp46) detected by ELISA.

high frequencies of circulating Tax11-19-reactive T cells in patients with HAM.

## Materials and Methods

### Subjects

Blood was obtained from four patients with typical HAM. The patients were residents of Jamaica, West Indies, and expressed the HLA-A2 phenotype. Blood was also obtained from healthy controls expressing the HLA-A2 phenotype. Human subjects approval for blood drawing was obtained from the Brigham and Women's Hospital and University of the West Indies, Jamaica Institutional Review Board committees. A summary of patient clinical data is presented in Table I.

### HLA-A2/Tax11-19 tetramer

HLA-A2 heavy chain and  $\beta_2$ -microglobulin were produced as inclusion bodies in *Escherichia coli* XA90 carrying either the pHN1-A2BT or the pHN1- $\beta_2$ -microglobulin plasmid. The inclusion bodies were purified and dissolved in urea denaturing buffer. The monomeric MHC-peptide complexes were formed by combining the A2BT,  $\beta_2$ -microglobulin, and the nonapeptide Tax11-19 in an arginine-folding buffer. The complex was purified using a Sephacryl S300 column (Pharmacia, Piscataway, NJ). The MHC-peptide complex was biotinylated enzymatically with BirA enzyme (Avidity, Denver, CO), and purified on a Mono-Q ion exchange column using a salt gradient (Pharmacia). Neutravidin-R-phycoerythrin (PE) conjugate (Molecular Probes, Eugene, OR) was added to form the tetrameric reagent, which was purified on a Sephacryl S300 column and concentrated.

### Isolation of PBMC

PBMCs were isolated by Ficoll/Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation followed by two washings in RPMI 1640 medium. Cells were resuspended at a concentration  $5 \times 10^6$ /ml in PBS medium containing 1% FBS (Sigma, St. Louis, MO). PBMC were stored in liquid nitrogen.

### CD8<sup>+</sup> T cell clones

The generation of HTLV-I and proteolipid protein reactive CD8<sup>+</sup> T cell clones used to assess the specificity of binding of HLA-A2/Tax11-19 tetramer has been previously described (13, 14).

### Staining and phenotypic analysis of Tax11-19-specific CD8<sup>+</sup> T cells

A total of  $2 \times 10^5$  PBMC were incubated with 1  $\mu$ g of PE-conjugated tetrameric complex at 4°C for 1 h. The mAbs used were: anti-CCR2, anti-

CXCR3, CXCR1-FITC, anti-CXCR2-FITC, anti-CCR5-FITC (R & D Systems, Minneapolis, MN), anti-CD45RO-FITC, anti-CD45RA-FITC, anti-perforin, anti-RANTES, anti-CD80-FITC, anti-CD8<sup>+</sup> Cy, (PharMingen, San Diego, CA), anti-CD2, anti-CD4, anti-CD25, anti-CD122, and anti-CD28 (all from Coulter, Immunotech, FL). When directly labeled Abs were used, cells were incubated with a mixture containing PE-labeled tetramer, FITC-labeled surface Ab, and cychrome-labeled anti-CD8. Indirect staining was completed by incubation of cells with goat anti-mouse IgG-FITC (Biosource, Camarillo, CA) and after washings by a 30-min incubation with anti-CD8-cychrome. Cells were then washed, resuspended in 1% formaldehyde (Sigma), and analyzed on a flow cytometer (Becton Dickinson, San Jose, CA). For staining of intracellular proteins, PBMC were first incubated with tetramer complex, washed, and fixed in 4% paraformaldehyde in PBS for 20 min on ice. After washing, PBMCs were permeabilized in a buffer containing 1% FBS and 0.1% saponin (permeabilization buffer) (Sigma) and incubated with anti-RANTES or anti-perforin Abs for 30 min at 4°C. After washing, PBMC were incubated with goat anti-mouse IgG-FITC F(ab')<sub>2</sub>. PBMCs were then washed and resuspended in the medium without saponin.

### TCR analysis

For analysis of variable TCR  $\beta$ -chain usage, PBMC were incubated with HLA-A2/Tax tetramer and specific TCR  $\beta$ -chain Ab. Abs recognizing TCR V $\beta$  2, 3, 5.1, 5.2, 5.3, 6.1, 8, 9, 11, 12, 13.1, 13.6, 14, 16, 17, 18, 20, 21.3, 22 and 23 were used, all from Immunotech (Marseille, France). PBMCs were initially incubated for 1 h at 4°C with HLA-A2/Tax tetramer conjugated with PE and after two washings, with the appropriate anti-TCR V $\beta$  Ab, as previously described (15). TCR V $\beta$  staining was visualized by incubation of cells with FITC-conjugated anti-mouse IgG F(ab')<sub>2</sub> (Biosource) for 30 min at 4°C. PBMC were then stained with anti-human CD8-cychrome.

## Results and Discussion

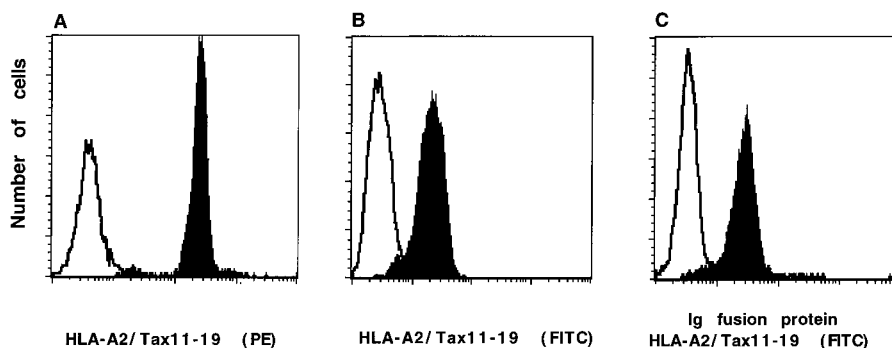
### Specificity of binding of HLA-A2/Tax11-19 complexes

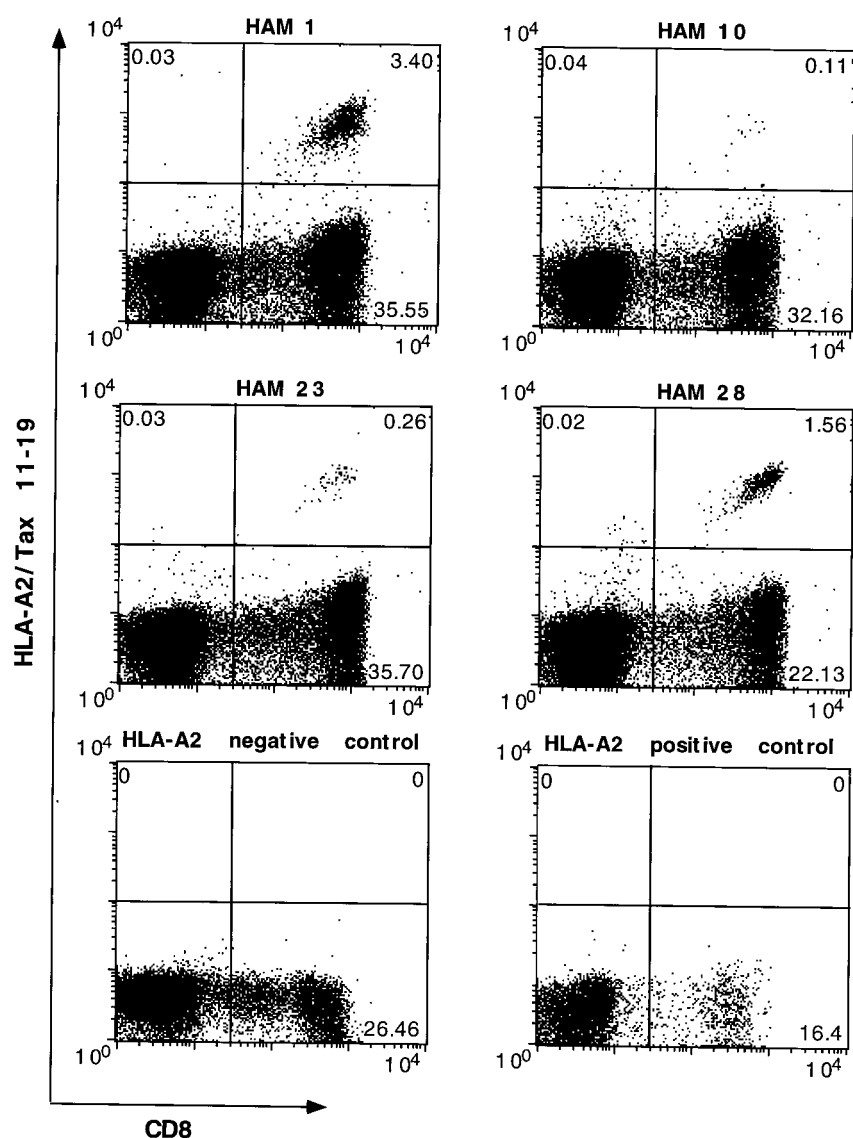
The specificity of tetramer binding to Ag-specific TCRs was compared using a HLA-A2-restricted CD8<sup>+</sup> T cell clone reactive with either Tax11-19 or a proteolipid protein-reactive T cell clone recognizing peptide 80–88. Whereas there was significant binding of the Tax11-19 PE tetramer to the Tax-specific T cell clone, there was no binding observed by the Tax11-19 tetramer to the proteolipid protein-reactive T cell clone (Fig. 1). Similarly, the Tax-reactive T cell clone bound an HLA-A2 Ig fusion protein loaded with Tax11-19, whereas no binding was observed when the HLA-A2 Ig fusion protein was loaded with an irrelevant peptide M1 (Fig. 1C). The HLA-A2/Tax11-19 complexes coupled to PE showed the highest intensity of staining and were used for the remainder of the experiments.

### Frequencies of Tax11-19-specific CD8<sup>+</sup> T cells in patients with HAM

Previous analyses of the frequency of Ag-specific T cells relied upon the ability to expand T cells or measure their function, or both, in LDAs. A more direct approach where the frequency of T cells was measured by PCR analysis of TCR  $\alpha$ - and  $\beta$ -chains suggested that assays based upon the ability to grow cells in vitro may

**FIGURE 1.** Specificity of binding of HLA-A2/Tax11-19 complexes. The specificity of binding was compared by using two A2-restricted CD8<sup>+</sup> clones reactive with either Tax 11-19 or proteolipid protein 80–88 (closed and open histograms, respectively). A, Staining with HLA-A2/Tax11-19 coupled to PE. B, Staining with HLA-A2/Tax11-19 coupled to FITC. C, Staining Tax11-19-specific CD8<sup>+</sup> clone with Ig fusion protein HLA-A2/Tax11-19 (closed histograms) and an Ig HLA-A2 fusion protein with an irrelevant peptide M1 (open histogram).





**FIGURE 2.** High frequencies of cells binding HLA-A2/Tax11-19 complexes in HAM patients. PBMC from four (1, 10, 23, and 28) HAM patients were stained with HLA-A2/Tax11-19 complexes (y-axis) and anti-CD8 mAb (x-axis). Numbers in the upper right corners show percentages of expression in all PBMC. PBMC from HLA-A2-positive and -negative individuals were stained with HLA-A2/Tax11-19 for comparison.

underestimate the frequencies of Ag-specific cells (6). The expansion of CD8<sup>+</sup> T cells in patients with HAM has thus far been based on LDA. Recently Gretten et al. (12), with the use of soluble HLA-A2/Tax11-19 Ig fusion proteins, reported high frequencies of virus-specific CD8<sup>+</sup> T cells in HAM/tropical spastic paraparesis patients. The use of HLA-A2/Tax11-19 complexes enabled us to confirm their observations and further characterize the population of CD8<sup>+</sup> T cells recognizing this epitope. In all patients examined, we detected a population of CD8<sup>+</sup> T cells that exhibited HLA-A2/Tax11-19 binding (Fig. 2). The frequency of cells that bound HLA-A2/Tax11-19 tetramer was high and ranged from 0.11 to 3.4% of all lymphocytes, indicating frequencies of between 1:8 to 1:30 of CD8<sup>+</sup> T cells. In marked contrast, HLA-A2/Tax11-19 tetramer staining of PBMC from HLA-A2-positive and HLA-A2-negative healthy subjects and HLA-A2-negative subjects with HAM did not demonstrate any detectable binding to the HLA-A2/Tax11-19 tetramer (Fig. 2).

#### Phenotypic analyses of Tax-specific CD8<sup>+</sup> T cells

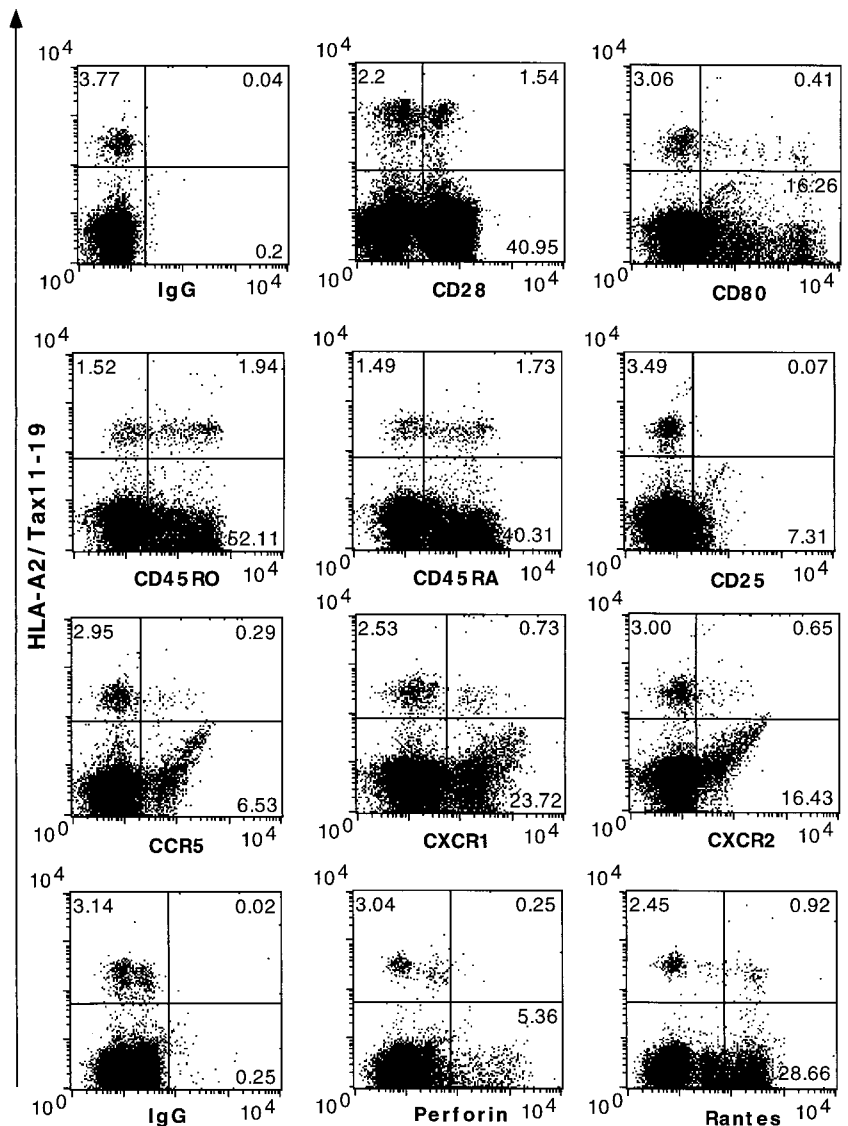
Because the HLA-A2/Tax11-19 tetramer allowed direct analysis of viral-reactive CD8<sup>+</sup> T cells without *in vitro* manipulation, we analyzed the phenotype of Tax11-19-specific CD8<sup>+</sup> T cells by

Table II. Summary of phenotypic analysis of Ag-specific CD8<sup>+</sup> T cells<sup>a</sup>

	HAM 1		HAM 10		HAM 23		HAM 28	
	Tax11-19	CD8 $\alpha$	Tax11-19	CD8 $\alpha$	Tax11-19	CD8 $\alpha$	Tax11-19	CD8 $\alpha$
CD2	100	99.7	100	98.6	100	97.6	100	99.6
CD25	0.1	0.2	0	3.7	3.7	1.3	0.2	1.2
CD122	4.5	2.2	57.9	8.1	50.0	9.0	20.3	14.8
CD80	13.2	4.1	34.5	18.5	57.1	10.9	9.2	3.6
CD45RO	47.7	37.6	69.4	54.6	37.5	20.5	75.1	24.2
CD45RA	54.4	44.9	9.1	45.4	55.8	69.5	14.9	48.6
CD28	42.5	55.6*	41.2	52.2*	77.5	47.1*	58.8	64.7*
CXCR1	18.8	2.2	21.7	19.4	8.3	8.8	14.9	11.1
CXCR2	3.6	0.8	29.2	21.4	4.2	7.8	8.4	0.1
CXCR3	8.5	1.4	11.7	20.8	11.7	22.9	64.2	12.6
CCR2	10.6	9.2	14.7	54.9	32.1	15.2	40.6	11.2
CCR5	11.9	0.5	41.7	15.0	57.3	7.7	0	0
RANTES	24.9	28.6*	53.1	10.6*	11.1	24.4*	23.1	19.2*
Perforin	5.8	5.3*	0	2.1*	0	5.2*	2.4	2.3*

<sup>a</sup> Results represent percentages of positive cells in the Tax11-19-specific CD8<sup>+</sup> T cells and in all CD8<sup>+</sup> population from the individual patient. Analyzed cells were gated on the basis of expression of CD8<sup>+</sup>  $\alpha$ -chain and HLA-A2/Tax11-19 binding for Tax-specific cells or CD8<sup>+</sup> only for CD8<sup>+</sup>  $\alpha$  T cells. \*, Results from all lymphocyte population when nondirectly labeled Ab was used.

**FIGURE 3.** Phenotypic analysis of HLA-A2/Tax11-19 specific CD8<sup>+</sup> T cells. Graphs represent dot plot of two parameter FACS analysis of PBMC from patient HAM 1. PBMC were stained with HLA-A2/Tax11-19 tetramer (PE labeled, y-axis) and surface marker (FITC-labeled, x-axis). Cells were gated on the basis of forward vs side scatter.



using mAbs directed against cell surface molecules associated with T cell functions, including their state of activation (Table II and Fig. 3).

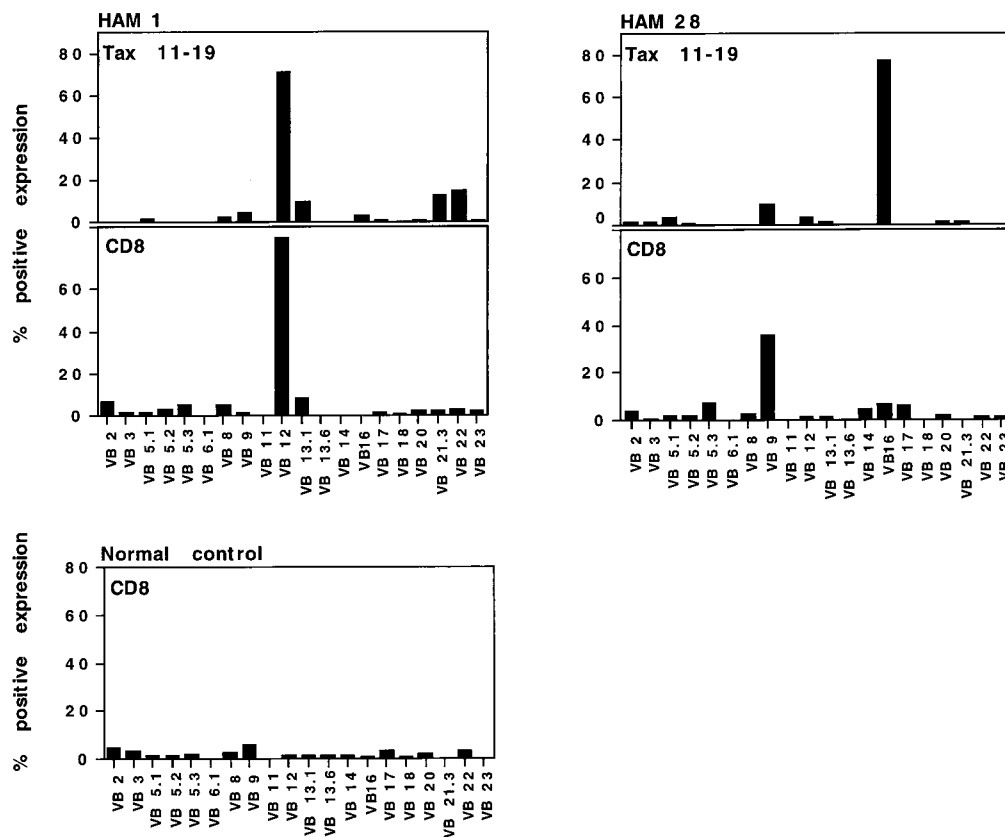
#### *IL-2R expression*

We first examined whether there was activation of the CD8<sup>+</sup> T cells specific for Tax11-19 as there is chronic stimulation of the viral-reactive T cells in the HTLV-I-infected patients. While very few of the CD8<sup>+</sup> T cells specific for Tax11-19 expressed the IL-2R  $\alpha$ -chain (CD25) in the four patients tested, they did express high levels of the IL-2R  $\beta$ -chain (CD122, Table II). To verify that IL-2R  $\alpha$ -chain expression could be increased with Ag stimulation in this population of T cells, PBMC were cultured for 24 h with Chinese hamster ovary cells cotransfected with HLA-A\*0201 and either CD80 (B7-1) or CD58 (LFA-3). Significantly increased expression of the IL-2R  $\alpha$ -chain was observed, indicating that the Tax11-19-specific CD8<sup>+</sup> T cells were capable of up-regulating IL-2R  $\alpha$ -chain upon Ag stimulation (data not shown). These results are consistent with recent data in murine models of viral infection, where expression of IL-2R  $\alpha$ -,  $\beta$ -, and  $\gamma$ -chains differed among CD4<sup>+</sup> and CD8<sup>+</sup> memory T cell populations (16). That is, the IL-2R  $\beta$ - but not the  $\alpha$ -chain was selectively expressed in

CD8<sup>+</sup>CD44<sup>high</sup> cells, a T cell population that is induced to proliferate by viruses. These data may indicate that in humans chronic viral activation of CD8<sup>+</sup> T cells is associated with expression of predominantly the IL-2R  $\beta$ - but not  $\alpha$ -chain. Thus, when Tax11-19-specific CD8<sup>+</sup> T cells are activated by the virus, the expression of CD25 may be more transient, consistent with the observation that CD8<sup>+</sup> T cells recruited to the brain after viral challenge express low levels of CD25 (17). Because CD122 may also bind IL-15, increased expression of CD122 by Tax11-19-specific CD8<sup>+</sup> T cells may be associated with the expression of the IL-15 receptor. This is of particular interest as Zhang et al. (16) have recently shown that IL-15 is one of the few cytokines that can cause selective stimulation of memory-phenotype CD8<sup>+</sup> T cells and thus might have a role in the maintenance of Tax11-19-specific CD8<sup>+</sup> T cells.

#### *CD28 and CD80 expression*

It has been suggested that CD8<sup>+</sup>CD28<sup>-</sup> T cells represent terminally differentiated effector cells. This is in part based on investigations of subjects with HIV infection where virus-specific CTLs were found within the CD28<sup>-</sup> subset of CD8<sup>+</sup> T cells (18). Direct examination of Tax11-19-reactive T cells revealed heterogeneous



**FIGURE 4.** T cell repertoire of HLA-A2/Tax11-19-specific CD8 T cells. Ag-specific CD8<sup>+</sup> cells were identified by staining with HLA-A2/Tax11-19 tetramer. The T cell receptor usage was verified by the use of panel of mAbs against major TCR-variable  $\beta$ -chains. Two patients with highest expansions of HLA-A2/Tax11-19-specific CD8<sup>+</sup> cells were analyzed (patients HAM 1 and HAM 28). Analysis of T cell repertoire in one healthy control is presented for comparison.

expression of CD28 with two distinct populations (Table II and Fig. 3). These results are thus consistent with recent data from patients with HIV infection where the same expanded clone is found within both the CD8<sup>+</sup>CD28<sup>+</sup> and CD8<sup>+</sup>CD28<sup>-</sup> T cell populations (19) and in simian immunodeficiency virus-infected rhesus monkeys where Gag-specific CD8<sup>+</sup> T cells identified by tetramer binding variably express CD28 (20). Thus, the phenotypic heterogeneity of CD8<sup>+</sup> T cells with a given Ag reactivity may indicate a functional difference between CD8<sup>+</sup>CD28<sup>+</sup> and CD8<sup>+</sup>CD28<sup>-</sup> T cell populations.

Increased expression of B7-1 (CD80), a ligand for CD28, was also observed on Tax11-19-reactive CD8<sup>+</sup> T cells. It has been shown that B7-1 is expressed on the surface of a subset of differentiated T cells (21, 22). Analysis of forward scatter of HLA-A2/Tax11-19 tetramer-binding cells demonstrated that cells were not in blastoid stage, implicating a differentiated phenotype.

#### Chemokine receptor expression

Because Tax11-19-specific CD8<sup>+</sup> T cells may migrate to inflammatory central nervous system lesions and thus may potentially be involved in the pathogenesis of HAM, we examined whether these cells expressed chemokine receptors. This was of interest as chemokine receptors are essential for recruitment of circulating leukocytes into sites of inflammation. Moreover, in addition to their role in cell diapedesis, expression of particular chemokine receptors is related to the activation state of the T cell and may also be associated with inflammatory responses.

The chemokine receptors were preferentially expressed on Tax11-19-specific CD8<sup>+</sup> T cells when compared with the total

CD8<sup>+</sup> population (Table II). The two patients with the highest frequency of Tax11-19-specific CD8<sup>+</sup> T cells (patients HAM 1 and 28, Table II) demonstrated a preferential increase in the expression of CXCR3 when compared with the total CD8<sup>+</sup> population in these patients. Indeed, CXCR3 expression in Tax11-19-specific CD8<sup>+</sup> T cells may characterize a subpopulation of clonally expanded cells with a high migratory potential. CXCR3 receptors are involved in T cell adhesion under conditions of flow (23) and are expressed on CD8<sup>+</sup> cells in perivascular lesions in the brains of macaques with simian immunodeficiency virus encephalitis (24). In addition, CXCR3 receptors appear to define activated effector/memory T cells and are expressed on Th0 and Th1 cells; in particular, Th1 cells that are involved in inflammatory reactions (25, 26). Moreover, patients HAM 1 and 28 also demonstrated increased expression of IL-8 receptors A and B (CXCR1 and CXCR2, respectively). IL-8 is a major mediator of acute inflammation, and the receptors CXCR1 and CXCR2 are restricted to CD8<sup>+</sup> T cells, NK cells, and monocytes, although expression can be variable between different individuals (27).

The Tax11-19-specific CD8<sup>+</sup> T cells demonstrated a relative increase in expression of CCR5 in patients HAM 1 and 10 and of CCR2 in HAM 23 and 28 (Table II). Previously activated peripheral blood T cells also express CCR5, a chemokine receptor for RANTES and macrophage inflammatory protein 1 $\alpha$  and  $\beta$ . CCR5 was described as a marker to distinguish a population of T cells with migratory capacities (25), is expressed on both Th1 and Th2 cells, and is markedly influenced by the presence of IL-2 (28). The Tax11-19-reactive CD8<sup>+</sup> T cells also expressed intracytoplasmic RANTES, a T cell chemoattractant considered to be an important

mediator of inflammation associated with a Th1 type immune response (29).

These experiments, in total, support data derived from HTLV-I-specific CTL clones isolated from patients with HAM, which have also been shown to secrete proinflammatory cytokines, chemokines, and matrix metalloproteinases *in vitro* (30). Thus, there may be a relation between activation, cytokine production, and migratory potential of Tax11-19-specific CD8<sup>+</sup> T cells. Moreover, the generally high levels of chemokine receptors expressed on Tax11-19-reactive CD8<sup>+</sup> T cells indicates that these cells are differentiated and have a potential to migrate into the peripheral tissues. The variable degree of expression among the different chemokine receptors for each subject is consistent with previously published observations of variability in chemokine receptor expression in the general population.

#### CD45 isoform expression

Significant numbers (40–75%) of Tax11-19-specific CD8<sup>+</sup> T cells expressed CD45RO, though in some patients there were also significant numbers of Ag-reactive T cells expressing CD45RA. Although naive T cells that do not respond to recall Ags express the CD45RA isoform of the CD45 complex and T cells recognizing recall Ags are found in the CD45RO population, activated and differentiated T cell clones frequently express both CD45RA and CD45RO Ags (31, 32). Thus, it is likely that at least some of the Tax11-19-reactive CD8<sup>+</sup> T cells coexpress both CD45 isoforms and represent activated T cells. These data are consistent with the observation that there is an accumulation of CD8<sup>+</sup>CD45RO<sup>+</sup> lymphocytes in the spinal cord lesions of HAM patients, which correlated with the duration of the disease (33, 34).

#### TCR repertoire of HLA-A2/Tax-specific CD8<sup>+</sup> cells

To examine whether Tax11-19-specific CD8<sup>+</sup> T cells preferentially used a particular TCR V $\beta$ -chain, PBMC from patients HAM 1 and HAM 28 were costained with the HLA-A2/Tax11-19 tetramer together with a panel of anti-TCR V $\beta$  mAbs (Fig. 4). In both patients, one type of TCR V $\beta$ -chain predominated: 86% of Tax11-19-reactive CD8<sup>+</sup> cells expressed TCR V $\beta$ 12 in patient HAM 1, and this was paralleled by expansion of TCR V $\beta$ 12 T cells in the total CD8<sup>+</sup> population (75%). In contrast, 77% of Tax11-19-specific CD8<sup>+</sup> cells expressed the TCR V $\beta$ 16 in patient HAM 28, whereas in the total CD8<sup>+</sup> population TCR V $\beta$ 9 predominated (35% of all CD8). In addition, minor populations of Tax11-19-specific cells expressed TCR V $\beta$ 8, 9, 21.3, and 22 in HAM 1, and TCR V $\beta$ 9, 12, and 5.1 in HAM 28.

Both patients showed dominance of one particular TCR V $\beta$ -chain in the CD8<sup>+</sup> population (Fig. 4). However, TCR V $\beta$  expansion of CD8<sup>+</sup> population can occur in a substantial number of healthy individuals (35), and these generally represent CD8<sup>+</sup> T cells recognizing MHC class I-restricted peptides. Thus, the expansion of CD8<sup>+</sup> T cells bearing V $\beta$ 9 observed in the CD8<sup>+</sup> population of patient HAM 28 may imply a response to other epitopes of either the Tax protein or of other HTLV-I-encoded proteins, or instead may represent CD8<sup>+</sup> T cells recognizing other unrelated Ags.

In summary, the use of HLA-A2/Tax11-19 tetramer complexes for visualization of Ag-specific CD8<sup>+</sup> T cells demonstrated a high frequency of Ag-reactive T cells in patients with HAM. As expected, there was an expansion of Ag-reactive T cells with preferential use of a particular TCR. Surprisingly, populations of CD8<sup>+</sup> T cells recognizing the Tax11-19 epitope were heterogeneous in terms of expression of CD28, CD45 isoforms, and different chemokine receptors. These data demonstrate the functional heterogeneity of Ag-reactive T cells in patients with a chronic viral infection.

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