High Human T Cell Lymphotropic Virus Type 1 (HTLV-1)-specific Precursor Cytotoxic T Lymphocyte Frequencies in Patients with HTLV-1-associated Neurological Disease

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Summary

The frequencies of human T cell lymphotropic virus type 1 (HTLV-1)-specific $CD8^+$ precursor cytotoxic T lymphocytes (pCTL) were quantitated from lymphocytes obtained from the peripheral blood and cerebrospinal fluid (CSF) of infected individuals with and without HTLV-1-associated neurological disease. An estimate of the pCTL was obtained by separating $CD8^+$ cells, plating these cells in limiting dilution, and testing wells for HTLV-1 specific lysis. Targets consisted of autologous lymphoblastoid cell lines (LCL) infected with vaccinia constructs expressing HTLV-1 gene products or LCL pulsed with HTLV-1 synthetic peptides. In patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), the frequency of HTLV-1 p40X-specific pCTL was at least 40-280-fold higher than in asymptomatic HTLV-1-infected individuals. All HAM/TSP patients (five of five) predominantly recognized HTLV-1 products encoded within the pX region. Lower pCTL to env were demonstrated in three patients, and only one of five HAM/TSP patients had pCTL to gag. A synthetic peptide corresponding to the tax region of HTLV-1 (peptide 11-19, amino acid sequence LLFGYPVYV) was recognized in association with human histocompatibility leukocyte antigen (HLA)-A2 in two HLA-A2 HAM/TSP patients with a high $CD8^+$ pCTL frequency of 1/325 and 1/265, respectively. A second immunodominant region of HTLV-1 tax (peptide 90-55, amino acid sequence VPYKRIEEL) was identified to be restricted by HLA-B14 in two HLA-B14 HAM/TSP patients with a $CD8^+$ pCTL frequency of 1/640 and 1/1,125, respectively. Lymphocytes from the CSF of a patient with HAM/TSP also showed a pCTL frequency against p40X of similar magnitude to that demonstrated from peripheral blood lymphocytes (PBL). The HLA-A2-mediated CSF pCTL activity to the immunodominant tax-specific peptide 11-19 was also comparable to pCTL from PBL. These results indicate that an extremely high pCTL frequency to HTLV-1 tax-encoded peptides may be related to pathogenesis of myeloneuropathy associated with HTLV-1.

The human T cell lymphotropic virus type I (HTLV-1)[1] is closely associated with a slowly progressive neurologic disease called HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (1-3). Perivascular mononuclear infiltrates in the central nervous system (CNS) of HAM/TSP patients and the presence of proviral DNA in spinal cord and medulla have been reported and strongly indicate a retrovirus-induced inflammatory process (4). However, the pathomechanism of this demyelinating myeloneuropathy is still not understood.

The vast majority of individuals infected by HTLV-1 remain asymptomatic, and <5% develop clinical disease including either HAM/TSP or the neoplastic disorder, adult T cell leukemia (ATL). These different outcomes of an HTLV-1 infection may be explained by the existence of different viral strains (5, 6), by genetic predisposition (7), or by differences in host-immune response (8, 9). Thus far, however, no unique sequence has been associated with any disease (10) or asymptomatic carrier state, and an association of a "disease susceptibility" allele is unclear.

The presence of HTLV-1-specific CTLs in the peripheral blood of HAM/TSP patients has been suggested as one ex-

[1] *Abbreviations used in this paper:* ATL, adult T cell leukemia; CNS, central nervous system; CSF, cerebrospinal fluid; pCTL, precursor CTL; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; HTLV-1, human T cell lymphotropic virus type 1; LCL, lymphoblastoid cell line; MRI, magnetic resonance imaging.

planation for the pathogenesis of this disease (11). Consistent with this hypothesis is the observation in HIV-1 infection, that retrovirus-specific CTL have been shown to have deleterious effects especially, in the CNS and lungs (12–14).

Preliminary data on HTLV-1–specific precursor frequencies of CTL (pCTL) from peripheral blood of two patients with HAM/TSP indicate that the number of such cells is extraordinarily high (15). These observations suggest that HTLV-1 induces a strong T lymphocyte immune response, and that the CTL precursor pool may be amplified by the chronic nature of the infection. Also, the amounts of pX and probably env DNA were found to be greater in the CNS tissue from HAM/TSP patients than in brain tissue from patients with ATL (4) which indicates increased viral load in HTLV-1–associated neurological disease.

In this report, limiting dilution analysis of lymphocytes from both peripheral blood and cerebrospinal fluid (CSF) of patients with HAM/TSP and asymptomatic HTLV-1–infected individuals has been performed in order to quantitate the frequencies of HTLV-1–specific $CD8^+$ pCTL. Both virus-specific pCTL as well as HLA-restricted peptide–specific pCTL responses were determined. The data extend our previous observations which demonstrated high CTL activity to the tax region of HTLV-1 in HAM/TSP patients, and serve further to increase our understanding of the role that these CTLs play in the pathogenesis of neurological HTLV-1 infection.

Materials and Methods

Subjects

The clinical characteristics of patients with HAM/TSP and asymptomatic HTLV-1–infected individuals are summarized in Table 1. PBL were obtained from five patients with HAM/TSP or five HTLV-1–infected asymptomatic individuals undergoing evaluation and treatment at the Clinical Center at the National Institutes of Health (NIH) (see Table 1). Four patients (Nos. 1–4) had a slowly progressive spastic paraparesis, and patient No. 5 (spouse of patient No. 4) had hyperreflexia in the lower extremities and extensor plantar responses indicative of corticospinal tract lesion(s). Subject No. 6 was an asymptomatic spouse of patient No. 1, and the remaining subjects (Nos. 7–10) were clinically asymptomatic. All HAM/TSP patients were HTLV-1 seropositive in serum and CSF. The CSF of all but one patient (No. 5) showed mild pleocytosis, elevated IgG levels, and/or the presence of oligoclonal bands. Magnetic resonance imaging (MRI) of three patients with HAM/TSP (Nos. 1–3) showed spinal cord shrinkage in the cervical or thoracic region. MRI of patient No. 4 was normal. No MRI data was available on patient No. 5. HAM/TSP patient No. 4 became infected with HTLV-1 through hemotransfusion. Histocompatibility typing was performed on PBL or EBV-transformed lymphoblastoid cell lines (LCL) by the Tissue Typing Laboratory at the NIH Clinical Center.

Cloning of Lymphocytes in Limiting Dilution

PBL. PBL isolated on Ficoll–sodium metrizoate gradients (lymphocyte separation media [LSM]; Organon Teknika, Rockville, MD) were $CD8^+$ sorted (>98% purity) by flow cytometry analysis (Epics, Coulter Corp., Hialeah, FL) and cloned in limiting dilution in 96-well plates (catalog No. 3799; Costar Corp., Cambridge, MA) at final concentrations of 100, 50, 25, 12.5, 6, and 3 cells per well in 0.2 ml of media containing RPMI 1640 (Whittaker Bioproducts, Walkersville, MD) plus 10% FCS (Gibco, Grand Island, NY). Irradiated (5,000 rad) allogeneic feeders from HTLV-1 seronegative donors were prestimulated with PHA (GIBCO BRL, Gaithersburg, MD) at a final concentration of 1 μg/well for 30 min at 37°C, and added to each well at a concentration of 1.0×10^5 cells/well in RPMI 1640 media containing 10% FCS. 24 h later, 100 μl of supernatant from each well was removed and replaced with 100 μl of fresh media containing RPMI 1640 plus 10% FCS, 10% purified IL-2 (Cellular Products, Inc., Buffalo, NY), and 100 U/ml of rIL-2 (Cetus Corp., Emeryville, CA). All wells were refed two times per week with IL-2 containing media. After 2–3 wk, cell buttons became prominent, and lines derived from this cloning were screened for cytotoxicity against ^{51}Cr-labeled autologous LCL infected with the HTLV-1 vaccinia-p40x, gag, env, or vaccinia–HA constructs, or pulsed with 10 μg/ml of peptides tax 11-19 (amino acid sequence LLFGYPVYV), or tax 90-55 (VPYKRIEEL).

CSF Lymphocytes. Lymphocytes were obtained from CSF by atraumatic lumbar puncture in which <1 RBC/mm^3 was present. As the number of cells was insufficient for $CD8^+$ sorting by flow cytometry analysis, unfractioned cells were cloned in limiting dilution and cultured as described above.

Peptides

Peptides were synthesized using a stepwise solid-phase approach on a peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA) at Medimmune Inc. (16).

Initial Screening of CTL Clones by CTL Assay

As targets, 2×10^6 autologous EBV-transformed LCL were infected with various HTLV-1 vaccinia recombinants as described previously (11) at a multiplicity of infection of 10.0 for 10–14 h and then incubated with 0.2 mCi of $Na_4{}^{51}Cr$ (Amersham Corp., Arlington Heights, IL) for 1.5 h. Target cells infected with the vaccinia-p40x expressed p40x (tax) and p21x; vaccinia–gag expressed p19, p24, and p15 internal structural proteins; vaccinia–env expressed gp62, gp46, and gp21 surface glycoprotein; and control vaccinia–HA expressed no HTVL-1 proteins. For initial screening, target cells plated at a final concentration of 3.5×10^3 cells/well were incubated with 20 μl/well of PBL or CSF effector cells in culture media for 4 h at 37°C in 5% CO_2.

Percent specific lysis was calculated by the following formula: $100 \times [(\text{test cpm} - \text{spontaneous cpm})/(\text{maximal cpm} - \text{spontaneous cpm})]$.

Precursor frequency analyses were determined by cloning cells in limiting dilution and testing wells for HTLV-1 specific lysis of autologous LCL infected with the HTLV-1 or vaccinia–HA control recombinants, HTLV-1 peptides, or irrelevant peptides. Individual wells demonstrating >25% specific lysis were screened as positive after subtraction of vaccinia–HA control.

Linear regression analysis was performed on limiting dilution cultures. The best straight line was determined using the least squares method. Results are plotted graphically (semilog plots) where the y-axis represents the negative natural logarithm of the fraction of nonresponding cultures and the x-axis represents the cell input (17). Experimental points fitting a straight line allow an estimate of the frequency of precursor cells because by interpolating at the level of 37% nonresponding cultures, the size of the sample containing an average of one precursor cell can be estimated (17). 95% confidence limits were calculated based upon true mean value of the dependent variable (cell input). Wells displaying

>25% specific lysis were expanded and retested. Precursor frequency for the total CSF or PBL population was calculated as the percentage of $CD8^+$ cells times the precursor frequency of the $CD8^+$ population.

Results

HTLV-1 p40X-specific pCTL Frequencies from PBL in HTLV-1 Seropositive Individuals with and without HAM/TSP. An estimate of the pCTL frequency was obtained by separating $CD8^+$ cells and plating these cells in limiting dilution. The HTLV-1-specific pCTL frequencies of patients with HAM/TSP and asymptomatic HTLV-1 seropositive subjects are summarized in Table 2, and representative plots for p40X specific lysis are shown in Fig. 1. The results show that all HAM/TSP patients had high HTLV-1-specific pCTL and that the frequencies of HTLV-1 p40X-specific $CD8^+$ pCTL from peripheral blood ranged between 1/75 and 1/320. In contrast, $CD8^+$ pCTL specific for HTLV-1 p40X were only detected in three of five asymptomatic HTLV-1-infected individuals and frequencies ranged between 1/2,900 and 1/20,850. These pCTL frequencies were 40–280-fold lower than in HAM/TSP patients (Fig. 1 and Table 2). A comparison of the number of HTLV-1 p40X-specific clones between asymptomatic No. 6 and HAM/TSP patient No. 1 is shown in Table 3. In two asymptomatic individuals, pCTL were too low to be determined.

pCTL Frequencies to Different Regions of HTLV-1. For estimating pCTL frequencies to various HTLV-1 proteins, the frequency of cells lysing autologous B cells infected with vac-

Table 1. *Characteristics of HTLV-1-infected Patients with and without HAM/TSP*

Patient No./ Clinical status	Country of origin	Age in yr/ sex	Duration of disease	HLA type				
				A	B	C	DR	DQw
			yr					
1. HAM/TSP	USA	51/M	14	2,28	18,44	6,7	5,7	2,3
2. HAM/TSP	Panama	31/F	7	11,24	7,14		1,7	2,5
3. HAM/TSP	Jamaica	45/F	8	28,32	14,70	3	1,7	1,2
4. HAM/TSP	USA	68/M	2	1,3	7,62	3	2,4	6,7
5. Corticospinal tract lesion	USA	70/F	1	1,2	37,60	3,6	11,15	6,7
6. Asymptomatic	Japan	46/F		11,31	54,60	1,3	4,9	3
7. Asymptomatic	Barbados	46/F		28,31	60,63	3	4,18	4,7
8. Asymptomatic	USA	43/F		2	53,	4		
9. Asymptomatic	Jamaica	39/F		2,30	43,43,		3,15	4,6
10. Asymptomatic	Jamaica	29/F		Not available				

Table 2. *HTLV-1-specific pCTL Frequencies of $CD8^+$ Cells from 10 HTLV-1-infected Subjects with and without HAM/TSP*

Patient No./Clinical status	p40X	Env	Gag
1. HAM/TSP	1/300 (1/228–372)	1/8140 (1/4,264–12,016)	1/4,337 (1/2,204–6,470)
2. HAM/TSP	1/265 (1/205–325)	1/4300 (1/2,497–6,103)	*
3. HAM/TSP	1/320 (1/270–370)	*	*
4. HAM/TSP	1/75 (1/20–130)	1/1590 (1/793–2,387)	*
5. HAM/TSP	1/186 (1/107–265)	*	Not available
6. Asymptomatic	1/2,900 (1/1,600–4,200)	*	*
7. Asymptomatic	1/3,620 (1/1,816–5,424)	*	*
8. Asymptomatic	*	*	*
9. Asymptomatic	1/20,850 (1/10,802–30,898)	*	*
10. Asymptomatic	*	*	*

* Too low to be determined. Of 360 wells screened for lysis on the respective targets, no well demonstrated any specific cytotoxicity. Values in parentheses represent 95% confidence limits of the calculated frequencies.

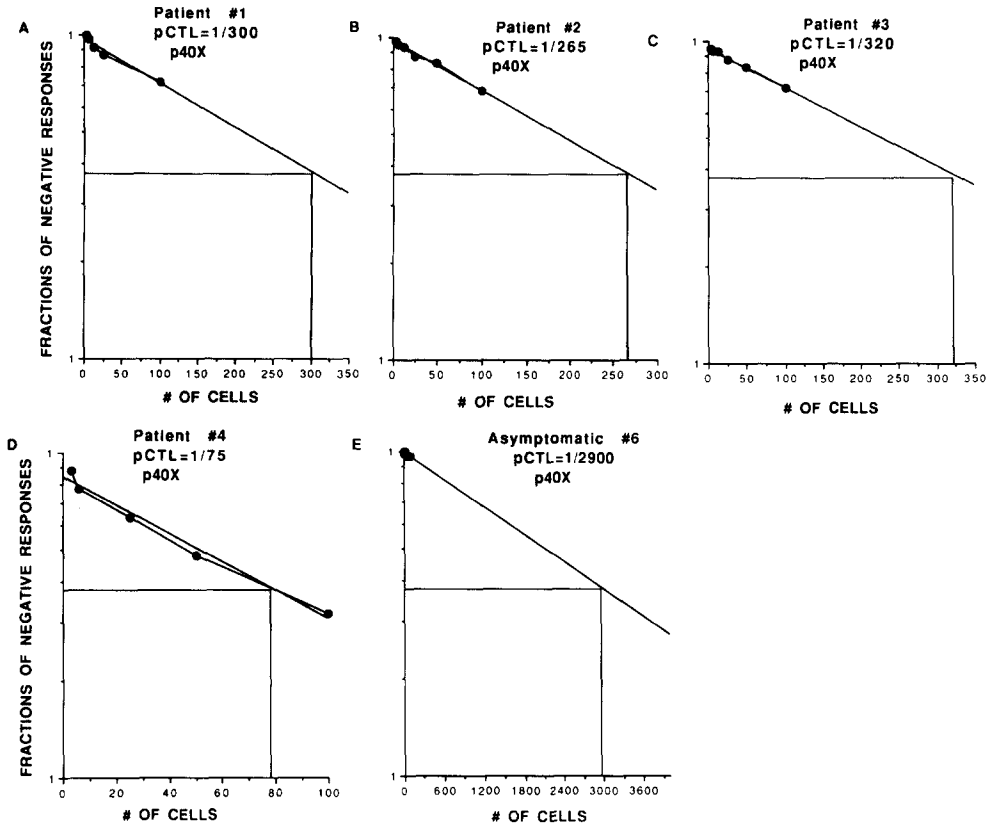

Figure 1. Quantitation of HTLV-1 p40X-specific CD8+ precursor CTL frequencies. Precursor frequency analyses were performed on freshly isolated, sorted, and in limiting dilution-cloned PBL from patients with HAM/TSP (*A–D*) or asymptomatic HTLV-1 infection (*E*) as described in the Materials and Methods section. Targets were Cr-labeled autologous LCL infected with the HTLV-1 vaccinia-p40X constructs. Effector cell frequencies were extrapolated as reciprocal of x variable where y equals 0.37. Individual wells demonstrating >25% specific lysis were screened as positive.

cinia constructs that expressed different HTLV-1 gene products were determined. Table 2 shows that in HAM/TSP, the frequencies of HTLV-1 p40X-specific CD8+ pCTL from peripheral blood were extremely high. However, some patients at the dilutions tested also showed some reactivity to HTLV-1 env (Nos. 1, 2, and 4) and HTLV-1 gag (No. 1) (Table 3), and estimates of pCTL to those regions are given in Table 2. In all cases, the pCTL to the p40X region were significantly greater than those to env and gag.

Table 3. *Cloning of CD8+ HTLV-1–specific CTL from Lymphocytes in the Peripheral Blood from Patients with HAM/TSP and Asymptomatic HTLV-1 Carriers (ASX)*

	Number of positive wells/60 wells tested			
	p40X		Env	Gag
Cells/well	HAM/TSP Patient No. 1	ASX Patient No. 6	HAM/TSP Patient No. 4	HAM/TSP Patient No. 1
100	24	2	ND	2
50	19	2	2	2
25	16	1	1	0
12.5	11	0	1	1
6	9	1	0	0
3	8	0	0	1
Precursor frequency	1/310	1/2,900	1/1,590	1/4,340

Although the magnitude of HTLV-1–specific pCTL response (i.e., frequencies of specific pCTL) in asymptomatic HTLV-1–infected individuals were markedly lower than in patients with HTLV-1–related neurologic disease, the recognition of viral products encoded within the pX region still predominated. The frequencies of gag- and env-specific pCTL were too low to be estimated. Of the 360 wells screened for lysis on the respective targets, no well demonstrated any specific cytotoxicity. This indicated that in HTLV-1 asymptomatic carriers, if lysis could be detected, all the HTLV-1–specific pCTL responses were directed against pX gene products and these responses were significantly lower than in HAM/TSP patients.

Precursor Frequencies of HTLV-1 Peptide-specific CTL. The fine peptide specificity of the CD8+ pCTL to the HTLV-1 p40X regions was determined. Previous studies had demonstrated that HTLV-1 tax synthetic peptides, tax 11-19, and tax 90-55 were recognized in the context of HLA-A2 and B14, respectively (18). The results in Fig. 2, *B* and *F* show that a high frequency of purified CD8+ cloned lymphocytes from the peripheral blood of HLA-A2 HAM/TSP patients Nos. 1 and 5 recognized HTLV-1 tax peptide 11-19. The frequency of tax peptide 11-19–specific pCTL was of similar mag-

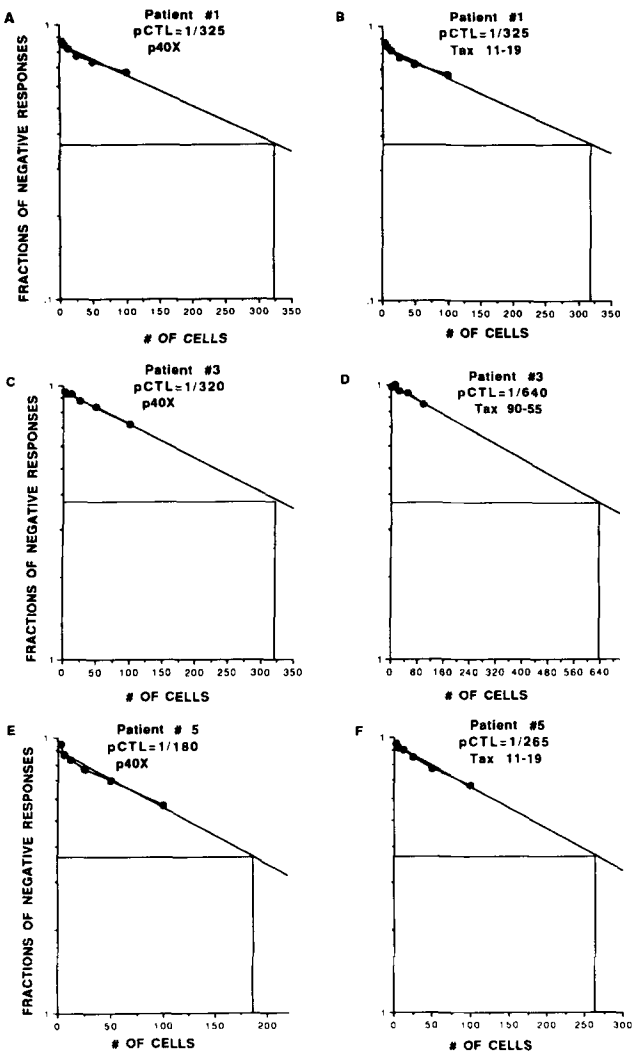

Figure 2. Quantitation of HTLV-1–specific CD8+ precursor CTL frequencies with HLA-A2 and HLA-B14 restriction of HTLV-1 peptides. An estimate of the pCTL was obtained and calculated as in Fig. 1. Targets were Cr-labeled autologous LCL infected with HTLV-1 p40x (*A*, *C*, and *E*) or pulsed with peptides tax 11-19 (FGYPV) (*B* and *F*) or tax 90–55 (VPYKRIEEL) (*D*).

nitude to that of p40X-specific pCTL (Fig. 2, *A* and *E*). Therefore, such high precursor frequencies to this peptide indicate that major responses within p40X in these subjects are directed towards tax peptide 11-19. In addition, Fig. 2 *D* demonstrates that the sorted and subsequently cloned CD8+ lymphocytes from the peripheral blood of HLA-B14 patient No. 3 had a high CD8+ pCTL frequency to tax peptide 90-55. The second HLA-B14 HAM/TSP patient No. 2 had a tax peptide 90-55–specific pCTL frequency of 1/1,125 (data not shown). These data indicate that the predominant response within p40X against tax peptide 90-55 is restricted by HLA-B14. Expansion of these clones and subsequent CTL assays confirmed the specificities of these responses.

HTLV-1 pCTL Frequencies in CSF. Because of low numbers of CSF leukocytes in patient No. 1, these cells were unable to be sorted into CD8+ T cells, and, therefore, they were directly cloned in limiting dilution. It is interesting that an initial screening of those CSF lymphocytes showed that there was a high pCTL frequency (1/145) against p40x (Fig. 3 *A*) which was of a similar magnitude (1/300) to that in PBL (Fig. 1 *A* and Table 2). Env pCTL could also be demonstrated (Fig. 3 *B*). The pCTL of these CSF lymphocytes from that HLA-A2 HAM/TSP patient were shown to be reactive against tax peptide 11-19, which was similar in magnitude to that in corresponding peripheral blood (Figs. 2 *B* and 3 *C*).

Discussion

The present data indicate that HTLV-1–specific CD8+ pCTL frequencies from lymphocytes obtained from the peripheral blood and CSF of patients with HAM/TSP are extremely high, whereas the magnitude of pCTL reactivity from subjects with asymptomatic HTLV-1 infection is clearly lower or absent. These pCTL predominantly recognized HTLV-1 products encoded within the pX region. In addition, we confirmed that the recognition of HTLV-1 tax epitopes 11-19 (LLFGYPVYV) and 90-55 (VPYKRIEEL) within the tax region of HTLV-1 is restricted by HLA-A2 and HLA-B14, respectively (18).

The presence of circulating HTLV-1–specific CTL in pa-

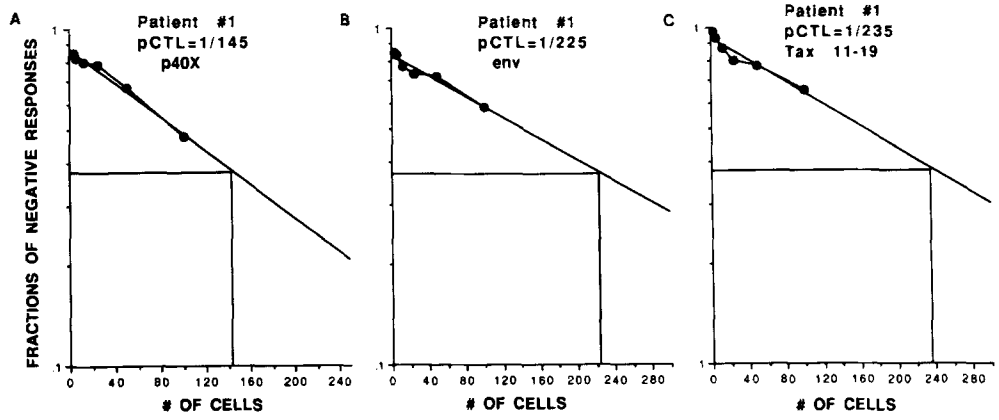

Figure 3. Quantitation of HTLV-1–specific pCTL frequencies in CSF. Lymphocytes were obtained from CSF by atraumatic lumbar puncture and cloned in limiting dilution. Targets were Cr-labeled autologous LCL infected with HTLV-1 p40x (*A*), env (*B*), or pulsed with tax peptide 11-19 (*C*). Vaccinia-HA constructs were used as controls. Effector cell frequencies were extrapolated as in Fig. 1. Individual wells demonstrating >25% specific lysis were screened as positive.

tients with HAM/TSP, and of lymphocytic infiltrates rich in $CD8^+$ T cells in spinal cord lesions of such patients has been reported (5, 11, 19, 20). Also, the HTLV-1 viral load in the CNS of these patients was found to be higher than in patients with ATL (4). Since in asymptomatic HTLV-1–infected individuals the responses of virus-specific CTL are lower or absent (11, 21), it may be that the high level of CTL activity in HAM/TSP is related to the persistence of HTLV-1 in CNS, and/or to chronicity of this infection associated with an increased viral load (4). For example, in HIV-1 infection, another retroviral disease frequently associated with CNS infection in the later stages of AIDS, systemic and local increase of the viral load is believed to lead to HIV-1–specific neuropathology (22). In HTLV-1 infection, the generation of pCTL may have dual consequences. These cells are generated presumably to suppress viral replication through the lysis of infected cells or through the production of cytokines with antiviral properties. In healthy HTLV-1–infected individuals CTL targeted to chronically infected T cells may decrease viral load and maintain clinically asymptomatic infection. On the other hand, infection of neural or glial cells followed by damage of these cells by CTL or by disturbance of their function by cytokine responses could contribute to the pathogenesis of neurologic disease associated with HTLV-1.

In HIV-1 infection, high frequencies of both HIV-specific CTL and pCTL have been detected even in asymptomatic individuals (23–25). The present results with HAM/TSP patients, however, indicate that in this disease, pCTL frequencies are at least 40-fold higher than in infection by HIV-1. It is interesting that whereas late stages of HIV infection characterized by immunological and neurological deterioration are accompanied by the decline of both CTL and their precursors, patients with neurological disease associated with HTLV-1 display significantly higher pCTL frequencies than asymptomatic carriers. This would imply that the presence of HTLV-1–specific CTL in HAM/TSP may be a disease-specific event, potentially contributing to the pathogenesis of this disorder.

In HAM/TSP patients, an infiltration of $CD8^+$ cells in spinal cord lesions, the presence of the HTLV-1 p19 core protein (19), HTLV-1 genomic sequences (20), and HLA class I molecules in such lesions (19), support the suggestion that these $CD8^+$ cells in HAM/TSP spinal cord lesions may cause lysis of infected cells in thoracic cord. Alternatively, cytokine responses could be induced that could adversely affect the function of uninfected neurons and glia within the CNS and contribute to the pathological effects observed in patients with HAM/TSP. The demonstration of HTLV-1–specific $CD8^+$ CTL directly from lymphocytes obtained from the CSF of patients with HAM/TSP (15) is consistent with this hypothesis and provides an explanation for the neurological manifestations of this disease. The results from the present study demonstrating similar pCTL frequencies in the CSF and blood, further support the view that CTL activity may be essential to the pathogenesis of HAM/TSP.

Although pCTL responses to HTLV-1 structural proteins were observed in some HAM/TSP patients, pCTL predominantly recognized HTLV-1 products encoded within the pX region. This confirms earlier data showing that CTL obtained directly from the peripheral blood of HAM/TSP patients predominantly lysed targets infected with the HTLV-1 p40x vaccinia constructs (9, 11). This indicates that a product of the HTLV-1 tax gene is recognized by both pCTL and CTL obtained directly from peripheral blood. Other HTLV-1 antigens, such as env or gag proteins, can also be recognized, but these responses occur with a lower frequency than the responses to the tax region of HTLV-1.

The observation that in HAM/TSP, pCTL from HLA-A2 patients and HLA-B14 patients, respectively, recognized tax peptide 11-19 and 90-55, indicates that small peptides of the HTLV-1 tax region can be recognized by specific pCTL in association with these HLA class I alleles. Therefore, HTLV-1–specific pCTL responses to the tax protein are not restricted to a particular haplotype, but rather to immunodominant peptide fragments that are associated with particular HLA alleles. HLA-restricted CTL to the tax protein have been found in association with HLA-A2, A3, and B14 (18). Moreover, since a strong CTL response to tax in HAM/TSP patients is related to a single immunogenic region in individuals with a given haplotype, this may be useful in developing strategies to interfere with peptide/HLA binding (26).

If HTLV-1–specific CTL play a central role in the development of HAM/TSP, then immunotherapeutic strategies that focus on eliminating these cells or inhibiting their activity, could be clinically significant. The information obtained from immunotherapy of neurological HTLV-1 infection, could be extended to other CNS diseases of retroviral origin, such as CNS infection by HIV-1, and possibly to other chronic-progressive neurological diseases such as multiple sclerosis.

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