

Demonstration of Human T Lymphotropic Virus Type I (HTLV-I) Tax-Specific CD8⁺ Lymphocytes Directly in Peripheral Blood of HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis Patients by Intracellular Cytokine Detection

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Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is an inflammatory neurologic disease caused by HTLV-I infection and has been associated with elevated levels of several proinflammatory cytokines in both serum and cerebrospinal fluid. It is unknown what kind of cells secrete these cytokines and if HTLV-I Ags are associated with this phenomenon. Here, we investigated the expression of cytokines in PBL from eight HAM/TSP patients, nine HTLV-I-infected asymptomatic carriers, and seven healthy controls by flow cytometry combined with intracellular cytokine staining. PBL were cultured with brefeldin A without mitogen and IL-2 for 14 h. Under these conditions, CD8⁺ cells produced proinflammatory cytokines including IFN- γ , TNF- α , and IL-2, which were significantly elevated in HAM/TSP patients. The proportion of CD8⁺ cells producing IFN- γ in HAM/TSP patients, asymptomatic carriers, and healthy controls were, on average, 4.9, 0.4, and 0.3%, respectively. IFN- γ production by these CD8⁺ cells was suppressed by anti-HLA-class I Ab. Purified CD8⁺ cells from an HLA-A2 HAM/TSP patient produced IFN- γ by cocultivation with autologous CD4 cells, the main reservoir of HTLV-I in vivo, or allogenic HLA-A2⁺ B cells pulsed with a known immunodominant HTLV-I tax peptide. These data suggest that high levels of circulating HTLV-I-specific CD8⁺ T lymphocytes have the potential to produce proinflammatory cytokines and may promote inflammatory responses to HTLV-I in HAM/TSP patients. *The Journal of Immunology*, 1998, 161: 482–488.

Human T lymphotropic virus type I (HTLV-I)³ is a human retrovirus well known as the causative agent for adult T cell leukemia/lymphoma (1) and a slowly progressive neurologic disorder, termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (2, 3). HAM/TSP patients have upper motor neuron signs and mild sensory and sphincter dysfunction (4). Pathologically, the disease is characterized by perivascular inflammatory cell infiltration with demyelination, which is prominent in the thoracic spinal cord (5, 6). Immunologic parameters, including high HTLV-I proviral load (7, 8), increased spontaneous lymphoproliferation (9, 10), and high Ab titers to HTLV-I both in sera and cerebrospinal fluid (4), are abnormal in HAM/TSP patients in comparison with HTLV-I-infected asymptomatic carriers and healthy controls. In addition, HAM/TSP patients show extraordinarily high levels of circulating HTLV-I-spe-

cific CD8⁺ CTL, specific for immunodominant peptides of HTLV-I in association with particular HLA (11, 12). Moreover, the frequency of these HTLV-I tax-specific CD8⁺ CTL in the peripheral blood of HAM/TSP patients can be as high as 1 in 75 (13). Immunohistochemical analysis of the affected spinal cord lesions in the early stage of patients with HAM/TSP reveals the presence of infiltrating CD4⁺ and CD8⁺ lymphocytes in which CD8 cells predominate with duration of illness (14). In such lesions, the expression of HLA class I Ags (15) and the existence of HTLV-I-specific CD8⁺ CTL have also been shown (16). Recent molecular biologic studies have demonstrated HTLV-I genome (17–19) and its expression in the affected lesions (20, 21). Collectively, this evidence supports the view that virus-specific CD8⁺ T lymphocytes may play a critical role in the immunopathogenesis of HAM/TSP.

In HAM/TSP, an increase in activated lymphocytes has been shown in PBL and cerebrospinal fluid (22, 23), and high levels of several inflammatory cytokines such as IFN- γ , TNF- α , and IL-6 in sera and cerebrospinal fluid have been reported (24–26). In addition, mRNA expression of IL-1 β , IL-2, TNF- α , and IFN- γ has been shown to be up-regulated in HAM/TSP PBL (27, 28). In the central nervous system (CNS), the expression of inflammatory cytokines, including IL-1 β , TNF- α , and IFN- γ , have been demonstrated in the spinal cord of HAM/TSP patients with a short duration of illness (29).

HTLV-I tax is a strong transactivator that can up-regulate host gene expression including several cytokines (30). Since HTLV-I is preferentially detected in CD4⁺ lymphocytes in vivo (31), CD4⁺ cells have been suggested to be a source of increased cytokine

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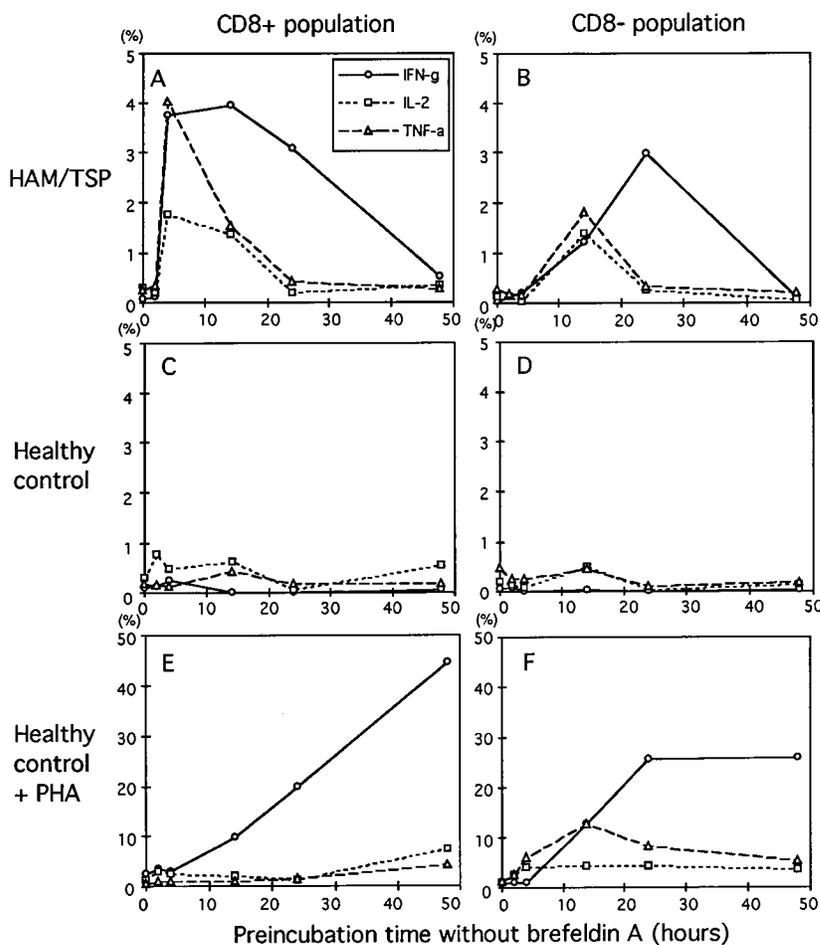
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³ Abbreviations used in this paper: HTLV-I, human T lymphotropic virus type I; HAM/TSP, HTLV-I-associated myelopathy/tropical spastic paraparesis; BFA, brefeldin A; PE, phycoerythrin; CNS, central nervous system.

FIGURE 1. Kinetic examination of cytokine production. One million PBL from a HAM/TSP patient and healthy control were precultured in medium for several hours (4 h to 48 h) without BFA in a 96-well round-bottom plate, then cultured with 10 $\mu\text{g}/\text{ml}$ of BFA for 10 h. Cultured PBL were harvested and stained with anti-CD8 Ab and anti-cytokine Abs. Graphs are representative of three different experiments and show the percentage of cytokine-expressing cells in the CD8-positive and -negative population vs preincubation time without BFA. A, CD8⁺ population from HAM/TSP PBL cultured without any stimuli; B, CD8⁻ population from HAM/TSP PBL cultured without any stimuli; C, CD8⁺ population from healthy control PBL cultured without any stimuli; D, CD8⁻ population from healthy control PBL cultured without any stimuli; E, CD8⁺ population from healthy control PBL cultured with 1 $\mu\text{g}/\text{ml}$ of PHA; F, CD8⁻ population from healthy control PBL cultured with 1 $\mu\text{g}/\text{ml}$ of PHA.



expression. However, it is unclear whether the highly immunoreactive HTLV-I-specific CD8⁺ T cells can also produce cytokines. To address these issues, we employed flow cytometric analysis for intracellular cytokine production from PBL of HAM/TSP patients, which allows us not only to identify individual cytokine-producing cells, but also to observe the cellular or humoral interactions associated with cytokine secretion.

In the present study, we demonstrated that peripheral CD8⁺ T lymphocytes produce IL-2, IFN- γ , and TNF- α in HAM/TSP patients and that the frequency of IFN- γ ⁺ CD8⁺ cells was significantly higher than that of asymptomatic carriers and healthy controls. This high IFN- γ production was shown to be associated with HTLV-I Ag-HLA class I complex on self-CD4 cells in HAM/TSP patients. These results suggest that high levels of circulating HTLV-I-specific CD8⁺ T lymphocytes, which have the potential to produce proinflammatory cytokines, may contribute to the immunopathogenesis of HAM/TSP.

Materials and Methods

Subjects

PBL from eight HAM/TSP patients, nine HTLV-I-infected asymptomatic carriers, and seven HTLV-I-noninfected healthy controls were tested. HTLV-I infection was confirmed by Western blot in serum of HAM/TSP patients and asymptomatic carriers. The diagnosis of HAM/TSP was made using the World Health Organization criteria and is based on the patients' neurologic symptoms and a serologic test for HTLV-I (32). PBL were isolated by Ficoll-Hypaque centrifugation and stored in liquid nitrogen until use.

Antibodies

Mouse anti-human CD3-phycoerythrin (PE)-conjugated mAb, anti-CD4-PE- or FITC-conjugated Ab or anti-CD8- TRI-COLOR-conjugated Ab

(Caltag Laboratories, South San Francisco, CA) was used to detect cell surface molecules of lymphocytes. Anti-TNF- α -PE-conjugated mAb, anti-IFN- γ -FITC-conjugated Ab, and anti-IL-2-PE-conjugated Ab (PharMingen, San Diego, CA) were utilized for intracellular cytokine staining. Mouse-IgG1-FITC-conjugated Ig and mouse-IgG1-PE-conjugated Ig (Caltag) were used as an isotype control for intracellular protein staining.

Staining of cell surface molecules, intracellular cytokines, and flow cytometric analysis

The method to detect intracellular cytokines was established by Sander et al. (33). Cultured PBL were harvested and washed in cold staining buffer containing 1% FCS and 0.1% sodium azide in PBS (pH 7.4). Cells were pretreated with 10 $\mu\text{g}/\mu\text{l}$ of human γ globulin at 4°C for 5 min to reduce nonspecific Ab binding and followed staining with 0.3 μg of mAbs to cell surface molecules in 10 μl of staining buffer at 4°C for 30 min. Cells were washed and fixed with 100 μl of 4% paraformaldehyde in PBS (pH 7.4) at 4°C for 20 min. After fixation, cells were washed and resuspended in 50 μl of permeabilization buffer that contained 0.1% saponin, 1% FCS, and 0.1% sodium azide in PBS (pH 7.4). Cells were pretreated with 2 $\mu\text{g}/\mu\text{l}$ of human γ globulin at 4°C for 5 min and stained with 0.5 μg of anti-cytokine Abs at 4°C for 30 min. Cells were washed with permeabilization buffer and finally resuspended in 200 μl of staining buffer. FACScan and CELLQuest computer software (Becton Dickinson, Mountain View, CA) were utilized for fluorescent signal detection and data analysis. Fifty thousand events were evaluated. Lymphocytes were gated on forward and side scatter image, and the proportion of cytokine-positive, CD8⁺, and CD8⁻ cells was calculated.

Culture condition of PBL

Frozen PBL were thawed, washed twice, and resuspended. The viability of the cells was over 90% by trypan blue staining. One million cells were transferred to a 96-well round-bottom plate in 200 μl of RPMI 1640 (Life Technologies, Gaithersburg, MD) containing 5% heat-inactivated human serum, 2.8 mM L-glutamine, 14 mM HEPES buffer, 40 U/ml penicillin, and 40 $\mu\text{g}/\text{ml}$ streptomycin. To optimize the detection of intracellular cytokines, conditions were initially established in which PBL were cultured for

various periods (4 h to 48 h) of time before the addition of brefeldin A (BFA) (10 μ g/ml) (Sigma, St. Louis, MO). BFA inhibits protein transportation from the Golgi apparatus to the cell surface, resulting in accumulation of protein in the Golgi (33). CD8⁺ and CD8⁻ cell populations from PBL of HAM/TSP patients and healthy controls were gated and analyzed for intracellular expression of IFN- γ , IL-2, and TNF- α . For the kinetic study of cytokine production, PHA was added to cells at a concentration of 1 μ g/ml. Based on these results, the culture conditions were optimized in which PBL were cultured in the absence of exogenous stimuli for 4 h before a 10-h incubation with BFA. These conditions were chosen for the following reasons: 1) the IFN- γ secretion appeared specific for HTLV-I-infected individuals; 2) detection of this cytokine, particularly in the absence of exogenous stimuli, may reflect dynamic events of cytokine production in T cell subsets *in vivo*; and 3) the percentage of cytokine-positive cells appeared high in HAM/TSP PBL, which facilitated further analysis. HLA-A2-binding peptide, HTLV-I tax 11–19 peptide (LLFGYPVYV), CMV gB peptide (IAGNSAYEYV), and influenza virus M1 peptide (GILGFVFTL) were used at the indicated concentration (34).

Blocking of IFN- γ production by anti-HLA Abs

To determine whether antigenic stimulation is involved in the elevated cytokine production in CD8⁺ cells from HAM/TSP patients, it was initially determined whether the expression of IFN- γ from CD8⁺ cells could be inhibited by anti-HLA Abs. Anti-HLA class I (W6/32) and class II (L243) mAbs were added to PBL from a HAM/TSP patient at a final concentration of 10 μ g/ml or 40 μ g/ml at the start of culture and the proportion of IFN- γ ⁺ cells in the CD8⁺ population was assessed by flow cytometry.

Cell-mixing experiment for IFN- γ production

To investigate if HAM/TSP CD4⁺ cells could induce cytokine expression from autologous CD8⁺ lymphocytes, cell-mixing experiments were performed. PBL from a HLA-A2 HAM/TSP patient were positively selected for CD8⁺ and CD4⁺ cells by immunomagnetic beads according to the manufacturer's instructions (DynaL, Lake Success, NY). The purity was greater than 96% in each cell population by flow cytometric analysis. Purified CD8⁺ cells (5×10^5) were cocultured for 4 h with autologous or allogenic CD4⁺ cells at CD4:CD8 ratios of 0.04, 0.2, and 1. BFA was then added and cells were cultured for an additional 10 h. Allogenic CD4⁺ cells were obtained from an HLA-A2-matched, HTLV-I-noninfected individual. In addition, Hmy-A2 cells (an HLA-A2-transfected B cell line) were pulsed with HTLV-I tax 11–19 peptide or influenza virus M1 peptide for 2 h at 37°C at the indicated concentration, and washed twice (34). Peptide-pulsed Hmy-A2 cells (5×10^5) were added to 5×10^5 purified CD8⁺ cells and cultured for 4 h before culture with BFA (10 μ g/ml) for an additional 10 h. The portion of IFN- γ ⁺ cells in the CD8⁺ population was assessed by flow cytometry.

Results

Kinetics of cytokine production

In Figure 1A, CD8⁺ cells from a HAM/TSP patient, in the absence of exogenous stimulation, produced IFN- γ , IL-2, and TNF- α at an optimal incubation time of 4 h preculture before the addition of a 10-h culture period in the presence of BFA. In the CD8⁻ population, an incubation time of 12 to 24 h preculture before the addition of BFA was required for maximal production of IFN- γ , IL-2, and TNF- α (Fig. 1B).

In marked contrast to the intracellular cytokine detection of IFN- γ , IL-2, and TNF- α in the CD8⁺ population from a HAM/TSP patient, expression of these cytokines was barely detectable from CD8⁺ or CD8⁻ cells from a healthy control (Fig. 1, C and D). Incubation with 1 μ g/ml of PHA in both the CD8⁺ and CD8⁻ cell populations from healthy controls increased the expression of all three cytokines although the kinetics of expression were different in these T cell subgroups (Fig. 1, E and F).

CD8⁺ cells from HAM/TSP express both TNF- α and IFN- γ

To determine whether there was an increase in the number of CD8⁺ cells from HAM/TSP patients that expressed IFN- γ and TNF- α , PBL were doubly stained with anti-cytokine Abs and Abs to CD8. As shown in a representative experiment in Figure 2, IFN- γ - and TNF- α -positive cells were detected at 14.0% and

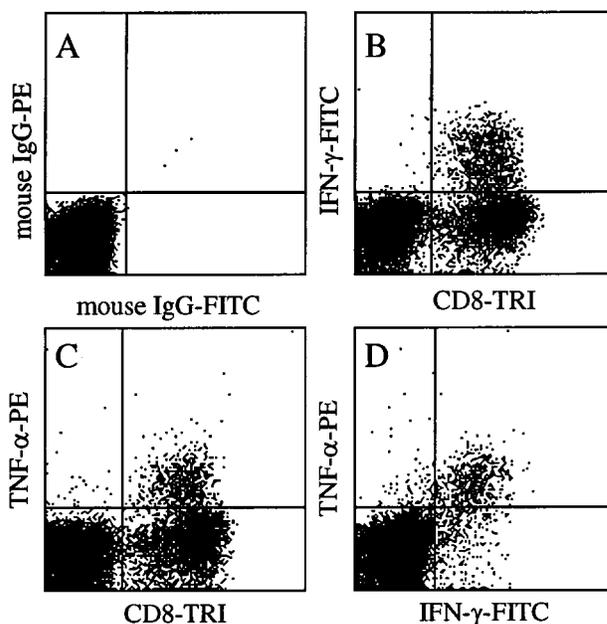


FIGURE 2. Double staining of the intracellular cytokines in PBL of a HAM/TSP patient. PBL were precultured without BFA for 4 h and cultured with BFA for 10 h. A, control staining with isotype-matched mouse anti-IgG. B, double staining with anti-CD8 and anti-IFN- γ Abs. IFN- γ -positive cells were 14.0% in the CD8 population. C, double staining with anti-CD8 and anti-TNF- α Abs. TNF- α -positive cells were 11.5% in the CD8 population. D, intracellular double staining with anti-IFN- γ and anti-TNF- α Abs. Of TNF- α -positive cells, 81.4% simultaneously produced IFN- γ .

11.5% in the CD8⁺ population, respectively (Fig. 2B and 2C). Importantly, 81% of the TNF- α -positive cells stained for IFN- γ , strongly suggesting that the TNF- α ⁺ CD8⁺ population in HAM/TSP PBL also produced IFN- γ (Fig. 2D).

IFN- γ production by CD8⁺ cells in PBL of HAM/TSP, asymptomatic carriers, and healthy controls

The proportion of CD8⁺ cells that express IFN- γ in PBL from HAM/TSP patients, HTLV-I seropositive asymptomatic carriers, and healthy controls were analyzed to determine whether the increased number of IFN- γ ⁺ CD8⁺ cells was related to infection with HTLV-I or the clinical status of the patient. Figure 3A is a representative flow cytometric analysis of IFN- γ -producing CD8⁺ cells from PBL of HAM/TSP patients, asymptomatic carriers, and healthy controls. A distinct subset of CD8⁺ cells that express IFN- γ could clearly be detected in PBL from HAM/TSP patients, while no comparable population was observed in healthy controls or HTLV-I asymptomatic carriers (Fig. 3A). In a survey of eight patients with HAM/TSP, nine asymptomatic carriers, and seven healthy controls, the percentage of IFN- γ -expressing cells in the CD8⁺ population was 4.9%, 0.4%, and 0.3%, respectively (Fig. 3B). The IFN- γ producing CD8⁺ cells was significantly greater in HAM/TSP patients compared with HTLV-I asymptomatic carriers or healthy controls. Indeed, in one HAM/TSP patient, as high as 14% of CD8⁺ cells expressed IFN- γ .

Inhibition of IFN- γ production in CD8⁺ cells by anti-HLA Abs

It was determined if the increased number of IFN- γ ⁺ CD8⁺ cells in HAM/TSP patients could be inhibited by anti-HLA Abs. As shown in Figure 4, only the anti-HLA class I but not HLA class II Ab suppressed the IFN- γ production in CD8⁺ cells.

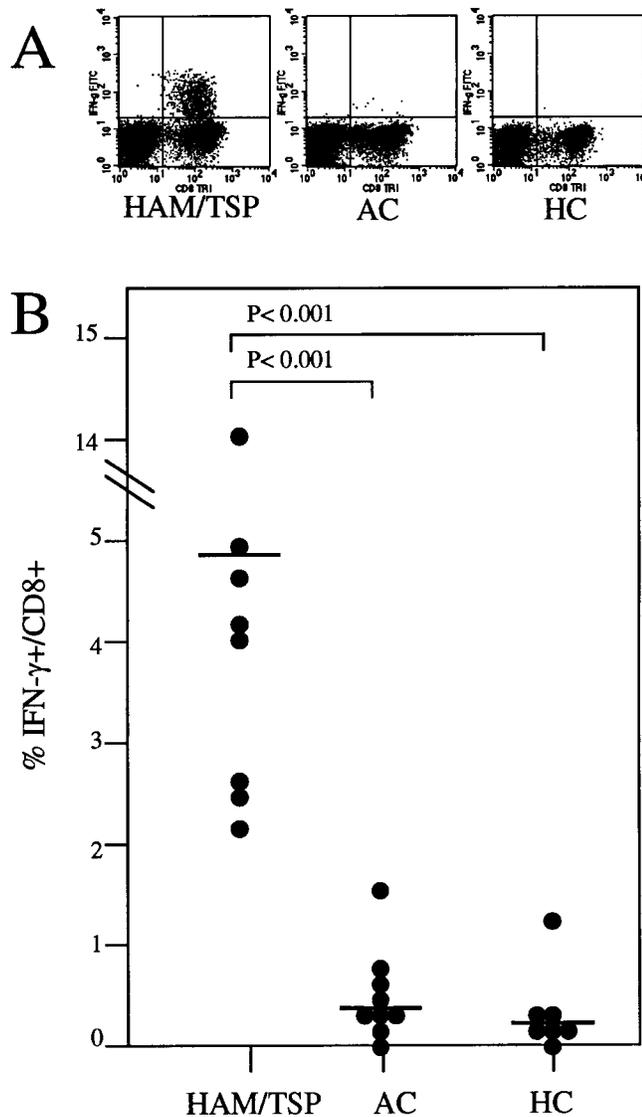


FIGURE 3. Representative flow cytometric analysis of IFN- γ ⁺ CD8⁺ cells in PBL of a HAM/TSP patient, an asymptomatic HTLV-I carrier (AC) and a healthy control (HC). *A*, PBL were precultured in medium without stimuli for 4 h before culture in BFA for 10 h. A distinct subset of IFN- γ ⁺ cells in CD8⁺ population could be detected in HAM/TSP PBL (14.0%), which could not be demonstrated in PBL from asymptomatic HTLV-I carriers (0.28%) and healthy controls (0.21%). *B*, IFN- γ production from CD8⁺ cells in PBL of eight HAM/TSP patients, nine asymptomatic carriers, and seven healthy controls were analyzed for the proportion of IFN- γ ⁺ cells in the CD8⁺ population. The mean percent positive of this subset for each group was 4.9, 0.4, and 0.3%, respectively. Statistical analysis was done by the Student *t* test.

Induction of IFN- γ by CD8⁺ cells is HTLV-I tax peptide dependent

The ability of the immunodominant HTLV-I tax 11–19 peptide to induce IFN- γ expression in CD8⁺ cells from a HAM/TSP patient was examined. As shown in Figure 5, culture conditions were chosen in which the expression of IFN- γ from CD8⁺ cells was dramatically reduced when BFA was added at the start of the culture period (Fig. 5; 0 h time point precultivation without BFA). Upon addition of the HTLV-I tax 11–19 peptide to HAM/TSP PBL, there was a dramatic induction of IFN- γ ⁺ CD8⁺ cells (8.1%), comparable (if not greater) than the optimal PBL culture condition for the induction of IFN- γ from CD8⁺ cells (Fig. 5, 4-h time point

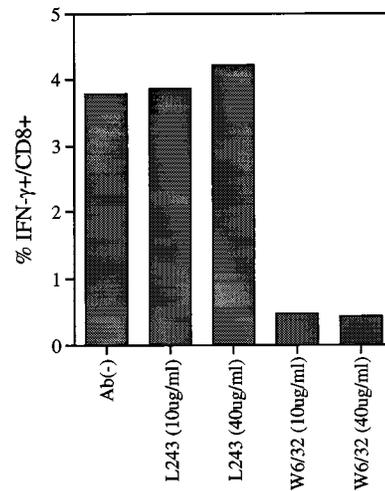


FIGURE 4. Inhibition of IFN- γ production in CD8⁺ cells by anti-HLA Abs. PBL from a HAM/TSP patient were cultured with or without anti-HLA Abs and the percentage of IFN- γ ⁺ cells in the CD8⁺ population was measured. W6/32 and L234 are anti-HLA-class I and class II Abs, respectively.

precultivation without BFA). A control CMV peptide, known to bind HLA A2, did not induce IFN- γ cytokine expression in CD8⁺ cells (Fig. 5).

Mixing of autologous CD4 cells and HTLV-I peptide-pulsed Hmy-A2 cells to purified CD8⁺ cells induced the expression of IFN- γ

Since we have shown that an immunodominant HTLV-I tax peptide could induce the expression of IFN- γ in CD8⁺ cells from HAM/TSP PBL, it was of interest to determine whether

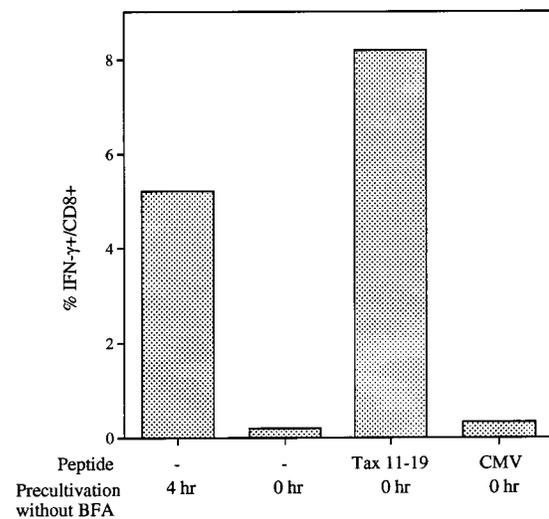


FIGURE 5. HTLV-I tax peptide-dependent IFN- γ production by CD8⁺ cells. Viral peptides, known to bind to HLA-A2, were added to HAM/TSP PBL at a final concentration of 1 μ M. From *left*, the first bar indicates the percentage of IFN- γ ⁺ cells in the CD8⁺ population of HAM/TSP PBL with no peptide when BFA was added after 4 h of culture (optimal culture conditions). The other bars indicate the percentage of IFN- γ ⁺ cells in the CD8⁺ population of HAM/TSP PBL with and without the addition of viral peptides at a suboptimal culture condition determined to induce low levels of IFN- γ expression. BFA was added at the start of the culture period (0 h time point precultivation without BFA).

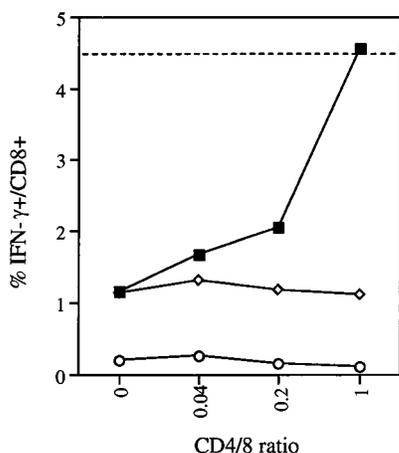


Figure 6. IFN- γ production in CD8⁺ cells by mixing autologous and allogenic CD4⁺ cells. Purified HAM/TSP CD8⁺ cells were cultured with the indicated concentrations of purified autologous CD4⁺ cells or purified CD4⁺ cells from an HLA-A2, non-HTLV-I-infected healthy control (HC). Cells were precultured in medium without stimuli for 4 h before culture with BFA for 10 h. The percentage of IFN- γ ⁺/CD8⁺ cells was measured. Black square: HAM/TSP CD8⁺ cells plus HAM/TSP CD4⁺ cells; diamond: HAM/TSP CD8⁺ cells plus HC CD4⁺ cells; circle: HC CD8⁺ cells plus HAM/TSP CD4⁺ cells; triangle: HC CD8⁺ cells plus HC CD4⁺ cells. The dashed line indicates the percentage of IFN- γ ⁺/CD8⁺ cells from unseparated HAM/TSP PBL.

autologous CD4⁺ cells, known to harbor HTLV-I in HTLV-I-infected individuals (31), could also induce cytokine expression. As shown in Figure 6, the addition of purified CD4⁺ cells from a HAM/TSP patient to purified HAM/TSP CD8⁺ cells resulted in a dose-dependent increase in the intracellular IFN- γ expression to the level observed from unseparated PBL from this patient. In contrast, the addition of purified CD4⁺ cells from an HLA-A2-matched HTLV-I seronegative donor to purified HAM/TSP CD8⁺ cells did not increase the expression of IFN- γ in these CD8⁺ cells (Fig. 6).

To determine whether this increase in IFN- γ in HAM/TSP CD8⁺ cells was HTLV-I tax peptide specific, Hmy-A2 cells (a B cell line transfected with HLA-A2) prepulsed with the HTLV-I tax 11–19 peptide were added to purified CD8⁺ cells. Increasing concentrations of HTLV-I tax 11–19 peptide-pulsed Hmy-A2 cells induced the expression of IFN- γ in purified HAM/TSP CD8⁺ cells, but not from purified CD8⁺ cells of an HLA-A2-matched HTLV-I seronegative donor (Fig. 7). As controls, Hmy-A2 cells prepulsed with influenza virus M1 peptide did not induce the expression of IFN- γ either from purified HAM/TSP CD8⁺ cells or from CD8⁺ cells of an HLA-A2-matched HTLV-I seronegative donor (Fig. 7).

Discussion

In this study, we demonstrated that, in HAM/TSP patients, circulating HTLV-I-specific CD8⁺ lymphocytes produce proinflammatory cytokines such as IFN- γ , TNF- α , and IL-2. Moreover, the proportion of these cytokine-expressing HTLV-I-specific CD8⁺ cells in total CD8⁺ cells was extraordinarily high. On average, 4.9% of HAM/TSP CD8⁺ cells expressed IFN- γ (which was as high as 14% in one patient). This is in marked contrast to the proportion of cytokine-expressing CD8⁺ cells that can be demonstrated in the peripheral blood of HTLV-I seropositive asymptomatic carriers (0.4%), a level comparable in PBL of normal healthy controls (0.3%). The ability to detect an increased frequency of cytokine-expressing CD8⁺ cells, particularly in patients with

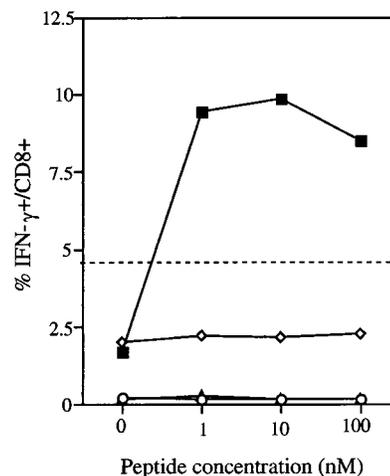


Figure 7. IFN- γ production from CD8⁺ cells by the addition of HTLV-I tax peptide-pulsed Hmy-A2 cells. Purified CD8⁺ cells from either a HLA-A2 HAM/TSP patient or a HLA-A2-matched healthy control (HC) were cultured with Hmy-A2 cells prepulsed with several concentrations of HTLV-I tax 11–19 or an influenza virus M1 peptide. Cells were precultured in medium with peptide for 4 h before culture with BFA for 10 h, and the percentage of IFN- γ ⁺/CD8⁺ cells was measured. Black square: HAM/TSP CD8⁺ cells plus Hmy-A2 cells prepulsed with the HTLV-I tax 11–19 peptide; diamond: HAM/TSP CD8⁺ cells plus Hmy-A2 cells prepulsed with the influenza M1 peptide; circle: HC CD8⁺ cells plus Hmy-A2 cells prepulsed with the HTLV-I tax 11–19 peptide; triangle: HC CD8⁺ cells plus Hmy-A2 cells prepulsed with the influenza M1 peptide. The dashed line indicates the percentage of IFN- γ ⁺/CD8⁺ cells from unseparated HAM/TSP PBL.

HAM/TSP, suggests that these CD8⁺ cells may play a role in the pathogenesis of HAM/TSP.

HTLV-I contains a unique *pX* gene coding for the HTLV-I tax protein, which is known to be a strong transactivator of many host genes (30). In vitro, cells infected with HTLV-I or transfected with HTLV-I tax constructs constitutively produce many cytokines including IL-1, IL-2, IL-6, TNF- α , TNF- β , IFN- γ , and granulocyte macrophage-CSF (30). However, it has not been determined which cells from HTLV-I-infected individuals in vivo produce these cytokines. In this report, CD8⁺ cells from PBL of HAM/TSP patients were shown to preferentially express IFN- γ and TNF- α , although we cannot exclude the possibility that HTLV-I-infected CD4 lymphocytes or other cell types such as macrophages may also express these cytokines. Because anti-HLA class I Abs were able to suppress the production of these cytokines from HAM/TSP CD8⁺ cells (Fig. 4) that are not infected with HTLV-I in vivo (31), it suggests that the expression of cytokines from CD8⁺ cells is a consequence of a virus-induced inflammatory process rather than trans-activation by the HTLV-I *pX* gene.

The inhibition of IFN- γ production from HAM/TSP CD8⁺ cells by HLA class I Ab suggests that cytokine expression may be associated with an interaction of the TCR/Ag/HLA trimolecular complex. The addition of purified CD4⁺ cells from a HAM/TSP patient to autologous CD8⁺ cells resulted in a dose-dependent increase in the intracellular IFN- γ expression to the level observed from unseparated PBL. The addition of CD4⁺ cells from a HTLV-I-noninfected individual did not induce IFN- γ production from HAM/TSP CD8⁺ cells (Fig. 6). This indicates that the CD8⁺ cells produced IFN- γ by recognition of some HTLV-I Ags on the infected CD4⁺ cells, the main reservoir of HTLV-I in vivo (31). Moreover, the production of

IFN- γ in CD8⁺ cells from an HLA-A2 HAM/TSP patient was up-regulated by the addition of the HTLV-I tax 11–19 peptide (Figs. 5 and 7). We have demonstrated previously that CTL in HLA-A2 HAM/TSP patients recognized this same immunodominant HTLV-I tax 11–19 peptide in strong association with HLA-A2 (12). Similarly, results in this study demonstrate that the production of IFN- γ in CD8⁺ cells from an HLA-A2 HAM/TSP patient can be up-regulated by the addition of the HTLV-I tax 11–19 peptide.

Recent molecular biologic studies provide evidence that the HTLV-I genome and its transcripts are present in the CNS of HAM/TSP patients (17–21). If HTLV-I-specific CD8⁺ cells in the affected lesions (16) recognize immunodominant HTLV-I peptides, this may lead to the production of proinflammatory cytokines such as IL-2, IFN- γ , and TNF- α . The expression of TNF- α by CD8⁺ lymphocytes from HAM/TSP patients is of considerable interest since TNF- α has been reported to be cytotoxic to oligodendrocytes in culture and induces demyelination (35). Moreover, in a disease like multiple sclerosis which is clinically similar to HAM/TSP, mRNA levels of TNF- α in PBL have been shown to correlate with the disease progression (36).

In HAM/TSP, there is a large body of information on HTLV-I pathology, disease association, and cellular immune reactivity, which allows for a number of hypothetical models of HAM/TSP pathogenesis. Three major models have been proposed: 1) recognition of HTLV-I gene products in the CNS by Ag-specific CD8⁺ effector CTL that result in lysis of glial elements and cytokine release (37), 2) an autoimmune process in which HTLV-I infection leads to activation of autoreactive T cells (19, 38), and 3) an autoaggressive bystander model mediated by immunocompetent virus-specific T cells releasing a cascade of cytokines that result in CNS damage (39). In all these models, HTLV-I-specific CD8⁺ lymphocytes are considered to play a crucial role in the pathogenesis of HAM/TSP, although it remains to be determined which cells in the CNS are preferential targets for these cells (18–21). Importantly, if HTLV-I-specific CD8⁺ lymphocytes play a central role in the development of HAM/TSP, immunotherapeutic strategies targeted to eliminate or inactivate these high levels of circulating HTLV-I-specific CD8⁺ cells expressing proinflammatory cytokines may be clinically beneficial in this disease.

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