CD8+ T cells are an in vivo reservoir for human T-cell lymphotropic virus type I

Masahiro Nagai, Meghan B. Brennan, Jill A. Sakai, Carlos A. Mora, and Steven Jacobson

It is thought that human T-cell lymphotropic virus type I (HTLV-I) preferentially infects CD4+ T cells in vivo. However, observations of high HTLV-I proviral load in patients with HTLV-I–associated myelopathy/tropical spastic paraparesis suggest that HTLV-I may infect other cell types in addition to CD4+ T cells. To identify in vivo T-cell tropisms of HTLV-I, real-time quantitative polymerase chain reaction (PCR) and intracellular protein staining were used. A high amount of HTLV-I proviral DNA was detected from purified CD8+ T cells by quantitative PCR (between 1.64 and 62.83 copies of HTLV-I provirus per 100 isolated CD8+ T cells). CD8+ T cells expressed HTLV-I–related antigens (HTLV-I Tax and p19 protein) after a short time in cultivation. These results demonstrate that CD8+ T cells are also infected with HTLV-I and express HTLV-I antigens at levels that are comparable to HTLV-I–infected CD4+ cells. Therefore, CD8+ cells are an additional viral reservoir in vivo for HTLV-I and may contribute to the pathogenesis of HTLV-I–mediated disorders.

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Patients and methods

Patients

Five patients with HAM/TSP (HAM-1 to HAM-5) were tested. The diagnosis of HAM/TSP was made according to neurologic assessment and serologic testing for anti–HTLV-I antibody. All patients had diagnostic cerebral and spinal magnetic resonance imaging. All patients had anti-HTLV-I antibodies in serum and cerebrospinal fluid and had a slowly progressive spastic paraparesis. Informed consent was obtained from all patients.

Cell preparation

PBMCs were isolated from peripheral blood samples on a density gradient with lymphocyte separation medium (ICN Biomedicals, Aurora, OH), and

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the cells were viably cryopreserved in liquid nitrogen until tested. CD4⁺ T cells or CD8⁺ T cells were negatively selected from PBMCs with magnetic beads (MACS CD4⁺ or CD8⁺ T-cell isolation kit; Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions.

**HTLV-I Tax and p19 expression in PBMCs**

PBMCs at 5 × 10⁶ were placed in a culture well (round-bottom 96-well plate) in 200 µL RPMI-1640 supplemented with L-glutamine, penicillin, streptomycin, and 5% human AB serum. Harvested cells were washed with phosphate-buffered saline containing 1% fetal calf serum and 0.1% NaN₃ and incubated with anti-human CD4-phycoerythrin (Caltag Laboratories, Burlingame, CA) and anti-human CD8-Tricolor monoclonal antibodies (mAbs) (Caltag Laboratories, San Diego, CA) for 20 minutes at 4°C. After washing with 0.1% saponin buffer (Perm/Wash solution; Pharmingen), the cells were incubated with anti-HTLV-I Tax mAb (the reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health; HTLV-I Tax hybridxDNA [168A51-42] from Dr Beatrice Langton) or anti-HTLV-I p19 mAb (Chemicon International, Temecula, CA) for 30 minutes at 4°C. After washing, fluorescein isothiocyanate–anti–human CD8-Tricolor monoclonal antibodies (mAbs) (Caltag Laboratories, San Diego, CA) for 20 minutes at 4°C. After washing, fluorescein isothiocyanate–anti–HTLV-I Tax mAb and anti–human CD8-Tricolor monoclonal antibodies (mAbs) (Caltag Laboratories, Temecula, CA) were used as second antibody for labeling anti–HTLV-I Tax mAb or anti–human CD8-Tricolor monoclonal antibodies (mAbs), respectively. Flow cytometric analyses were performed using a FACS Calibur (Becton Dickinson, Mountain View, CA).

**Quantitative PCR**

HTLV-I proviral load was measured using an ABI PRISM 7700 Sequence Detector (Applied Biosystems) as described previously. DNA was extracted from 1 × 10⁷ cells with the Puregene DNA Isolation Kit (Genta, Minneapolis, MN) and was adjusted to 10 ng/µL. PCR conditions were as follows: 10 µL DNA solution was added to 40 µL reaction mixture containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 10 mM EDTA; 60 µM of each primer; 0.1 µM saponin buffer (Perm/Wash solution; Pharmingen), the cells were incubated with anti-HTLV-I Tax mAb (the reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health; HTLV-I Tax hybridxDNA [168A51-42] from Dr Beatrice Langton) or anti-HTLV-I p19 mAb (Chemicon International, Temecula, CA) for 30 minutes at 4°C. After washing, fluorescein isothiocyanate–anti–HTLV-I Tax mAb and anti–human CD8-Tricolor monoclonal antibodies (mAbs) (Caltag Laboratories, San Diego, CA) were used as second antibody for labeling anti–HTLV-I Tax mAb or anti–human CD8-Tricolor monoclonal antibodies (mAbs), respectively. Flow cytometric analyses were performed using a FACS Calibur (Becton Dickinson, Mountain View, CA).

**Provincial load in purified T-cell subsets**

To determine the T-cell tropism of HTLV-I in vivo, we isolated CD4⁺ and CD8⁺ T cells from PBMCs of 5 HAM/TSP patients (purity of each T-cell subset is shown in Table 1) and quantified the amount of HTLV-I proviral DNA in each cell fraction by Taqman PCR. As shown in Figure 1, PBMCs from all HAM/TSP patients contained HTLV-I tax sequences in both CD8⁺ and CD4⁺ T cells. Between 1.64 and 62.83 copies of HTLV-I provirus per 100 isolated CD8⁺ T cells were observed (Figure 1). HTLV-I proviral DNA could not be amplified from HTLV-I–seronegative donors. This result strongly indicates that the HTLV-I–positive PCR signals of CD8⁺ T-cell fractions resulted from natural HTLV-I infection of CD8⁺ T cells in vivo. Given the high degree of purity (Table 1) and the extent of CD8⁺ infection, contaminating HTLV-I–infected CD4⁺ T cells in the purified CD8⁺ population (even assuming every CD4⁺ T cell was infected) could not account for the high HTLV-I proviral load observed in HAM/TSP CD8⁺ cells. Even CD8⁺ T cells from HAM/TSP patient no. 4, with the lowest proviral load (1.72 copies per 100 PBMCs), contained 1.64 copies of HTLV-I per 100 CD8⁺ T cells.

**HTLV-I Tax and p19 expression in PBMCs**

To address the question of whether HTLV-I–infected CD8⁺ cells express HTLV-I antigen, we cultured PBMCs from HAM/TSP patients for a short time and measured the expression of HTLV-I Tax and p19 (HTLV-I Gag) proteins using an intracellular protein-staining technique. As reported previously, detection of HTLV-I antigen-expressing cells in uncultured HAM/TSP PBMCs was negligible (Figure 2, time 0) and was consistent with HTLV-I RNA analysis of fresh PBMCs from HAM/TSP patients. However, after briefly cultivating these PBMCs in vitro, we could detect HTLV-I Tax and p19 protein in both CD4⁺ and CD8⁺ T cells (Figures 2, 3). Maximum viral antigen expression occurred after 12 hours of cultivation and then declined (Figure 2). The HTLV-I Tax expression time course of CD4⁺ populations was similar to that of CD8⁺ cells, and all patients exhibited the same trend in protein expression. As expected from the HTLV-I proviral load data

**Results**

**HTLV-I proviral load in purified T-cell subsets**

To determine the T-cell tropism of HTLV-I in vivo, we isolated CD4⁺ and CD8⁺ T cells from PBMCs of 5 HAM/TSP patients

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<th>Table 1. Purity of isolated CD4⁺ or CD8⁺ T-cell fractions</th>
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Figure 1. HTLV-I proviral load in purified T-cell subsets. CD4⁺ T cells or CD8⁺ T cells were negatively selected from PBMCs. The purity of isolated populations is shown in Table 1. HTLV-I proviral load was measured using quantitative PCR for HTLV-I pX region and human β-actin. The gray bars indicate HTLV-I proviral load per 100 PBMCs, the striped bars indicate proviral load per 100 purified CD4⁺ T cells, and the black bars indicate proviral load per 100 purified CD8⁺ T cells.
The expression of Tax in CD4+ or CD8+ T cells was measured at each time point. Graphs show Tax expression in CD4+ or CD8+ T cells over time. The open circle (dotted line) indicates percentage of Tax expressing CD4+ T cells in total CD4+ T cells. The open square (solid line) indicates percentage of Tax expressing CD8+ T cells in total CD8+ T cells.

The open square (solid line) indicates percentage of Tax expressing CD8+ T cells.

Percentage of HTLV-I infected CD4+ and CD8+ T cells expressing Tax

As an estimate of HTLV-I viral activity (viral protein expression) within these different T-cell populations, the proportion of peak Tax expression per infected cell was calculated for isolated CD4+ and CD8+ cells (Figure 4). Surprisingly, this analysis suggested that in 3 of 4 HAM/TSP patients, the amounts of HTLV-I expressed per HTLV-I-infected CD4+ or CD8+ T cell were similar, and in one HAM/TSP patient (patient no. 4), the amount of HTLV-I expressed was even higher in CD8+ cells than in CD4+ cells (Figure 4).

Discussion

Although HTLV-I has been thought to preferentially infect CD4+ T cells in vivo,15 in vitro CD8+ cells could also be infected and immortalized.30,31 In this study, we demonstrate that in PBMC of HAM/TSP patients, CD8+ T cells have a high amount of HTLV-I proviral DNA (Figure 1). CD8+ T cells also express HTLV-I-related antigens (HTLV-I Tax and p19 protein) after a short time in cultivation (Figures 2, 3). These results strongly indicate that HTLV-I infects CD8+ T cells in vivo and these naturally infected CD8+ T cells have the ability to produce HTLV-I antigens. Similar observation that short-cultured CD8+ T cells from HTLV-I-infected individuals (HAM/TSP patients and asymptomatic HTLV-I carriers) expressed HTLV-I Tax protein has also been recently reported.32 Interestingly, it has been shown that both CD4+ and CD8+ T cells subsets were equally susceptible to HTLV-2 infection.33

High HTLV-I proviral loads have been demonstrated in patients with HAM/TSP34 and have been correlated with high immune responses, such as HTLV-I–specific cytotoxic T lymphocytes (CTLs)14 and anti–HTLV-I antibody.27 This elevated HTLV-I proviral load and high immune response have been suggested to play a role in HTLV-I–associated disease pathogenesis.35,36 It was originally believed that these high HTLV-I–specific immune responses were solely driven by HTLV-I–infected CD4+ T cells, which are elevated in patients with HAM/TSP.27 In vivo, HTLV-I–infected CD4+ T cells (helper T cells) were thought to become activated and present immunodominant viral peptides37 that stimulate virus-specific CD8+ CTLs. This high frequency of circulating HTLV-I–specific CTLs could then lyse these expanded, activated HTLV-I–infected CD4+ T cells, keeping the circulating viral infection in check.28 If virus-specific CTL recognition of antigen were to occur in a target organ containing HTLV-I–infected cells (hitherto thought only to be inflammatory HTLV-I–infected CD4+ T cells), then an immunopathologic process could ensue.35,36,38

The results in this report provide evidence that, similar to HTLV-I–infected CD4+ T cells, CD8+ T cells in PBMCs of HAM/TSP patients are infected with HTLV-I and are capable of...
expressing viral protein, including HTLV-I Tax. This protein is known to transactivate both viral and host genes, including IL-2, IL-2 receptor (IL2r), 22 and IL-15. 39 The transactivation of IL-15 by HTLV-I Tax is of particular interest because this newly described cytokine has been reported to be involved in the maintenance and expansion of memory CD8+ T cells. 40 HTLV-I–infected CD8+ T cells may therefore have a role as a significant viral reservoir in vivo and may also drive the high HTLV-I–specific immune response observed in patients with HAM/TSP. It remains to be seen whether comparable levels of HTLV-I–infected CD8+ cells are also observed in ATL patients. This newly reported CD8+ cell tropism should be studied to clarify the pathogenesis of HTLV-I–associated disease and has implications for the therapy of HTLV-I–mediated disorders and other human retroviruses.

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References


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