Patterns of *In vitro* Lymphoproliferative Responses Among HTLV-1-infected Subjects: Upregulation by HTLV-1 During HIV-1 Co-infection

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Abstract

*The present study evaluated the *in vitro* response to different mitogens and a candidin antigen (CMA) in Human T-cell lymphotropic virus type 1 (HTLV-1) and co-infected HIV-1/HTLV-1 patients, to identify if this co-infection may modify the spontaneous lymph proliferative response. Peripheral blood mononuclear cells from 72 healthy seronegative controls, 75 asymptomatic HTLV-1-infected carriers, 42 HAM/TSP cases, 33 solely HIV-1-infected subjects and 24 HIV-1/HTLV-1 patients were assayed in the presence and absence of mitogens (PHA, PWM and OKT3) and CMA. The HAM/TSP group had the highest proliferation rate at 3 and 6 days after culture. HAM/TSP cases showed decreased response to PHA, compared with asymptomatic HTLV-1 subjects, and most important, the co-infected HIV-1/HTLV-1 cases presented a similar response to HTLV-1-infected subjects after 3 days of culture. The singles HIV1-infected group had decreased *in vitro* response. It appears that during co-infection, the HTLV-1 regulatory proteins overwhelm the action of HIV-1 regulatory proteins.

Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1) has been recognized as the aetiologic agent of a mature adult T-cell leukemia/lymphoma (ATL) and a chronic myelopathy known as HTLV-1 tropical spastic paraparesis (TSP)-associated myelopathy (HAM) [1–2]. Less than 5% of HTLV-1-infected carriers develop symptoms, usually after 30–40 years of incubation [3]. HAM/TSP is considered to be an immune-mediated disease, and pro-inflammatory cytokines play an important role in this immune deregulation. The virus promotes T-cell proliferation by usurping several signalling pathways central to immune T-cell function, such as antigen stimulation and receptor–ligand interaction, suggesting that extracellular signals are important for HTLV-1 oncogenesis or inflammatory properties [4].

Several studies have indicated that immune activation may account for the pathogenesis seen in ATL development and HAM/TSP cases [5]. Large amounts of IL-2, IL-2r, IL-15, IFN-gamma and TNF-α have been detected in supernatant fluids of peripheral blood mononuclear cells (PBMC) or cerebral spinal fluid (CSF) of HAM/TSP patients.

Human T-cell lymphotropic virus type 1 infection is endemic in certain geographical areas, including Japan [6], the Caribbean Islands [7] and parts of Africa [8] and Brazil [9–14]. In Brazil, almost 10% of human immunodeficiency virus type I (HIV-1)-infected subjects are co-infected with HTLV-1 [15], which may indicate higher risk for HAM/TSP development, HIV disease progression [16], or immune disturbance. In fact, while HIV infection leads a progressive decrease in lymphocyte proliferation [17], HTLV-1 acts directly on T cells to induce cell activation, a boost in cytokine synthesis and clonal expansion [18]. A few studies have been published on the role of T-cell proliferative capacity during co-infection with HTLV-1 and HIV to our knowledge [15]. In the present study, we evaluated the *in vitro* response to different mitogens and antigen during co-infection for a better understanding of the immune interaction between these viruses. We hypothesized that HTLV-1 infection may lead to an increased spontaneous T-cell proliferation capacity among HIV-1-co-infected subjects.

Material and methods

Patients and controls. Human T-cell lymphotropic virus type 1-infected subjects were recruited from the HTLV-1 out-patient clinic of the Institute of Infectious Diseases 'Emílio Ribas', São Paulo, Brazil, and HIV-1-infected patients were recruited from the out-patient clinic of the Secondary Immunodeficiency Clinic of the 'Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo'. The subjects were divided as follows: group 1: 72 healthy seronegative controls; group 2: 75 asymptomatic HTLV-1-infected carriers; group 3: 42 HAM/TSP cases; group 4: 33 solely HIV-1-infected subjects; group 5: 24 HIV-1/HTLV-1 co-infected patients. The participants had support from nurses, nutritionists and physical therapists, as do all patients in follow-up, and the Institute of Infectious Diseases 'Emílio Ribas ethical board approved the protocol. Written informed consent was obtained from all participants.

Peripheral blood mononuclear cell cultures. Isolation of PBMC was done as described in detail elsewhere [19]. Briefly, 10 ml of peripheral heparinized blood was collected from each patient and control, and PBMC were isolated using Ficoll-Hypaque (Pharmacia, Piscataway, NJ, USA) gradient, washed two times in sterile saline and resuspended in RPMI-1640 (Cultlab, Campinas, Brazil). For phytohemagglutinin (PHA, Difco, Detroit, MI, USA), anti-CD3 monoclonal antibody (OKT3, Institute Karolinska, Sweden), pokeweed, (PWM, Sigma, St Louis, MO, USA) and candidin antigen (CMA, Institut Pasteur, Paris, France) culture assays, PBMC from patients and controls, 2×10^6 cells/ml in RPMI with 10% fetal calf serum (Laborclin, Campinas, Brazil) were incubated at 37 °C and 5% CO_2 for 3 days with PHA and OKT3 and 6 days with PWM and CMA in triplicate in 96-well plates (Costar, Cambridge, MA, USA). Cells were pulsed with tritiated thymidine (0.5 μCi/ml, Amersham International, Little Chalfont, UK) 18 h before harvest in a semi-automatic cell harvester (Flow Laboratories, Irvine, UK) and counted in a β-counter (Beckman, Fullerton, CA, USA) as described [19]. The mean counts per minute (CPM) of the triplicate samples were calculated and the results were expressed as the difference between the cpm of stimulated and non-stimulated cultures. The stimulation index was measured by the ratio between spontaneous/stimulated results.

The HTLV-1 proviral load was quantified by real-time PCR using TaqMan probes for the pol gene in two million PBMC. The albumin gene was the internal genomic control and MT2 cells were used as positive control [20].

Statistical analysis. Differences in patient characteristics or laboratory values from the groups were evaluated with a one-way Mann–Whitney test. In both cases values of $P < 0.05$ were considered statistically significant.

Results

Overall, the mean age was 40 years, with more women among the healthy HTLV-I carrier group. T CD4 cell counts were similar in the control and HTLV-1-infected patients. All HIV-1/HTLV-I patients were under anti-retroviral therapy. In the HIV-infected group, the T CD4 cell counts were lower and the T CD8 cell counts were higher, compared with the control group, asymptomatic HTLV-I-infected subjects and HAM/TSP cases ($P = 0.005$, 0.0004 and 0.0007, respectively, Figs 1 and 2). The HTLV-I DNA proviral was 208, 971 and 118 copies/10^4 PBMC for the HTLV-I-infected carriers, HAM/TSP cases and HIV-1/HTLV-I co-infected subjects respectively.

The T-cell background proliferation was higher in HAM/TSP than in asymptomatic HTLV-1 carriers after 3 or 6 days of culture ($P = 0.002$). Also, the HTLV-1-infected carriers had higher proliferation than the controls and those patients with HIV-1 infection ($P < 0.0001$ in all comparisons, Fig. 3). HAM/TSP cases showed a decreased response to PHA, compared with HTLV-1-infected carrier subjects, and the co-infected
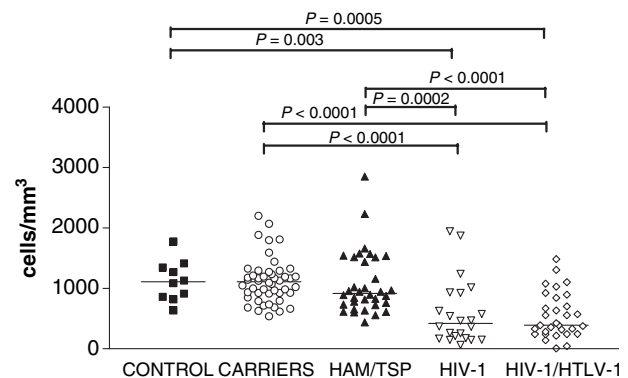

Figure 1 Mean of CD4$^+$ T cells count among HTLV-1-infected patients and HIV-1-infected subjects.
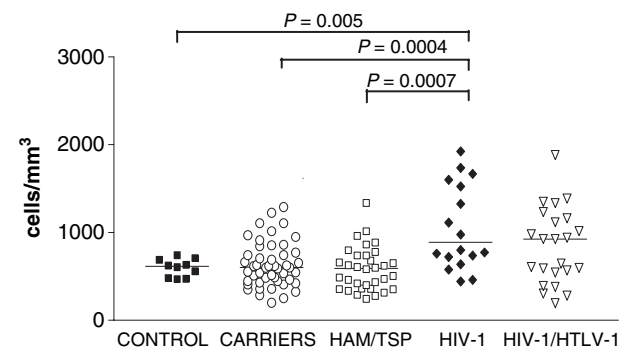
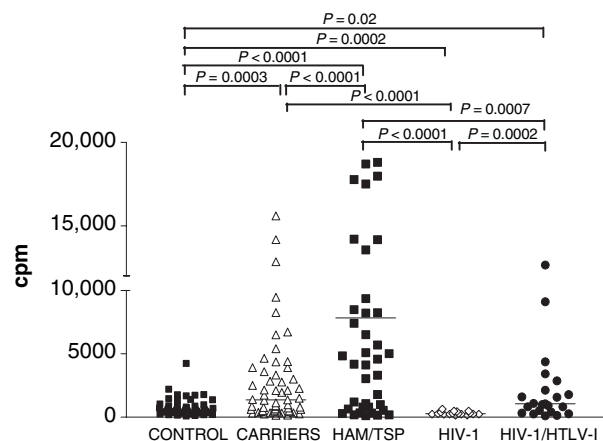

Figure 2 Mean of CD8$^+$ T cells count in the groups.

Figure 3 T-cell proliferation after 3 days of culture, without stimulation (background proliferation).

Figure 4 The stimulation index for proliferation after candidin antigen stimulation.

HIV-1/HTLV-I cases presented a diminished response after 3 days of culture (*P* < 0.0001, Table 1).

Fig. 4 shows the stimulation index. After 6 days of culture, the HTLV-I-infected subjects (more evident among HAM/TSP patients), showed a lower capacity for proliferation after CMA stimulation. HAM/TSP cases and co-infected HIV-1/HTLV-1 patients showed the lowest ratio (*P* < 0.0001 and *P* < 0.0001 respectively).

Discussion

A high spontaneous T-cell background proliferation was noted during HTLV-1 infection, especially among HAM/TSP cases, and the T-cell counts did not exponentially increase with stimulation. These findings are expected, as tax protein (or other regulatory HTLV-1 proteins) induced this effect [21]. In addition, we noted in HIV-1/HTLV-1 co-infection an upregulated T-cell proliferation pattern. It is possible to infer that HIV-1/HTLV-1 co-infection may not suppress the upregulation of tax/rex proteins, which affects T-cell proliferation when using polyclonal stimulation. Thus, during co-infection, the
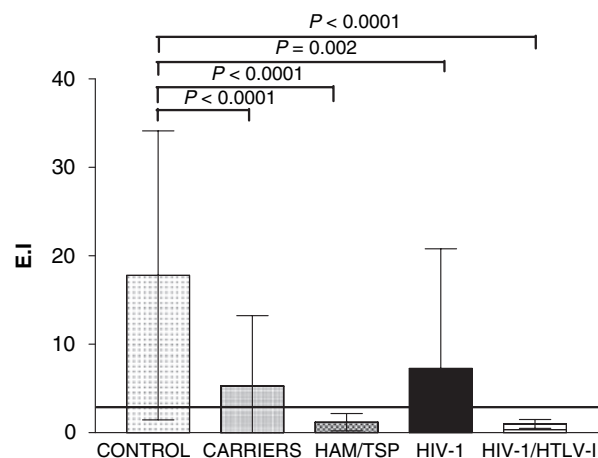
HIV-1 regulatory proteins could not overwhelm HTLV-1 action upon Tax and/or p12 proteins, but further studies should be performed to assess this hypothesis.

Human T-cell lymphotropic virus type-1-infected subjects had a higher spontaneous rate, and their gain after stimulation was lower than in control or HIV-infected patients. These findings are expected, as the tax protein is implicated in the induction of over-expression of IL-2r/IL-2 [22]. This may affect the progression of ATL, a neoplasic pathway, as well HAM/TSP pathogenesis, an inflammatory pathway [5–18].

During HIV disease progression, immune activation can also act as a major factor for T CD4 cell loss. However, the role of HTLV-I during HIV natural history has been very controversial. Some authors believe that survival is increased during co-infection [23, 24], but other authors showed opposite findings [25]. However, this issue was not the focus of our study design.

In our findings, it seems that HAM/TSP progression is also related to T-cell activation in the spinal cord, leading to the inflammatory process and demyelination.

Table 1 T-cell proliferation after 3 days of culture.

	Control (*n* = 72)	Carriers (*n* = 75)	HAM/TSP (*n* = 42)	HIV-1 (*n* = 33)	HIV1/HTLV-I (*n* = 24)
Background proliferation 3 days	799	2997	8828	1268	1809
PHA	53,870	60,960	40,780	43,440	34,410
OKT3	26,380	27,460	24,650	20,950	20,270
Background proliferation 6 days	437	9401	16,280	468	8044
PWM	28,560	26,890	29,470	13,320	19,660
CMA	5666	12,030	18,080	2972	6650

Background proliferation: T-cell proliferation without stimulus. Control: healthy seronegative for HIV-1 and HTLV infection. PHA: phytohemagglutinin; OKT3: anti-CD3 monoclonal antibody; PWM: pokeweed; CMA: candidin antigen.

One possible hypothesis to explain higher immune activation would be the presence of higher DNA proviral load among HAM/TSP patients [20]. Alternatively, these findings may be related to the time of disease, as we studied a broad range of TSP/HAM cases, or to tax activity. Therefore, to assess this question, tax activity should be evaluated using luciferase assay in co-infected subjects.

During co-infection, HIV-1 infection may not induce downregulation of T-cell proliferation and this finding may explain a lower survival rate among these patients [23]. Perhaps decreasing T-cell proliferation could be a feasible strategy to avoid HAM/TSP development or improvement of its symptoms, such as the use of corticosteroids. In addition, new compounds, like quinolones can induce inhibition of HTLV-1-infected cells [26, 27], which might be an alternative to corticosteroids and the accompanying side effects.

Acknowledgment

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