ORIGINAL ARTICLE

EXPRESSION OF INTERLEUKIN-2Rα CHAIN (CD25) ON PERIPHERAL BLOOD LYMPHOCYTES IN HTLV-I ASSOCIATED MYELOPATHY /TROPICAL SPASTIC PARAPARESIS PATIENTS


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Abstract

Background-Mashhad, a city in northern Iran, is a newly recognized endemic area for a retrovirus, the Human T-cell Lymphotropic Virus type I (HTLV-I). This virus is the causative agent of a chronic slowly progressive cord syndrome called HTLV-I associated with Human T-cell virus type I-associated myelopathy or/tropical spastic paraparesis (HAM/TSP) and has tropism for CD4+ T-cells, which results in T-cell activation that escape the autocrine pathway.

Objective-The purpose of this study was to determine the immuno-phenotypic features of peripheral blood lymphocytes of HAM/TSP patients, especially differential expression of interleukin-2 receptor α (IL-2Rα) chain on the surface of the T-cells of HAM/TSP patients, HTLV-I carriers and healthy controls.

Methods-Subjects in this case-control study included 20 HAM/TSP patients, 14 HTLV-I carriers and 12 healthy controls. The absolute white blood cell count and the differential cell count were determined by hematologic analysis and the relative and absolute number of peripheral blood CD3+ CD25+ T-cells were determined by flowcytometry.

Results-The relative number of lymphocytes (36±9%), relative number of CD3+ cells (74±7%) and the relative and absolute number of CD25/3+ cells (21±8%, 0.309±0.155 x10⁹/1) were significantly higher in HAM/TSP patients than healthy controls (29±7%, 68±4%, 13±3%, 0.187±0.065 x 10⁹/1) (p<0.05). The relative lymphocyte (36±5%) and relative CD25/3+ (18±5%) cell counts were significantly higher in carriers in comparison with controls. No significant differences were present in these parameters between carriers and HAM/TSP patients.

Conclusion-This differential pattern of T-cell activation markers among the study groups may have striking diagnostic and therapeutic implications.

Keywords • HTLV-I • tropical spastic paraparesis • asymptomatic carrier • Interleukin2-Rα chain

Introduction

It is estimated that about 15-20 million individuals are infected with Human T-cell Lymphotropic Virus type I (HTLV-I) throughout the world.¹ Small clusters of high endemicity are located in areas where the virus is rare or absent. Highly endemic areas include regions localized in South America, intertropical Africa and Malaysia. In the Middle East, Mashhad, a city in northeastern Iran, appears to be an important reservoir of HTLV-I infection, with 2-3% seropositivity.² The association of spastic paraparesis with HTLV-I was demonstrated independently in two different areas, the Caribbean basin and Japan. HTLV-I has tropism for CD4+ T-cells, which results in infection of up to 10% of the circulating pool of T-cells.³ A large amount of information exists on the pathogenesis of HTLV-I that advocates the immunologic
Peripheral Blood Lymphocytes of HAM/TSP Patients

Table 1. A summary of demographic features of the study groups.

<table>
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<tr>
<th></th>
<th>Control</th>
<th>Carrier</th>
<th>HAM/TSP</th>
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<tbody>
<tr>
<td>Age (mean±SD) years</td>
<td>41±12</td>
<td>43±11</td>
<td>46±14</td>
</tr>
<tr>
<td>Male : Female</td>
<td>10 : 2</td>
<td>8 : 6</td>
<td>5 : 15</td>
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</table>

damage of the CNS, especially the thoracic spinal cord.\(^4\) Having more complete knowledge about the possible differences in immunologic parameters of HAM / TSP patients compared with normal individuals or a more appropriate control group, the HTLV-I asymptomatic carriers, will dictate new diagnostic and therapeutic strategies for disease intervention. Therefore, the present study, aimed at characterizing the immunologic features of the peripheral blood lymphocytes of HAM/TSP patients and evaluating any possible differential expression of the T-cell surface activation marker (CD25) among HTLV-I carriers and healthy age-matched controls.

Materials and Method

HAM/TSP patients
Twenty adult patients (15 females and 5 males, mean age: 46, ranging from 21 to 69 years) were selected from consecutive patients of the Neurology or Immunology Departments according to the Kagoshima guidelines. The exclusion criteria included recent use of steroids or any other medication and history of any medical condition such as autoimmune disorders which might lead to an increase in CD25+ cells.

HTLV-I carriers
Fourteen asymptomatic seropositive subjects, 8 women and 6 men (mean age of 43, ranging 18 to 52 years) who were referred from Mashhad blood bank with no history of recent illnesses or intake of medication were selected as HTLV-I carriers.

Healthy controls
Twelve healthy age-matched adult volunteers, 10 men and 2 women, who had no recent medical problems were chosen as the control group.

Blood collection and flowcytometry
Peripheral blood (2mL) was collected into a sterile heparinized tube by venipuncture. Fresh samples were immediately processed by the hematologic analyzer (Coulter counter) and the absolute leukocyte count and the differential cell count were determined. The absolute number of lymphocytes was calculated from the absolute number of leukocytes and the relative number of lymphocytes.

Flowcytometry
Twenty-five µL of whole blood was stained with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated mouse monoclonal antibodies against the following surface molecules:
- PE-CD3 (B-D, San Jose, CA, USA)
- FITC-CD25 (B-D San Jose, CA, USA)

The cells were then incubated for 20 minutes in dark room temperature. After incubation, 155 µL of lysing buffer (B-D) was added to each tube and the cells were incubated for another 10 minutes. After three washing steps and adding buffered saline solution, the samples were immediately analyzed on a fluorescence activated cell sorted (FACS) Caliber (B-D, CA, USA) flowcytometer. A total number of 10,000 events were collected from each sample in list mode. Data was then analyzed with the Cell Quest software.

Statistical analysis
Intergroup comparison of each hematologic or immunophenotypic parameter was performed by the ANOVA F test and p values less than 0.05 were considered as significant. The SPSS software, version 10.2 was used for data analysis.

Results
A total of 46 subjects (23 women, 23 men) were enrolled in three study groups: HAM/TSP patients, asymptomatic carriers and healthy controls. Table 1 shows a brief demographic comparison of the study groups. Clinical and epidemiologic features of HAM/TSP patients are demonstrated in Table 2. The presence of CSF antibodies to HTLV-I was evaluated in three suspected patients. There were no significant differences in the total WBC count, relative and absolute polymorphonuclear, absolute lymphocyte, relative and absolute CD3+ cells and absolute CD23/3+ cell counts of carriers as compared to
Table 2. The demographic and clinical features of HAM/TSP patients.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>Number of cases</td>
<td>20</td>
</tr>
<tr>
<td>M : F</td>
<td>5 : 15</td>
</tr>
<tr>
<td>Mean breast feeding duration</td>
<td>22 months</td>
</tr>
<tr>
<td>HTLV-I seropositivities in patients’</td>
<td>8 (40)</td>
</tr>
<tr>
<td>family (%)</td>
<td></td>
</tr>
<tr>
<td>Mean age of symptom onset</td>
<td>42 years</td>
</tr>
<tr>
<td>Mean duration to wheelchair bound state</td>
<td>10 years</td>
</tr>
<tr>
<td>Spasticity or lower extremities hyper-</td>
<td>100%</td>
</tr>
<tr>
<td>reflexia</td>
<td></td>
</tr>
<tr>
<td>Lower limb muscle weakness</td>
<td>90%</td>
</tr>
<tr>
<td>Low back pain</td>
<td>60%</td>
</tr>
<tr>
<td>Sensory disturbances</td>
<td>34%</td>
</tr>
<tr>
<td>Urinary bladder problems</td>
<td>30%</td>
</tr>
</tbody>
</table>

Healthy controls. But, relative lymphocyte and CD25/3+ cells were significantly higher in carriers as compared to controls. No significant differences were found to exist in the absolute and relative monocyte, absolute lymphocyte and absolute CD3+ cell counts between HAM/TSP patients and controls. Total WBC counts in HAM/TSP patients tends to be lower than controls. However, probability values were marginal (p=0.07). Relative lymphocyte, CD3+ cells and relative and absolute CD25/3+ cells were significantly higher in HAM/TSP patients in comparison with controls (Figure 1, 2). A dual parameter histogram of lymphocytes in healthy controls and HTLV-I infected subjects stained with CD3 and CD25 is depicted in Figure 3.

There were no significant differences in these immunophenotypic parameters between HAM/TSP patients and HTLV-I carriers.

![Figure 1](Image1.png)

**Figure 1.** Intergroup comparison of relative number of CD 25/3+ cells in study group.

![Figure 2](Image2.png)

**Figure 2.** Intergroup comparison of absolute CD 25/3+ cell counts in study groups.

**Discussion**

The majority (95%) of HTLV-I infected individuals remain asymptomatic and less than 5% go on to develop clinical disease. Why one group of HTLV-I-seropositive individuals develops neurologic symptoms, and another develops a neoplastic condition while the majority remain asymptomatic, is of fundamental importance. A number of possibilities have been proposed to explain these vastly different outcomes of HTLV-I infection. Some individuals may be genetically predisposed to develop the disease. Studies from Japan have suggested a disease association with HLA complex. We also found that certain HLA haplotypes were over- or under-represented in HAM/TSP patients as compared to healthy Iranian controls (unpublished data). Another explanation for this different clinical outcome is that various molecular strains of HTLV-I may be disease specific, but sequence analysis have shown no unique sequences in association with any HTLV-I related disease. The third explanation may be the existence of an environmental factor i.e., coinfection with other retroviruses, other microbial or parasitic infestations. Finally, investigations concerning different host predisposing factors i.e., polymorphism in cytokine genes or cellular immune responses have made considerable progress.

In this study, we found that HTLV-I infection is characterized by a number of abnormalities of the hematologic and immunophenotypic parameters and interestingly it is accompanied by an increase of activated (CD25+) peripheral T-cells. These findings are in agreement with the findings of other investigators. Early observations pointed to the causative role of HTLV-I in T-cell activation.
Mononuclear cells (MNCs) from patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), incorporate \[^{[3]}H\] thymidine \textit{in vitro} in the absence of exogenous growth factors (spontaneous proliferation) in marked contrast to MNCs from uninfected subjects. Moreover, a high proportion of freshly isolated MNCs from patients with HAM/TSP bear activation markers such as the p55 α chain of the interleukin-2 (IL-2) receptor (IL-2Rα) and MHC class II, indicating ongoing \textit{in vitro} activation, even in uninfected cells.\(^{13}\) This phenomenon has also been described in the peripheral blood lymphocytes of asymptomatic HTLV-I seropositive individuals and in individuals infected with HTLV-II, although the magnitude of the response is higher in HAM/TSP patients.\(^{13}\) Gessian, et al also showed the cell surface phenotype in 12 T-cell lines derived from the peripheral blood and CSF of patients with HAM/TSP, most of which exhibited a pattern characteristic of CD4+ cells associated with a strong density of Tac and DR molecules. Jacobson, et al showed an increase in the number of the CD3+ cells that also express a marker of T-cell activation such as HLA-DR and IL-2R molecules.\(^{11}\) Infection by the human T-cell lymphotropic virus type I (HTLV-I) causes T-cell activation by at least two separate mechanisms.

One mechanism involves activation of T-cells harboring the virus and is exemplified by \textit{in vivo} infected nonimmortalized T-cell clones that display a prolonged state of activation. The phenotype of this pathway is consistent with hyperactive IL-2R pathway or CD28 pathway, indicating that HTLV-I may contribute a costimulatory signal to infected T-cells. As a separate mechanism, T-cells infected by this virus can induce activation of uninfected T-cells via T-cell interaction mediated by the LFA-3-CD2 pathway. This may induce IL-2 production from the uninfected T-cells, leading to a more generalized activation of the immune system that could potentially provide a basis for some of the diseases associated with HTLV-I.\(^{14}\) Whatever the mechanism of HTLV-I induced T-cell activation, these activated T-cells with increased expression of the adhesion molecules, can easily enter the CNS. The recognition of these cells by CD8CTLs leads to CNS damage. Alternatively, HTLV-I- induced T-cell activation may allow autoreactive T-cells in the periphery to escape self-tolerance mechanisms and to migrate to the CNS resulting in self-protein recognition such as processed myelin antigens and cytokine-mediator induced inflammatory process in the CNS.

Given the possible key role of HTLV-I induced T-cell activation in the pathogenesis of the HTLV-I associated diseases, it seems completely rational to think about the inhibition of this pathway in order to decrease progression or even prevent HAM/TSP patients with anti-Tac. In the present
study, the mean activated peripheral blood T-cell counts were higher in HAM/TSP patients versus HTLV-I asymptomatic carriers, but the differences were not significant. There are however two issues this study did not address. First, it was not determined whether T-cell surface activation marker density is different between patients and carriers and second, it was not stated whether other HTLV-I associated diseases also demonstrate a state of T-cell activation. We suggest further studies be performed addressing these questions and establishing the possible efficacy of anti-Tac in the management of HAM/TSP and other HTLV-I associated diseases.

Acknowledgment

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References