Peripheral Blood Mononuclear Cells from Individuals Infected with Human T-Cell Lymphotropic Virus Type 1 Have a Reduced Capacity To Respond to Recall Antigens

Rita Elizabeth Mascarenhas,1,4 Cláudia Brodskyn,2 Geisa Barbosa,1,4 Jorge Clarencio,2 Antônio Souza Andrade-Filho,3,4 Frederico Figueiroa,3,4 Bernardo Galvão-Castro,1,4 and Fernanda Grassi1,4*

Advanced Laboratory of Public Health/Gonçalo Moniz Research Center (CPqGM), Oswaldo Cruz Foundation (FIOCRUZ), Salvador, Bahia, Brazil; Microbiology and Immunoregulation of Integrate Laboratory/Gonçalo Moniz Research Center (CPqGM), Oswaldo Cruz Foundation (FIOCRUZ), Salvador, Bahia, Brazil; Neurology and Neurosurgery Foundation, Salvador, Bahia, Brazil; and Bahiana School of Medicine and Public Health, Salvador, Bahia, Brazil

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Evidence indicates that human T-cell lymphotropic virus type 1 (HTLV-1) infection leads to chronic immunosuppression and a greater susceptibility to infectious diseases. Spontaneous in vitro proliferation of peripheral blood mononuclear cells (PBMC) is an important immunological feature of HTLV-1-infected individuals. However, the association between spontaneous proliferation and immunosuppression is not clear. In this study, we evaluated the cellular immune responses of PBMC from 58 asymptomatic HTLV-1-infected individuals with PBMC showing or not showing spontaneous proliferation. Individuals with PBMC that spontaneously proliferated had increased proportions of CD4 T cells expressing CD45RO and dramatically reduced responses to recall antigens. In addition, frequencies of positive responses to recall antigens were also decreased in HTLV-infected individuals without spontaneous proliferation of PBMC. There was a polyclonal expansion of multiple T-cell receptor Vβ families of CD4+ T lymphocytes in patients with spontaneous proliferation. We observed that HTLV-1 induced an immunosuppression characterized by a decrease in the stimulation index to a recall antigen, even in individuals who did not present spontaneous proliferation. On the other hand, only patients with PBMC presenting spontaneous proliferation showed polyclonal activation and increased proportion of CD4 T cells expressing CD45RO.

Human T-cell lymphotropic virus type 1 (HTLV-1) is a human retrovirus that has infected 10 to 20 million people worldwide (3). There are areas in Japan, the Caribbean, Melanesia, central and western Africa, and South America that are large endemic foci (17, 35). In Brazil, the overall prevalence of HTLV-1 infection in blood donors is 0.45%, and the city of Salvador (located in the northeast) has the highest prevalence (1.35%) in the country (4). Recently, a population-based cross-sectional study performed in Salvador, Brazil, found an HTLV-1 seroprevalence of 1.76% (2).

Two diseases are clearly associated with HTLV-1 infection: adult T-cell leukemia/lymphoma (5, 26, 34) and HTLV-1-associated myelopathy/tropical spastic paraparesis (5, 24). Although 95% of infected people remain asymptomatic (7), there is evidence that HTLV-1 has a much broader spectrum of disease manifestations, such as uveitis (20), arthritis (8, 12), polymyositis (22), lymphocytic interstitial pneumonia (31), and infective dermatitis in children (15). Severe immunosuppression is well documented in patients with adult T-cell leukemia/lymphoma (10, 19). However, a growing body of literature suggests that many HTLV-1-infected individuals show chronic immunosuppression, even in the absence of malignant disease. HTLV-1-infected individuals are more susceptible to several infectious diseases, such as stronglyloidiasis (1, 30), Hansen’s disease (11), and tuberculosis (21, 25). In addition, they have reduced cutaneous delayed-type hypersensitivity responses to purified protein derivative (PPD) of Mycobacterium tuberculosis (14, 23, 33, 36).

The immunologic hallmark of HTLV-1-infected individuals is a spontaneous in vitro proliferation of their peripheral blood mononuclear cells (PBMC) (without any added antigen) observed in approximately fifty percent of patients (13, 27, 29). The memory CD4+ CD45RO+ T cell population involved in this phenomenon (29). In this study, we evaluated the cellular immune responses of HTLV-1-infected individuals (with or without PBMC spontaneous proliferation) against candidin, cytomegalovirus (CMV), PPD, and tetanus toxoid (TT) recall antigens.

We observed that HTLV-1-infected individuals had reduced stimulation indexes to recall antigens, even when their PBMC did not spontaneously proliferate. On the other hand, only the individuals with spontaneous PBMC proliferation (SP+) had polyclonal T-cell activation (as shown by the study of T-cell receptor Vβ [TCR-Vβ] families) and an increase in the proportion of CD4 T cells expressing CD45RO.

MATERIALS AND METHODS

Patients. Fifty-eight asymptomatic HTLV-1-infected individuals studied at the HTLV-1 Reference Development of Science Foundation (Salvador, Bahia, Brazil) were included in the study. The group consisted of 47 women (81%) and 11 men (19%) and had a mean age of 43 years. Blood samples from 12 healthy
individuals from the Bahia State Blood Bank (HEMOBA) were used as controls. All samples were screened for HTLV-1/2 antibodies by an enzyme-linked immunosorbent assay (ELISA) (A/B-Capture ELISA test system; Ortho-Clinical Diagnostics, Inc., Raritan, New Jersey), and results were confirmed by Western blot assay (HTLV Blot 2.4; Genelabs Technologies, Singapore). Informed consent was obtained from all enrolled patients, and the Oswaldo Cruz Foundation (FIOCRUZ) ethics committee approved this study.

**Media and reagents.** RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) was supplemented with 2 mM l-glutamine, 1% nonessential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, and pooled human AB serum (10%) (Sigma).

**Cells.** PBMC from HTLV-infected individuals and healthy blood donors were obtained from heparinized venous blood samples by Ficoll-Hypaque density gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden). All experiments were performed with freshly isolated PBMC.

**Flow cytometry.** For direct labeling, 50-μl portions of whole-blood samples from HTLV-infected individuals and healthy blood donors were incubated with antibodies for 30 min at room temperature. Erythrocytes were subsequently lysed with fluorescence-activated cell sorting (FACS) lysing solution (Becton-Dickinson Immunocytometry System, San Jose, CA). Cells were then washed three times in 2 ml of phosphate-buffered saline containing 1% bovine serum albumin. After a final wash, cells were fixed in phosphate-buffered saline containing 4% paraformaldehyde. In order to quantify CD4 and CD8 T cells, the following monoclonal antibodies were used: fluorescein isothiocyanate (FITC)-labeled anti-CD3 (Immunotech, a Beckman Coulter Company), phycoerythrin (PE)-labeled anti-CD4 (BD Pharmingen Technical), and phycoerythrin-cyanin-labeled anti-CD8 (Immunotech). To analyze CD4 T-cell memory and naïve subsets, cells were stained with phycoerythrin-cyanin-CD4, PE-labeled anti-CD8, and FITC-labeled anti-CD4 (BD Pharmingen Technical), and phycoerythrin-cyanin-labeled anti-CD8 (Immunotech). To analyze CD4 T-cell memory and naïve subsets, cells were stained with phycoerythrin-cyanin-CD4, PE-labeled anti-CD8, and FITC-labeled anti-CD4 (BD Pharmingen Technical), and phycoerythrin-cyanin-labeled anti-CD8 (Immunotech). To analyze CD4 T-cell memory and naïve subsets, cells were stained with phycoerythrin-cyanin-CD4, PE-labeled anti-CD8, and FITC-labeled anti-CD4 (BD Pharmingen Technical), and phycoerythrin-cyanin-labeled anti-CD8 (Immunotech). To analyze CD4 T-cell memory and naïve subsets, cells were stained with phycoerythrin-cyanin-CD4, PE-labeled anti-CD8, and FITC-labeled anti-CD4 (BD Pharmingen Technical), and phycoerythrin-cyanin-labeled anti-CD8 (Immunotech). To analyze CD4 T-cell memory and naïve subsets, cells were stained with phycoerythrin-cyanin-CD4, PE-labeled anti-CD8, and FITC-labeled anti-CD4 (BD Pharmingen Technical), and phycoerythrin-cyanin-labeled anti-CD8 (Immunotech).

**TABLE 1. Spontaneous proliferation of PBMC from HTLV-1-infected individuals**

<table>
<thead>
<tr>
<th>PBMC</th>
<th>No. of HTLV-1-infected individuals</th>
<th>Mean cpm ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP+</td>
<td>39</td>
<td>1,656 ± 956 (520-4,629)</td>
</tr>
<tr>
<td>SP−</td>
<td>19</td>
<td>217 ± 152 (18-476)</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

* The mean counts per minute of nonstimulated PBMC from uninfected controls were 15±138 cpm (range, 6 to 462) for HTLV-1-infected individuals, the mean cpm from nonstimulated cells was 500±550 cpm at day 6 for HTLV-1-infected individuals with SP+, while it was ≤500 cpm at day 6 for HTLV-1-infected individuals with SP− PBMC.

**RESULTS**

PBMC spontaneous proliferation. As shown in Table 1, spontaneous PBMC proliferation was observed in 39 out of 58 (67%) HTLV-1-infected individuals (1,656 cpm ± 956 cpm; range, 520 to 4,629 cpm).

On the other hand, no spontaneous PBMC proliferation was observed in 19 out of 58 (33%) HTLV-1-infected individuals (217 ± 152 cpm; range, 18 to 476). In this case, the mean counts per minute for asymptomatic HTLV-1-infected individuals did not differ from the mean counts per minute for unin-

**TABLE 2. Percentages of T-cell subsets from HTLV-1-infected individuals and uninfected controls**

<table>
<thead>
<tr>
<th>Group and parameter</th>
<th>Group A (SP+ HTLV-infected subjects [n = 19])</th>
<th>Group B (SP− HTLV-infected subjects [n = 10])</th>
<th>Group C (uninfected controls [n = 10])</th>
</tr>
</thead>
<tbody>
<tr>
<td>% T cells</td>
<td>CD4+</td>
<td>CD45RA+</td>
<td>CD8+</td>
</tr>
<tr>
<td>Mean</td>
<td>41</td>
<td>29</td>
<td>79</td>
</tr>
<tr>
<td>SD</td>
<td>14</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Median</td>
<td>44</td>
<td>30</td>
<td>79</td>
</tr>
<tr>
<td>Range</td>
<td>10-61</td>
<td>10-53</td>
<td>55-92</td>
</tr>
</tbody>
</table>

*CD4+ cells expressing CD45RA.

*CD4+ cells expressing CD45RO.

* Differences between the values for SP+ and SP− HTLV-1-infected groups and uninfected controls were compared by the Kruskal-Wallis test (group A versus group B versus group C). Differences between two groups were analyzed by the Mann-Whitney U test (group A versus group B, group A versus group C, and group B versus group C). Differences between the values for SP+ and SP− HTLV-1-infected groups and uninfected controls that were statistically significant are shown in bold type. **
fected controls (159 ± 138 cpm; range, 6 to 462) (P = 0.32 by the Mann-Whitney U test).

HTLV-1-infected individuals have an expansion of the memory CD4⁺ CD45RO⁺ T-cell subset. As shown in Table 2, the percentage of circulating CD4⁺ T lymphocytes in the HTLV-1-infected group with PBMC with spontaneous proliferation (41% ± 14%) was similar to those of HTLV-1-infected individuals without spontaneous PBMC proliferation (43% ± 9%) and of uninfected controls (42% ± 9%) (P = 0.84). However, the memory CD4⁺ CD45RO⁺ T-cell subset was expanded in HTLV-1-infected individuals with spontaneous PBMC proliferation (79% ± 10%) compared to that in HTLV-1-infected individuals without spontaneous proliferation (67% ± 18%) (P = 0.04) and uninfected controls (67% ± 11%) (P = 0.01). Additionally, the proportion of CD4 T cells expressing CD45RO was positively correlated to spontaneous proliferation of PBMC in culture (Spearman’s r = 0.37; P = 0.04) (data not shown).

There were no significant differences in the frequencies of naive CD4⁺ CD45RA⁺ and CD8⁺ T-cell subsets in HTLV-infected individuals (with or without spontaneous PBMC proliferation) and in uninfected controls.

PBMC from HTLV-1-infected individuals have a lower capacity to proliferate in response to recall antigens. HTLV-1-infected individuals with spontaneous proliferation showed the lowest stimulation indexes to all antigens tested (candidin, PPD, tetanus toxoid, and cytomegalovirus antigens) compared to HTLV-1-infected individuals without spontaneous proliferation and uninfected controls. Only 5.3% (1 out of 19) responded to candidin, while 4.8% (1 out of 21) responded to CMV or TT (Fig. 1). Besides that, HTLV-1-infected SP patients also showed decreases in stimulation indexes: 50% of them (4 out of 8) responded to candidin, 33% (4 out of 12) responded to PPD, 42% (5 out of 12) to cytomegalovirus, and 50% (6 out of 12) to tetanus toxoid antigen. Almost all uninfected controls had positive responses to recall antigens: 90% to candidin (9 out of 10), 83% to PPD (10 out of 12), 100% to tuberculin (11 out of 11), and 67% (8 out of 12) to CMV.

In addition, PBMC from HTLV-1-infected individuals re-

FIG. 1. Proliferative responses to recall antigens from HTLV-1-infected asymptomatic individuals with PBMC with and without spontaneous proliferation, respectively, and uninfected controls. PBMC were cultured in the presence of candidin, PPD, tetanus toxoid, and cytomegalovirus. At day 5 of culture, cells were pulsed overnight with 1 μCi [3H]thymidine. Results are expressed as stimulation indexes (average counts per minute in the presence of antigens/average counts per minute obtained without antigen). Tests were carried out in triplicate. A stimulation index of ≥3 was considered positive for proliferative responses (above dashed lines). Bars depict median values. Differences were considered significant when P was <0.05 (Mann-Whitney U test).
responded to fewer recall antigens than uninfected controls: while 83% of uninfected controls had specific PBMC proliferative responses to three or more different antigens, PBMC from HTLV-1-infected SP+ individuals were unable to recognize more than one antigen (P < 0.0001). Moreover, only 33% of HTLV-1-infected individuals without spontaneous PBMC proliferation responded to three or more antigens (P = 0.038).

**TCR-Vβ analysis disclosed polyclonal expansion only in HTLV-1-infected individuals with spontaneous proliferation of PBMC.** As shown in Fig. 2A, polyclonal expansion of CD4+ cells (TCR-Vβ families 2, 3.1, 6.1, 8, 11, 12, 16, 17, 20, 21.3, and 22) was observed in patients with spontaneous PBMC proliferation. In contrast, HTLV-1-infected individuals without spontaneous PBMC proliferation had CD4 TCR-Vβ repertoires similar to those of uninfected individuals (Fig. 2B).

**DISCUSSION**

Spontaneous proliferation is an immunological hallmark of PBMC from HTLV-1-infected individuals. This phenomenon is observed in up to 50% of cultures from PBMC from HTLV-1 carriers (28). In this study, we evaluated the cellular immune responses from HTLV-1-infected individuals with PBMC showing and not showing spontaneous proliferation. Compared to uninfected controls, HTLV-1-infected individuals with PBMC presenting spontaneous proliferation had an increased proportion of CD4 T cells expressing CD45RO. Moreover, TCR-Vβ analysis showed a polyclonal expansion of almost all Vβ families studied concerning CD4 T-cell subsets. In addition, HTLV-1-infected individuals with spontaneous PBMC proliferation had markedly reduced stimulation indexes for candidin, PPD, tetanus toxoid, and cytomegalovirus.
Therefore, responses to these recall antigens could not be detected by cell proliferation.

The absence of detectable cellular immune responses to recall antigens in individuals with spontaneous PBMC proliferation was expected, since high levels of spontaneous proliferation would mask a specific response to the recall antigens evaluated by the stimulation index. However, previous findings support the idea that there could be different levels of T-cell anergy among HTLV-I-infected individuals.

One of the most important observations of this study was that cells from HTLV-I-infected individuals with PBMC without spontaneous proliferation showed significant decreases in the stimulation indexes to candidin, PPD, and tetanus toxoid. In addition, only 33% were able to recognize more than two antigens. In those individuals, decreases in the stimulation indexes to candidin, PPD, and tetanus toxoid were expected, since high levels of spontaneous proliferation showed significant decreases in the stimulation indexes to candidin, PPD, and tetanus toxoid.

Therefore, responses to these recall antigens could not be detected by cell proliferation.

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