Immunophenotyping of circulating T cells in a mucosal leishmaniasis patient coinfected with HIV

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ABSTRACT
HIV coinfection modifies the clinical course of leishmaniasis by promoting a Th2 pattern of cytokine production. However, little information is available regarding the lymphocytic response in untreated coinfected patients. This work presents the immunophenotyping of Leishmania-stimulated T cells from a treatment-naive HIV+ patient with ML. Leishmania braziliensis antigens induced CD69 expression on CD3+CD4+ and CD3+CD8+ cells. It also increased IL-4 intracellular staining on CD3+CD4+GATA3+ population and decreased the percentage of CD3+CD4+IL-17+ cells. This suggests that modulations in the IL-4R/STAT6 pathway and the Th17 population may serve as parasitic evasion mechanisms in HIV/ML. Further studies are required to confirm these results.

Keywords: Mucosal leishmaniasis. Leishmania braziliensis. HIV coinfection.

INTRODUCTION

Human protection against localized cutaneous leishmaniasis (LCL) due to Leishmania (Viannia) braziliensis (Lb) is dependent on an efficient Th helper lymphocyte 1 (Th1) response, whereas susceptibility is associated with an increased Th2 and T regulatory (Treg) profile. Mucosal leishmaniasis (ML) is defined as an uncontrolled Th1-type inflammation of the oropharyngeal region, presenting a disfiguring facial lesion and can occur after unsuccessful healing of a previous LCL. Recent data demonstrate that HIV coinfection is crucial to unbalancing the immune response and could favour the occurrence of reactivated leishmanial lesions. The report discusses some data concerning the anti-Leishmania specific cellular immune response of an HIV+ ML patient.

CASE REPORT

A 36-year-old male from Itaporã City (State of Mato Grosso do Sul, Brazil) was admitted to the Hospital das Clínicas of the Triângulo Mineiro Federal University on December 2009, with a 5-years progressive mucosal lesion on his right septum. At the time of admission, physical examination revealed a sepal perforation and a roundish scar on his right ankle originating from an untreated clinically resolved ulcerative lesion 15 years previously. Chest radiography and electrocardiography (ECG) were normal, with no historical record of altered blood pressure or diabetes events among close relatives. The patient reported past illicit drug use. Laboratory analysis revealed negative results for Mycobacterium leprae bacilloscopy, fungi and mycobacteria cultures, serology for Trypanosoma cruzi, hepatitis B and C viruses and Treponema pallidum (FTA-Abs). Serology for HIV was repeatedly positive (ELISA), with a viral load of 25560 RNA copies/ml, a CD4+/CD8+ ratio of 0.87 (377 CD4+ cells/mm³; 432 CD8+ cells/mm³) and 1430 CD45+ cells/mm³. A tissue fragment collected from the scar on the right ankle revealed negative histology for fungi and mycobacteria, while a fragment from the nasal septum showed chronic granulomatous inflammation and amastigote forms of Leishmania sp. Computerized tomography revealed a concentric mucosal hypertrophy of the ethmoidal and maxillary sinuses and the presence of fluidic discharge in the inferoventral portion of the nasal septa and the inferior turbinate.

Venous blood was collected before any treatment regimen began. Peripheral blood mononuclear cells (PBMC) were separated using Ficoll-Paque Plus gradient (GE Health Care, Uppsala, Sweden) and cultured in RPMI 1640 (GIBCO, Grand Island, NY, USA) medium alone or in the presence of Leishmania (V.) braziliensis antigens (AgLb) in a 5% CO₂ atmosphere at 37°C for 24h, as described elsewhere. Cells were then harvested, suspended in 100µl Hanks’ balanced salt solution (Sigma, St. Louis, MO, USA) at a final concentration of 5x10⁹ cells/mL and proceeded to immunophenotyping. Briefly, the cells were incubated with 5µl of...
fluorochrome-conjugated antibodies (BD Pharmingen, San Diego, CA, USA) against the following surface markers: CD3-PE (clone S4.1), CD3-APC (clone HIT3a), CD4-PE-Cy7 (clone RPA-T4), CD8-FITC (clone HIT8a), CD8-PE-Cy5 (clone HIT8a), CD69-PE (clone FN50) and CD25-FITC (clone PC61). For intracellular staining, cells were permeabilized with BD Cytofix/Cytoperm™ Plus (BD Biosciences) and then incubated with 10µl of the following antibodies: FoxP3-PE (clone 259D/C7), IL-17-Alexa 488 (clone S4.1), CD3-APC (clone HIT3a), CD4-PE-Cy7 (clone RPA-T4), CD8-FITC (clone HIT8a), GATA-3-PE (clone L50-823), IL-4-FITC (clone MP4-25D2) and GATA-3-PE (clone L50-823). Multiparameter flow cytometry was performed using a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA) compensated with single fluorochromes. Data was analyzed using Cell Quest Pro software (Becton Dickinson) and the results were plotted (Table 1). Dead cells were omitted by side scatter/forward scatter (SSC/FSC) gating, and isotype-matched control antibodies were used to determine background levels of staining.

<table>
<thead>
<tr>
<th>T lymphocyte population</th>
<th>Labelled PBMC (%)</th>
<th>Nonstimulated</th>
<th>AgLb-stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+CD69+</td>
<td>0.30</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>CD3+CD4+CD25+Foxp3+</td>
<td>0.31</td>
<td>0.90</td>
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<tr>
<td>CD3+CD4+IL-10</td>
<td>28.74</td>
<td>26.96</td>
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<tr>
<td>CD3+CD4+IFN-γ</td>
<td>28.71</td>
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</tr>
<tr>
<td>CD3+CD4+GATA3+IL-4-</td>
<td>28.26</td>
<td>28.61</td>
<td></td>
</tr>
<tr>
<td>CD3+CD4+GATA3+IL-4-</td>
<td>0.60</td>
<td>2.77</td>
<td></td>
</tr>
<tr>
<td>CD3+CD8+IL-17</td>
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<td>1.49</td>
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<tr>
<td>CD3+CD8+CD69+</td>
<td>7.89</td>
<td>19.01</td>
<td></td>
</tr>
<tr>
<td>CD3+CD8+IFN-γ</td>
<td>0.69</td>
<td>0.78</td>
<td></td>
</tr>
</tbody>
</table>

PBMC: peripheral blood mononuclear cells; 24h: after 24h in vitro cell culture; medium alone.

After blood sampling, a delayed-type hypersensitivity test (Montenegro skin test) was performed and resulted in a 12mm positive induration. The patient was then followed and treated in accordance with standard Brazilian Ministry of Health clinical practice.

**DISCUSSION**

Very little data is available regarding the immunological response in HIV+ ML patients. It has been demonstrated that cells from HIV ML patients present a strong anti-Leishmania specific TNF-α and IFN-γ production, with a concomitant decrease in IL-10 and TGF-β levels. In a late stage AIDS-associated ML patient, TNF-α and IFN-γ production, with a concomitant decrease in IL-4 levels, were observed in the CD4+ cells was associated with an increase in the expression of transcription factor Foxp3, characteristic of a Treg cell phenotype, and in intracellular staining of the Th2 cytokine IL-4 in the GATA-3 subgroup, which indicates up-regulation in the IL-4R/STAT6 pathway. Concomitantly, a decrease in CD4+IL-17A+ cells was observed. The exact role of the Th17 response in human parasitic diseases remains unclear, but seems to be related to the in situ inflammatory milieu observed in ML patients and could be down-modulated during the course of HIV, favoring parasite evasion and the establishment of infection. Studies by Bottrel et al showed that the CD8+ cell population revealed only a slight increase in IFN-γ intracellular staining following stimulation with Lb antigens, which suggests a secondary role of this cell population in host protection and infection clearance.

These results show the existence of some modulating mechanism in this HIV coinfected ML patient and brings to light some new aspects concerning effector T helper cell involvement during *Leishmania (V.) braziliensis* infection. Further case-control studies are required to confirm these results.

**FINANCIAL SUPPORT**

Postgraduate training fellowships were provided to L.R.C. and J.R.M. by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and to M.L. and M.V.S. by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

**REFERENCES**