Case Report/Relato de Caso

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

Immunofenotipagem de células T circulantes em um paciente com leishmaniose mucosa co-infectado com 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-naïve 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.

RESUMO

A co-infecção por HIV modifica o curso clínico da leishmaniose ao promover aumento no perfil Th2 de produção de citocinas. No entanto, há pouca informação a respeito da resposta linfocitária em pacientes co-infectados sem tratamento. Neste trabalho, foi realizada a imunofenotipagem de células T estimuladas com antígenos de *Leishmania braziliensis* em paciente não tratado HIV$^+$ e com leishmaniose mucosa. Os resultados mostraram aumento na expressão de CD69 em células CD3$^+$CD4$^+$ e CD3$^+$CD8$^+$. Além disso, foi observado aumento de IL-4 na população de linfócitos CD3$^+$CD4$^+$GATA3$^-$ e diminuição no percentual de células CD3$^+$CD4$^+$IL-17$^+$. Estes resultados sugerem que a modulação da via IL-4R/STAT6 e da população de células Th17 funcione como mecanismo de evasão parasitária em HIV/LM. Estudos futuros são necessários para confirmar estes resultados.

Palavras-chaves: Leishmaniose mucosa. *Leishmania braziliensis*. Co-infecção por HIV.

INTRODUCTION

Human protection against localized cutaneous leishmaniasis (LCL) due to *Leishmania (Viannia) braziliensis* (Lb) is dependent on an efficient T helper lymphocyte 1 (Th1) response, whereas susceptibility is associated with an increased Th2 and T regulatory (Treg) profile[1,2]. 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[3]. Recent data demonstrate that HIV coinfection is crucial to unbalancing the immune response and could favour the occurrence of reactivated leishmanial lesions[4,5]. 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 septal 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/mm^3; 432 CD8$^+$ cells/mm^3) and 1430 CD45$^+$ cells/mm^3. 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% CO_2 atmosphere at 37ºC for 24h, as described elsewhere[1]. Cells were then harvested, suspended in 100µl Hanks' balanced salt solution (Sigma, St. Louis, MO, USA) at a final concentration of 5x10^5 cells/mL and proceeded to immunophenotyping. Briefly, the cells were incubated with 5µl of

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Received in 07/10/2010
Accepted in 01/02/2011

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 N49-653), IFN-γ-FITC (clone 4S.B3), IL-10-PE (clone JES3-9D7), 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 1 - Frequency of T cell populations in peripheral blood of a mucosal leishmaniasis patient coinfected with HIV$^+$.

	Labelled PBMC[a] (%)	
T lymphocyte population	Nonstimulated[b]	AgLb-stimulated
$CD3^+CD4^+CD69^+$	0.30	1.62
$CD3^+CD4^+CD25^+Foxp3^+$	0.31	0.90
$CD3^+CD4^+IL\text{-}10^+$	28.74	26.96
$CD3^+CD4^+IFN\text{-}\gamma^+$	28.71	26.92
$CD3^+CD4^+GATA3^+IL\text{-}4^+$	28.26	28.61
$CD3^+CD4^+GATA3^-IL\text{-}4^+$	0.60	2.77
$CD3^+CD4^+IL\text{-}17^+$	2.53	1.49
$CD3^+CD8^+CD69^+$	7.89	19.01
$CD3^+CD8^+IFN\text{-}\gamma^+$	0.69	0.78

PBMC: peripheral blood mononuclear cells, [a]after 24h *in vitro* cell culture, [b]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[3]. In a late stage AIDS-associated ML patient, the low lymphocyte proliferative response and IFN-γ production were restored after the first specific immunochemotherapy course. Apparently, this restoration was dependent on predominating $CD8^+$ rather than $CD4^+$ responding T cells[6]. Another HIV$^+$/ML case series showed that circulating $CD4^+$ T cell count was < 150 cells/mm^3 and that the leishmaniasis clinical outcome showed strong variability among the patients[5]. To our knowledge, no data is available regarding the effects of early HIV infection on the cytokine profile of ML patients. Here, observation verified that the ML patient responded to AgLb and that this response was dependent on both $CD4^+$ and $CD8^+$ T cells, with increased expression of the $CD69^+$ cellular activating surface marker (**Table 1**). The activation state observed on 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 $GATA\text{-}3^-$ subpopulation, which indicates up-regulation in the IL-4R/STAT6 pathway. Concomitantly, a decrease in $CD4^+IL\text{-}17A^+$ 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[7] 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[8].

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 trainning 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).

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