Case Report: Tegumentary Leishmaniasis as the Cause of Immune Reconstitution Inflammatory Syndrome in a Patient Co-infected with Human Immunodeficiency Virus and Leishmania guyanensis


Fundação de Medicina Tropical do Amazonas, Manaus, Amazonas, Brazil; Núcleo de Doenças Infecciosas, Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil

Abstract. We report a case of immune reconstitution inflammatory syndrome (IRIS) in a 32-year-old man infected with human immunodeficiency virus and Leishmania guyanensis. Three months after initiation of highly active antiretroviral therapy (HAART), the patient had disseminated cutaneous leishmaniasis and started anti-leishmanial therapy. The patient’s leishmaniasis manifestations during HAART ranged from an anergic response (46 CD4+ T cells/µL) to a disseminated cutaneous leishmaniasis (112 CD4+ T cells/µL). Eight weeks later (168 CD4+ T cells/µL), skin biopsy specimens showed inflammatory infiltrates with no detectable amastigotes. The patient then became comatose. Prednisone therapy (60 mg/day) was initiated with a significant improvement within 48 hours. Three months later (CD4+ T cell count = 184 cell/µL), localized, classic, cutaneous leishmaniasis developed in the patient and anti-leishmanial treatment was re-introduced. On that occasion, frequency of T regulatory cells was 1.82% of all CD4+ cells. Our data suggest a pivotal role for CD4+ T cells in the onset of IRIS and lesion ulceration and their association with a low frequency of T regulatory cells.

Morbidity and mortality associated with acquired immunodeficiency syndrome has been significantly reduced since the introduction of highly active antiretroviral therapy (HAART), which causes strong suppression of viral replication and helps reconstitute quantitatively and qualitatively the immune system. However, clinical deterioration is frequently reported in approximately 10–25% of patients with advanced, symptomatic disease and low CD4+ cell counts, who had started HAART, regardless of an immunologic improvement and a reduction in plasma human immunodeficiency virus-1 (HIV-1) loads. This phenomenon is known as immune reconstitution inflammatory syndrome (IRIS) and is believed to be a result of an inflammatory response to hidden or pre-existing but partially treated opportunistic infections. The spectrum of pathogens associated with IRIS continues to increase, with a predominance of infections with mycobacteria, Cryptococcus neoformans, and herpesviruses, but also Leishmania major, Toxoplasma gondii, Schistosoma mansoni, and Strongyloides stercoralis. Recently, two cases of IRIS caused by Leishmania spp. were reported in Brazil. To date, 14 cases have been reported worldwide. We report a case of IRIS induced by L. guyanensis.

CASE REPORT

A 32-year-old man, from Manaus, Amazonas, Brazil, was admitted to the Hospital of the Fundação de Medicina Tropical do Estado do Amazonas in mid-October 2007, with chronic diarrhea, neurologic manifestations, and cutaneous lesions (Figure 1). Infection with HIV-1 was diagnosed on admission by serologic analysis. Neurotoxoplasmosis was suspected to be the cause of the observed neurologic alterations. However, presence of this disease was not confirmed after evaluation of cranial computed tomography (CT) scans of the patient. Fluid analysis showed that all inflammatory parameters evaluated were within normal ranges. The patient was not infected with mycobacteria, C. neoformans, T. gondii, or Histoplasma capsulatum.

Because of the low CD4+ cell count (16 cells/µL) quantified at the time of admission, prophylaxis for neurotoxoplasmosis (clindamycin, 300 mg and pirimetamine, 25 mg) was started. Test results of skin lesion biopsy specimens were compatible with a diagnosis of histoplasmosis (Figure 2A and B), and treatment with amphotericin B (total dose = 445 mg) was also initiated. HAART (lamivudine, tenofovir, and efavirenz) was introduced in early November 2007, but patient was lost to follow-up until February 2008. At that time, he returned to the hospital and reported erythematoviolaceous plaques (isolated and confluent) for the past three months. He was referred to the Dermatology Division of our hospital. Lesion biopsy specimens showed a moderate inflammatory infiltrate with multiple, intracellular Grocott-negative, parasitic elements. These findings ruled out a diagnosis of histoplasmosis.

Results of a polymerase chain reaction (PCR) for Leishmania DNA were positive for skin lesions. Typing was conducted by PCR and restriction fragment length polymorphism (RFLP) analysis as described. Observed RFLP patterns were consistent with infection by L. guyanensis. Results of PCR and RFLP analysis were also positive for L. guyanensis in skin lesion samples collected in October 2007, at the time when infection with HIV was diagnosed and the patient was admitted mission to the hospital.

To investigate the presence of visceral leishmaniasis, bone marrow aspirates were obtained and analyzed. Results of analysis were negative for either Leishmania spp. or mycobacteria. By March 2008, his skin lesions worsened, and confluent erythematoviolaceous plaques were detected on his face, chest, back, upper and lower limbs, and scrotum (Figure 2C). Biopsy specimens of skin lesions showed a significant increase in inflammatory infiltrates, with the presence of Leishmania amastigotes (Figure 2D).
At this time, patient had received HAART for four months. His CD4+ and CD8+ T cells counts were 46 cells/µL and 190 cells/µL, respectively (CD4:CD8 ratio = 0.24). The patient was re-admitted to our hospital and treatment with amphotericin B (total dose = 1.5 g) was re-initiated; this treatment produced only mild clinical and parasitologic responses. Pentavalent antimony therapy was introduced, with a dramatic healing improvement, with regression of cutaneous lesions, resulting in flat, hyperpigmented lesions, within 20 days of treatment (Figure 1E).

By June 2008, *Leishmania* amastigotes were not detectable in biopsy specimens of skin lesions (Figure 1F). Physical examination confirmed a progressive reduction of hyperchromic patches. Serial skin lesions biopsy samples taken at different time points were immunohistochemically stained for CD4+ T cells. As HAART progressed and lesions healed, an increase in CD4+ T cells numbers at lesion sites was observed and this finding showed a correlation with the cell concentration in peripheral blood (Figure 1B, D, F, and H).

The patient was re-admitted to our hospital in June 2008 with mental confusion and seizures; he then became comatose (Figure 3A). To investigate the origin of the observed neurologic manifestations, complete blood counts, a chest radiograph, fluid analysis, a cranial computed tomography (CT) scan were obtained. As observed at the time of diagnosis of infection with HIV, results of the cranial CT were within normal standards. Fluid analysis of liquor showed that the central nervous system was negative for mycobacteria, *C. neoformans*, *T. gondii*, *H. capsulatum*, and aerobic bacteria. Results of serologic tests were negative for IgM and IgG specific for cytomegalovirus and syphilis but positive for IgM and IgG (400 U/mL) specific for toxoplasmosis. The CD4+ and CD8+ T cell counts were 112 cells/µL and 464 cells/µL, respectively (CD4:CD8 ratio = 0.24). New bone marrow aspirate samples were obtained and were negative for *Leishmania* spp. and mycobacteria.

Because an infectious origin was not confirmed, we evaluated other parameters such as the level of potassium in the blood, which was consistently lower than the reference value (Figure 3B). Because of the rapid increase in CD4+ T cell count from 16 cells/µL to 112 cells/µL in less than six months (increase = 700%), IRIS was suspected as the most probable

<table>
<thead>
<tr>
<th>CD4 (cells.µL⁻¹)</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 = 16</td>
<td>Baseline (Nov, 2007) - Hospital admission &amp; diagnosis of HIV-1 infection. Suspicion of disseminated histoplasmosis, Amphotericin B (1300mg total dose) initiated. HAART was introduced.</td>
</tr>
<tr>
<td>CD4 = 46</td>
<td>Day 129 (Mar., 2008) - Skin lesions worsened, <em>Leishmania</em> amastigotes found in biopsy, <em>Leishmania guyanensis</em> identification by PCR-RFLP. Bone marrow aspirates negative for <em>Leishmania</em> and mycobacteria.</td>
</tr>
<tr>
<td></td>
<td>Day 141 (Mar., 2008) - Pentavalent Antimonial (1275mg/day for 20 days); initiated</td>
</tr>
<tr>
<td></td>
<td>Day 196 (May, 2008) - Second round of Pentavalent Antimonial (1275mg/day for 30 days)</td>
</tr>
<tr>
<td>CD4 = 112</td>
<td>Day 220 (Jun., 2008) - Admitted to ward, with neurological alterations</td>
</tr>
<tr>
<td></td>
<td>Day 223 (Jun., 2008) - Comatose. Prednisone started (60mg)</td>
</tr>
<tr>
<td></td>
<td>Day 225 (Jun., 2008) - Out of coma.</td>
</tr>
<tr>
<td></td>
<td>Day 228 (Jun., 2008) - Skin biopsy negative for <em>Leishmania</em>, mycobacteria, and fungi. Bone marrow aspirates negative for <em>Leishmania</em> and mycobacteria.</td>
</tr>
<tr>
<td></td>
<td>Day 239 (Jul., 2008) - New skin biopsy negative for <em>Leishmania</em>, Mycobacteria, and fungi.</td>
</tr>
<tr>
<td></td>
<td>Day 252 (Jul., 2008) - Skin lesions healing</td>
</tr>
<tr>
<td>CD4 = 189</td>
<td>Day 305 (Sep., 2008) - Classical cutaneous leishmaniasis lesions. Skin biopsy positive for <em>Leishmania</em> with the presence of granulomas</td>
</tr>
<tr>
<td></td>
<td>Day 317 (Sep., 2008) - Third round of Pentavalent Antimonial (1275mg/day for 20 days)</td>
</tr>
<tr>
<td></td>
<td>Day 338 (Out., 2008) - Cutaneous leishmaniasis lesions healing, but still active.</td>
</tr>
<tr>
<td></td>
<td>Day 373 (Nov., 2008) - Cutaneous leishmaniasis lesions still active Fourth round of Pentavalent Antimonial (1275mg/day for 30 days.)</td>
</tr>
</tbody>
</table>

Figure 1. Timeline of patient clinical evolution from time of diagnosis of infection with human immunodeficiency virus to the onset of ulcerative cutaneous leishmaniasis lesions.
Changes in observed dermatologic manifestations (A, C, E, and G) and immunohistochemical detection of peroxidase-labeled CD4+ T cells (black arrowhead pointing to brown T CD4+ cells) (B, D, F, and H) in skin lesion biopsy specimens of the patient compared with recovery of peripheral blood CD4+ T cell numbers. A and B, October 2007 (before highly active anti-retroviral therapy; 16 CD4+ T cells/µL) Insert in B, hematoxylin and eosin–stained *Leishmania guyanensis* amastigotes (white arrowheads, magnification × 1,000). C and D, March 2008 (46 CD4+ T cells/µL). E and F, June 2008 (112 CD4+ T cells/µL). G and H, September 2008 (189 CD4+ T cells/µL). This figure appears in color at www.ajtmh.org.
diagnosis (Figure 3A). Treatment with prednisone (60 mg/day) was initiated and resulted in a significant clinical improvement in the overall condition of the patient. The patient regained consciousness within 48 hours.

Three months later (September 2008), the patient returned to the dermatology outpatient clinic with classic cutaneous leishmaniasis lesions on the forearm, leg, ears, and genital area (Figure 2G). Biopsy specimens showed scarce Leishmania amastigotes and an active inflammatory infiltrate with increases in CD4+ (Figure 2G), CD8+, and CD68+ cell counts, which confirmed a diagnosis of localized cutaneous leishmaniasis. Patient’s peripheral blood CD4+ and CD8+ T cell counts were 184 cells/µL and 599 cells/µL, respectively (CD4:CD8 ratio = 0.31) (Figure 3A). Pentavalent antimony therapy was re-introduced for 20 days and resulted in a reduction of lesions. At that time, the frequency of peripheral blood T regulatory cells (CD4 + CD25 + high FOXP3 + cells) was determined by flow cytometry (FACSort; Becton Dickinson, Franklin Lakes, NJ). The frequency of these cells was 1.82%, and the patient had a peripheral blood CD4+ cell count of 184 cells/µL (Figure 3C).

**DISCUSSION**

We report a case of IRIS in a 32-year-old man from Manaus, Brazil, who was co-infected with HIV and *L. guyanensis*. To our knowledge, this is the third case of IRIS associated with cutaneous leishmaniasis in Brazil and the first to identify *L. guyanensis* as the causative agent. After initiation of HAART, a progressive and disseminated manifestation of cutaneous leishmaniasis was observed before the onset of IRIS, which was triggered as the CD4+ T cell counts increased to 112 cells/µL. This increased cell count was sufficient to elicit a systemic inflammatory response, which may have caused the patient to become unconscious.

It has been demonstrated that IRIS occurs with a higher frequency among patients with CD4+ T cell counts < 50 cells/µL and who have either subclinical infections or suppressed responses to clinical disease but also retain the capacity for rapid increments in immune function. Although IRIS can develop within the first 1–2 weeks of HAART even before any detectable increase in circulating CD4+ T cell numbers, most cases develop in the first three months of HAART, which
corresponds to the first phase of immune reconstitution in which a rapid increase in circulating CD45RO+ memory cell frequency and CD4+ T cell function are normally observed.7-9

Recirculation of this previously sequestered cell population may provide an adequate opportunity for pathogen-specific T cells to gain access to sites of infection and engage in the inflammatory response of the host to foreign antigens. IRIS is considered the result of an exaggerated host inflammatory response to soluble antigen, live organisms (in the case of subclinical or partially treated infections) or dead organisms (in the case of treated infections).10-13

The presence of CD4+ T cells and their response to Leishmania parasites or antigens are mandatory for development of skin lesions during experimental cutaneous leishmaniasis.10 Mice lacking functional CD4+ T cells are highly resistant to lesion development after experimental infection with L. amazonensis. Additionally, as recently demonstrated in vivo for persons infected with HIV, antigen may persist on dendritic cells for long periods despite successful HAART. Therefore, it is possible that sustained high antigen concentration, such as that observed after successful pentavalent therapy, may play an important role in increasing the risk and severity of IRIS.11,14

In our patient, the onset of IRIS after initiation of HAART, the CD4+ cell counts < 50 cells/µL, and the comatose state of the patient patient’s coma, which was probably caused by cerebral edema elicited by a systemic inflammatory response, were rapidly reversed when corticoid therapy was introduced. Although the patient was hypokalemic, we believe that low potassium levels were not the only cause of the comatose state, especially if considers that potassium levels were lower before, during and after the comatose state (1.9, 2.0, and 2.0 mmol/L, respectively) (Figure 3B). Three months after been released from the hospital (September 2008), the patient had a CD4+ T cell count of 184 cells/µL and had active ulcerated lesions on his forearm, leg, ears, and genital area.

Immunohistochemical investigation showed Leishmania amastigotes associated with an inflammatory infiltrate rich in CD4+, CD6+, CD68+ cells. These increases in cell counts are consistent with cases of human cutaneous leishmaniasis with non-ulcerative lesions frequently observed in patients with acquired immunodeficiency syndrome and low CD4+ T cell counts.10 Frequent augmentation of CD4+ T cell counts after initiation of HAART, and the pivotal role of CD4+ T cells for responses to Leishmania parasites and ulcer formation in cutaneous lesions during experimental leishmaniasis.12,13

As CD4+ T cell counts increased, a strong immune response to Leishmania antigens may have been mounted, which contributed to the onset of IRIS and culminated in development of ulcerated cutaneous lesions. Our findings support this hypothesis by showing that the paucity of CD4+ T cells correlated with peripheral blood CD4+ cell counts and that their increase over time also correlated with the worsening of skin lesions (Figure 2).

Bone marrow aspirations obtained at the time of diagnosis of infection with HIV and at the time of onset of the comatose state were consistently negative for Leishmania parasites. Although investigated more than once, infections with mycobacteria, C. neoformans, T. gondii, H. capsulatum, cytomegalovirus, and Treponema pallidum were not confirmed, which support our hypothesis that IRIS was triggered by L. guyanensis and was the most probable cause for the reported comatose state.

It is known that T regulatory cells play a critical role in down-regulating inflammatory responses and restricting immunologic damage associated with increased immune responses.14 Additionally, it has been shown that numbers of naturally occurring Foxp3+ T regulatory cells are increased in patients in which IRIS does not develop, whereas patients in which IRIS develops normally elicit Th-0 cell and effector T cells responses, which produce pro-inflammatory cytokines in response to cell-signaling factors.15 Our data show that the frequency of CD4+ CD25+ FOXP-3+ T cells in peripheral blood was low (1.82%) when compared with levels in healthy persons (5–10%). Therefore, although T regulatory cells were present, they were not sufficient to control establishment of ulcerated lesions or onset of systemic manifestations, which culminated in a comatose state. Whether IRIS reported in our patient was the result of a response to a high antigen burden, an excessive response by the recovering immune system, exacerbated production of pro-inflammatory cytokines, or a lack of immune regulation caused by an inability to produce regulatory cytokines remains to be determined.

Received February 9, 2009. Accepted for publication July 2, 2009.


REFERENCES


