Bronchopulmonary and Mediastinal Leishmaniasis: An Unusual Clinical Presentation of Leishmania donovani Infection

Ben G. Marshall, Pascale Kropf, Kate Murray, Colin Clark, Adrienne M. Flanagan, Robert N. Davidson, Rory J. Shaw, and Ingrid Müller

From the Departments of Respiratory Medicine (National Heart and Lung Institute), Immunology, Histopathology, and Tropical Medicine/Infectious Diseases, Imperial College School of Medicine, St. Mary’s Campus, and Department of Clinical Immunology, St. Mary’s Hospital NHS Trust, London, United Kingdom

We describe a case of unusual leishmaniasis in a Sudanese man with a history of progressively enlarging granulomatous mediastinal lymphadenopathy, worsening hemoptysis, and an intense mucosal granulomatous inflammatory response in the large bronchi. Leishmania donovani DNA was detected in bronchial biopsies by polymerase chain reaction. This is a novel description of human leishmanial infection in an immunocompetent patient involving this anatomical site. The patient's condition improved clinically, spirometrically, and radiologically after a course of treatment with amphotericin B. The cell-mediated immune response was analyzed before, during, and after successful antileishmanial chemotherapy.

The disease spectrum of leishmaniasis ranges from self-healing cutaneous lesions to fatal systemic disease, depending on the species of parasite and the host immune response [1]. Visceral leishmaniasis (kala-azar), which is caused by Leishmania donovani in Sudan, is the most serious form of leishmaniasis and has been a major health problem in Sudan [2]. Mucosal involvement due to L. donovani and Leishmania infantum occurs rarely in immunocompetent patients and is confined to the upper respiratory tract (nose, upper lip, tongue, and mouth) [3]. Cases of oral, nasal, nasopharyngeal, and laryngeal leishmaniasis have been recorded in the Sudan and other parts of Africa [3, 4]. Lower respiratory tract involvement in immunocompetent individuals is rare; interstitial pneumonitis with fibrosis has been reported to occur in only 1 series, involving HIV-seronegative persons with New World visceral leishmaniasis but without mediastinal lymphadenopathy [5]. Pulmonary involvement by Leishmania in HIV-infected patients, however, has been described [6–8].

The immunologic basis for the different manifestations of human leishmania infections is not well understood. In experimental models of Leishmania major infections, healing is associated with a polarized Th1 response, characterized by IL-2 and IFN-γ secretion, whereas a Th2 response (IL-4, IL-10) is associated with nonhealing disease [9]. In humans, however, the situation is less clear, and patients with visceral leishmaniasis can have mixed Th1 and Th2 cytokine profiles. Active infection with L. donovani, L. infantum, or L. chagasi is accompanied by impaired IFN-γ production [10] and by elevated IL-10 levels, which decline when healing occurs [11, 12]. Parasitized macrophages exposed to IFN-γ will be activated to kill intracellular parasites, and there is evidence in both mice and humans that IFN-γ is a central component of cellular immunity against Leishmania [9–11].

We describe here a case of leishmaniasis involving the bronchial tree of a Sudanese patient and outline the kinetics of his cellular response to Leishmania antigen during antileishmanial treatment.

Methods

Case History

A 39-year-old Sudanese man presented because of cough and exertional dyspnea. The patient had been a resident of the United Kingdom since 1991; before this he had lived in Yemen and southern Sudan. His illness was characterized by gradually increasing bilateral hilar and mediastinal lymphadenopathy (on serial CT scans) and splenomegaly. He had a recurring mucosal glossal lesion and a discharging metatarsal sinus. Histologic examination of biopsy specimens from these lesions revealed noncaseating granuloma. Stains for fungi and mycobacteria were negative. Empirical antituberculcc chemotheraphy was unsuccessful.

After 2 life-threatening episodes of hemoptysis, he underwent successful right-bronchial-artery embolization; bronchoscopy had shown severe inflammatory changes throughout the bronchial tree and lower trachea. However, the patient remained otherwise symptomatic, and mediastinal adenopathy and splenomegaly persisted. Table 1 summarizes other clinical tests.

Initial antileishmanial treatment was given iv amphotericin B. However, the patient's renal function deteriorated, and he was switched to liposomal amphotericin B (AmBisome; NeXstar Pharmaceuticals, San Dimas, CA).
Clinical Investigations

Bone marrow, bronchoalveolar lavage, and endobronchial specimens were cultured in Novy-MacNeal-Nicolle medium. DNA was extracted from fresh bronchial biopsy specimens and bronchoalveolar lavage cells, and PCR was performed with use of primers specific for *Leishmania* kinetoplast minicircle DNA, as described elsewhere [13].

Serum was tested for antibody to *Leishmania* by direct, indirect, and latex agglutination tests. The leishmanin skin test antigen (Institute Pasteur, Tehran, Iran) was administered intradermally at a dose of 0.1 mL and read at 72 h.

**Lymphocyte separation.** Blood was taken before, during, and after treatment, and peripheral blood mononuclear cells (PBMC) were isolated over a Hypaque-Ficoll gradient for immunologic analysis. An age- and sex-matched control, seronegative for *Leishmania*, served as a negative control at each time point.

**Leishmania antigen.** *L. donovani* LV9 antigen was obtained by fixing 10^9 parasites/mL in paraformaldehyde 2% (pH, 7.4) in PBS for 30 min on ice. The fixed parasites were washed and adjusted to 2 × 10^7/mL, and an equal volume of glycerol was added. All preparations were stored at −20°C. Before use, the parasites were washed in Dulbecco’s modified Eagle’s medium.

**Stimulation of cytokine production and proliferation.** Cells (4 × 10^5) were cultured for 3 days with *L. donovani* antigen at 37°C in 5% CO2 in air, in 96-well Costar plates (Costar, Bucks, UK), in a final volume of 1 mL. The culture supernatants were then collected and frozen before cytokine measurement. For the proliferation, cells were pulsed with 0.5 Ci 3H-thymidine (Amersham, Bucks, UK) during the last 18 h of culture. Unstimulated cells were used as a control.

**Flow cytometry.** After 3 days of stimulation, cells were harvested and labeled with 20 L of anti-CD25 (clone 2A3; Becton Dickinson, Cockeysville, MD). After 20 min of incubation, cells were washed and resuspended in 0.5 mL of paraformaldehyde 1% in PBS and kept overnight at 4°C before analysis with a FAC-Scalibur (Becton Dickinson).

**Figure 1.** Molecular evidence of *Leishmania*. Agarose gels of PCR products from the patient’s respiratory specimens with use of *Leishmania* kinetoplast minicircle DNA-specific primers. *A*, Before treatment: lane 1, blank; lane 2, Φ × 174 marker; lane 3, patient’s histologic extract; lane 4, *L. donovani* control; lane 5, *L. major* control; and lane 6, negative control. *B*, 3 months after treatment: lane 1, Φ × 174 marker; lane 2, patient’s histologic extract, in saline; lane 3, patient’s histologic extract, dry; lane 4, bronchoalveolar lavage fluid in *L. donovani* complex; lanes 5–8, nil; lane 9, *L. donovani* control; and lane 10, *L. major* control. The arrow indicates the *L. donovani* PCR product (800 bp).
Cytokine measurement. IFN-γ, IL-4, IL-5, and IL-10 were measured by 2-site ELISA using antibody pairs, standards, and protocols supplied by the producer (PharMingen, San Diego, CA).

Results

Leishmania PCR. PCR for DNA of the L. donovani, L. infantum, or L. chagasi complex was positive on bronchial tissue and bronchoalveolar lavage cells before treatment (figure 1A). Three months after treatment, bronchial biopsies were PCR-negative for the L. donovani complex, but a bronchoalveolar lavage specimen remained positive (figure 1B). Five months after treatment, all specimens were PCR-negative (data not shown).

Histopathology. Before treatment, mediastinal lymph node biopsies showed nonnecrotizing granulomata, multinucleated giant cells, and focal fibrosis. Endobronchial biopsies revealed severe chronic and moderate acute inflammation with extensive loose granulomatous inflammation (figure 2). Ziehl-Neelsen, Giemsa, and diastase periodic acid–Schiff stains were negative for all specimens.

Culture of clinical specimens. No Leishmania parasites could be cultured from or detected in clinical specimens.

Clinical response to therapy. The patient’s symptoms had abated and he had gained weight 12 months after completion of therapy. His spirometry values improved substantially (forced expiratory volume at 1 s increased from 1 to 3.05 L). The bronchoalveolar lavage lymphocyte count fell from 60% of the total cell count (pretreatment) to 28%. CT of the thorax showed a reduction in mediastinal lymphadenopathy and splenomegaly in comparison with findings from a pretreatment scan (figure 3).

PBMC proliferation and CD25 expression. PBMC were stimulated for 3 days in the presence or absence of L. donovani antigen at different time points before, during, and after treatment. As shown in figure 4, a strong proliferative response (stimulation index, 112) was observed when the PBMC were stimulated with L. donovani antigen. The PBMC from a seronegative individual were used as a control and had a low proliferative response (stimulation index, 2.7) to L. donovani antigen. Expression of the activation marker CD25 in PBMC from the patient and the control was analyzed. In the unstimulated cultures, the PBMC purified from both individuals had low expression of CD25. A strong increase in the expression of CD25 (compared with that of the control and the unstimulated cultures) was observed when the lymphocytes from the patient were stimulated with L. donovani antigen (figure 4).

Cytokine production by PBMC. The production of Th2 cytokines in response to antigenic activation was measured. IL-10 was produced by the PBMC purified from the patient in response to L. donovani at all time points analyzed, and no change was observed before and after the treatment (figure 5). No IL-10 was detected in the supernatants obtained from the control and from the unstimulated lymphocytes from both individuals (data not shown). The production of IL-4 and IL-5 by the lymphocytes from both individuals was not detectable at any time point analyzed (data not shown).

To characterize further the immune response of the patient
Figure 3. Radiographic demonstration of mediastinal lymphadenopathy. Contrast-enhanced CT images of thorax at the level of carina demonstrating marked mediastinal and hilar lymphadenopathy (arrow).

during and after treatment, the levels of IFN-γ secreted by his PBMC were measured. PBMC isolated from the patient produced IFN-γ in response to L. donovani antigen. It is interesting that the production of IFN-γ was elevated when measured at the 2 time points after completion of treatment. Unstimulated PBMC were used as a control, and no IFN-γ was detectable before or after treatment (data not shown). The lymphocytes isolated from the negative control were also stimulated with L. donovani antigen, and as expected, no production of IFN-γ was detected (figure 5).
Figure 4. Proliferative response and CD25 expression of peripheral blood mononuclear cells (PBMC). PBMC from the patient and from the negative control were isolated by Ficoll gradient and stimulated at a concentration of $4 \times 10^6$ cells/mL with $L. donovani$ antigen ($1 \times 10^6$ parasites/mL). For the proliferation, cells were pulsed after 2 days with 0.5 Ci of $^3$H-thymidine; 18 h later, cells were harvested and the proliferative response was measured. For the flow cytometric analysis, cells were harvested after 3 days, and the expression of CD25 was analyzed. Unstimulated cells were used as a control. Medium indicates no antigen added.

Discussion

We present here a report of a patient from a Leishmania-endemic region with an atypical, possibly unique form of leishmaniasis involving the mucosa of the lower respiratory tract and draining lymph nodes. The diagnosis rested on immunologic evidence of previous leishmaniasis (serology and leishmanin skin test were positive) and a positive PCR for the $L. donovani$ complex in bronchial tissue, which subsequently became negative after successful anti-Leishmania therapy. He had an excellent clinical response to amphotericin B (an antibiotic with activity against fungi and Leishmania).

We also considered a diagnosis of histoplasmosis, but this was excluded by negative serology and lack of response to a 6-week trial of itraconazole. The clinical forms of leishmaniasis that most resemble this patient’s illness, namely, mucocutaneous leishmaniasis and post-kala-azar dermal leishmaniasis (PKDL) with mucosal involvement, are similarly characterized by an exuberant host inflammatory response with parasites that are scanty or undetectable. On balance, our patient’s disease clinically fits a previously unrecognized form of PKDL. The finding of splenomegaly, which persisted despite successful treatment, suggests that he could have had either visceral leishmaniasis or (at least) a visceral response to leishmaniasis. Splenic aspiration was not performed, so this assumption could not be tested.

We analyzed the patient’s immune response to further support the clinical diagnosis and to evaluate the response to a trial of antileishmanial therapy. The capacity of his PBMC to be activated, to proliferate, and to produce cytokines was determined in response to specific activation (figures 4 and 5). The clear specific proliferative response of the patient’s PBMC and the expression of the high-affinity IL-2R (CD25) after activation with Leishmania antigen is in contrast to the anergy seen in patients with active visceral leishmaniasis but is similar

Figure 5. IL-10 and IFN-γ production by peripheral blood mononuclear cells (PBMC) in response to $L. donovani$ antigen. At the indicated time points, PBMC from the patient and from the negative control were isolated by Ficoll gradient and stimulated at a concentration of $4 \times 10^6$ cells/mL with $L. donovani$ antigen ($1 \times 10^6$ parasites/mL). After 3 days, the supernatants were harvested and tested for their content of IL-10 and IFN-γ by ELISA. Unstimulated cells were used as a control. ND, not detectable.
to the responses seen after recovery from the disease [14, 15] and in PKDL [14-17].

Analysis of the cytokine profile indicated that IFN-γ and IL-10 were produced in response to specific activation, whereas IL-4 and IL-5 were not detected (figure 5 and data not shown). The levels of specific IL-10 were unaltered before, during, and after treatment (figure 5). Thus, the IL-10 profile of the patient with this unusual form of leishmanial infection differs from that usually observed in visceral leishmaniasis. Patients cured of active visceral leishmaniasis have shown reduced expression of IL-10, as compared to the high levels found in patients with active visceral leishmaniasis [12, 18]. PBMC isolated from patients with acute visceral leishmaniasis have been noted to produce IL-10 in response to leishmania antigen, whereas after treatment, no IL-10 was detectable [12]. It has also been shown that mRNA for IL-10 was present in a lesion from a patient with PKDL [12]. Thus, the stable levels of IL-10 (figure 5) further indicate that our patient had a form of leishmaniasis more akin to PKDL than to visceral leishmaniasis.

IFN-γ is an important cytokine in disease pathogenesis; it is one of the key cytokines that activates macrophages to kill the intracellular Leishmania parasite [19, 20]. Specific IFN-γ production was detectable at all time points analyzed (figure 5). Moreover, an increase in the levels of specific IFN-γ was observed during and after treatment. An increased and sustained production is associated with clear amelioration of the disease in patients with active visceral leishmaniasis, a form of disease usually characterized by an inability to produce IFN-γ in response to leishmania antigen [12, 14, 15]. The capacity to produce IFN-γ is also restored after successful therapy.

The concomitant production of IFN-γ and IL-10 (figure 5) that was observed during clinical improvement further indicates that this was not visceral leishmaniasis with pulmonary involvement. The possibility cannot be excluded that the sustained levels of these cytokines observed in the present study were due to the use of in vitro restimulation of PBMC. However, it has been shown that lower IL-10 production from PBMC specifically restimulated ex vivo was associated with healing [15].

In summary, this report documents an unusual clinical and immunologic form of leishmaniasis, occurring in a Sudanese patient presenting with pulmonary granulomatous disease.

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References


