Leishmaniasis Recidivans
Recurrence after 43 Years: A Clinical and Immunologic Report after Successful Treatment

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We describe a patient with very late recurring leishmaniasis recidivans from whom lesional biopsy samples were obtained during and after topical steroid treatment that demonstrated the ability of the host to contain the parasite in the absence of therapy. Combination therapy with intralesional sodium stibogluconate and oral itraconazole was successful and immunologic data suggest that both CD4+ and CD8+ T cell subsets had roles in this disease process.

An unusual clinical variant of cutaneous disease caused by Leishmania tropica is leishmaniasis recidivans. Leishmaniasis recidivans typically recurs at the site of an original ulcer, generally within 2 years and often within the edge of the scar [1]. Commonly, children are affected with these recurrent lesions, which can be notoriously difficult to treat—hence the name “chronic relapsing cutaneous leishmaniasis.” Many practitioners continue to use sodium stibogluconate, but the long list of treatment options speaks to the lack of a single, reliable therapy. We report the clearance of such a recurrent lesion after treatment with a 2-drug regimen (intralesional stibogluconate and oral itraconazole) and discuss the immunologic changes associated with healing.

Materials and methods. We manually searched for previously reported cases of leishmaniasis recidivans in the National Institutes of Health (NIH) library microfiche of Tropical Medicine Bulletin for the years 1900–1966. Computerized searches were done for the years 1966–2000.

Peripheral blood mononuclear cells (PBMCs) were obtained from blood drawn from the patient at 3 different time points (designated “pretreatment,” “during treatment,” and “post-treatment” samplings) and were cryopreserved before use. For lymphocyte proliferation assays, PBMCs were plated at 10⁶ cells well in triplicate in a 96-well plate (Costar). The cells were stimulated with 3 different Leishmania antigens that had been prepared from 2 strains of L. tropica (an Afghanistan isolate and a Desert Storm isolate) and 1 strain of Leishmania donovani (from India) at 5 μg/mL. Purified protein derivative (PPD; World Health Organization) was used at 10 μg/mL and pokeweed mitogen (Sigma) was used at 2.5 μg/mL. Supernatants were collected on day 5 for cytokine analysis. Immediately thereafter, the cells were pulsed with [³H]thymidine and harvested onto scintillation mats 18 h later to measure thymidine incorporation. Supernatants, which were stored at −70°C until assayed, were assessed for IFN-γ exactly as described elsewhere [2]. PBMCs were depleted of CD8+ T lymphocytes by use of beads (Dynal), according to the manufacturer’s guidelines. About 99% of the CD8+ T cells were depleted from the PBMCs.

For flow cytometry, the following monoclonal antibodies were used (Becton Dickinson): CD3-phcoerythrin (PE), CD3–fluorescein isothiocyanate (FITC), CD4-FITC, CD8-PE, CD14-PE, CD16-FITC, and CD20-PE. The cells were stained and fixed in 2% paraformaldehyde, and 10,000 events were acquired on a FACSCalibur (Becton Dickinson) and analyzed by use of CellQuest software.

Case report. A 50-year-old Pakistani man presented with a crusted, papular lesion on the wrist. It was located on the edge of a healed scar that had resulted from a childhood ulcer acquired 43 years earlier in Pakistan. The childhood ulcer healed after 2 years, leaving a depressed, hypopigmented scar. In September 1997, the patient bought a new metallic watchband that irritated his wrist enough for him to seek medical advice in July 1998. The wrist lesion was treated with topical antibiotics, followed by topical steroids, without improvement. A biopsy sample obtained during steroid treatment showed unusually large numbers of intracellular Leishmania species. He was referred to the NIH in November 1998, where he presented with a crusted lesion with peripheral induration. A second specimen was obtained for biopsy (steroid treatment had been dis-
continued for a 2-week period), which revealed a granuloma without obvious parasites. However, cultures of this second biopsy specimen yielded flagellates that were typed as *L. tropica* by use of both isoenzyme analysis (authors' unpublished data) and typing with species-specific monoclonal antibodies [3].

Initial therapy with iv sodium stibogluconate (20 mg/kg) was discontinued after 9 days, because the patient experienced generalized urticaria. He then received a lipid formulation of amphotericin B (300 mg given iv on alternating days; total dose, 1200 mg). At this point, there was a 50% reduction in the lesion's diameter. After 3 months without significant continuing improvement, and after receiving negative results of serological tests for HIV, he was treated with intralesional injections of sodium stibogluconate (250 mg given on alternating days; total dose, 750 mg) and an 8-week course of oral itraconazole (400 mg per day) with an excellent clinical response. Itraconazole levels remained in the therapeutic range, and the patient did not experience side effects. He has been monitored closely, and thus far there has been no evidence of recurrence 1 year after treatment.

**Results.** The ulcerated leishmanial lesion steadily cleared once the combination therapy was begun in March 1999. Figure 1 shows the evolution of the lesion. The initial histological examination of the biopsy specimen (during topical steroid treatment; figure 1A) demonstrates both an abundance of intracellular parasites and an extremely thin epidermis. The pretreatment lesion presented in figure 1B (after steroid treatment had been discontinued for 2 weeks) shows the location within the edge of the scar. The corresponding histology for the period after discontinuation of steroid treatment is shown in figure 1C. Note the absence of parasites and the regeneration of the epidermis. Figure 1D shows the cleared lesion after the patient received combination therapy. At presentation, skin testing with a *Leishmania major* antigen yielded negative results; in March 1999, the results of skin tests were positive.

The cutaneous leishmaniasis lesion is reported to be rich in CD8⁺ T lymphocytes [4]. Furthermore, in patients with leishmaniasis recidivans, there has been a reported decrease in the ratio of CD4⁺ cells to CD8⁺ cells (<2.0) in peripheral blood [5]. We found a pretreatment ratio of CD4⁺ cells to CD8⁺ cells of 1.40, a ratio during treatment of 1.52, and a posttreatment ratio of 1.80. This increase in the ratio was entirely due to the increase in the percentage of CD4⁺ cells that occurred during treatment, because the percentage of CD8⁺ cells remained constant. This observation, in conjunction with the literature, which suggests a role for CD8⁺ cells in immunity to leishmaniasis [6], led us to test the effects of CD8⁺ cell depletion on proliferation and IFN-γ responses. Figure 2A shows the

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**Figure 1.** Chronology of events in the course of late-onset leishmaniasis recidivans. Arrows and dates indicate important events in the patient's history. The times when blood was drawn for immunologic monitoring are indicated in the boxes above the time line; the results of skin tests and HIV serological tests are indicated below the time line; and photographs include both histological and clinical changes observed during the course of treatment. A, Biopsy sample obtained during steroid treatment. B, National Institutes of Health (NIH) presenting lesion. C, Biopsy specimen of NIH presenting lesion after cessation of steroid treatment. D, Cleared lesion (bar size, 25 μm). Abx, antibiotics; AMB, lipid formulation of amphotericin; Bx, biopsy; Cx, culture; Rx, treatment; On-Rx, during treatment; Sb, antimony; −, negative; +, positive.
Figure 2. Lymphocyte proliferation (A) and IFN-γ production (B) in response to Leishmania antigens or purified protein derivative (PPD) in peripheral blood mononuclear cells (PBMC) obtained from patient at indicated time points (pretreatment [pre], during treatment [on], or posttreatment [post]). Data are expressed as counts per minute (cpm; A) and picograms per milliliter (pg/mL; B), in response to antigens listed. Open bars represent media controls; black bars represent antigen stimulation. Left side of each graph in panel B shows data with unmodified PBMC; right side shows data obtained from CD8⁺ cell–depleted PBMC. L. donovani, Leishmania donovani; L. tropica, Leishmania tropica.

results of the lymphocyte proliferation assays after stimulation with various leishmanial antigens. Note the low proliferative response before treatment and the increase in proliferative response that occurred during treatment. Also, note the relative species specificity: the best responses were seen for L. tropica, and very little (and transient) response was seen for L. donovani. Similar results, although of greater magnitude, were seen in the CD8⁺ cell–depleted PBMCs, probably because of the increase in absolute numbers of CD4⁺ T cells per well (not shown). Of interest, we observed similar increases in the proliferative responses to PPD that occurred during treatment. The mitogenic response to pokeweed mitogen was robust and unaffected by therapy (data not shown). The IFN-γ protein measured in the supernatants that were collected during the antigen stimulation assays (figure 2B) increased over time, which paralleled the increases in antigen-specific proliferation. Note the markedly enhanced IFN-γ production in the CD8⁺ cell–depleted PBMCs that occurred when they were stimulated with Leishmania antigens or PPD. As expected, there was an increase in the absolute number of CD4⁺ T cells in the well after CD8⁺ cell depletion (pretreatment, from 35,000 to 46,000 cells/10⁵ PBMCs; during treatment, from 38,000 to 52,000 cells/10⁵ PBMCs; and posttreatment, from 45,000 to 51,000 cells/10⁵ PBMCs).

Discussion. We believe that this is the longest documented
delay in recurrence of leishmaniasis recidivans yet reported (a 31-year delay was reported by Gitelzon [in Russian] in 1933, which is discussed in [1]). Most cases of leishmaniasis recidivans result from infection with *L. tropica*, but a few cases have been reported in association with *Leishmania braziliensis* [7]. Implicit in the phenomenon of recurrent lesions, particularly those recurring in the exact site of the scar, as in this case, is the presumption that the parasite remains there over time and is reactivated by some stimulus. Reports suggest that local trauma contributes to the reactivation of leishmaniasis [8]. Perhaps the microenvironment that results from the trauma or events involved in wound healing enable the parasite to thrive. We did not find any evidence of the inhibitory cytokine IL-10 in the supernatant (data not shown). Another immunomodulatory cytokine that may be involved, but that is not studied here, is transforming growth factor-β (TGF-β), which is involved in wound healing [9], expressed in leishmanial lesions, and provides a parasite escape mechanism [10].

There are few reported data, however, regarding the effects of topical steroids on the clinical course of leishmaniasis. We believe that, in this case, steroid use contributed to the ability to make the diagnosis, at the very least. The combination of local trauma (new metallic watchband) and topical steroids, the lack of underlying medical illnesses or overt immune suppression, and the parasitological clearance with the discontinuation of the steroids reinforces this point. The rapid regeneration of the epidermis during the 2-week period in which steroid treatment had been discontinued indicates that the steroid effect had indeed been diminished at the structural level. Associated with this steroid removal was the dramatic ability of the skin to contain the parasite, as indicated by the absence of any demonstrable parasites in the second biopsy sample (figure 1C), although they could be cultured.

Despite the urticaria that developed during treatment with iv sodium stibogluconate, we were able to safely deliver the drug locally. In combination with prolonged itraconazole therapy, the patient’s lesion appears to be fully reepithelialized and without induration or nodules, which suggests complete inactivity. During the progression from active, recurrent leishmaniasis to a quiescent, healed state, increases were seen in both T cell proliferation and IFN-γ production in response to leishmanial antigens, notably *L. tropica* antigens, and PPD, but without changes in the mitogenic response. This state of antigen-specific hyporesponsiveness has been reported in other parasitic diseases and may be induced by either IL-10 or TGF-β [11]. A common thread linking the local trauma, wound healing, leishmaniasis reactivation, and hyporesponsiveness may be an alteration in local TGF-β production in favor of the parasite. Because we have only the results from 1 patient, however, the underlying mechanism remains to be elucidated. This report upholds the theory that leishmaniasis may never really be cured, and that it is only held in check by a delicately balanced immune system. The enhanced cytokine production in the absence of CD8+ T cells is an intriguing observation that extends to both leishmanial and mycobacterial antigens. These results suggest a role for both CD4+ and CD8+ cells in this patient’s disease process.

**Acknowledgments**

We gratefully acknowledge the expert nursing provided by Melissa Law and the privilege of caring for this patient.

**References**