Influence of Helminth Infections on the Clinical Course of and Immune Response to Leishmania braziliensis Cutaneous Leishmaniasis

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Background. Helminth infections influence the clinical outcome of and immune response to certain immune-mediated diseases.

Methods. We conducted a cohort study of 120 patients to examine the role that intestinal helminth infection plays in the clinical course of and immune response to cutaneous leishmaniasis (CL) treated with pentavalent antimony.

Results. Patients coinfected with Leishmania braziliensis and helminths took longer to heal (relative hazard for healing, 0.47 [95% confidence interval, 0.26–0.85]; P = .01) than patients with CL without helminths, with 70% of coinfected patients being cured at 90 days, compared with 92% of helminth-free patients. Coinfected patients had an immune response shifted toward the T helper 2 type, with increased total immunoglobulin E levels (P < .06) and a tendency toward increased interleukin-5 levels, compared with helminth-free patients with CL.

Conclusions. Helminths influence both the clinical outcome and the immune response of patients with CL. These results may have clinical implications for the care of patients with CL caused by Leishmania braziliensis, because screening for and treatment of helminths may improve responses to treatment and possibly reduce the risk of progression to mucosal disease.

Cutaneous leishmaniasis (CL) is a vectorborne protozoal disease that is classically characterized by ≥ 1 well-demarcated cutaneous ulcer with raised borders. More than 1.5 million people are infected annually [1] with lesions that can be destructive and disfiguring and that occasionally advance to involve the mucosa [2]. Standard treatment of Leishmania braziliensis CL is 20 days of intravenous pentavalent antimony, a therapy that is moderately toxic and is difficult to administer in a resource-poor area. Response rates vary from 50% to 90% [3, 4], depending on the study quoted, and the reasons behind this variability are not fully understood. Subtle differences in the host, parasite, and vector could all play a role in determining the rate of treatment resistance. We examined whether coinfection with intestinal helminths can affect the clinical outcome after treatment with antimony.

A Th1-type immune response is crucial to controlling leishmania infection—the release of interferon (IFN)–γ is necessary for the activation of macrophages and, ultimately, the nitric oxide (NO)–mediated killing of the intracellular parasite. However, excessive inflammation caused by Th1 cytokine release has also been implicated in the pathogenesis of CL. Several lines of evidence [5, 6] support the role of the Th1 immune response in the pathogenesis of ulcers in CL: (1) parasites are absent or scarce in the lesions [7]; (2) the development of the ulcer is preceded by granulomatous vasculitis with lymphocytic infiltration [8]; (3) the use of antimony during the early preulcer phase of CL does not prevent lesion formation [8]; (4) increased IFN-γ and tumor necrosis factor–α levels are observed during...
Table 1. Clinical characteristics of patients with cutaneous leishmaniasis at the time of presentation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with helminths (n = 106)</th>
<th>Patients without helminths (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female ratio</td>
<td>1.9:1</td>
<td>1:1</td>
<td>.25a</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>28.7 ± 11.9</td>
<td>33.6 ± 14.9</td>
<td>.16b</td>
</tr>
<tr>
<td>Skin-test reaction, median (IQR), mm²</td>
<td>204 (132–306)</td>
<td>224 (144–380)</td>
<td>.44c</td>
</tr>
<tr>
<td>No. of lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, no. (%)</td>
<td>75 (70.8)</td>
<td>12 (85.7)</td>
<td>.35a</td>
</tr>
<tr>
<td>2, no. (%)</td>
<td>31 (29.2)</td>
<td>2 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Size of lesion, median (IQR), mm²</td>
<td>280 (132–525)</td>
<td>400 (260–750)</td>
<td>.21c</td>
</tr>
<tr>
<td>Serum ferritin level, median (IQR), ng/mL</td>
<td>68 (49–124)</td>
<td>121 (94–147)</td>
<td>.27c</td>
</tr>
</tbody>
</table>

NOTE. IQR, interquartile range.

a Fisher’s exact test.
b Unpaired t test.
c Wilcoxon rank sum test.

the active phase of the lesion, and these decrease after treatment [5, 9]; and (5) treatment with immune modulatory drugs and antimony accelerates healing in patients with CL and mucosal leishmaniasis [10, 11].

Helminth infection is highly prevalent in Brazil [12, 13], but, because clinical manifestations are mild, continuous exposure to infection is common, and patients remain infected by worms for long periods of time. Recent attention has focused on the ability of intestinal helminths to modulate the human immune response, thereby altering the clinical presentation of immune-related diseases, including asthma [14, 15], human T lymphotropic virus (HTLV)—associated myelopathy (HAM)/tropical spastic paraparesis (TSP) [16], and Crohn disease [17]. This effect may be directly related to the ability of helminths to induce secretion of the regulatory cytokine interleukin (IL)–10 [18], to shift the overall cellular response toward a more dominant Th2 profile [19–21], or to expand the population of regulatory T cells [22]. Because the immune response plays a central role in the pathogenesis of CL, we evaluated the effect of helminth coinfection on the presentation, progression, and response to treatment of patients with CL who were enrolled in a prospective cohort study.

PATIENTS, MATERIALS, AND METHODS

Study Area

Patients were enrolled in a prospective cohort study at the health post in the village of Corte de Pedra, in the southeast portion of the state of Bahia, Brazil [2]. This clinic is a referral center for the diagnosis and treatment of leishmaniasia; it serves a population of 300,000 people living within an ~30-km radius. This region has the highest prevalence of CL in the state of Bahia, and the clinic sees an average of 800 new cases per year. Given the rural agricultural setting, the prevalence of intestinal helminth infections was expected to be high.

Patient Selection and Follow-Up

The criteria used for the diagnosis of CL were the presence of a typical ulcerated lesion and a positive Montenegro antigen skin test (>5-mm induration at 48–72 h). Patients with this diagnosis were then selected on the basis of the following inclusion criteria: (1) age 13–60 years, (2) a maximum of 4 ulcers with ≤2 body regions involved, and (3) a period of 15–90 days from the onset of the first ulcer. Patients with a history of CL or antimony use, patients with evidence of mucosal or disseminated disease, pregnant or breast-feeding women, and patients with diabetes mellitus were excluded. A total of 127 patients were enrolled in the study, 7 were excluded, and 120 were used in the final analysis. Of the 7 excluded patients, 2 were lost to follow-up, 2 stopped antimony treatment prematurely, and the remaining 3 developed evidence of disseminated disease during the course of the study.

At enrollment, all patients were treated with 20 mg/kg/day intravenous antimony for 20 days. Patients provided 3 stool samples for parasitological assay at study entry and returned to the health post 30, 60, and 90 days after the initiation of antimony therapy for follow-up. Bidirectional measurements were taken of the patients’ lesions at each visit, and the area involved was calculated as the product of the 2 measurements. All lesions were also categorized as either active or healed at each visit. Only lesions with complete reepithelialization—without raised borders or scabs—were considered to be healed. Patients with helminth infections were treated with the appropriate oral regimen on the day-60 visit, to provide a window of time during which the immune response to L. braziliensis could develop in the presence of active helminth infection. Written, informed consent was obtained from all adult patients and from parents or guardians of minors. The study was approved by the ethics committee of the Hospital Universitário Professor Edgard Santos, Salvador, Brazil, and by the institu-
Table 2. Prevalence of helminths, by species, in patients with cutaneous leishmaniasis from Corte de Pedra, Bahia, Brazil.

<table>
<thead>
<tr>
<th>Helminth</th>
<th>Overall %</th>
<th>Sex, no. (%)</th>
<th>Age, no. (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13–25 years</td>
<td>26–40 years</td>
<td>40 years</td>
</tr>
<tr>
<td><em>Ancylostoma duodenale</em></td>
<td>80.8</td>
<td>66 (68.0)</td>
<td>31 (32.0)</td>
<td>.09</td>
</tr>
<tr>
<td><em>Trichuris trichiur</em></td>
<td>52.5</td>
<td>42 (66.7)</td>
<td>21 (33.3)</td>
<td>.57</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>48.3</td>
<td>36 (62.1)</td>
<td>22 (37.9)</td>
<td>.71</td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>16.7</td>
<td>14 (70.0)</td>
<td>6 (30.0)</td>
<td>.62</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>10.0</td>
<td>9 (75.5)</td>
<td>3 (25.0)</td>
<td>.53</td>
</tr>
</tbody>
</table>

* Fisher’s exact test.

Laboratory Diagnosis

**Intradermal skin test.** The *Leishmania* antigen used for intradermal skin testing was obtained from a *L. amazonensis* strain (MHOM-BR-86BA-125). The volar surface of the left forearm was injected with 25 mg of antigen in 0.1 mL of distilled water, and the largest diameter of induration was measured at 48–72 h [23]. The test was considered to be positive for indurations >5 mm.

**Parasitologic assays.** The parasitologic assay of feces used sedimentation, Baermann, and Kato-Katz methods for all 3 samples. A single slide was prepared for sedimentation and Baermann, and 2 slides/sample were prepared for the Kato-Katz reading [24]. Helminth burdens were calculated for *Ancylostoma duodenale, Ascaris lumbricoides, Trichuris trichiura,* and *Schistosoma mansoni* species and were expressed as the number of eggs per gram of stool.

Immunologic Studies

Because of the limited number of available biological samples, immunologic testing was performed in a subgroup of patients chosen randomly. The sample population for cytokine determination consisted of 34 patients with CL who were coinfected with helminths and 6 helminth-negative patients with CL. For serologic testing, the sample size consisted of 75 helminth-positive patients with CL and 9 helminth-negative patients with CL.

**Cytokines.** Levels of cytokines (IFN-γ and IL-5) in supernatants of peripheral blood mononuclear cells (PBMCs) stimulated with *L. braziliensis* antigens were measured using ELISA. PBMCs were obtained by density-gradient centrifugation using lymphocyte separation medium (Organon Teknika). After being washed in saline, the cells were adjusted to $3 \times 10^6$ cells/
mL in RPMI 1640 medium (Gibco) supplemented with 10% AB+ serum that contained 100 U of penicillin/g and 10 μg/mL streptomycin. All cultures were stimulated with *L. braziliensis* antigens and incubated for 72 h at 37°C in 5% CO₂. Supernatants were collected and stored at −20°C. Levels of IFN-γ (Genzyme) and IL-5 (PharMingen) were measured using the ELISA sandwich technique, and the results were expressed in picograms per milliliter on the basis of a standard curve generated by use of recombinant cytokines.

**Laboratory tests.** Total IgE levels were determined by enzyme-linked fluorescent assay using the CAP System (Pharmacia), and the results were expressed in international units per milliliter. Serum levels of iron and ferritin were determined using dry chemistry and chemiluminescence, respectively.

**Statistical Analysis**

Data were analyzed using Stata statistical software (version 7; StataCorp). Continuous variables were compared using the Wilcoxon rank sum test. For confirmation, unpaired Student’s *t* tests were also performed with log transformation of skewed variables. Because of similar results, only the rank sum *P* values are reported. Fisher’s exact test was used to analyze categorical data. The cumulative probability of healing, stratified on the presence of helminth coinfection, was estimated using the Kaplan-Meier method, and the log-rank test was used to compare curves. Univariate and multivariate adjusted Cox proportional-hazard models were used to analyze the association between helminth coinfection and the risk of healing. *P* = .05 was taken to indicate statistical significance.

**RESULTS**

Patient characteristics in the study roughly follow known demographic patterns for CL in this region, with young males being at the greatest risk of acquiring the disease. Patients ranged in age from 13 to 60 years (mean, 29.3 ± 12.3 years), with a male:female ratio of 1.9:1. The majority of patients presented with a single lesion (72.5%). There was no significant difference between helminth-positive and helminth-negative groups with regard to age, sex, number of lesions, or skin-test area (table 1). The lesion with the greatest area at presentation was considered to be the main lesion. Patients with helminths tended to have smaller main lesions, although this trend did not reach statistical significance: the median size of lesions in the helminth-positive group was 280 mm² (interquartile range [IQR], 132–525 mm²), compared with 400 mm² (IQR, 260–750 mm²) for their helminth-negative counterparts.

Helminth infections were extremely prevalent in this population, with 88.3% of patients infected with at least 1 species. Polyparasitism was the rule, with 73% of patient harboring multiple helminths. Patients with schistosomiasis had the intestinal form of the disease, and those with strongyloidiasis were asymptomatic or had mild symptoms, such as intermittent diarrhea. For all helminth species, the prevalence was higher in the subgroup of patients 13–25 years old, but only infection with *T. trichiura* and *A. lumbricoides* reached statistical significance (*P* < .001). Only 14 patients were negative for helminths after a series of 3 stool samples. Further details about prevalence by species can be found in table 2. The burden of helminth...
infection was calculated for *A. duodenale*, *A. lumbricoides*, *T. trichiura*, and *S. mansoni* species. Although not presented here, these data reflected a pattern typical for areas where helminths are endemic. The majority of patients harbored relatively mild to moderate worm burdens, and only a small minority had an egg output reflecting heavy helminth infection. Of the helminth-negative patients, 7 (50%) reported having received antihelminthic drugs within the preceding 12 months. In the helminth-positive group, 32 patients (30.2%) reported antihelminthic use within the preceding 12 months.

The median level of IFN-γ in supernatants of lymphocyte cultures was not significantly different between patients with CL with and without helminth infection (figure 1). There was a trend for the median IL-5 level to be higher in helminth-positive patients (364 pg/mL; IQR, 212–956 pg/mL) than in their helminth-negative counterparts (184 pg/mL; IQR, 69–488 pg/mL) (*n* = 6; *P* = .19). There was a larger sample size (*n* = 84) for comparison of total IgE levels in patients with CL with and without helminths (figure 2). The median total IgE level was higher in patients with CL who were coinfected with helminths (1578 IU/mL; IQR, 560–2526 IU/mL) than in those free of helminths (427 IU/mL; IQR, 93–1499 IU/mL) (*P* = .06).

At the 90-day study end point, 30.2% (*n* = 32) of patients coinfected with helminths had persistent lesions, compared with 7.1% of patients (*n* = 32) in the helminth-negative group. By Kaplan-Meier analysis (figure 3), patients with helminths took longer to heal their lesions than their helminth-negative counterparts (*P* < .01, log-rank test). We used both crude and adjusted Cox proportional-hazards models to quantify the effect of multiple variables on lesion healing over time. In these models, a hazard ratio (HR) < 1 means that the variable was associated with delayed lesion healing. In a univariate model, the presence of helminth coinfection was associated with delayed lesion healing (HR, 0.47 [95% confidence interval (CI), 0.26–0.85]; *P* = .01). The presence of larger ulcers before treatment was also associated with longer healing times (HR, 0.80 [95% CI, 0.64–1.01]; *P* = .06). Immunologic markers—including IL-5 (HR, 1.00), IFN-γ (HR, 1.01), and total IgE (HR, 1.01)—showed no effect on time to lesion healing. There was also no effect seen for age, sex, number and duration of lesions, serum iron and ferritin levels, or helminth burden on lesion healing (data not shown). The helminths were also subdivided into invasive (*S. mansoni* and *Strongyloides stercoralis*) and non-invasive (*A. lumbricoides*, *A. duodenale*, and *T. trichiura*) species subgroups. There was no difference in lesion healing between the invasive and noninvasive subgroups (data not shown). Finally, because of its potential confounding effect on time to healing, we adjusted for the main lesion size in the multivariate model. In the adjusted model, the association between helminth infection and delayed lesion healing was strengthened (HR, 0.41 [95% CI, 0.22–0.76]; *P* < .01).

**DISCUSSION**

CL in northeastern Brazil is predominantly caused by *L. braziliensis* and is typically limited to a few ulcers on the lower extremities. However, the appearance of mucosal lesions is observed in up to 3% of the patients, and it can occur even after cutaneous ulcers have resolved [2]. Therapeutic failure in CL has been predominantly associated with an increased duration of disease, the presence of multiple lesions, and larger lesions [25]. Helminths are highly prevalent in tropical and subtropical countries, especially in rural poor populations. Although this distribution coincides closely with the areas where leishmaniasis...
is endemic, no previous studies have evaluated the influence of helminths on the clinical course of leishmaniasis. Our results demonstrate that helminth coinfections alter both the clinical course and, possibly, the immune response of patients with CL. Clinically, although coinfected patients presented with smaller lesions, their time to healing was approximately twice that of patients without coinfection. Coinfected patients had stronger Th2-type immune responses, as reflected by higher IgE and IL-5 levels.

Helminth infections have been shown to influence the outcome of disease in both experimental models and humans. *S. mansoni* infection decreases the frequency of type 1 diabetes mellitus in nonobese diabetic mice [26]. In experimental autoimmune encephalitis, *S. mansoni* infection decreases the inflammatory response and improves clinical outcome [27]. In humans infected with HTLV-I, the presence of *S. mansoni* coinfection appears to protect against the development HAM/TSP [28]. Coinfection with *S. mansoni* also limits the severity of asthma [15]. In addition, ingestion of live *Trichuris suis* eggs has been shown to improve the gastrointestinal manifestations of Crohn disease [17]. Although the benefits of helminth infection in attenuating inflammation and autoimmune diseases have been well documented, helminths may increase the incidence of or worsen the course of other infections. For instance, patients with *S. mansoni* are more prone to chronic *Salmonella* infection [29]. The persistence of the hepatitis C virus and the development of chronic hepatitis are also increased in patients coinfected with *S. mansoni* [30].

A type 1 immune response is critical for the control and cure of *Leishmania* infection. In the absence of an effective T cell response to *Leishmania* antigen, the parasite disseminates throughout the body, resulting in diffuse CL [31] and visceral leishmaniasis [32]. However, in areas of *L. braziliensis* transmission, progression from infection to disease and the severity of disease are related to an exaggerated and poorly modulated immune response to *L. braziliensis* [6, 31, 33]. With their ability to induce a dominant type 2 immune response, helminth coinfection could therefore improve or worsen the outcome of *L. braziliensis* infection.

Helminths induce a type 2 immune response characterized by high IL-4, IL-5, and IL-13 levels and high total and parasitic-specific IgE levels [20]. As expected, in our study, high total IgE levels were found in helminth-infected patients. In addition, coinfection with helminths tended to shift the immune response to *Leishmania* antigen toward a type 2 response, as shown by a trend toward increased IL-5 levels. Although previous studies have shown the ability of helminths to downmodulate both type 1 and type 2 immune responses to a bystander antigen [34], we did not find a decrease in IFN-γ levels in stimulated PBMC cultures from patients dually infected with *L. braziliensis* and helminths. It is possible that an overwhelming induction of Th1-type responses by the *L. braziliensis* antigen [6] may have prevented any modulating effects of the helminth infection. Small sample numbers from helminth-free patients were an overall limitation of our study, and this have limited our ability to detect a down-regulated Th1 response.

We have shown that, although patients coinfected with helminths tend to present with smaller ulcers than patients without helminths, they are less likely to heal after treatment with antimony. This effect does not appear to be explained by iron deficiency, although we cannot rule out confounding by other nutritional factors or unmeasured differences that might be associated with both helminth infection and CL healing; nor does this effect appear to be explained by changes in the acquired T cell immune response. It is also interesting that this effect was observed, despite 50% of helminth-negative patients having received antihelminthic therapy during the preceding year. Macrophages play a central role in the control of leishmaniasis, and it is conceivable that macrophages from coinfected patients are less efficient at killing *Leishmania*. The results of a study of *L. major* and *S. mansoni* coinfection in C57BL/6 mice supported this possibility. In that case, BL/6 mice coinfected with *S. mansoni* and *L. major* produced lower levels of NO, which translated into decreased macrophage killing of parasites in vitro [35].

In summary, the present study shows that patients with CL and helminth coinfection are less likely to respond to antimony therapy, which often results in repeat courses of this toxic and difficult-to-administer 20-day therapy. Therapeutic failure of antimony is associated with an increased risk of parasite dissemination and the development of mucosal disease. Therefore, screening for and treatment of helminth infections may prove to be efficacious in improving response rates to antimony therapy. This simple intervention could have a significant effect on the treatment of CL worldwide where coinfection with helminths is common.

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