MAJOR ARTICLE

Failure of Early Treatment of Cutaneous Leishmaniasis in Preventing the Development of an Ulcer

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The clinical characteristics and treatment outcome were determined for 26 patients who presented with early-stage cutaneous leishmaniasis. Illness duration ranged from 8 to 20 days, and the commonest clinical presentation was the presence of a papule with small central crust on a lower extremity. Prominent regional adenopathy was found in 22 (85%) of 26 patients. The results of an intradermal skin test for *Leishmania* were positive for 96% of those patients, and results of serologic testing were positive for 61% of patients tested. Ten (46%) of 22 patients for whom follow-up data were available developed enlargement and ulceration of the lesion despite early antimony therapy and required additional courses of treatment. Histopathological studies of samples from the lesions of 3 patients showed vasculitis. These data show that early therapy for cutaneous leishmaniasis does not prevent the development of an ulcer in one-half of patients. This unfavorable outcome underlines the relevance of local exacerbated inflammatory and immune response in the pathogenesis of the disease.

American cutaneous leishmaniasis is characterized by a spectrum of variable clinical manifestations, ranging from a single ulcerated lesion that is sometimes self-healing to diffuse cutaneous leishmaniasis, for which treatment is a challenge [1, 2]. Localized ulceration is the most common presentation, but vegetative, verrucous, sporotricoid, lupoid, and disseminated forms also have been described [3–5]. This heterogeneous clinical picture is well described in the literature, but very few data are available for the initial manifestations of the disease. Even in areas of endemicity, diagnosis is rarely made early in the disease, and most patients present 30–60 days after the appearance of a lesion, at which point an ulcer is quite evident.

Regional lymphadenopathy is seen in the majority of patients with cutaneous leishmaniasis caused by *Leishmania braziliensis* [6]. Although lymphadenopathy has been considered to be an early manifestation of the disease, the frequency and relevance of this symptom have not been determined. The histopathology of samples from cutaneous leishmaniasis lesions is characterized by an inflammatory reaction, composed of lymphocytes, plasma cells, and macrophages, and, in many cases, a granulomatous reaction [7]. The treatment of choice is administration of pentavalent antimony (Sb^V), and the cure rate in patients with classic ulcers ranges from 60% to 90%. However, reepithelization may take as long as 90 days [8, 9].

In the present study, we describe the clinical picture and histopathological findings for 26 patients with early-stage cutaneous leishmaniasis (<20 days after the

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appearance of the first lesion). The majority of the patients included in the study were in the preulcerative stage of cutaneous leishmaniasis and presented with papular or nodular lesions. We took advantage of early diagnosis and evaluated the response to early antimony therapy in these patients.

PATIENTS AND METHODS

Patients. Patients were recruited in Corte de Pedra (located 260 km southeast of Salvador, the capital of Bahia, Brazil), an area in which *L. braziliensis* infection is endemic and where clinical and immunological studies of leishmaniasis have been conducted since the early 1980s. The diagnosis of early-stage cutaneous leishmaniasis (<20 days of evolution) was made in 26 patients who presented with suspicious skin lesions, and the diagnosis was confirmed by parasite isolation or at least 2 of the following: (1) positive results of skin testing, (2) positive results of serologic testing, and (3) histopathological findings indicative of *Leishmania* infection. None of the patients had received antileishmanial therapy. The cutoff point of 20 days was chosen because patients with a longer course of illness present with classic ulcers. The study was approved by the Ethical Committee of the Hospital Universitário Prof. Edgard Santos, University of Bahia, and informed consent was obtained from all patients or their guardians. The guidelines for human experimentation of the Federal University of Bahia were followed in conducting the clinical research. All patients were treated with intravenous Sb^V in a conventional schedule (Sb^V, 20 mg/kg/day for 20 days). All patients who had enlargement of a lesion at 60 days or a lesion that persisted at 90 days after initiation of therapy received a second 20-day course of Sb^V. A third course of Sb^V was administered to patients in whom the infection had not resolved at 60 days after initiation of the second course.

Laboratory diagnosis. The *Leishmania* antigen used for intradermal skin testing was obtained from an *Leishmania amazonensis* strain (MHOM-BR-86BA-125), and 25 μg of antigen in 0.1 mL of distilled water was injected into the forearm of each patient [10]. The largest diameter of the induration was measured after 48 h, and the reaction was considered to be positive if the diameter was >5 mm.

Needle aspiration of a skin lesion or lymph node was performed, and aspirates were cultured in Nicolle-McNeal-Novy medium overlaid with modified liver infusion triptase medium. Cultures were kept at 25°C and examined twice weekly. Isolates were characterized by use of a panel of monoclonal antibodies [11].

Antileishmanial antibodies were detected by an indirect immunofluorescence assay. A promastigote *L. amazonensis* strain [12] was used as antigen. An antibody titer of 1:16 or higher was considered to be a positive result.

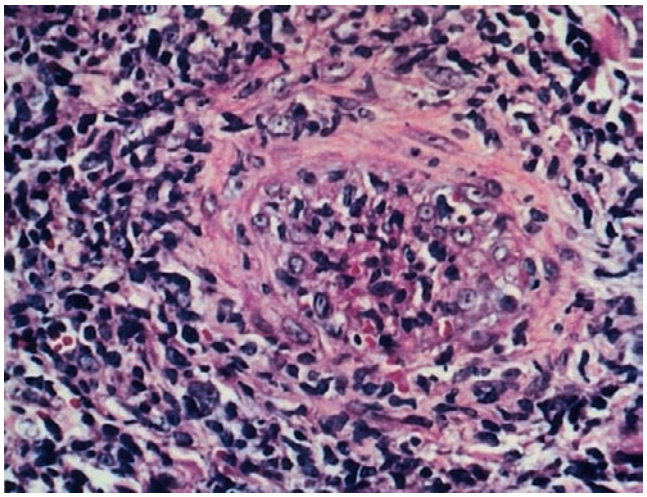

Figure 1. Arteriole with thickened wall and proliferative endarteritis obliterating the lumen in a patient with cutaneous leishmaniasis. Mononuclear cell infiltration is present in the lumen and around the vessel. (Hematoxylin-eosin stain; original magnification, ×320.)

Skin biopsies were performed with a 4-mm punch after local anesthesia was induced with 1% lidocaine, and biopsy specimens were fixed in buffered formaldehyde. Sections of paraffin-embedded tissue were stained with hematoxylin-eosin.

Statistical analysis. The Mann-Whitney test was used to analyze continuous variables, and Fisher's exact test was used to analyze categorical data, with InStat, version 3.00 for Windows 95 (GraphPad).

RESULTS

Clinical and demographic characteristics. Patients with early-stage cutaneous leishmaniasis presented with several types of lesions, including nodules and superficial ulcers. The most common presentation was a papule with a small central crust, often localized to the lower limbs, followed by findings of superficial ulceration. Patient age ranged from 12 to 45 years, and 19 male patients and 7 female patients were included. The time between appearance of the lesion and diagnosis ranged from 8 to 20 days (mean ± SD, 14 ± 8.7 days), but the majority of patients (88%) had <15 days of evolution. The number of lesions varied from 1 to 3: 23 patients presented with 1 lesion, 2 with 2 lesions, and only 1 with 3 lesions.

Lymph node enlargement was found in 22 (85%) of 26 patients, predominantly in the inguinal and crural regions; cutaneous lesions were seen mainly on the lower limbs. Most patients complained of slight tenderness and pain, despite great lymph node enlargement (diameter, >3 cm). Lymph node en-

Table 1. Treatment outcomes for patients with early-stage cutaneous leishmaniasis treated with pentavalent antimony (Sb^V).

Variable	Patients with favorable outcomes[a] (n = 12)	Patients with unfavorable outcomes[b] (n = 10)	P
Age, mean years ± SD	27 ± 10.3	23 ± 6	.25[c]
Sex, female/male	4/8	1/9	
No. (%) with 2 lesions	0	2 (20)	
No. (%) with 3 lesions	0	1 (10)	
Size of lesion on presentation, mean mm^2 ± SD	105.8 ± 148.1	150.1 ± 127.2	.27[c]
Duration of disease, mean days ± SD	15.4 ± 2.6	12.2 ± 3.6	.07[c]
No. (%) with lymphadenopathy	8 (67)	10 (100)	.09[d]
No. with positive results of skin testing	11	10	1.0[d]
No. with positive results of serologic testing[e]	5	6	.15[d]

[a] Patients who were cured in ⩽90 days with 1 course of Sb^V and remained without disease after 1 year of follow-up.
[b] Patients who had enlargement or ulceration of the lesion at 60 days or persistence or reactivation at 90 days after initiation of treatment.
[c] Calculated using the Mann-Whitney test.
[d] Calculated using Fisher's exact test.
[e] Serologic testing was performed for 11 patients who had favorable outcomes and 7 patients who had unfavorable outcomes.

largement preceded the appearance of the first cutaneous lesion in 7 of 26 patients and occurred simultaneously with appearance of the first lesion in 14 patients. The length of time between enlargement of a lymph node and appearance of the first clinical lesion ranged from 7 to 40 days. Lymphadenopathy was always observed on the same side as the lesion and in the proximal lymphatic chain. In 4 patients, no lymphadenopathy was found on physical examination or reported in the patient's medical history.

Laboratory data. The results of skin testing for *Leishmania* were negative in only 1 patient, and positive results varied from induration of 5 to 75 mm. Ulceration was seen in 1 patient. Positive results were found in 11 (61%) of 18 patients for whom serologic testing was performed. *L. braziliensis* was identified in all patients from whom a parasite was isolated.

Histopathological examination of samples from 3 patients who had a papular or an ulcerated lesion revealed a mononuclear infiltrate around and in the wall of the dermal vessels, which is characteristic of a vasculitic process (figure 1). In 1 patient, leukocytoclastic vasculitis also was observed. Amastigotes, macrophage infiltration, and cytolitic necrosis were found in 2 patients. We did not find pseudocarcinomatous epidermal hyperplasia or a granulomatous reaction in the infiltrate, which would be expected in cases of classic cutaneous leishmaniasis. Because biopsy was performed on a small number of patients, no correlation could be made between histopathological data and response to therapy.

Treatment outcome. All patients were treated with Sb^V, 20 mg/kg/day for 20 days. Clinical data were recorded every 15 or 30 days for at least 1 year of follow-up. Enlargement or ulceration of the lesion at 60 days or persistence or reactivation at 90 days after initiation of treatment was considered to indicate treatment failure, and a second course of Sb^V was administered. Table 1 shows clinical characteristics and treatment outcomes. In the subgroup of 22 patients who received standard treatment and for whom sufficient follow-up data were available, 12 patients (55%) had resolution of infection at 30–90 days (mean ± SD, 55 ± 22 days) after initiation of a single course of Sb^V, and no reactivation was documented during at least 12 months of follow-up. Infection resolved in 10 (46%) of those 22 patients only after ⩾2 courses of Sb^V: in 1 patient, a new lesion developed; in 6 patients, a lesion became enlarged; and in 3 patients, a papule or a nodule developed into a large and classic ulcer. Additionally, 2 of these 10 patients had reactivation in the borders of the scar and needed a third course of Sb^V before the infection was cured. No mucosal disease was documented in any patient. The clinical evolution of the disease in one of the patients who did not respond to a single course of Sb^V is shown in figure 2. A 14-year-old boy with a 15-day history of a papular lesion in his thigh (figure 2*A*) developed enlargement and ulceration of a lesion (figure 2*B*) despite early administration of a course of Sb^V. The lesion healed (figure 2*C*) only after a second course of therapy.

No correlation between the diameter of induration on skin testing, the duration of illness, and the size of the lesion and response to therapy was observed (table 1). Although there was no difference in the frequency of lymphadenopathy in the 2 groups of patients (those with favorable and those with unfavorable outcomes), resolution of infection was observed after

a single course of Sb^V in all 4 patients for whom no lymph node enlargement was documented.

DISCUSSION

American cutaneous leishmaniasis is clinically described as an ulcerative lesion with a well-delimited and raised border [1]. Although the presence of a papule before the development of an ulcer has been reported in the literature [1, 3], this common manifestation of this disease has not been recognized in relation to active infection, and most often the diagnosis is made and therapy initiated only when a typical ulcerated lesion is found. The present study demonstrates that a papular lesion associated with regional tumoral lymphadenopathy in patients from an area in which the organism is endemic is highly associated with *L. braziliensis* infection. In fact, regional lymphadenopathy and a papular lesion are the initial signs of clinical cutaneous leishmaniasis.

Few data are available on the relevance of regional adenopathy in cutaneous leishmaniasis. We have found previously that regional lymphadenopathy is documented in as many as 66% of patients with classic ulcers [6] and that lymphadenopathy can develop before the cutaneous ulcer is evident [13]. In our study, lymph node enlargement was an earlier finding than a cutaneous lesion in 27% of patients, but lymphadenopathy developed concomitantly with the lesion in the majority of patients. The presence of tumoral lymphadenopathy in 85% of our patients who had early and small skin lesions highlights the participation of the immune system in the course of *Leishmania* infection. It is interesting to note that, in all 4 of the patients who had no regional lymphadenopathy, the infection resolved after a single course of Sb^V. Ongoing studies are being performed that evaluate the immune response in patients with tumoral lymphadenopathy and in patients without adenopathy, to better clarify the role of the immune response in the clinical outcome for patients with early-stage cutaneous leishmaniasis.

Early diagnosis and therapeutic intervention clearly are associated with a higher cure rate in most infectious diseases. Our data show a surprising rate of treatment failure (46%) among patients with early-stage cutaneous leishmaniasis, despite use of a standard schedule of administration of Sb^V that currently results in cure for as many as 90% of patients with classic cutaneous leishmaniasis [8, 14]. In the present study, some patients developed a large ulcer, and the infection resolved only after a second or third course of treatment. Additionally, histopathological findings for samples from 3 patients showed vasculitis, which suggests that local inflammatory mechanisms play an important role in the development of the disease. These data suggest that tissue inflammation leading to ulcerative lesions may be related to the host's immune response and not only to the presence of the parasite. The observation that administration of a TNF-α inhibitor (pentoxifylline) in association with antimony therapy improves the healing of mucosal leishmaniasis supports this hypothesis [15].

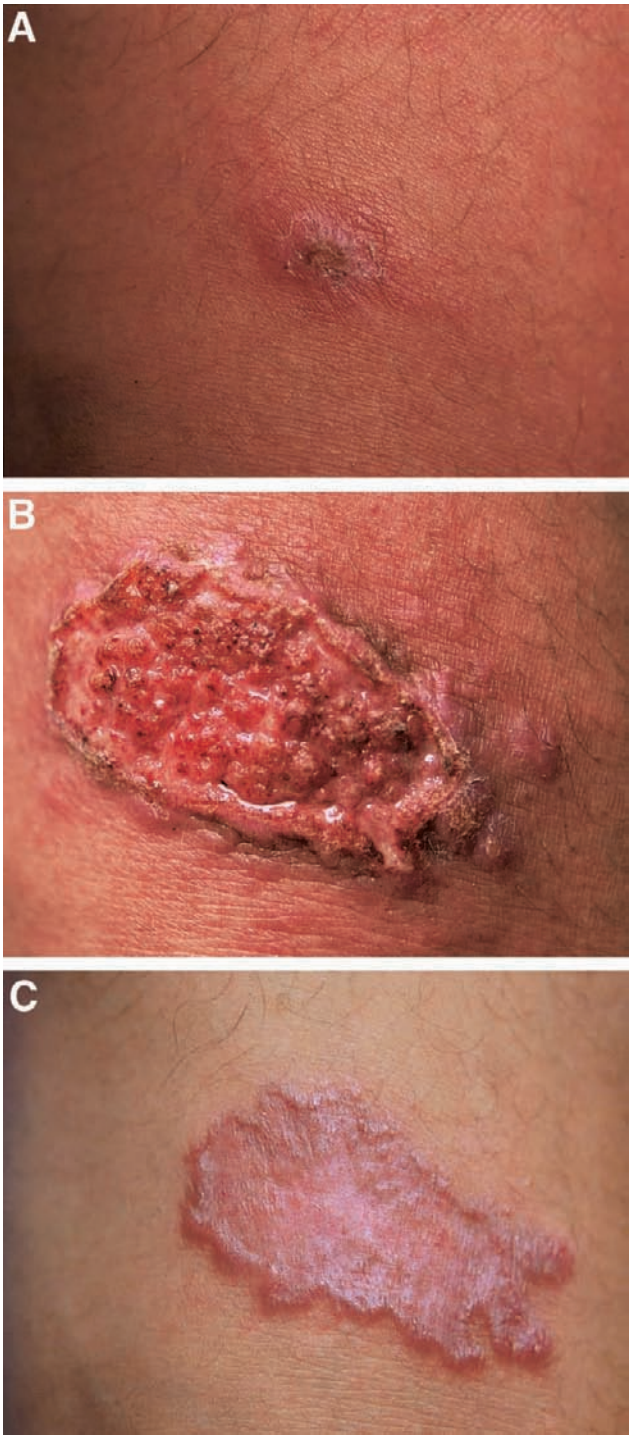

Figure 2. *A,* Initial superficial lesion of cutaneous leishmaniasis. *B,* Ulcerated and enlarged lesion 90 days after initiation of treatment with pentavalent antimony (Sb^V). *C,* Atrophic scar with infiltration in the borders 60 days after initiation of a second course of Sb^V.

Our results show that, in cutaneous leishmaniasis caused by

L. braziliensis, early therapy does not guarantee the healing of lesions. Therefore, for selected patients and patients with refractory cases of cutaneous leishmaniasis, the possibility of use of an immunomodulator in addition to antimony therapy should be considered.

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