REPORT

POLAR AND SUBPOLAR DIFFUSE CUTANEOUS LEISHMANIASIS IN BRAZIL: CLINICAL AND IMMUNOPATHOLOGIC ASPECTS

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Abstract

Background. Diffuse cutaneous leishmaniasis (DCL) is a rare manifestation of human leishmaniasis, characterized by multiple, slowly progressive nodules or plaques without ulceration, involving almost the entire body. It has been suggested, that DCL results from a lack of cell-mediated immunity to leishmanial antigen, leading to uncontrolled parasite growth.

Methods. We have performed detailed clinical, histopathologic, and immunologic investigations in six patients with DCL. Biopsies were taken from the nodules, processed, and examined for determination of the macrophagic pattern present, based on the intensity of vacuolation and the frequency of vacuolated cells, the parasite index, and the presence of eosinophils. Immunologically, patients were evaluated by their response to intradermal skin test to PPD or leishmania antigen, determination of antileishmania antibodies by immunofluorescent assay, and lymphocyte proliferation assay.

Results. There seemed to be a negative relation between nodules and skin ulcerations, whereas the highest number of parasites were observed in patients with the greatest number of vacuolated macrophages. The delayed hypersensitivity skin test to leishmanial antigen was negative, and antileishmania IgG antibodies were positive in all patients.

Conclusions. Although all cases fulfill the criteria for being classified as DCL, they present a wide spectrum. Three cases were clearly at the unresponsive pole, and three other cases belonged to the subpolar form of DCL, exhibiting varying weak signs of antiparasite responsiveness.

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Clinical presentations of American cutaneous leishmaniasis (ACL) depend on genetic factors of the host and the virulence of the parasite. American cutaneous leishmaniasis ranges from hyperresponsive, largely ulcerative and destructive mucocutaneous leishmaniasis to unresponsive diffuse cutaneous leishmaniasis (DCL). The latter was first described in Brazil,[1] and then in Bolivia[2] and Venezuela.[3] Cases have also been reported in Kenya, the Dominican Republic, Ethiopia, Honduras, Costa Rica, Mexico, and the United States.[4,5]

Diffuse cutaneous leishmaniasis, as classically described, is a rare manifestation of leishmaniasis, characterized by multiple, slowly progressive nodules or plaques without ulceration, involving the entire body, except for the scalp, axillae, inguinal folds, palms, and soles.[6] It is generally admitted that DCL results from a lack of cell-mediated immune response to parasite antigens leading to uncontrolled parasite growth.[7,8] Based on the intensity of the lymphocytic infiltration, a histologic classification for Oriental DCL was proposed,[6] with similarities to Ridley and Joplin's criteria for leprosy.[9]

In the present study, we report six cases of DCL from Brazil in which clinical, histopathologic, and immunologic parameters led us to divide them in the classic, totally unresponsive polar DCL, and in a subpolar form in which weak signs of responsiveness are observed.

Materials and Methods

Patient Population: Since 1986, six patients with DCL have been studied at the University of Bahia Hospital and in the Hospital dos Servidores do Estado do Maranhão. The study included a clinical history and physical examination, an intradermal skin test with purified protein derivative (PPD) or leishmania antigen,[10] determination of antileishmanial antibodies by immunofluorescence (IFA),[11] and a lymphocyte blastogenesis test.[12] The biopsies were taken from the nodules and ulcers, when present. Case 6 had no prior treatment; all other cases had been treated with several courses of pentavalent antimony with only marginal clinical improvement. All patients had been without treatment for the previous 6 months at the time of the beginning of the study. The study was approved by the Human Rights Committee of the University of Bahia.

Parasite Isolation, Cultivation, and Characterization: Parasites were isolated by puncture aspiration of the lesions[13] and were cultured in tubes of NNN blood agar overlaid with a modified liver infusion tryptose (LIT) medium. Cultures

Table 1. Major Clinical Findings in Diffuse Cutaneous Leishmaniasis

			Lesions									
								Body Distribution of Lesions				
Patients (No.)	Age/Sex (yr)	Duration of Disease (yr)	Nodules	No. of Ulcers	Ulcerations	IP	Number	Face	MMSS	MMII	Abdomen	Thorax
1	28/M	4	++++	0	+	+	>500	++++	++++	++++	++++	++++
2	31/M	9	+++	0	+++	+	168	++	++	+++	+++	++
3	16/M	12	++	0	++	+	20	++	++	++	–	–
4	16/M	14	+++	6	+++	++	51	++	++	+++	–	–
5	21/M	15	+++	2	++	–	86	+++	++++	++++	–	–
6	12/F	5	++	0	+	++	40	+	++	+++	–	+

+ = few; ++ = moderate; +++ = intense; ++++ = very intense.
IP = infiltrate plaques; MMSS = upper extremities; MMII = lower extremities.

were expanded for growth in modified LIT medium and kept at 25°C. Procedures for both serodeme analysis with monoclonal antibodies and 15-enzyme electrophoresis have been reported previously.[14]

Histopathologic Examination: Skin biopsies were performed for diagnostic purposes with a 4 mm punch after local anesthesia. The material obtained was fixed in buffered formalin, embedded in paraffin for hematoxylin and eosin staining. Parasite index was determined according to Ridley's criteria and only the well preserved amastigotes were counted.[9] The frequency of eosinophils was also estimated by counting the number of eosinophils in 30 fields of 1,000 × magnification, applying the following score: less than 10 cells = rare, 10–100 cells +, 100–200 cells ++, and > 200 cells +++. The degree of macrophagic pattern was considered, taking into account the intensity of vacuolation and frequency of vacuolated cells.

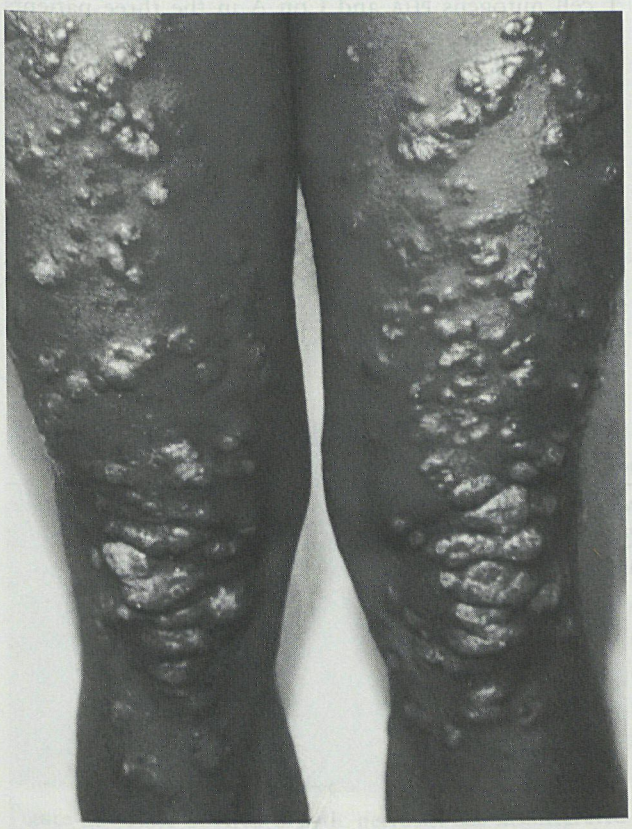

Figure 1. Case 1: Widespread nonulcerated papillar and nodular lesions.

RESULTS

Clinical Findings

The major clinical findings in the six patients studied is shown in Table 1. The age ranged from 12 to 31 years. All cases but one were men, and the duration of their illness ranged from 4 to 15 years. Multiple nodular lesions were seen in all patients. Lesions ranged from 20 to more than 500 nodules, but there were clinical differences regarding the ulcerative lesions. Case 1, with the highest number of nodules, had no ulceration (Fig. 1). Cases 2, 3, and 6 presented with crusted ulcerations over infiltrated plaques. Such lesions differed from the deep ulcers with a necrotic center and elevated borders, resembling the ulcers of classic cutaneous leishmaniasis observed in cases 4 and 5 (Fig. 2). Case 6 presented a clear asymmetry of lesions, some of them presenting a coppery discoloration or a "punched out" aspect (Fig. 3).

None of the patients had evidence of systemic involvement, based on the absence of both symptoms and

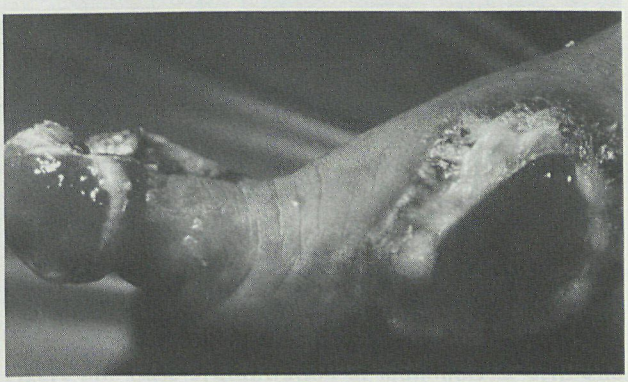

Figure 2. Case 5: Two deep lesions on the foot with infiltrated borders.

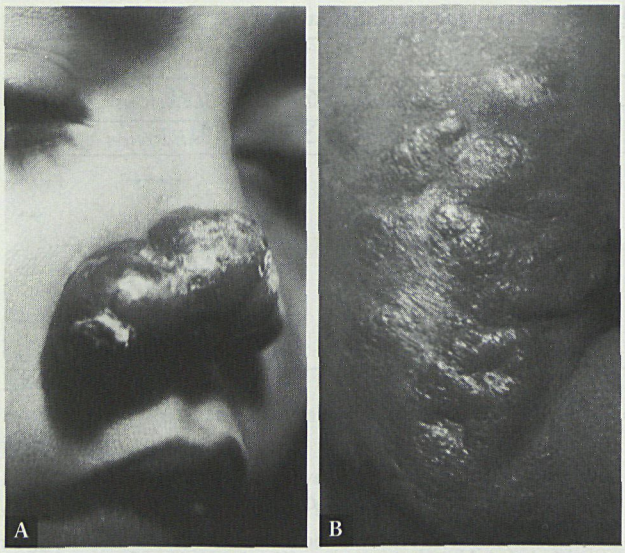

Figure 3. Case 6: *A*, Copper-colored tumoral lesion on the nose with a small ulceration at right. *B*, Infiltrated erythematous plaques on the right arm with "punched-out" aspect.

hepatosplenomegaly. Five had superficial infiltration of the nasal mucosa, without ulceration or destruction.

Histopathology

The histologic picture of the nodular lesions (Table 2) showed a spectrum in parasite frequency (both in terms of parasite numbers and parasitized cells), and in the intensity of vacuolation of macrophages (Fig. 4). Cases 1, 2, and 3 presented the highest numbers of parasites and a clear macrophagic pattern; cases 4, 5, and 6 had the lowest number of vacuolated macrophages and of parasites. Conversely, cytolytic necrosis (Fig. 5) was observed in high intensity in cases 4 and 5, and only case 6 exhibited a granulomatous reaction (Fig. 6). This reaction consisted of a few disorganized granulomas, with giant and epithelioid cells. Other aspects, such as intensity of lymphoplasmocytic infiltrate, Unna's band, presence of eosinophils, and acanthosis did not exhibit a clear spectral distribution. The true ulcerative lesions observed in cases 4 and 5 were characterized by marked cytolytic necrosis and reduction of intracellular parasites. A moderate degree of acanthosis was observed in both ulcers and in the nodular lesions of cases 2 and 3. The acanthosis was restricted to the papillar dermis. In case 5, the ulcer showed a marked infiltration of eosinophils. No granulomatous reaction was observed in the ulcers. The lymphoplasmocytic infiltration was always focal with predominance of plasma cells and usually present in the deep areas of the dermis.

Immunologic Evaluation

Delayed hypersensitivity skin test to leishmanial antigen (Montenegro test) was negative in all patients, whereas all of them were positive for PPD (indurated area more than 10 mm in diameter). In three patients (cases 4, 5, and 6) an occasional weak response was observed. Results of lymphocyte proliferation and antileishmania antibody titers are shown in Table 3.

All cases had a persistent negative lymphocyte proliferative response to leishmanial antigen. Blastogenesis to unrelated antigens, such as PPD and *Candida albicans*, was documented in three patients. Lymphocytes from all patients had proliferative responses to phytohemagglutinin (PHA), Concanavalin A and pokeweed mitogen (PWM) but to different degrees. While the response to the T cell mitogens PHA and Con A in the three patients with the polar DCL were 49,194 ± 2,771 and 78,904 ± 5,688, in the patients with the subpolar form these responses were 123,356 ± 25,079 and 110,918 ± 12,188. The response to PWM of the patients with the polar form was 47,679 ± 1,922, whereas in the patients with the subpolar form it was 39,704 ± 1,602.

Antileishmania IgG antibodies were present in all patients, with titers ranging from 1:512 to 1:16,000. The highest antibody titers were observed in polar patients (cases 1 and 2) who histologically had a dominant macrophage pattern.

Table 2. Histopathologic Aspects

Patient (No.)	*Biopsied Lesion*	*Macrophagic Pattern*	*PI*	*LP Infiltration*	*Frequency of Eosinophils*	*Cytolytic Necrosis*	*Parasites in Interstice*	*Acanthosis*	*Unna's Band*
1	N	++++	6	–	0	–	–	Atrophy	+
2	N	++++	6	++	+	+	+	++*	–
3	N	+++	5	+	0	–	–	++	–
4	N	++	5	+	0	–	–	–	+
4	U	+	3	++	+++	+++	+	++	–
5	N	+	3	+	+	+++ eosinophilic	++	+	+
5	U	–	†	++	+	++++	+++	++	–
6	N	+	3	+	++	+	+	+	+

N = nodule; U = ulcer; PI = parasite index; LP = lymphoplasmacytic; * = parasites within the epidermis; † = great quantity of amastigotes with rare preserved forms.

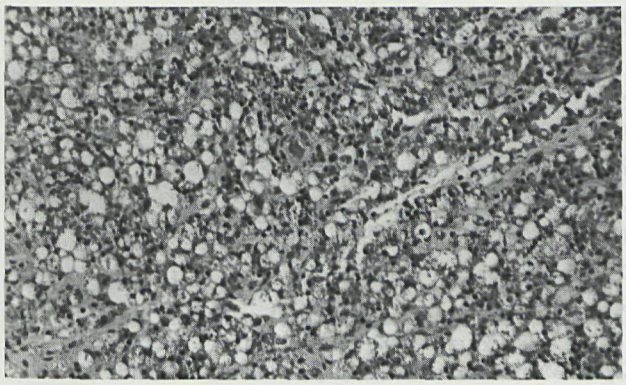

Figure 4. Case 1: Diffuse infiltration of the dermis by vacuolated and parasitized macrophages. (hematoxylin and eosin, magnification × 160)

Parasite Characterization

Parasites were easily obtained from all six cases and were characterized as *Leishmania amazonensis*. Promastigotes were typed using species-specific monoclonal antibodies, and in three cases also by isoenzymes and karyotype analysis. Such speciation was confirmed in all cases by in situ immunoperoxidase staining in the paraffin-embedded sections of lesions using species-specific antiamastigote monoclonal antibodies.

DISCUSSION

The cases described here fulfill the accepted criteria for their classification as DCL. They present: (1) multiple nodules and infiltrated plaques; (2) slow but progressive spread of lesions; (3) no evidence of visceralization; (4) negative skin test response to leishmanin (Montenegro test); (5) absence of destructive mucosal lesion; (6) lesions with vacuolated macrophages with heavy parasite burden; (7) resistance to pentavalent antimonial therapy and other chemotherapeutic agents. Clinically they are easily differentiated from post–kala-azar dermal leishmaniasis, and they also differ from the disseminated cutaneous leishmaniasis, a condition with multiple lesions but in which an immunologic responsiveness to leishmanial antigens is present. Disseminated cutaneous leishmaniasis develops in a short time (a few weeks), the lesions are small, acneiform, and less frequently appear as small ulcers. The patients respond to antimonial therapy; histologically, parasites are rare or absent, and the classical lymphoplasmacytic infiltration in the absence of the macrophagic pattern is observed.

Although all the cases presented here fulfill the criteria for being classified as DCL, they do show a spectral distribution in many regards. A more precise description of the cases would be obtained by defining a clearly unresponsive pole (cases 1, 2, and 3) and a subpolar form (cases 4, 5, and 6) with varying degrees of antiparasite responsiveness. The presence of polar and subpolar anergic disease is also observed in lepromatous leprosy, a disease with a spectral distribution similar to leishmaniasis. Case 6 presented clinical characteristics similar to those observed in borderline leprosy, such as coppery discoloration, punched-out lesions, asymmetric distribution of lesions, and a microscopically granulomatous reaction.

With a large variation in parasite numbers and with polar DCL cases (classical) having the highest parasite burden, all the cases presented here have many more parasites than classic cutaneous leishmaniasis. The high frequency of marked vacuolated macrophages, a hallmark of DCL, shows a variation closely related to the degree of parasitism. Lytic necrosis and granulomatous reaction appear only in the cases more distinct from the polar DCL picture.

All DCL cases reported here had antileishmanial IgG antibodies at titers much higher than those observed in CL cases.[12] Bittencourt et al. have shown, in contrast to previous reports in the literature, that there was no defect of humoral immunity in DCL.[15] One of the polar forms of DCL (case 1) exhibited an extremely elevated

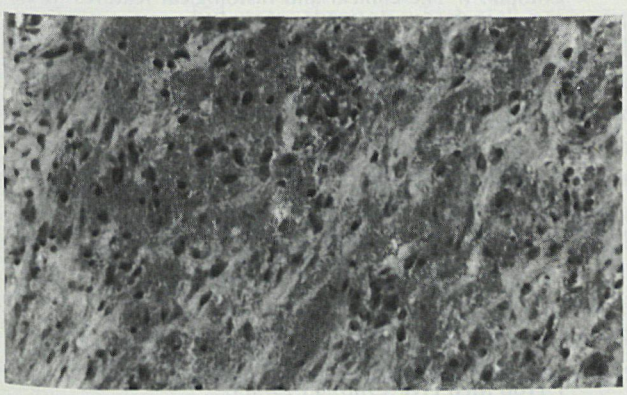

Figure 5. Case 5: Area of lytic necrosis. See disintegrated macrophages and a finely granular, eosinophilic material among them. (hematoxylin and eosin, magnification × 256)

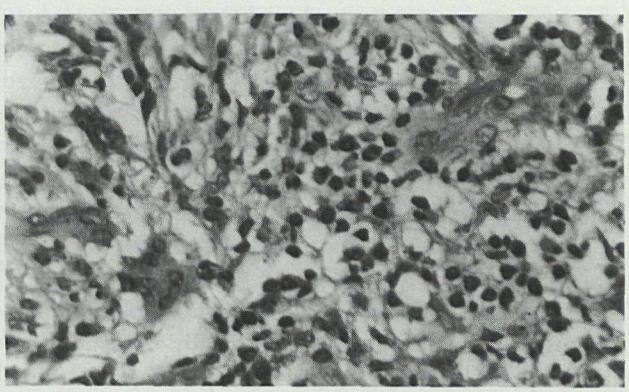

Figure 6. Case 6: Infiltration of mononuclear cells and some eosinophils. At right, three multinucleated giant cells. (hematoxylin and eosin, magnification × 400)

Table 3. Immunologic Findings

Patient (No.)	PHA*	Con A*	PWM*	Candida*	PPD*	Leishmania		
						CPM**	E/C†	IFA‡
1	86.5	157.3	40.7	11.1	7.7	2488 ± 833	2.2	>16000
2	64.7	151.1	22.2	1.2	5.5	3065 ± 180	1.2	2048
3	118.6	139.9	20.9	ND	ND	2616 ± 156	1.0	1024
4	565.2	377.4	12.3	5.1	6.0	2833 ± 790	0.7	512
5	424.6	417.1	85.5	ND	ND	1577 ± 766	1.9	512
6	195.3	234.5	24.8	0.9	ND	552 ± 65	1.0	1024

E/C = experimental/control candida at 10 µg.
*Blastogenesis expressed as E/C; Phytohemagglutinin (PHA) used at 1:100; Concanavalin A (Con A) at 10 µg/mL; Pokeweed mitogen (PWM) at 1:100; Candida at µg/mL; PPD at 2 µg/mL.
**Blastogenesis with leishmania antigen (10 µg/mL) expressed as counts per minute.
†Blastogenesis with leishmania antigen (10 µg/mL) expressed as E/C.
‡Immunofluorescence assay (inverse titer).

titer, only observed in visceral leishmaniasis. Our observations of elevated antibody titers in the absence of cell-mediated immunity are similar to those found in several other conditions, such as visceral leishmaniasis[16] and lepromatous leprosy; this imbalance probably reflects the predominance of Th-2 over Th-1 lymphocytes.[17,18]

The antileishmanial skin test is usually negative in DCL, but sporadic positive responses occur in patients who regularly have negative skin tests to leishmanial antigen.[7] A similar observation was made in the present series about the three patients who are more removed from the polar DCL classification. Absence of proliferative responses of lymphocytes to leishmania antigen was observed in all patients, as expected in DCL. The observation of lower mitogen-driven T cell responses in polar patients suggests that the abnormalities in cell-mediated immunity were more evident in this group.

Lymphoplasmacytic infiltration may be either present with variable frequency or absent in classic DCL.[19] Such differences were related to the stage of development of lesions, and the appearance of lesions is not necessarily the same in all lesions of the same patient. This is also evidenced by the spontaneously healing of some DCL lesions.[19]

CONCLUSIONS

The present report shows that some DCL patients may show aspects correlated with resistance in their clinical-histologic pictures, such as ulcerations, reduction in parasitism, marked cytolytic necrosis, and the presence of granulomatous reaction. Because in all forms of leishmaniases, recovery from and resistance to disease is dependent on effective T cell responses, the classification of DCL patients into polar (totally unresponsive) and subpolar (weakly responsive) forms may contribute to the understanding of their disease course and outcome; however, such differences are only demonstrable through a careful clinical and pathologic evaluation because polar and subpolar forms of DCL generally present with no antileishmania cell-mediated immune responses as measured by *in vitro* tests performed with peripheral blood lymphocytes.

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On Bathing

To many persons the descriptions and explanations below may seem unnecessarily minute, but they will not, I think, to him who has had much experience in giving instructions for home-treatment. He who has seen persons attempt to take sitz-baths in wash-bowls, to take half-baths without undressing, to give a dripping-sheet by wetting one corner of the sheet in cold water, or to give hot fomentations with a small linen towel, or a bit of flannel as large as his two hands, has learned how crude are the notions of the people in regard to the whole matter of water-treatment. A vast deal of injury has been done in this method of treatment, as well by the bungling use of appliances, which, if skilfully used, would have been entirely proper, as by the use of such as were wholly unsuited to the person to whom they were administered.

We do not give *heroic* treatment. We do not believe in it. Our baths are all mild, and given at not very frequent intervals. The first thing to be done when a bath is to be given, is to prepare the room, making it a *comfortable temperature*. The second is to prepare the bath, using *soft water*, and making it of the *right temperature, as indicated by a thermometer*. Persons sometimes ask us to explain what we mean by certain temperatures, so that they can get along without a thermometer. This is impossible. The terms hot, cold, warm, tepid, are so indefinite, and convey so different impressions to different persons, as to be entirely unreliable in giving directions. What is hot to one person is cold to another, in the morbid states through which sick persons pass. And the sensations of healthy persons are so variable, that they cannot be relied upon to temper baths by the touch for those with whom a slight change is of consequence. Of course the line where cold passes into tepid, or tepid into warm is inappreciable, but in general terms I should consider a bath at 75° Fahrenheit, cold; at 85°, tepid; at 95°, warm; and at 105°, hot. The idea that the hotter a person is the colder should be his bath, is productive of great mischief. The true rule is exactly the reverse of this. That is, a person in a *high fever* should have his bath at a *higher temperature* than if he had no fever; for what, in the latter case, would be a pleasant temperature to him, might be shockingly cold in the former. So, while, in such conditions, a bath at 90° would subdue the fever, one at 75° would be likely to produce violent reaction, and in half an hour the fever would be higher than before. *From Austin HN. Baths, and how to take them. New York, 1873:3.*