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BORDERLINE CUTANEOUS LEISHMANIASIS

Clinical, Immunological and Histological Differences from Mucocutaneous Leishmaniasis

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SUMMARY

A case of borderline cutaneous leishmaniasis is described from Bahia, Brazil. The patient had disseminated lesions of long standing, an atypical intradermal response to leishmanial antigen, and moderate to rare numbers of leishmania in 5 of 6 biopsies examined. He showed no delayed hypersensitivity to any of 6 other recall antigens tested and could not be sensitized to DNCB. These results are contrasted with those obtained in 6 clinically typical mucocutaneous leishmaniasis patients from the same geographical area. Examination of Epon-embedded histological sections demonstrated that basophil type delayed hypersensitivity does not occur in chronic cutaneous leishmaniasis lesions.

INTRODUCTION

Cutaneous leishmaniasis is a disease whose clinical form is influenced not only by the species and subspecies of the parasite, but also by the immunological response of the host. BRYCESON [3], in a study of African cutaneous leishmaniasis, classified disease forms in a system similar to that applied in leprosy. The spectrum, according to histopathological features, includes: MM, macrophage, an anergic form similar to lepromatous leprosy; MI, macrophage-intermediate; II, intermediate, analogous to borderline leprosy; IT, intermediate tuberculoid, and TT, tuberculoid. As in leprosy, the spectrum is associated with varying cellular immune responsiveness to the parasite.

Classic South American mucocutaneous leishmaniasis conforms to the TT classification of BRYCESON [1]; and South American cases of diffuse cutaneous leishmaniasis of an anergic form at the opposite (MM) pole of the spectrum have been described [2,4]. However, intermediate forms which would serve to substantiate this immunologically associated disease spectrum have not been previously documented from Brazil.

Many questions still remain to be answered concerning mucocutaneous leishmaniasis. Though previous studies have shown that patients with typical South American leishmaniasis produce positive serological [6], immediate hypersensitivity [12], and delayed hypersensitivity [12] responses to various leishmanial antigens, no detailed investigations have been made of the general responsiveness of the immune system of such patients. In addition, the possible involvement of basophil type delayed hypersensitivity [5] in cutaneous leishmaniasis has not been investigated, since routine fixing and staining techniques are not adequate for such a study, and special techniques are necessary [5,10]. Finally, the absence of described intermediate forms and the paucity of comparative studies of immunologically different

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forms from the same geographical area have made it difficult to determine which pathological features might be associated with the cellular immune response of the host and which might be related to other factors.

In this report, a case of borderline or II form cutaneous leishmaniasis is described from Bahia, Brazil. Clinical, immunological and histopathological features of this case are compared with those of other mucocutaneous leishmaniasis patients from the same geographical area.

MATERIAL AND METHODS

Selection of cases

Subjects were examined as they became available as inpatients in participating hospitals. A general history and history of leishmanial infection were taken using a standard questionnaire form.

Intradermal testing

A battery of nine intradermal tests was administered in predetermined locations of the patient's upper back. The antigens used included: leishmanin (Instituto Adolfo Lutz, São Paulo); PPD, 2 UT (National Anti Tuberculosis Campaign Reference Laboratory, Brazil); coccidioidin, 1:100 (Cutter Laboratories, USA); purified phytohaemagglutinin (PHA), 10 μg/ml (Burroughs Wellcome, England); trichophyton (Laboratório de Alergia e Imunopatologia, Rio); levedurin (Lab. de Alergia e Imunopatologia, Rio); histoplasmin (Parke Davis, USA); mumps (Eli Lilly, USA); and schistosome adult worm (National Center for Disease Control, USA). Wheal or induration areas were recorded and measured at 15 min, 4, 10, 24, and 48 hr. An immediate (15 min) reaction area of 1.2 cm^2 or greater and a delayed reaction area of 0.6 cm^2 or greater were considered positive. An Arthus reaction was defined as a response at 4 and/or 10 hr which had disappeared by 24 hours.

DNCB sensitization

Contact sensitization with 2,4 dinitrochlorobenzene (DNCB) was carried out by a modification of previously described techniques [13]. Sensitization was made using a patch with 2 mg DNCB, a patch with 100 μg DNCB being applied simultaneously to test for possible previous sensitization. Patches were left undisturbed for 48 hr.

The patient was challenged between 2 and 4 wk later by aplication of a patch with 100 μg DNCB, applied near the site of the original patches. After occlusion for 48 hr, the patch was removed and the reaction was read at 48 and 72 hr. Reactions were scored as: no visible reaction (−); erythema (+); erythema and induration (++); erythema, induration and vesiculation (+++).

Leishmanial antigens

A strain of **Leishmania mexicana** from Fundação Gonçalo Moniz, Salvador, Brazil, was maintained in culture by previously described techniques [7].

The supernatant of a crude saline extract of washed promastigotes was used for double diffusion studies. Protein nitrogen, determined by Lowry technique using a BSA standard, was 400 μg N/ml. The solution was lyophilized and stored at -20°C until reconstituted to the original volume for use.

Formalinized promastigotes for the indirect fluorescent antibody test (FAT) were prepared by methods recommended by GUIMARÃES et al. [6].

Serological methods

Micro-Ouchterlony double diffusion in gel (DD) was performed by previously described techniques [9].

For the FAT, 2 fold dilutions of sera were examined starting with a 1:20 dilution, each slide including a control for the fluoresceinated anti-human IgGAM (Hyland Laboratories, USA) used. Slides were examined immediately on a Zeiss fluorescence microscope with HBO 200 W/4 mercury vapor lamp, exciter filters BG38 and BG12 and barrier filters 53 and 44. The result was considered positive if bright homogeneous fluorescence was present around the entire periphery of the parasites.

The intradermal and serological techniques used with leishmanial antigens produced

no positive reactions either in normal human controls or in a case with non-leishmanial ulcerative skin lesions.

Biopsies

Punch or elliptical biopsies were taken under local anesthesia from 5 of the 7 patients. Tissue was fixed in buffered neutral 10 per cent formalin, and paraffin-embedded sections were stained with haematoxylin-eosin, Prussian blue and methyl green pyronin.

In addition, within 30 sec of biopsy small fragments were fixed in cold 2 per cent gluteraldehyde in 0.15 M cacodylate buffer, pH 7.4, post fixed in 1 per cent OsO_4 and embedded in Epon. One micron sections cut on a Reichert OMU2 ultramicrotome were permanently mounted on glass slides following staining with toluidine blue [11]. Vascular alterations and the presence of basophils and mast cells were analyzed in these Epon-embedded sections.

RESULTS

Clinical findings

Six of the seven cases studied presented clinically typical forms of mucocutaneous leishmaniasis (Table I), including one case (No. 3) with a primary ulcer and the others (No. 2, 4-7) with cutaneous or mucosal secondary lesions. Patient No. 1, however, had disseminated nodular and papular lesions over the face, upper limbs and trunk, while the lower legs and feet showed large ulcerovegetative lesions. There was invasion of nasal mucosa without destruction of the nasal septum. This patient had an impalpable liver and normal liver function tests, and a bone marrow aspirate was negative for leishmania.

TABLE I
Clinical findings in leishmaniasis patients

Case No. Age/Sex	Clinical Form of Disease	Total Duration	History of Previous Treatment
1 75/M	Disseminated nodular and papular lesions, ulcerovegetative on lower legs	18 mo	none
2 55/M	mucocutaneous, with ulcerovegetative malar lesions	5 yr	prev. treatmt; relapse
3 34/M	ulcerated primary lesion	5 mo	none
4 35/M	mucocutaneous; destruction of larynx	7 yr	prev. treatmt; relapse
5 39/F	ulcerated relapse of primary lesion; 2 distant cutaneous metasteses	2 yr	none
6 68/F	extinct mucocutaneous form; scars of primary and mucosal lesions	15 yr	denies prev. treatment
7 61/M	mucocutaneous; destruction of larynx	10 yr	prev. treatmt; relapse

Immunological responses

Of the two serological tests employed to detect anti-leishmanial antibodies (Table II), the indirect FAT showed greater sensitivity than DD. However, titers in the FAT were not high and were not obviously related to the clinical form of the disease. Positive DD results, in contrast, were found only in subjects with mucosal involvement. Serology was repeated in patients No. 3 and 5 after one month of specific treatment, without alteration of results.

The intradermal test with leishmanial antigen (Table II) was positive at 48 hr in the 6 patients with clinically typical leishmaniasis. Reactions were also present at 4 hr in 4 patients, but could only be distinguished as an Arthus reaction in one case (No. 3) in which the reaction was no longer present at 10 hr. The responses of patients No. 2 — No. 7 to other intradermal antigens demonstrated that in general the effector mechanisms of the various types of hypersensitivity response were intact. All these patients could be sensitized to DNCB.

TABLE II
Immunological responses of leishmaniasis patients

Case No.	Responses to Leishmanial Ag					Other Intradermal Responses(*)
	Serological		Intradermal			
	FAT	DD	4hr	24hr	48hr	
1	1:80	—	—	+	—	IHS (Sch); PHA (+); DNCB (—)
2	1:160	1 line	+	+	+	IHS (Sch); Arthus (Sch); DHS (PPD); PHA (+); DNCB (+++)
3	1:40	—	+	+	+	IHS (Sch); Arthus (Sch); DHS (hist); PHA (+); DNCB (+)
4	1:40	1 line	—	+	+	DHS (hist); PHA (+); DNCB (+++)
5	1:160	—	—	+	+	IHS (Sch); Arthus (Sch); DHS (PPD); PHA (+); DNCB (+)
6	1:20	—	+	+	+	IHS (Sch); Arthus (Sch); DHS (PPD); PHA (+); DNCB (+++)
7	1:40	1 line	+	+	+	IHS (Sch); PHA (+); DNCB (+++)

(*) DHS — delayed hypersensitivity; hist — histoplasmin; IHS — immediate hypersensitivity; Sch — schistosome adult worm antigen.

Patient No. 1 showed positive serological reactivity to leishmanial antigen in the indirect FAT. His immediate hypersensitivity response to schistosomal antigen demonstrated that the expression of this type of humoral immune response was also intact. However, expression of cellular immunity was evidently deficient in this patient. The transient intradermal response (1.1 cm^2) to leishmanial antigen at 24 hr, disappearing before 48 hr, is atypical; the patient would have been considered as non-reactive in routine testing in which the reaction is only examined at 48 hr or later. In addition, he showed no positive delayed intradermal response to any other antigen tested and could not be sensitized to DNCB, though the response to PHA was positive.

Histopathology

In biopsies of four patients with positive cellular immune responses to leishmanial antigen, no parasites were found in the lesions (Table III). An intense and diffuse granulomatous inflammatory reaction with plasma cells, histiocytes, lymphocytes and a few neutrophils, together with epithelioid and giant cells, was observed. Plasma cells predominated in the infiltrates; and in case 3 fibrinoid necrosis was also observed. Marked pseudoepitheliomatous hyperplasia and exocytosis were seen in all the four cases. The histological aspects placed these four cases in the tuberculoid or TT form in Bryceson's classification.

In Case No. 1, in contrast to the other cases, leishmania parasites were identified in five of six biopsies. In these biopsies with parasites, some lymphocytes and plasma cells were seen mixed with histiocytes, but without granulomatous reaction (Fig. 1). In biopsy c (Table III), inflammatory infiltrate was found only in the deep dermis and subdermis. Histologically these lesions conformed to Bryceson's II classification, though the number of parasites varied from moderate to rare. In one lesion (f), no parasites were found and a well circumscribed granulomatous reaction with epithelioid and giant cells was found in the dermis (Fig. 2). Histologically this lesion conformed more to the TT classification of Bryceson; however, the epidermis was not ulcerated. None of the lesions showed the intense proliferation of heavily parasitized histiocytes or subepidermal clear zone which are found in the anergic form of the disease.

In 1 micron Epon-embedded sections, basophils were rarely seen, though mast cells were occasionally observed. These results were confirmed by electron microscopy [8]. Vascular

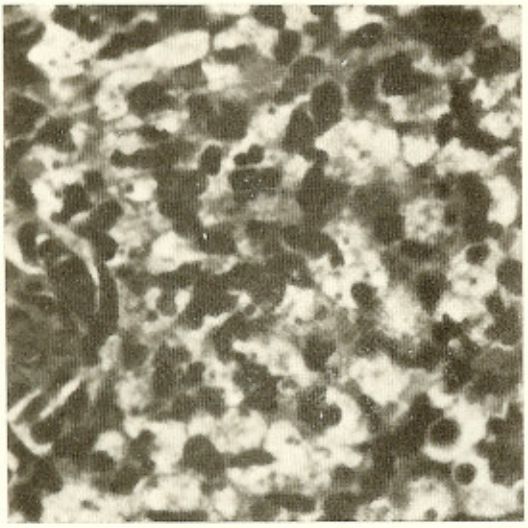

Fig. 1 — Patient 1, biopsy d. Presence of numerous vacuolated histiocytes containing leishmania. H.E., 400 X

alterations, including congestion, were observed and were similar for all cases. Arterioles, meta-arterioles, and capillaries were observed, and the majority showed hyperplasia, edema and tumefaction of endothelial cells, with projection of nuclei into the vascular lumen. In the subendothelial zone of the vessels, edema was present, along with deposits of homogeneous material in irregular distribution. In the media, muscle cells were altered, with blurring of cell margins; increase of intercellular substance, edema, and deposits of homogeneous material were also present. Occasional inflammatory cells were encountered in the vascular wall.

DISCUSSION

Patient No. 1 presented clinical, immunological and histological features which clearly distinguished this case from typical cases of

TABLE III
Histopathology of leishmaniasis lesions

Case No.	Appearance of Lesion(*)	Presence of Leishmania	Predominance of Plasma Cells	Granulomatous Reaction	PSE(**) Hyperplasia
1. a.	UN	++	+	—	+
b.	V	+	+	—	+
c.	N	rare	—	—	—
d.	IM	++	—	—	—
e.	IM	rare	—	—	—
f.	N	—	—	+	—
2, 3, 4, 5	2—UV; 3—U; 4—IM; 5—V; U	—	+	+	+

(*) IM — infiltrated mucosa; N — nodular; U — ulcer or ulcerated; V — vegetative.
(**) PSE — pseudoepitheliomatous

leishmaniasis from the same geographical region. Widely disseminated nodular lesions of long duration, not seen in typical mucocutaneous leishmaniasis, are found in the rare anergic form of diffuse cutaneous leishmaniasis already described from this region [2], as well as from other areas of South America and Africa [3]. However, in Case No. 1 some lesions assumed an ulcerovegetative appearance, which is not a clinical feature of the anergic form. Detailed examination of immunological and histological aspects also demonstrated differences from both the mucocutaneous and the anergic form. A transient intradermal response was present at 24 hr, and though most lesions contained leishmania, the parasites were never superabundant. Post Kala-Azar dermal leishmanoid was ruled out by the lack of visceral or bone marrow involvement and the absence of previous treatment. Histologically, immunologically and clinically this case represents a borderline form between the pole of anergic diffuse cutaneous leishmaniasis and that of chronic mucocutaneous leishmaniasis. The histological features conformed to the II form of Bryceson's classification.

In our patients with classic mucocutaneous leishmaniasis, expression of the various types of immune response was intact, includ-

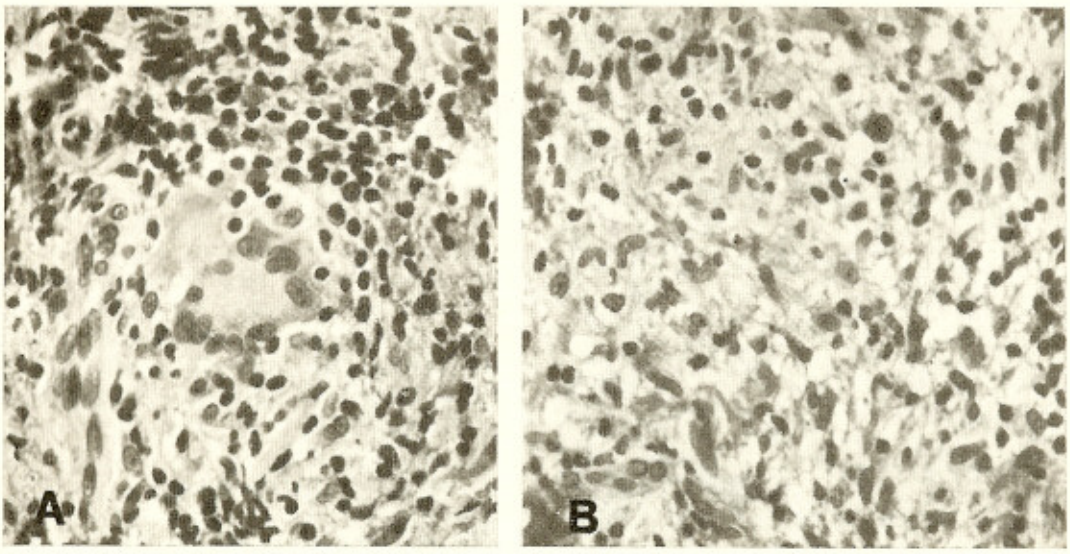

Fig. 2 — Patient 1, biopsy f. A) Granulomatous reaction with giant cell. B) Granulomatous reaction with epithelioid cells. H.E., 200 X

ing the ability to mount a response to a new contact sensitizing agent, DNCB. The cellular response in the lesions resembled classic cellular immunity, rather than basophil type hypersensitivity. DVORAK et al. [5], in a quantitative study of inflammatory reactions in delayed hypersensitivity tests, reported a more frequent association of basophil infiltration with contact type hypersensitivity than with classic delayed hypersensitivity to protein antigens such as tuberculin. The effectiveness of basophil type hypersensitivity as a host defense mechanism has not been specifically investigated.

The absence of basophil type hypersensitivity in chronic mucocutaneous leishmaniasis suggests that the lesions do not represent a contact type delayed response to residual parasite antigens linked, perhaps, to host tissue. Since intact parasites are very difficult or impossible to locate in the lesions, it was considered of interest to investigate this hypothesis.

Vascular alterations in our cases were similar to those described by DVORAK et al. [5] in delayed hypersensitivity reactions. However, similar alterations were present to an equal degree in Patient No. 1, in whom delayed hypersensitivity was not fully expressed; thus other or additional mechanisms may have produced vascular damage. The presence in the infiltrates of numerous plasma cells, which are not found in delayed hypersensitivity reactions [5], indicates that humoral immune responses might also contribute to the complex pathogenesis of mucocutaneous leishmaniasis lesions.

Our results re-emphasize a feature which distinguishes Brazilian from Old World cutaneous leishmaniasis. In the Brazilian form, chronic secondary lesions and even relapse after therapeutically induced clinical remission often occur in the face of an active humoral and cellular immune response. The immune response may determine the clinical form of the disease, but does not necessarily produce clinical cure.

RESUMO

Leishmaniose cutânea tipo "borderline" Diferenças imuno e histológicas em relação à leishmaniose muco-cutânea

Neste trabalho é descrito um caso de leishmaniose cutânea, forma "borderline", procedente da Bahia, Brasil. O paciente apresentava lesões disseminadas de longa evolução e uma resposta intradérmica atípica ao antígeno da leishmânia. Em cinco das seis biopsias realizadas havia parasitismo de raro a

moderado. O paciente não apresentou hipersensibilidade retardada a nenhum dos vários tipos de antígenos testados e não foi sensibilizado pelo DNCB. Estes resultados contrastaram com os obtidos em seis casos típicos de leishmaniose cutâneo-mucosa da mesma área geográfica. Ficou comprovado que na leishmaniose cutânea não ocorre reação de hipersensibilidade mediada por basófilos.

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