

## DIFFUSE CUTANEOUS LEISHMANIASIS WITH ATYPICAL ASPECTS

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### Abstract

A 16-year-old man had long-standing diffuse cutaneous leishmaniasis with the following characteristics: diffuse infiltrated lesions rich in amastigotes, absence of mucosal involvement, and lack of parasite-specific cell-mediated immune response. *In situ* identification of *Leishmania mexicana amazonensis* was achieved by the use of monoclonal antibodies. Clinically, as an atypical finding there was deep and extensive ulceration in the lower limbs. Histologically, an atypical characteristic was the presence of a high number of eosinophils in the infiltrate predominantly in the ulcerated lesion. Ultrastructurally, parasitized and lysed eosinophils with dispersion of their granules were seen in the vicinity of parasitized or lysed macrophages.

Diffuse cutaneous leishmaniasis (DCL) is a polar form of cutaneous leishmaniasis characterized by largely disseminated nodules, abundance of parasites throughout the course of disease, and absence of parasite-specific cell-mediated immune response. Microscopically, DCL is represented by a monomorphic cellular infiltrate of vacuolated and heavily parasitized macrophages, sometimes with foci of lymphoplasmacyte infiltration.<sup>1-5</sup> We present here an atypical case of DCL, which exhibited extensive ulcerations and, microscopically, an intense eosinophil infiltrate, a characteristic seen for the first time in this form of human leishmaniasis.

### Case Report

A 16-year-old man presented with a history of several nodular ulcerated lesions involving the whole body since the age of one year. He was born in Pinheiro (Maranhão State, Brazil). He had previously received several courses of pentavalent antimonial (Glucantime) and amphotericin B with-

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out improvement. The dermatologic examination showed erythematous nodule lesions disseminated over the whole body, being more numerous on the upper and lower extremities. In the ears, the nodules show confluence, and in the malar region of the face, there are infiltrated plaques. The lower half of the nose is severely infiltrated conferring a tumor-like appearance (Fig. 1A). On the feet, two ulcerated tumor-like lesions measure 8x7 cm and 5x5 cm. There are six other large ulcerations, with infiltrated borders and granulomatous bottom, the larger measuring 8x5 cm and the smaller 3.5x3 cm, located on the feet and the anterior aspect of the left leg (Fig. 1B). There is also enlargement of the volume of all pododactyles, and thick and rough nails are observed. Immunoperoxidase staining using anti-*Leishmania mexicana amazonensis* amastigotes-specific monoclonal antibodies<sup>6</sup> showed a positive reaction confirming the species

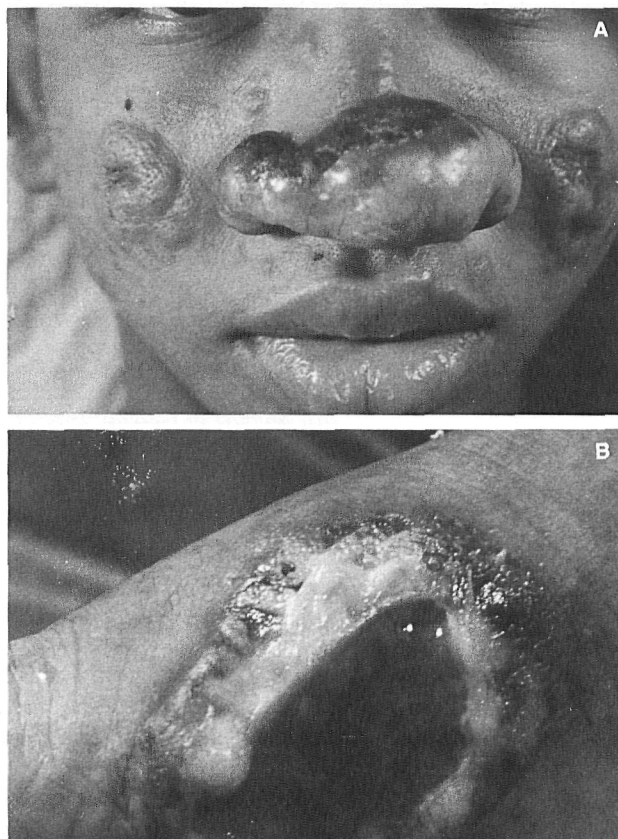


Figure 1. A, tumor-like lesion on the nose and infiltrated plaques in the malar regions. B, a deep ulcer on the foot.

of the parasite present. Anti-*Leishmania* cell-mediated immunity, as tested by delayed type hypersensitivity reaction (Montenegro's test) and by lymphocyte transformation test<sup>7</sup> was negative.

## RESULTS

### Pathological Study

**Ulcer border.** Sections exhibit moderate acanthosis and parakeratosis. In the dermis, there is infiltration of lymphocytes, plasma cells, macrophages, and eosinophils, and small focal areas of vacuolated and parasitized macrophages surrounded by mononuclear cells and eosinophils (Fig. 2A). In local areas, there is a predominance of eosinophils, many of them showing degranulation and parasites free in the interstice (Fig. 2B).

**Nodule.** The epidermis and a subjacent strip of connective tissue have a normal appearance. The dermis is diffusely infiltrated by vacuolated and parasitized macrophages with scattered plasma cells. Most of the macrophages have a moderate number of parasites, whereas some are heavily parasitized. Few parasites are present in the interstitial tissue.

### Ultrastructural Study

Histopathologic differences were observed between the ulcerated lesion and the nodule. It is noteworthy that in ulcerated lesions there was a predominance of eosinophils among inflammatory cell infiltrate, which was also composed of macrophages, scarce lymphocytes, and plasma

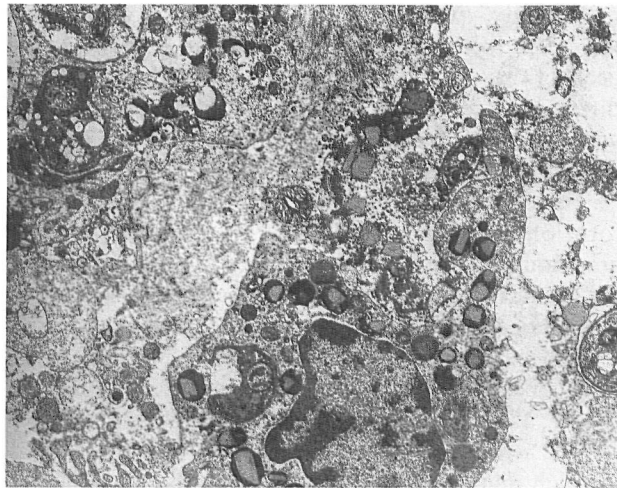


Figure 3. Ulcer. Eosinophils with damaged amastigotes. Parasites and granules are seen in the interstice. (original magnification  $\times 7000$ )

cells. Many eosinophils were lysed, and their granules were seen in the interstitial space, sometimes close to macrophages. Few eosinophils contained amastigotes within parasitophorous vacuoles. Most of the parasites appeared degenerated in the interstitial tissue or phagocytized by macrophages. Generally such macrophages were lysed showing rupture of the cytoplasmic membrane. In the nodular lesion, the parasitism was more marked than in the ulcerated lesion, and macrophages predominated over eosinophils. A lot of degenerated or lysed amastigotes were seen in the interstice. Some eosinophils contained parasites within cytoplasmic vacuoles. Only a few macrophages were lysed.

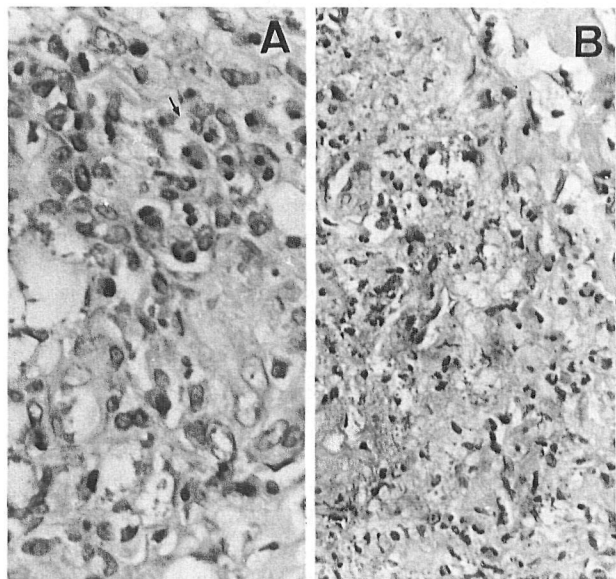


Figure 2. Ulcer. A, vacuolated and parasitized macrophages, some plasma cells, and on the right, eosinophils (arrow). B, amastigotes and eosinophil granules in the interstice associated with disintegrated eosinophils. (hematoxylin and eosin stains, original magnifications  $\times 320$ (A),  $\times 192$ (B))

## DISCUSSION

This patient presented most of the characteristic aspects of DCL, such as nodular and infiltrative lesions disseminated throughout the body with absence of mucosal involvement, a high parasite load in the lesions, lack of cell-mediated immune responses to *Leishmania* antigen, and a poor response to antimicrobial treatment. But different from the classical aspect of DCL, the patient presented large and deep ulcers and a great number of eosinophils in the inflammatory infiltrate. Ulcerations, when present in DCL, are superficial and small, but without the histopathologic aspects observed here, and appear on the surface of infiltrated lesions exposed to trauma.<sup>1</sup> Considering the sites of the ulcerated lesions in the present case (feet and anterior aspect of the leg) and the fact that the patient has a history of disease dating from his first year of life (a child is considerably more exposed to trauma), these ulcerations could be related to trauma. Other factors may be involved in the pathogenesis of these lesions because they are very large and persistent and do not represent secondary lesions.

Recently, two other cases of DCL with ulcerations were described, both having the immunopathologic aspects of this polar type of leishmaniasis.<sup>8,9</sup> As antigenic differences can occur among isolates from patients with DCL,<sup>10</sup> we can speculate if these atypical aspects are related to these antigenic differences.

These patients cannot be considered as borderline form of leishmaniasis, because they did not present granulomatous reaction.<sup>11</sup> Similarly to other cases with the classical presentation of DCL, a predominance of degenerated and dead parasites, most of them in the interstice, were observed ultrastructurally. The presence of lysed amastigotes indicated a cell-mediated immune response, although it was unable to control the disease.<sup>12</sup>

One aspect never before observed in human DCL was the high number of eosinophils, being the predominant cell type in the ulcer and many of them exhibiting degranulation. The association of lysed parasitized macrophages, dead parasites, and eosinophils was frequently seen and may indicate a parasitocidal role of eosinophils. In the experimental infection of mice by *L. amazonensis* and *L. mexicana*, eosinophils can predominate in the infiltrate.<sup>13,14</sup> The presence of these cells does not correlate with immune status, as they are observed in resistant (C57BL16) and susceptible BALB/c strains of mice and also in immunized and nonimmunized BALB/c.<sup>14</sup> Several yet untested hypotheses have been put forward concerning the parasitocidal action of eosinophils, either by phagocytosis, extracellular granule release, or by cooperation between eosinophils and macrophages.<sup>13,14</sup> It has been suggested that the immune response in the mouse is dependent on the balance of the two helper T cells with largely diverse functional activities, Th1 or Th2 cells.<sup>15</sup> In mice, high numbers of eosinophils are correlated to high levels of IL-4 and IL-5, lymphokines synthesized by Th2 lymphocytes<sup>15</sup> and such cells are activated upon the infection of susceptible mouse strains by *L. amazonensis*.<sup>16-18</sup> Although these types of clones have not been described in humans such a scenario would help to understand the presence of a partial unresponsiveness, but not anergy, in DCL patients, responsible for a very limited immune response, which may vary from lesion to lesion. A predominance of Th2 type cells would also explain the occasional findings of a predominant eosinophil infiltration in lesions from DCL patients.

#### CONCLUSIONS

This case demonstrated that ulceration can occur in DL not only related to trauma, and it is associated with a marked infiltration of eosinophils and ultrastructural indication of a parasitocidal action of these cells.

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