

IN SITU IDENTIFICATION OF *LEISHMANIA AMAZONENSIS* ASSOCIATED WITH DIFFUSE CUTANEOUS LEISHMANIASIS IN BAHIA, BRAZIL

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Diffuse cutaneous leishmaniasis (DCL) is the generalized spread of non-ulcerating cutaneous nodules. Immunologically, there is a positive antibody response, but a negative cellular or delayed type hypersensitivity response to leishmanial antigen. The parasitism is typically intense with a heavy accumulation of parasitized and vacuolated macrophages in the lesions and there is a poor response to treatment (R. Medina & J. Romero, 1962, *Arch. Venez. Pathol. Parasitol. Med.*, 4: 349-353; A. Bittencourt et al., 1968, *Med. Cut. ILA*, 2: 395-402). In the New World, DCL has been associated with different parasite species of the *Leishmania mexicana* complex (for review see G. Grimaldi et al., 1989, *Am. J. Trop. Med. Hyg.* In press). In Brazil, however, the disease has only been associated with *L. amazonensis* infection, based on data from the Amazon region (M. Miles et al., 1980, *Trans. R. Soc. Trop. Med. Hyg.*, 74: 248-252; G. Grimaldi et al., 1987, *Am. J. Trop. Med. Hyg.*, 36: 270-287). The current concept is that cutaneous leishmaniasis is rarely due to *L. amazonensis*, but approximately 30% of human infection with this parasite evolve to DCL (R. Lainson, 1983, *Trans. R. Soc. Trop. Med. Hyg.*, 77: 569-596). In contrast, data from other regions in Brazil show that this anergic form of the disease is indeed unusual, representing only a few reported cases (A. Bittencourt & N. Guimarães, 1968, *Med. Cut. ILA*, 2: 395-402; A. Fonseca et al., 1981, *Med. Cut. ILA*, 9: 317-322; F. Nery Guimarães, 1951, *Hospital*, 40: 11-24; A. Silva et al., 1981, *Rev. Inst. Med. Trop. S. Paulo*, 23: 31-35; H. Portugal & A. Porto Marques, 1960, *Hospital*, 57: 813-823). Confirming this view, we recently demonstrated that despite the relative high frequency of

L. amazonensis infection in Bahia, only one case of DCL was associated with this parasite (A. Barral et al., 1989, *Am. J. Trop. Med. Hyg.*, in press). Here we extend this study, detecting three more cases of DCL due to *L. amazonensis* infection, by *in situ* identification of the parasite, employing an immunocytochemical technique and an appropriate monoclonal antibody. All cases from Bahia (from the cities of Itacaré, Mutuípe and Santarém) analyzed in this study represent paraffin-embedded biopsed sample material, obtained from the lesions more than 20 years ago. Those samples were initially treated with a 3 M urea solution for 5 minutes, to improve the reactivity of the antigen determinant with the monoclonal antibody (G. Grimaldi et al., 1983, *X Reun. An. Pesq. Bas. D. Chagas, Caxambu* — MG, Brazil). After rinsing, the sections were incubated with the reactive monoclonal antibody (XCIII-3D8-D8, kindly provided by Dr Diane McMahon-Pratt, Yale University School of Medicine, New Haven, CT, USA) and stained with a revealing probe consisting of an immunoperoxidase (avidin-biotin) reaction, using as substrate 3,3' diaminobenzidine tetrahydrochloride (DBA) or aminoethylcarbazole in acetate buffer (A. Barral et al., *loc. cit.*). This method was shown to be simple and efficient in the *in situ* differentiation of parasites; the sections showed a heavy parasitism, represented by clusters of amastigotes stained in brown when DAB was used as substrate and in red when carbazol was the substrate.

Although the monoclonal antibody used in this study was produced against *L. pifanoi* amastigotes, it also reacts with *L. amazonensis* amastigotes (A. Pan & D. McMahon-Pratt, 1988, *J. Immunol.*, 140: 2406-2414). Considering that *L. pifanoi*, a parasite originally associated with DCL in Venezuela (R. Medina & J. Romero, *loc. cit.*) is presently not recog-

nized as a species distinct from *L. mexicana* (for review see G. Grimaldi et al., *loc. cit.*) the positive reaction obtained with this monoclonal antibody was related with *L. amazonensis* infection. In addition, molecular characterization of promastigotes forms,

obtained by cultivating biopsy samples from one of the DCL cases, also identified this parasite as *L. amazonensis*. In conclusion, this study indicates that all cases of DCL so far detected in Bahia were due to *L. amazonensis* infection.