

FREQUENCY OF Leishmania-REACTIVE T CELLS IN LESIONS OF AMERICAN MOCUCUTANEOUS LEISHMANIASIS (AMCL) PATIENTS. ITS RELEVANCE IN THE PROCESS OF HEALING OR AGGRAVATION OF THE DISEASE.

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In the endemic area of Jacarepaguá in Rio de Janeiro, where the majority of patients studied live, Leishmania braziliensis braziliensis (Lbb) has being considered the only circulating Leishmania parasitizing men and dogs (Momen et al., 1983). The disease produced by Lbb has two main clinical forms: 1- localized cutaneous leishmaniasis (LCL), characterized by a single or multiple skin lesions usually ulcerated localized in areas exposed to the bite of the insect vector (sandfly). This is a mild form of the disease with a tendency to cure after antimonial therapy or even self-healing. A well modulated cellular immune response seems to be operative in the process of healing of those lesions; 2- mucosal leishmaniasis (ML), a severe form of the disease with methastatic mucosal lesions mainly in the face, which appear even several months after the cure of the primary skin lesions. This chronic form of the disease is usually resistant to antimonial therapy and associated with an exacerbated cellular immune response (DTH and lymphoproliferative response to Leishmania-antigens - Lbb-LPR) as well as an extreme scarceness of parasites in the lesion.

In this connection the exacerbated T cell-dependent immune response of those ML patients (Castes et al., 1983 and 1984; Carvalho et al., 1985) may play an important role in the mechanisms of aggravation of the lesion (Coutinho et al., 1987). Similar explanation has been postulated for the severe cases of tuberculoid leprosy where an enhanced cell-mediated immune response is also present in the absence of bacilli in the lesion (Bloom, 1986).

Studies on mice infected with Leishmania major have

shown aggravation of the lesions when Leishmania-reactive T cells were adoptively transferred (Titus et al., 1984) in order to produce an enhanced T cell dependent immune response. Probably, these observations can not be extended to human infection with Lbb because the process of aggravation in the mouse is associated with an augmented parasite load in the lesions in contrast to what occurs in ML human lesions.

In order to study the mechanisms involved in the cellular immune response in a quantitative basis, our group had successfully adapted a limiting dilution analysis to estimate the Leishmania-reactive T cell frequencies in peripheral blood of American cutaneous leishmaniasis patients (Dórea et al., 1988).

In this work we utilized this limiting dilution technique to quantitate Leishmania-reactive T cells in peripheral blood (18 patients) and in the skin or mucosal lesions (14 patients). The T cell-dependent immune response was also studied by lymphoproliferative response to Lbb antigens (Lbb-LPR), the magnitude of Montenegro skin test-MST (Delayed Type Hipersensitivity) and the histopathological picture of the lesions.

Previous results from our group (Conceição-Silva, et al., 1987) have shown that the frequencies of Leishmania-reactive T cells in mucosal lesion are significantly higher than those ones encountered in localized cutaneous lesions from leishmaniasis patients with active disease (means respectively 1:67.5 and 1:357.9;  $p < 0.02$ ). However, when these frequencies were estimated in peripheral blood of the same ML and LCL patients no significant differences were detected (means respectively 1:31,830 and 1:42,943;  $p > 0.05$ ). In order to study the association between the frequencies of Leishmania-reactive T cells in the blood and/or lesions with other parameters for cellular immune response, the MST, the Lbb-LPR and the histopathological picture of the lesions were analysed.

No significant association was observed between the Leishmania-reactive T cell frequencies in peripheral blood

and the magnitude of the MST, or the histopathological features or the frequencies of Leishmania-reactive T cells in the lesion. However, a significant positive correlation ( $p < 0.05$ ) was observed between the Lbb-LPR and the frequency of Leishmania-reactive T cells in peripheral blood.

A significant positive correlation was observed between the high frequencies of Leishmania-reactive T cells in the lesions and the high magnitude of MST ( $p < 0.01$ ) as well as with the presence of granulomatous reaction and fibrinoid necrosis in the lesions ( $p < 0.05$ ). No positive correlation was observed between the Leishmania-reactive T cell frequencies in the lesions and the Lbb-LPR.

The present results point to the possibility that the most relevant cell-mediated operational events for healing or aggravation of the disease occur in the site of the lesions and might be different from those detectable in the blood. In fact, the high frequencies of Lbb-reactive T cells in lesions associated to an exacerbated DTH, as well as to an augmented fibrinoid necrosis reaction may provide aggravation of the lesions. It is necessary to investigate if an enhanced production of lymphokines at the site of those lesions is a major effective mechanisms for aggravation of the disease. Furthermore, a lack of association was observed between the frequencies of Leishmania-reactive T cells in the blood and the severity of the disease, DTH and histopathological features.

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#### REFERENCES

- BLOOM, B.R., 1986. Learning from leprosy: a perspective on immunology and the third world. J.Immunol., 137: I-X.
- CARVALHO, E.M.; JOHNSON, W.D.; BARRETO, E.; MARSDEN, P.O.; COSTA, J.L.M.; REED, S. & ROCHA, H., 1985. Cell mediated immunity in American cutaneous and mucosal leishmaniasis. J.Immunol., 135: 4144-4148.

- CASTES, M.; AGNELLI, A.; VERDE, O. & RONDON, A.J., 1983. Characterization of the cellular immune response in American cutaneous leishmaniasis. Clin.Exp.Immunol., 27: 176-186.
- CASTES, M.; AGNELLI, A. & RONDON, A.J., 1984. Mechanisms associated with immunoregulation in human American cutaneous leishmaniasis. Clin.Exp.Immunol., 57: 279-286.
- CONCEIÇÃO-SILVA, F.; SCHUBACH, A.O.; NOGUEIRA, R.S. & COUTINHO, S.G., 1987. Quantitation of T cells which recognize Leishmania braziliensis braziliensis (Lbb) antigens in lesions and peripheral blood of cutaneous or mucosal leishmaniasis patients by limiting dilution analysis. Abstract. Mem.Inst.Oswaldo Cruz, 82(Suppl.): 118.
- COUTINHO, S.G.; PIRMEZ, C.; MENDONÇA, S.C.F.; CONCEIÇÃO-SILVA, F. & DÓREA, R.C.C., 1987. Pathogenesis and immunopathology of Leishmaniasis. Mem.Inst.Oswaldo Cruz, 82 (Suppl.): 214-228.
- DÓREA, R.C.C.; COUTINHO, S.G.; SABROZA, P.C.; HENDRIKS, J.; CYSNE, L. & CONCEIÇÃO-SILVA, F., 1988. Quantification of Leishmania-specific T cells in human American cutaneous leishmaniasis (Leishmania braziliensis braziliensis) by limiting dilution analysis. Clin.Exp.Immunol. 71: 26-31.
- MOMEN, H.; GRIMALDI G. & MARZOCHI, M.C.A., 1983. Identification of New World Leishmania isolated by agarose gel electrophoresis and polyacrilamide gell isoelectrofocusing. J.Cell Biochem., 70 (Suppl. 7A): 28.
- TITUS, R.G.; LIMA, G.G.; ENGERS, H.D. & LOUIS, J.A., 1984. Exacerbation of murine cutaneous leishmaniasis by adoptive transfer of parasite-specific helper T cell populations capable of mediating Leishmania major-specific delayed-type hypersensitivity. J.Immunol., 133: 1594-1600.