

CH-87-9

**SERUM-MEDIATED IMMUNOSUPPRESSION IN AMERICAN VISCERAL
LEISHMANIASIS**

Aldina Barral, M. Barral-Netto, Silvane Braga, R. Badaro & E.M. Carvalho.

Laboratorio de Imunologia, Hospital Prof. Edgard Santos;
Universidade Federal da Bahia.
Rua Joao das Botas, s/n; 41.140 Salvador - Bahia.

Bacterial infections are an important cause of death in VL patients and its origin is probably linked to immunosuppression. The absence of T cell response in VL is specific for leishmanial antigen, with preservation of cellular proliferation to unrelated antigens. In contrast the serum from VL patients is able to mediate a potent nonspecific suppression of cell-mediated immunity in vitro tests (Carvalho & Bacellar, 1983). We have shown that immunosuppression mediated by VL sera inhibits even mitogen-driven lymphocyte proliferation, causing reductions of the responses to Con A, PHA or PWM in the order of 67.2%, 71% and 75%, respectively (mean from determinations in several AVL sera in comparison to sera from normal volunteers). Serum from AVL does not interfere to the Con A binding to cell surfaces. The suppressive effect is not due to either cytotoxicity or inadequate nutritional support. An altered kinetics of DNA synthesis in the presence of AVL serum is also not responsible for the observed difference between cultures performed with VL or normal human serum (Barral et al, 1986).

We have determined that PHA-stimulated Interleukin-2 (IL-2)-production by normal human peripheral blood mononuclear cells (PBMC) is impaired in the presence of VL serum. Cells were stimulated in the presence of 10% VL serum or in 10% normal human serum (NHS) and the IL-2 production was tested on a IL-2 dependent CTLL maintenance assay. At a dilution of 1:4 supernatant from cultures with NHS gave 2,628 + 613 CPM, whereas those in VL serum gave 451 + 413 CPM. The number of cells expressing IL-2 receptors was not affected by VL serum. Cells were stimulated in the presence of NHS or VL serum and then submitted to indirect immunofluorescent assay using anti-Tac antibodies. The percentage of reactive cells was 54 + 9.5% with VL serum and 61.8 + 12.9 % with NHS. Additionally it was possible to restore the Con A responsiveness of cells cultivated in the presence of 10% VL serum by adding exogenous IL-2. One hundred units of IL-2 increased 4.4 to 7.3 times the proliferation of such cultures, and by only 0.6 to 1.1 similar cultures in NHS. Such results show that serum from VL impair the production of IL-2. The effect of AVL sera on the induction of IL-2 receptors was evaluated on the percentage of cells expressing the receptors, without evaluating changes in density or affinity of the

receptors. However the responsiveness of PBMC to exogenous IL-2 in the presence of AVLS does not suggest a major effect on receptor function. The addition of IL-1 or GM-CSF, (1 or 10 ng/ml), both in the form of human recombinant products, ^{did not} revert the suppressive effect of sera from AVL on normal mitogen-stimulated PBMC.

Serum from VL also affects the Interferon production by Con A stimulated human PBMC. Cultures with NHS gave $7.7 + 0.58$ (208) Units/ml (log mean + S.D. and geometric mean in parenthesis) and cultures with VL serum reduced it to $5.7 + 0.58$ (52) U/ml. Addition of IL-2 reverted the suppressive effect. In two experiments, cultures receiving 100 IL-2 U/ml produced equal amounts of Interferon ($6.33 + 0.58$ U/ml) regardless of serum source. We therefore concluded that impairment of Interferon production induced by VL serum is secondary to the defect on IL-2 production.

The nature of the serum suppressive factor is poorly characterized. We have no indication for the participation of parasite products in the immunosuppression mediated by AVLS. The capacity to suppress mitogen-driven responses argues against the possibility of the stimulant being complexed by antibodies; and, furthermore, the binding of Con A to PBMC is not affected by VL serum. Sera from VL patients exhibit a remarkable hypertriglyceridemia, and lipids have been implicated in immunosuppression in other situations (Chisari, 1977; Curtiss & Edington, 1981). Another possibility which deserves consideration is the suppressive participation of immunoglobulin, either alone or as immunocomplexes, since this has been documented (Mehra et al., 1979; Tsuyugushi, 1980), and there is extremely elevated immunoglobulin levels in VL sera. Recently we have obtained evidence that IgG depleted VL sera (by absorption to Protein A-Sepharose) partially lose its suppressive activity. Cultures performed in post-column sera exhibited proliferation indexes higher than cultures maintained with the same sera before the passage through the column. In six out of eight tested AVL sera the index of post column/pre-column Con A stimulated proliferation varied from 9.95 to 52.41; in the other two the indexes were 1.08 and 3.98. The same procedure performed in six NHS resulted in post-column/pre-column indexes varying from 0.13 to 2.42. It is most likely that the suppressive effect observed in VL sera represents the end result of several different factors.

REFERENCES

Barral, A.; Carvalho, E.M.; Badaro, R.; Barral-Netto, M., 1986. Suppression of lymphocyte proliferative responses by sera from patients with American visceral leishmaniasis. Am. J. Trop. Med. Hyg. 35 : 737-742.

Carvalho, E.M. & Bacellar, O., 1983. Lymphocyte reactivity to mitogens in American visceral leishmaniasis. *Braz. J. Med. Biol. Res.* **16** : 35-41.

Chisari, F.V., 1977. Immuno regulatory properties of human plasma very low density lipoproteins. *J. Immunol.* **119** : 2129-2136.

Curtiss, L.K. & Edgington, T.S., 1981. Immuno regulatory plasma low density lipoprotein: the biological activity and receptor-binding specificity independent of neutral lipids. *J. Immunol.* **126** : 1008-1012.

Mehra, V.; Manson, L.H.; Fields, S.P. & Bloom, B.R., 1979. Lepromin-induced suppressor cells in patients with leprosy. *J. Immunol.* **123** : 1813-1817.

Tsuyuguchi, I.; Skiratsuchi, H.; Teraoka, O. & Hirano, T., 1980. Increase in T cells bearing IgG Fc receptor in peripheral blood of patients with tuberculosis by in vitro stimulation with purified protein derivative. *Am. Rev. Respir. Dis.* **121** : 951-957.