SY-6 IMUNO REGULATION IN AMERICAN VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis (VL) is a chronic protozoan disease characterized oy perturbed immunological responses. These abnormalities include: 1. depressed lymphocyte reactivity to leishmanial antigen, 2. absence of Interleukin-2 and gamma interferon production when lymphocytes are stimulated with leishmanial antigen, 3. polyclonal B cell activation and high plasma levels of immune complexes, 4. Presence of serum immunosupressor factor(s), 5. Decreased neutrophil number. These abnormalities of the immune response have been documented during clinical disease but return to normal after succesfull therapy. The mechanisms of abnormal immunoregulation during acute L. donovani infection and the association of immunological abnormalities to acquision of disease and to disease progression are areas of active investigation. In the past ten years we have studied the immune response in patients with visceral leishmaniasis. We have followed children who live in Jacobina an endemic area, for leishmaniasis to better understand the clinical course of the infection, the risk factors related to the development of classic visceral leishmaniasis and the role of the cell mediated immune responses in the control of leishmania infection. To define the immune response of VL we sought to determine the cells involved in the immunosupression to leishmanial antigen and the mechanism(s) for serum mediated immunosuppression.

Association between lymphocyte reactivity to leishmania antigen and ability to control L. donovani

infection. Epidemiological studies in Jacobina, have determined a seasonal variation in vector and cases of leishmaniasis. The vector is most prevalent during the months of June and July, and there is a clustering of visceral leishmaniasis cases during the period of August through December. In this endemic area we have followed for 3 to 5 years 29 children who became acutely infected with L.donovani. The infection was diagnosed by seroconversion, the presence of anti leishmania antibodies detected by the ELISA technique. To define their immunologic response we measured skin test and lymphocyte blastogenic response to leishmanial antigen. Intradermal skin test performed just after infection showed that only 5 of 29 infected children had delayed hypersensitivity response to L.donovani antigen. In response to leishmanial antigen, lymphocyte blastogenesis, determined by 3H-thymidine uptake, was positive in 17 of the 29 children. A follow-up of these infected children allowed us to divid them into two groups. Asymptomatic cases (7 children who remained asymptomatic) and subclinical cases (22 children who after L.donovani infection developed hepatomegaly, low weight gain and frequent respiratory tract infections). Some (13/29) of these children with subclinical infection became healtny after months of follow up. In 9 of 29 children with subclinical infection, classical disease developed over a period ranging from 3 weeks to 17 months after infection. We have examined lymphocyte blastogenesis in the group with subclinical infection. Of the 22 children with subclinical infection, lymphocyte reactivity to leishmania antigen was documented in 13 children (responder group) but was absent in 9 children (non-responder). In the responder group only

1 (7%) of the 13 children progressed to visceral leishmaniasis. This 3 years old boy developed chikenpox three months after seroconversion. On evaluation, he had splenomegaly, anemia and leukopenia. Amastigotes of leishmania were demonstrated in the bone marrow aspirate. In the non-responder group of 9 children with subclinical infection 4 (45%) developed visceral leishmaniasis. Based in these studies, infected children with depressed lymphocyte reactivity to leishmania antigen are 65 times more likely to develop clinical desease than children with a lymphocyte proliferative response to leishmania antigen.

Cell Mediated Immune Response in American Visceral Leishmaniasis. Sera from Visceral Leishmaniasis patients suppress the proliferative response of lymphocytes obtained from normal donors. The suppression of the in vitro proliferative lymphocyte response to antigen and mitogens in the presence of V.L. sera is as high as 70%. This suppression is not due to a toxic component in the sera and can be overcomed by the addition of Interleukin-2. In addition to the suppression mediated by sera, we have defined abnormal cellular respones to leishmanial antigen. Cells from visceral leishmaniasis patients when cultured in media containing normal AB sera do not proliferate when stimulated by leishmanial antigen. The depressed immune response is specific to leishmania antigen, since the lymphocytes from VL patients react normally to PPD, C. albicans and mitogens. In addition to suppressed proliferative response, lymphocytes from visceral leishmania patients do not produce interleukin-2 and gamma interferon when stimulated with L.donovani antigen. Confirming this observation is the finding that the

supernatants of cultures of visceral leishmaniasis lymphocytes stimulated with leishmania antigen are unable to activate macrophages to kill leishmania. We investigated whether exogenous IL-1 or IL-2 or GM-CSF may restore the immunosuppresion in visceral leishmaniasis. Recombinant products were added to lymphocytes of patients with VL and then stimulated with leishmania antigen. All three lymphokines failed to restore the lymphocyte proliferavie response to leishmanial antigen. To determine whether a particular cell population may be responsible for the immunosuppresion in visceral leishmaniasis, mononuclear cells of these patients were depleted of specific cell population such as, monocytes, B cells, K cells, T4 cells, and T8 cells. Depletion of each of these population did not restore the lymphocyte reactivity to leishmania antigen. Since successful therapy of kala-azar restores lymphocyte proliferation to leishmania antigen we decided to perrormed co-culture experiments with cells obtained before and after therapy. Cells obtained before therapy were frozen in liquid nitrogen. These cells do not respond to leishmanial antigen. In three patients we perrormed co-culture experiments using cells obtained before and after therapy. 3H-Thymidine incorporation by mononuclear cells obtained from patients before therapy was 460±76 cpm. Mononuclear cells obtained after successful treatment incorporated 4293±1442. Co-cultivation of T cells from before treatment with cells obtained after successful treatment reduced the incorporation of 3H-thymidine to 530±149. This represent an 80% reduction of proliferative response to leishmanial antigen the phenotype of these T cells will be determined in future experiments.