RELATIONSHIP OF PARASITE SPECIFIC ANTIBODIES AND HEART SPECIFIC AUTOANTIBODIES IN MICE INFECTED WITH TRYPANOSOMA CRUZI. Edwin C. Rowland, Kelly R. Spears and Thomas S. McCormick. Department of Basic Sciences, College of Osteopathic Medicine, Ohio University Athens, OH 45701, USA

Immunization of inbred mice with the Corpus Christi strain of T. cruzi increases resistance to Brazil strain challenge as indicated by enhanced survival in C3H and lowered parasitemia in C3H and C57 mice. Previous adoptive transfer studies have shown this protection is a B cell mediated phenomenon. Mice which survive acute infection develop characteristics of cardiac histopathology, such as inflammation, muscle necrosis and connective tissue replacement, up to day 300 post infection. Examination of parasite immunogen specific antibodies in the sera from these mice by Western Blot analysis reveals reactions to a variety of parasite extract antigens. Of interest, sera from mice surviving acute infection consistently show a strong reaction to a 75-77 Kd glycoprotein suggesting a protective function.

To examine the presence of autoantibody in the antisera, an ELISA was performed using normal syngeneic cardiac tissue extract as the target antigen. Sera from C3H and C57 mice contain anti-heart antibodies during acute and chronic infection. The specificity of this response was indicated by C3H sera reacting with cardiac and striated but not smooth muscle preparations, and C57 sera reacting only with cardiac muscle. This autoantibody reactivity consists of both IgG and IgM isotypes throughout infection. Immunoaffinity purified heart-specific antibodies were found to cross-react with parasite extract in an ELISA. The use of these purified antibodies in Western Blots indicated that they do not bind to the 75-77 Kd antigen of the parasite extract. The specific heart antigens bound by the mouse antisera were shown to be a 150, a 120 and, most prominently, a 67 Kd polypeptide by SDS PAGE examination. The possibility that the autoantibodies were directed toward the beta adrenergic receptor, also reportedly 67 Kd, was examined using Turkey erythrocytes (TRBC), which are rich in beta receptors. Infected mouse sera was found to react strongly with TRBC in ELISA. Similarly, adsorption of this sera with TRBC reduced its reactivity to heart extract. Addition of the beta blocker alpenolol inhibits antisera binding to TRBC and heart extract in a dose dependent manner. Similar results found with sheep RBC controls suggested a carbohydrate moiety as the target of the autoantibodies. The involvement of carbohydrates was further indicated by the ability of N-acetylgalcosamine or galactose to decrease the binding of the mouse antisera to heart, TRBC or parasite antigens. Similarly, the cleaving of carbohydrate from heart extract by N-glycanase treatment resulted in a reduction of antisera binding in ELISA.

The results suggest that some of the autoantibodies present in the infected mice are directed against a carbohydrate epitope on a heart protein, perhaps the beta adrenergic receptor. This glycosylation is likely similar to one found on a nonprotective T. cruzi glycoprotein, which induces the production of the cross-reactive autoantibodies during infection. This work is supported by NIH grant AI 23704.
IMUNO REGULATION IN AMERICAN VISCERAL LEISHMANIASIS


Visceral Leishmaniasis (VL) is a chronic protozoan disease characterized by 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 immunosuppressor factor(s), 5. Decreased neutrophil number. These abnormalities of the immune response have been documented during clinical disease but return to normal after successful therapy. The mechanisms of abnormal immunoregulation during acute L. donovani infection and the association of immunological abnormalities to acquisition 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 immunosuppression 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 healthy 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 chickenpox 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 disease 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 overcome by the addition of Interleukin-2. In addition to the suppression mediated by sera, we have defined abnormal cellular responses 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 immunosuppression 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 proliferative response to leishmanial antigen. To determine whether a particular cell population may be responsible for the immunosuppression 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 performed 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 performed 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.