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AUTOIMMUNITY IN CHAGAS' DISEASE: IMMUNOMODULATION OF AUTOIMMUNE AND *T. CRUZI*-SPECIFIC IMMUNE RESPONSES

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Although the involvment of autoimmunity in the chronic myocarditis of Chagas' disease is well established, the quantitative importance of autoaggression on the maintenance of the inflammatory process resulting in cardiac lesions is still unknown. The definition of a true organ-specific autoimmune nature for Chagas' disease myocarditis awaits definition of discrete organ-specific autoantigens. Data pertaining to defined autoantigenic targets in heart tissue at the level of cellar immunology are scarce in the literature. The involvement of of contractile proteins in other states of heart-specific autoimmunity, as in rheumatic carditis, makes them likely targets. The immunoregulatory derangements that lead to the expression of autoimmunity in this setting are also worthy of study.

We report here our findings pertaining to lymphocyte proliferative responses to striated muscle contractile proteins and *T. cruzi* infected mice, and the immunomodulation of these anti-self and anti-parasite immune responses.

1. Lymphocytes from *T. cruzi*-infected mice proliferate in response to striated muscle myosin and *T. cruzi* soluble antigen (TCSA).

BALB/C mice were infected with three IP injections of 10^7 cloned Albuquerque stock epimastigotes 15 days apart, followed 1 month later by IP injection of 10^4 *in vitro* derived trypomastigotes (1). Sixty to 120 days later, the proliferative responses of the cells from the popliteal and inguinal lymph nodes were assayed against rabbit skeletal muscle actin (2), myosin (3) and *T.cruzi* soluble antigen, TCSA (4). Significant proliferative responses were found against myosin and TCSA, but not to actin in any of the concentrations tested. Lymphocytes from naive mice did not proliferate against any of the antigens tested. In order to exclude the possibility that myosin reactivity could be a result of heart antigen carry-over by LIT culture-medium epimastigotes, in a particular strain of mice, we performed the same assay in CBA mice injected IP with 500 blood-derived Berenice stock trypomastigotes. Again, myosin and TCSA stimulated significantly the proliferation of lymphocytes from chronically infected CBA mice.

2. Antigen-specific immunomodulation by antisera generated against *in vitro* expanded antigen-responsive cells.

Antisera against myosin or TCSA-responsive cells were raised by IV injection in naive, syngenic mice, of 10^6 *in vitro* antigen-expanded cells from infected mice. Antiserum against myosin-responsive cells significantly inhibited lymphocyte proliferation against myosin but not against TCSA or Concanavalin A (Con A). Conversely, antiserum against TCSA-responsive cells significantly inhibited lymphocyte proliferation against TCSA,but not against myosin. These results suggest that it is possible to induce serum factors that specifically inhibit *in vitro* different compartments of the immune response associated with chronic *T. cruzi* infection of the mouse.

3. Potentiation of antigen-specific serum suppressive activity by simultaneous injection of antigen-pulsed spleen adherent cells.

IV injection of 10^5 myosin-pulsed spleen adherent cells (Ag-SAC) from naive mice simultaneous to myosin-expanded cells as described above potentiates antigen-specific serum suppressive activity, reaching significantly higher titers in Ag-SAC-injected mice than non-injected mice. These results are consistent with two possibilities: a) Ag-SAC expand injected or recruited antigen-specific cells, generating a boosted clonotype-centered suppressive circuit; b) Ag-SAC *per se* might be the initiators of an antigen-centered supressive circuit in the non-infected mice.

4. Antigen-specific serum suppressive activity is found in both IgG2a and non-protein A-binding serum fractions.

The sera of mice injected with syngeneic myosin or TCSA-activated lymphocytes was filtered through a Protein A-Sepharose 4B column. Suppressive activity for the appropriate antigen was found both in the pass-through and eluted fractions, suggesting that suppressive serum activity might be due to IgG and non-IgG molecules. When Protein A-bound fraction was subject to differential elution (5), it was found that all suppressive activity could be eluted at the IgG2a region, while no activity was recovered in the IgG1 and IgG2b fractions.

We further filtered the IgG2a eluate on a monoclonal anti-IgG2a-Sepharose 4G column. We found that the activity could be recovered in the eluted fraction of the column. These data suggest the existence of an isotypic restriction of IgG suppressive responses.

5. Serum suppressive factor activity is subject to genetic restriction.

Searching for an element of genetic restriction in the suppressive factor's interaction with antigen-responsive cells, we studied the effect of allogeneic antisera on antigen-specific proliferative responses of an allogeneic strain of mice. It was found that BALB/C anti-myosin responsive cell antisera had no effect on CBA myosin-specific responses. Conversely, CBA antisera also had no effect on BALB/C myosin-specific responses.

The data summarized above represent a first attempt to characterize the immunoregulatory derangements associated to autoimmunity in chronic *T. cruzi* infection. Although myosin reactivity was demonstrated, it must be kept in mind that the pathogenetic role of myosin-responsive cells has not been clarified yet. However, it has been demonstrated that one can modulate an autoimmune response without interfering in the parasite-specific response in chronic *T. cruzi* infection in the mouse. The perspective of antigen-specific immunomodulation as a means for controlling the progression of the autoimmune heart lesions in chronic Chagas' disease remains thus an open possibility.

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