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Lymphocyte subpopulations and clonal repertoires participate in immune response to acute *T.cruzi* infection.

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Many different laboratories have been provinding demonstrations of "natural" autoantibody production by normal individuals. Autoimmunity can, therefore, be considered as an essential characteristic of the normal immune system, associated with some levels of natural immune activities in unmanipulated individuals. Furthermore, it has been repeatedly demonstrated that the interaction of natural antibodies with ligands is frequently "degenerate" and typically "multireactive". Studies on the interactions between pathogens, such as parasites, bacteria or virus, and their hosts, may be improved by the consideration of those new concepts : internal immune activities, self-reactivity, multispecificity and natural antibodies.

Experimental T.cruzi research has essentially focused on the characterization of parasite-membrane antigens and the specific immune responses against them, with the main perspective of vaccine development. Much work has also been dedicated to the putative auto-immunopathogenicity of the chronic phase of T.cruzi infection. The current explanation involkes antigenic cross-reactivities between parasite determinants and auto-antigens. This hypothesis, however, has been contested by some authors who argue that formal proof for cross-reaction is yet to be produced and, if such clones exist, that they are stimulated by T.cruzi infection. Given the autoreactive specificities of many natural antibodies, it is plausible to consider that perturbations in their production or repertoires induced by parasite infection, were relevant to this problem. This could be the case, even in T.cruzi infection would not "specifically" stimulate such clones, since it has been stablished in other situations of microorganism infection conditions that "nonspecific" activation is a major immune reaction.

Our initial studies have been primarily directed at analysing the overall lymphoid activity in normal and infected animals, regardless of "specificities" at clonal levels. We have later conducted preliminary analyses of the specificity repertoires that are lymphocytes activated after parasite inoculation.

The first stricking finding was the very extensive blast transformation of B and T lymphocytes, in both spleen and lymph nodes, early after infection. The intensity of these responses indicates that immunocompetent cells are activated polyclonally, by mechanisms independent of parasite-specific recognition, and characterize them as the major immunobiological phenomenon associated with infection. All lymphocyte classes are differentiated to effector functions, namely, immunoglobulin production by secreting cells (accompanied by hypergammaglobulinemia), helper and cytolytic T-cell activities.

The range of specificities in the B cell responses was analysed by deriving hybridomas from lymph nodes of acutely infected mice. The study of an unselected pannel of such monoclonal antibodies revealed that the large majority of the B lymphocytes activated in T.cruzi infection is essentially "nonspecific". Interestingly, many of the antibodies are self-reactive, but their isotype demonstrates that their production is a consequence of infection. These "nonspecifically" induced antibodies may well be related with the auto-immune pathology associated with chronic infection.

The polyclonality of antibody responses to infection was demonstrated by studies on the V_H-gene families expressed by activated B cells. Both by the analysis of RNA isolated from the spleen and the V_H repertoires of CFU-B produced by B cell blasts isolated from the spleen and lymph nodes of infected animals. These studies revealed that B cell responses to T.cruzi are actually panclonal. Interestingly, V_H-gene family representation in activated B cells from infected animals follow very closely that of "naturally" activated B cells in normal syngeneic mice. This finding could suggest that B lymphocyte activation in acute phase is essentially an expansion of natural

activities, rather than the stimulation of resting B cell clones. The hypothesis is supported by the "natural antibody" reactivities of the monoclonal antibodies isolated in infected mice and by the mechanisms which participate B cell activation. Thus, we have been unable to detect strong T or B-cell directed mitogens in T.cruzi extracts and have demonstrated that the polyclonal antibody response is entirely dependent upon CD4+ T cell activity, suggesting again that T.cruzi infection simply reinforces ongoing mechanisms of cooperative cell interaction.

Some other recent findings seem to further support this interpretation, namely the selective participation of peculiar lymphocyte sub-populations in the polyclonal responses to T.cruzi infection. Recent studies on B-lymphocyte differentiation, have indicated the existance of two lineages of B lymphocytes: conventional B cells (+, CD5-), and the socalled Ly1B cells (+, CD5+), respectively. While the first set of B cells are implicated in "conventional" responses to "foreign" antigens, Ly1B cells seem to be responsible for the production of most "naturally secreted" IgM, including several of the IgM autoantibodies in normal and auto-immune mice, and anti-idiotypic antibodies produced by normal animals. More importantly this set of cells are naturally activated in normal mice and seem to partiocipate in the internal lymphocyte activity. They are rarely detected in spleen and lymph nodes, but represent 30-40% of total B cells in the peritoneum. Furthermore, whereas lethally irradiated and bone-marrow reconstituted animals, can be repopulated on conventional B cells, Ly1B lymphocytes are hardly recovered after this procedure, and are largely depleted, shortly after reconstitution in those animals. We have now used this experimental system to investigate Ly1B cell participation in the antibody responses to T.cruzi infection. As expected, infected animals show a signifficantly enhanced proportion of Ly1B cells in the spleen. More strickingly, the study of depleted and infected animals, revealed that the polyclonal B lymphocyte activation was abolished, and could be partially restored by reconstituting the animals with peritoneal cell suspensions from normal donors. These observations demonstrate that Ly1B cells are implicated in T.cruzi responses, but also indicate that "something else" is missing in reconstituted animals. In fact, normal responses can be

obtained when splenic T-cells are used to supplement the peritoneal cell reconstitutions. It could be envisaged that the T-cell compartment was not fully restored in bone-marrow reconstituted animals by the time of infection, but normal levels of CD4+, CD8+ and CD3+ lymphocytes were found before infection. A T cell defect could be considered only if we were dealing with a minor cell population, such as double negative T cells.

Thus, it has been reported that mice with T-cell dependent autoimmune disorders,such as lpr/lpr and gld/gld also show an expansion of an unusual T cell subset. These cells have classical T cell markers, such as Thy-1 or CD3, but in contrast to most peripheral T cells of normal individuals, are CD4- CD8-. Recent studies on T cell receptor expression in autoimmune mice show a correlation between the T cell recptor expression in such double negative T cells and autoimmunity. It is interesting, therefore, that this minor lymphocyte population -DNT3+ cells- which is very little represented in normal spleen, is expanded in T.cruzi infected animals. Since most DNT3+ cells seem to express -type receptors, we have used appropriate molecular probes, to demonstrate that the spleen of infected animals contains increased levels of the corresponding RNA in the first week of T.cruzi infection.

If suggestive, these observations do not directly implicate Ly1B cells and DNT cells in the immunopathology of chronic T.cruzi infection, since their activation could simply accompany other aspects of host reaction and have no consequences for the establishment of auto-immunity. Given the known repertoires and state of activation of this minor lymphocyte subsets in normal animals, and the possibility that T.cruzi infection amplifies ongoing "natural" immune activities, however, it is tempting to postulate that the study of Ly1b and DNT cells in infected individuals will clarify important aspects of Chagas' disease. Experiments are in progress to assess this possibility.