

ROLE OF L3T4⁺ T CELLS IN THE IMMUNOPATHOLOGY
OF CHRONIC EXPERIMENTAL CHAGAS' DISEASE

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During the chronic phase of Trypanosoma cruzi infections as well in humans as in experimental hosts, there is a large extent of inflammatory lesions affecting specially muscles and nervous tissues (Santos-Buch, 1981, Barreira, 1981, Said, 1985, Palmieri 1984). At that time, none or very few parasites are detectable in the host although parasitic antigens may be revealed by indirect immunofluorescence in about 10% of the tissular lesions (Ben Younes-Chennoufi, 1987). The pathogenesis of these lesions has been for a long time attributed to a cell-mediated immunity (Koberlé, 1968) and more recently to autoimmunity (Santos-Buch, 1981, Hudson, 1985, Joskowicz, 1985). However, until now the nature of cells involved in this autoimmunity has not been yet defined.

Inflammatory infiltrates observed in chronically infected mice are mainly composed of monocytes and few lymphocytes (Said, 1985). Staining of the cellular infiltrates with monoclonal antibodies against surface markers of lymphocytes shows that only 5% of the cells are Thy-1+ and among them 4% have the Lyt1+ phenotype (Ben Younes-Chennoufi, 1987b). Presence of numerous degranulating mast cells in tissues of chronic mice indicates therefore that delayed-type hypersensitivity-mediating T cells could play a role in the chronic lesions.

Experiments of transfer of lymphocytes from C3H/HeJ chronically infected mice to naive recipients show that only Lyt2⁻ cells are able to reproduce inflammatory infiltrates in recipients. Surprisingly, cellular infiltrates have been principally observed in the liver of recipients, at a lesser

degree in muscles and occasionally in sciatic nerves (Said, 1985, Hontebeyrie-Joskowicz, 1987). Because of the possible presence of parasites into the inoculum of lymphocytes, we decide to derive T cell lines from T.cruzi chronically infected mice. Lymph nodes and peripheral blood lymphocytes have been cultured according usual conditions for the establishment of T cell lines. However, macrophages and Lyt2⁻ T cells have been previously removed before the beginning of culture to avoid the presence of parasites and/or of suppressor cells. In vitro stimulation has been performed by adding T.cruzi extract (freeze-thawed culture trypomastigotes) and IL2. T cell lines have been obtained that have the capacity to transfer a local delayed-type hypersensitivity reaction when they are injected into naive recipients with the relevant antigen. They have the L3T4⁺ phenotype of the T helper/DTH subset. One of the lines derived from the lymph nodes and named G-05, is able to transfer a local DTH reaction in presence of T.cruzi extracts but also in presence of peripheral nerve extract and of sheep-red-blood-cells. This result suggests a) T.cruzi extract and peripheral nerve may have a common epitope recognized by G-05 line, b) G-05 line may be an autoreactive T cell line mainly activated by class-II MHC molecules (Ia). These hypotheses have been tested by transfer of G-05 cells in naive recipients. Inoculation of the cells by intraneural or intravenous (i.v.) route leads to the development of granulomatous inflammatory infiltrates. In the sciatic nerves, infiltrates are accompanied with demyelination that also occurs in chronic mice (Said, 1985). Following i.v. inoculation, infiltrates are mainly located in the liver. It is possible that

this localization is determined by the recognition of Ia molecules on Kupffer cells in the liver that is the first organ where injected blastic cells may circulate (Hontebeyrie-Joskowicz, 1987).

In order to define the specificity or the autoreactivity of G-05 T cell line, we performed experiments of proliferative responses in presence of antigen (T.C extract) and antigen presenting cells (APC). We therefore observed proliferation of G-05 cells occurs on syngeneic APC in absence of T.cruzi extract, and addition of antigen to APC does not increase the proliferation of G-05 cells. This is an additive argument that G-05 line may be an autoreactive T cell line activated in an autologous mixed lymphocyte reaction (Glimcher, 1981).

To further study the characteristics of G-05 line and its involvement in the chronic experimental Chagas'disease, we have explored the helper function of this line. We have co-cultured in vitro T cells from G-05 line with unprimed normal B cells and assessed for the proliferation and diferenciation of B cells, after 4 days of culture. B cells have been induced to proliferate (measured by ³H-thymidine incorporation) and to differentiate into Ig-secreting B cells (measured by reverse Protein A-plaque assay). In vivo, transfer of G-05 cells in naive recipients gives a polyclonal activation of splenic B cells, in a range similar to that observed in chronically infected mice (D'Imperio Lima, 1986). Both in vitro and in vivo B cell activation lead to an identical isotypic pattern showing a majority of IgM- and IgG2b-secreting cells (Spinella-Jaegle, unpublished results). This is

in perfect accordance to the B cell polyclonal activation observed in chronically infected mice.

In this work, we have demonstrated that L3T4⁺ T cells derived from lymph nodes of chronically infected mice may play a major role in the immunopathology of chronic experimental Chagas' disease. First, these L3T4⁺ T cells are able, through a delayed-type hypersensitivity reaction, to reproduce inflammatory infiltrates similar to those observed in infected mice and probably following an autologous recognition mediated by Ia molecules. Second, these L3T4⁺ T cells have the capacity to induce unprimed B cells to differentiate into Ig-secreting cells showing an isotypic pattern of the same type than observed in chronically infected mice. These results strongly suggest that autoreactive L3T4⁺ T cells are developing during the chronic phase of the disease. Why and how these autoreactive T cells escape to the immune control of self-reactivity is not yet understood. However, we can do the hypothesis that triggering of autoreactive T cells depends of the extensive activation of T cells occurring in the acute phase of the disease and may be of the high degree of cytotoxic activity of the Lyt2⁺ T cells (Minoprio, 1986 a & b). Evidence of the role of L3T4⁺ T cells in the induction of polyclonal B cell activation during the acute phase of the disease has been recently demonstrated (Minoprio, 1987).

REFERENCES

BARREIRA ANTUNES A., SAID G., KRETTLI A.U., 1981. Multifocal demyelinating lesions of peripheral nerves in experimental

chronic Chagas'disease. Trans. R. Soc. Trop. Med. Hyg., 75, 751.

BEN-YOUNES-CHENNOUFI, M. HONTEBEYRIE-JOSKOWICZ, H. REYNES, V. TRICOTTET, H. EISEN, & G. SAID. Persistence of T.cruzi antigens in the tissular infiltrates during the chronic experimental Chagas'disease. Trans. Roy. Soc. Med. and Hyg. (in press).

BEN YOUNES-CHENNOUFI A., G. SAID, H. EISEN, A. DURAND & M. HONTEBEYRIE-JOSKOWICZ. 1987. Cellular immunity of Trypanosoma cruzi is mediated by helper T cells (CD4). Trans. Roy. Soc. Med. and Hyg. (in press).

D'IMPERIO LIMA R., H. EISEN, P. MINOPRIO, M. JOSKOWICZ & A. COUTINHO. 1986. Persistence of polyclonal B cell activation with undetectable parasitemia in late stages of experimental Chagas'disease. J. Immunol., 137, 353.

GLIMCHER L.H., D.L. LONGO, I. GREEN, R.H. SCHWARTZ. 1981. Murine syngeneic mixed lymphocyte response. I- Target antigens are self Ia molecules. J. Exp. Med. 154, 1652.

HONTEBEYRIE-JOSKOWICZ M., G. SAID, G. MILON, G. MARCHAL, & H. EISEN. 1987. L3T4⁺ T cells able to mediate parasite-specific-delayed-type hypersensitivity play a major role in the pathology of experimental Chagas'disease. Eur. J. Immunol., 17, 1027.

HUDSON L. 1985. Autoimmune phenomena in chronic chagasic cardiopathy. Parasitol. Today, 1, 6.

JOSKOWICZ M. 1985. Chagas' disease and Chagas' syndromes: The pathology of American trypanosomiasis. Adv. Parasitol., 6, 63.

MINOPRIO P., H. EISEN, L. FORNI, M.R., D'IMPERIO LIMA, M. JOSKOWICZ, & A. COUTINHO. 1986. Polyclonal lymphocytes responses to murine T. cruzi infection, I. Quantitation of both T and B responses. Scand. J. Immunol., 24, 661.

MINOPRIO P., A. COUTINHO, M. JOSKOWICZ, M.R. D'IMPERIO LIMA, 1 H. EISEN. 1986. Polyclonal lymphocyte response to murine T.cruzi infection. II. Cytotoxic T lymphocytes. Scand. J. Immunol., 24, 669.

MINOPRIO P. H. EISEN, M. JOSKOWICZ, P. PEREIRA & A. COUTINHO. 1987. Suppression of polyclonal antibody production in Trypanosoma curzi infected mice by treatment with anti-L3T4⁺ antibodies. J. Immunol., 139, 545.

PALMIERI J.R., LACHANCE M.A., CONNER D.H. Parasitic infection of the peripheral nervous system. In: Dyck P.J., Thomas P.K., Lambert P.H., Bunge Eds, Peripheral Neuropathy, vol. 2, Philadelphia: Saunders, 1984, 1988.

SAID G., JOSKOWICZ M., A. BARREIRA ANTUNES & H. EISEN. 1985. Neuropathy associated with experimental Chagas' disease. Ann. Neurol., 18, 176.

SANTOS-BUCH C.A., 1981. American Trypanosomiasis: Chagas' disease. Int. Rev. Exp. Pathol. 19, 63.