SOME ASPECTS OF THE CELLULAR IMMUNE RESPONSE IN EXPERIMENTAL AND HUMAN CHAGAS' DISEASE: A SUMMARY

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Chagas' disease is a major public health problem in South America. Infection by *Trypanosoma cruzi*, the causal agent of the disease, is characterized by an acute phase with intracellular parasite multiplication and high parasitemias which are eventually suppressed, but not cured, by the immune system of the host. Patients that recover from the acute phase, may evolve to the chronic phase of the disease with cardiac and/or digestive manifestations, which seem to be caused by immunopathological destruction of muscular and/or nerve cells (Brener, 1980; Andrade & Andrade, 1979). Both humoral and cellular immune responses may participate in the immunoprotective and immunopathological phenomena (Scott & Snary, 1982).

The present paper discusses some aspects of the cellular immunity in Chagas' disease. The cellular immune response is T lymphocyte dependent. Such T cells recognize antigen in the context of histocompatibility molecules on the surface of antigen-presenting cells (McDevitt & Benacerraf, 1969). This recognition restriction is an acquired phenomena determined by the MHC genotype of the thymus where the T cells differentiate (Zinkernagel et al., 1978). The T lymphocytes are not only the effector cells in some immune responses but are also responsible for the immunoregulatory circuits (Green, Flood & Gershon, 1983). Contrary to the B cells whose antigen-receptor is an immunoglobulin molecule with an already known structure, the T cell antigen-receptor has been only very recently defined from a molecular point of view (Hedrick et al., Yanaki et al., Saito et al., 1984).

In our laboratory we have been studying the cellular immune response to glycoconjugates purified from epimastigotes of *T. cruzi*. In this study we present some features of the T lymphocyte response to a purified glycoconjugate (Gp-25) extracted from this parasite. Gp-25 is a heat-resistant soluble glycoconjugate with a molecular weight of 25000 daltons. It bears a carbohydrate portion containing galactose, mannose, glucose and xylose at a molar ratio of 35 : 13 : 1 : 1. Three main reasons made us decide to study this molecule: its degree of purity (one single band in PAGE) which had enabled the definition of its amino-acid and sugar constitution (Mendonça-Previato et al., 1983); the possibility of obtaining the molecule in sufficient quantities to perform all the studies and mainly the fact that the vast majority of Chagas' disease patients have specific serum antibodies while normal individuals and patients with unrelated parasitic infections lack immune reactivity to Gp-25, indicating that this molecule is recognized in the course of the natural *T. cruzi* infection (Scharfstein et al., 1983).

MATERIAL AND METHODS

Studies in mice

a) Animals — Mice of both sexes, 6-8 weeks old were used. The strains C57B1/10 (B10), B10.A, B10.Br, Balb/c, C_3H/Hej and (B10XB10.A) F_1 were employed. All mice were raised in our own facilities.

b) T cell proliferation assay — Mice were immunized in the hind foot-pads with the relevant antigen. One to two weeks after immunization, the draining lymph nodes were removed and single cell suspensions of whole lymph node cells (LNC) were prepared. Fractionation of LNC was carried out by incubation over nylon wool columns. The resulting T-cell-enriched, non-adherent lymphocytes are referred to as lymph node lymphocytes (LNL). Culture medium consisted of RPMI-1640 (Grand Island Biological Co., New York, NY) supplemented with gentamycin ($10 \mu g$/ml), 5-Fluorocytosine ($1 \mu g$/ml), L-Glutamine (2mM), 2-mercaptoethanol (5×10^{-5} M) and 2,5% human serum. Cells were cultured at 4×10^5 cells/well in 96-well round bottom microtiter plates (Linbro, Hamden, Connecticut) with the appropriate antigen concentration, for 3-4 days in a humid environment containing 5% CO_2, at 37ºC. Eighteen hours before harvesting, $1 \mu ci$ of tritiated thymidine (^{3}HTdR, specific activity 6.7 Ci/mmol, New England Nuclear, Boston, Ma.) was added to each well. Cultures were harvested with the aid of a semiautomated harvesting device onto fiber glass filters and the amount of incorporated ^{3}HTdR was measured by liquid scintillation spectroscopy.

c) Generation of short term T cell lines specific for Gp-25 — Lymph node lymphocytes were cultivated as described above at a concentration of 2.5×10^6 cells/ml in the presence of 0.5×10^6 Mitomycin-C treated spleen cells. After 7 days in culture the non-adherent viable T cell blasts were recovered, washed and restimulated with fresh, syngeneic accessory spleen cells in the presence or absence of antigen.

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d) Characterization of the cells involved in the response — A rat anti-Thy 1.2 monoclonal antibody (clone 30-Hiz Becton-Dickinson, Mtn. View, Ca.) was used to characterize the T cell dependence of the antigen specific proliferative response. Whole immune LNC were incubated with the monoclonal antibody $(1 \mu g / 10^6$ cells) for 45 minutes at 4^oC. LNC were then washed and ressuspended in a $1:5$ dilution of previously absorbed rabbit complement (0.4 ml of fresh serum were absorbed with a whole mouse spleen on ice for 1 hour) and incubated for 45 minutes at 37^oC. LNC were washed 3 times, counted and cultured at 4×10^5 viable cells/well, with or without antigen.

A monoclonal anti-Ia antibody $(I-E^k/I-C^k)$ was used continuously in culture, in order to show the Ia dependence of antigen presentation.

Studies in humans

a) Patients — Patients with chronic chagasic cardiomyopathy were studied. The diagnosis of Chagas' disease was made by conventional serology in addition to a radioimmunoassay for Gp-25. All the patients were submitted to a 12 lead conventional eletrocardiogram in rest and to X-ray studies in posterior-anterior and left-lateral positions. The diagnosis of chagasic cardiomyopathy was established when one of the characteristic conduction disturbances was found in the ECG: complete or incomplete right bundle branch block, left-anterior hemiblock or the various types of atrioventricular conduction defects. These could be associated or not with radiological signs of heart enlargment or congestive heart failure. Control population was constituted mainly of patients from the same out-patient clinic with non-chagasic cardiopathies and of normal volunteers.

b) Characterization of the peripheral blood mononuclear cell (PBMC) sub-population profile — Peripheral blood mononuclear cells were purified by Ficoll/Hypaque gradient. Monoclonal antibodies of the OKT series (Ortho Pharmaceutical Co.) specific for monocytes (OKM_1), total T cells (OKT_3), helper/inducer (OKT_4) and cytotoxic/suppressor cells (OKT_8) were used (Breard et al., 1980; Reinherz et al., 1980). The percentage of each of the cell sub-populations was defined by indirect immunofluorescence employing a biotin/avidin system.

c) Measurement of the lymphoproliferative response — After Ficoll/Hypaque purification, PBMC were washed in Hank's-ballanced salt solution, counted and cultured in RPMI-1640 medium supplemented as described for the murine cells, in the presence of 5% heat inactivated foetal calf serum or normal human serum. Cells were cultivated for 5 to 6 days at a concentration of 1.2×10^6 cells/ml. Thymidine incorporation was measured as described for the murine cells. T-cell enriched populations were obtained by passage of the cells over a nylon-wool column. In some experiments indomethacyn (Sigma Chemical Co.)., a cyclo-xigenase inhibitor was added to the cultures at a final concentration of $2 \mu g/ml$.

RESULTS

Studies in mice

a) Mice immunized with whole extracts of epimastigotes, respond to the purified glycoconjugates irrespective of their H-2 haplotype $(H-2^{a, b, d, k})$. So, there is no obvious H-2 linked genetic control of the immune response to those antigens. The treatment of the cells with anti-Thy 1.2 plus complement, drastically reduces the proliferative response to Gp-25 thus characterizing its T cell dependence. The murine T cell response to those antigens is accessory cell dependent, MHC restricted and Ia dependent.

b) The T cell clone that recognizes Gp-25 is not the same that recognizes epitopes in antigenic molecules extracted from mouse heart. If T cell cross-reactivity does exist between heart antigens and *T. cruzi* antigens, the glycoconjugate Gp-25 is not the molecule that bears the cross-reactive epitope.

Studies in humans

a) No significant differences have been found in the frequency of the different lymphocyte sub-populations when PBMC from chronic patients and controls were compared. However, the OKT_4/OKT_8 ratio in chronic chagasic patients is abnormally high in females and abnormally low in males (Gattass, Albanesi Fº & Barcinski, 1984). Those findings suggest that immuno-regulatory disfunctions in the course of the chronic disease may involve both helper/inducer and suppressor/cytotoxic T cell populations.

b) PBMC of chronic chagasic patients give little or no proliferative response to Gp-25. The un-responsiveness can be reverted by addition of indomethacyn to the cultures or by depleting the PBMC of monocytes. These results suggest that a monocyte dependent suppressor mechanism operates in the response to the glycoconjugate during the chronic phase of the disease.

DISCUSSION

The series of experiments described here characterize, in immunized mice, some of the genetic and cellular aspects of the T cell response to a purified glycoconjugate from *T. cruzi*. There are no evidences that this antigen operates under H-2 dependent genetic control. However, as for any other soluble antigen,

this molecule is presented to immune T cells by Ia positive macrophages. Gp-25 which is able to induce an antibody response in chronic chagasic patients is not, at least in the mouse, responsible for cross-reactivity with heart antigens, if such a phenomenon indeed exists. This same glycoconjugate fails to elicit a marked blastogenic response in patients with chronic Chagas' disease unless indomethacyn is added to the cultures. Depletion of adherent cells from the mononuclear cell population is also able to reconstitute the blastogenic response to the molecule. Those results suggest that a PGE_2-dependent suppressive mechanism operates in patients with chronic Chagas' disease.

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