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T CELL RESPONSES IN MICE INFECTED AND IMMUNIZED WITH TRYPANOSOMA CRUZI

Ises A. Abrahamsohn, Laura C. Barbosa de Oliveira, Maria A. Curotto & Gloria M. Collet de A. Lima

Instituto de Ciencias Biomedicas, Universidade de S. Paulo, Departamento de Imunologia, C. Postal 4365, S. Paulo, CEP 01051, Brasil.

The understanding of the mechanisms underlying the immune responses to **Trypanosoma cruzi**, not to mention their possible role in the pathogenesis of Chagas' disease is a formidable task.

During the course of **T. cruzi** infection in mice several alterations of the immune response occur. These are more intense during the acute phase and comprise severely impaired responses to T dependent and T independent antigens, along with T and B polyclonal cell activation. In spite of these alterations, specific antibody and cell mediated immunity ensue and decrease the parasite load, conferring protection against reinfection to the individuals which survived the acute phase. **T. cruzi** specific antibody and cell mediated responses were shown to be strictly T cell dependent.

We report herein some aspects of the parasite specific T cell responses in infected mice and in mice immunized with **T. cruzi**.

A) T. cruzi specific proliferative responses and IL-2 (TCGF) production in infected mice.

A/Sn (susceptible) and C57 Bl/10J (resistant) mice were infected by the intraperitoneal (i.p.) route with 5,000 blood forms (Y strain). All B10 mice survive this inoculum while all A/Sn mice die between the 15th to 17th day after infection. Parasitemia peaks on the 8th day (7-9 x 10 to the 5 / ml) and maximal splenomegaly was observed around the 14th day in both strains.

The proliferative responses of splenic, and of periaortic and mesenteric lymph node (LN) cells to tissue culture trypomastigotes antigen (T-Ag, frozen/thawed parasites) and to Con-A were measured by 3H-Thymidine incorporation at different times after infection. Proliferating cells (blasts isolated in Percoll gradients) had their phenotypes determined by immunofluorescence. IL-2 in the supernatants of the

cultures was quantified by the proliferation of the CTLL-2 cell line.

The proliferative response to T-Ag was abolished by anti-Thy 1.2 mediated T cell lysis and suppressed by the monoclonal antibody GK 1.5 added to the cultures; 63% of the blasts in cultures stimulated with T-Ag were L3T4+, 16% were Ig+ and 3% Ly2+ cells. Therefore, the proliferative response to T-Ag was considered as an adequate assay to evaluate the amplification of parasite specific T lymphocyte populations of the L3T4 phenotype, along the **T. cruzi** infection.

LN cells of resistant and susceptible strains of mice proliferated to T-Ag and to Con-A all along the infection. B 10 spleen cells responses to T-Ag were suppressed between days 10-14 after infection and recover by day 21. A/Sn responses were also suppressed during this period.

The responses by LN and spleen cells to Con-A along the infection followed the general pattern observed for antigen specific responses, except that LN cells from resistant mice were moderately suppressed and following the acute phase spleen cell responses recovered only partially.

The proliferative responses of spleen cells stimulated with T-Ag or Con-A and of LN cell cultures stimulated with Con-A were parallel to the production of IL-2. In contrast, supernatants of LN cells stimulated with T-Ag showed very low or undetectable levels of IL-2, in spite of a vigorous proliferative response. The low levels of IL-2 could not be correlated with the presence of active infection, since LN cells from mice immunized with T-Ag developed good specific proliferative responses in cultures, whose supernatants exhibited very low levels of IL-2. Neither the T-Ag used to stimulate the cultures nor the LN cells themselves inhibited IL-2 activity.

Following i.p. infection, specific stimulation appeared initially in the spleen and later in the LN. LN cells respond to specific T-Ag all along the infection. The responses observed during the initial phase of infection were of the same magnitude in resistant and susceptible mouse strains. However, higher levels of IL-2 were consistently detected in the cell cultures of resistant mice than in cultures of susceptible mice cells.

The above results suggest that lymphoid compartments other than the spleen are responsible for the maintainance of the parasite specific T cell responses during the acute phase of the infection.

We investigated next how high (300,000) and low (5,000) parasite loads injected either by the intraperitoneal or by the subcutaneous (s.c.) route would affect the spleen and lymph nodes T cells parasite specific proliferative responses.

Spleen cell responses were always suppressed during the acute phase of infection independently of the size of the inocula, inoculation route and mouse strains. Suppression of LN cells proliferation was only observed in the lymph nodes draining subcutaneous sites inoculated with 300,000 parasites. The responses of LN distant from the site of infection were not affected. Suppressed LN cell responses could not be restored by the addition of IL-2.

Therefore, suppression of parasite specific responses in LNs is a restricted phenomenon which depends on the intensity of the initial local parasite load and does not occur in the presence of high systemic parasite loads.

The infection of susceptible mice with **T. cruzi**, by itself, led to much higher levels of intrinsic activation of LN cells, than those observed in LN of the resistant strain, irrespectively to the size of the inocula and infection routes.

Our results show that parasite specific responses by L3T4 lymphocytes can be detected all along the acute phase of infection with **T. cruzi** in lymphoid cell compartments other than the spleen. These findings challenge the concept of a generalized suppression of the cellular immune responses during the acute phase of infection and justify previous results showing that the spleen is not relevant to the control of the infection [1]. The role of L3T4+ cells as mediators of the resistance to infection was recently demonstrated [2,3].

B) Functional analysis of cell-mediated immunity generated by immunization with T. cruzi

It has been shown that the transfer of T cells from infected mice confers partial protection to the recipients against a challenge with **T. cruzi**. On the

other hand, it has also been shown that T cells can mediate inflammatory lesions v.g. the peripheral neuritis transferred by cells from **T. cruzi** infected mice [4], or aggravation of the disease upon challenge with parasites as reported for **Leishmania major**[5,6] and **Plasmodium berghei**[7] infections.

The dual role of T cells as mediators of protection or pathology deserves intense investigation, particularly in the diseases for which vaccination is being contemplated.

We started our work by establishing optimal immunization conditions leading to expression and detection of **in vivo** specific cell-mediated immunity (CMI) to **T. cruzi** antigens. C57 Bl/10J mice were immunized by the s.c. route with Y strain tissue culture trypomastigotes (T-Ag, 8-10 X frozen/thawed parasites), using saponin as adjuvant. CMI was evaluated by the delayed hipersensitivity (DTH) footpad test at different times after immunization. Optimal adjuvant and antigen concentrations for immunization and test were obtained from dose response curves. The DTH nature of the reaction was confirmed by the adoptive transfer of cells. Specificity was controlled by challenging both normal and immunized mice with T-Ag antigen and an unrelated antigen (ovalbumin-OVA).

The non-infective nature of T-Ag was checked by cultivation in LIT medium over a period of 30 days and challenge of immunized mice with high inocula of **T. cruzi** blood forms. No parasites could be detected in the cultures and the mice developed parasitemia and died.

Next we tested CMI **in vitro** of LN cells from immunized mice by the blast transformation assay, upon stimulation of the cultures with T-Ag. Cells from T-Ag immune mice proliferated to T-Ag and to frozen/thawed antigens prepared from epimastigotes, spleen and liver isolated amastigotes, metacyclic trypomastigotes but did not respond to LLC-MK2 cells (used for cultivation of trypomastigotes) and to OVA. None of the antigens were mitogenic for normal LN cells in the optimal doses used in the proliferative assays.

LN cells from T-Ag immune mice were cultivated in 24 wells plates for 4 days in the presence of T-Ag. The blasts were separated and expanded in TC flasks for additional 3 days in the presence of EL-4 cell culture supernatants as a source of IL-2. Stimulation indexes for the LN cells at the moment when they were first

cultivated and at the end of the expansion cultures were respectively 7-12 and 67-87. The majority of the cells were blasts of the L3T4 phenotype.

Normal syngeneic mice were transferred (I.V.) with the blasts and tested 24 h later for DTH with T-Ag. One day later, the mice were challenged (i.p.) with 200,000 living blood forms. Parasitemia and mortality were followed for a period of 50 days.

In all four experiments performed, the recipients of T-Ag blasts developed ten-fold higher mean parasitemia levels than normal or normal footpad tested mice infected at the same time (control groups). In three experiments the 20 days mortality rates were 80% for the transferred mice vs. 0% in the control groups; the 50 days mortality rates were 100% vs. 0-17% respectively.

Our results indicate that within the repertoire of clones of T cells stimulated by **T. cruzi** antigens, during **in vivo** immunization, populations of cells may develop, which instead of protecting the host against the disease would rather aggravate the infection.

There is also the danger that these " unwanted " populations might be expanded by immunization boosters. On the other hand, it is possible that only some **T. cruzi** antigenic molecules have the potential to stimulate deleterious T cell clones. Both hypothesis deserve careful investigation whenever vaccination protocols are being considered.

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