Modification of T-cell proliferation and interleukin 2 production in mice infected with Trypanosoma cruzi (autoimmunity/suppression)

ANNICK HAREL-BELLAN*, MIREILLE JOSKOWICZ†, DIDIER FRADELIZI*, AND HARVEY EISEN†

*Laboratoire des Interactions Lymphoïtaires, Institut National de la Santé et de la Recherche Medicale, U93, Centre Hayem, Hôpital St. Louis, 2 Place du Dr Fournier, 75475 Paris Cedex 10 France; and †Unité d’Immunoparasitologie, Institut Pasteur, 28 rue du Dr. Roux, 75015 Paris, France

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ABSTRACT Acute infection of mice with Trypanosoma cruzi results in severe immunodepression and the appearance of autoimmune symptoms. In vitro, concanavalin A-stimulated T cells from spleens of infected animals could neither produce nor respond to interleukin 2. Interleukin 2 production was not restored by addition of exogenous interleukin 1, and proliferative response to concanavalin A was not restored by exogenous interleukin 2. A population of Thy-1-negative cells in the spleen of infected animals was shown to suppress the concanavalin A proliferative response and, to a lesser extent, the production of interleukin 2. These and other symptoms of T. cruzi-infected mice are similar to the immune dysfunction of autoimmune lpr/lpr mice. These findings are discussed in relationship to the pathology of Chagas disease.

Trypanosoma cruzi causes Chagas disease in man. The disease is characterized by a variable acute phase during which trypanastigotes circulate, followed by a lifelong chronic phase in which parasites are not detected in circulation but rather appear to be sequestered in the tissues (1). The parasites persist in the chronically infected individuals in spite of the presence of high levels of circulating anti-T. cruzi antibodies. The mouse has been used as a laboratory model for Chagas disease because injection of parasites gives rise to both acute and chronic infections.

Severe perturbations of the host immune system accompany T. cruzi infections in the mouse (1–4). On one hand, autoantibodies directed to various host antigens are induced. On the other hand, severe immunodepression has been reported to occur during the acute phase of the infection, and cellular responses to mitogens, parasite antigens, or irrelevant antigens are depressed. These results suggest that the parasites perturb the host immune system at the level of the T cells. We have studied this possibility by examining T-cell proliferation and the production of T-cell regulatory molecules in T. cruzi-infected mice.

Interleukin 2 (IL-2) appears to play a key role in the regulation of lymphocyte immune responses (5, 6). It is produced by T lymphocytes in the presence of interleukin 1 (IL-1) and mitogen or antigen, and it sustains the proliferation of helper and effector T lymphocytes and thus amplifies the effector phase of the immune response. Because of this central role of IL-2 in regulating the immune response, we have examined its production and activity in T. cruzi-infected mice.

We report here that acute infection with T. cruzi results in a severe depression of IL-2 production which parallels the depressed proliferative response to the mitogen concanavalin A (Con A). Furthermore, Thy-1+ spleen cells from infected animals are able to suppress the proliferative response to Con A and, to a lesser extent, IL-2 production by spleen cells from normal animals.

MATERIALS AND METHODS

Mice and Infection. All experiments were performed with pools of three to eight C3H/HeJ F1 male mice (Pasteur Institute, Paris). Mice were 6–8 weeks of age at the time of infection. They were infected intraperitoneally by injection of 2–3 × 10^5 bloodstream forms of Y (7) or CL (8) T. cruzi strain. Routinely, the acute phase of Y strain- and CL strain-infected mice showed peak parasitemias at 7 and 10 days, respectively. The infected mice were in chronic phase 60 days after infection.

Culture Media, Mitogen, and Reagents. Spleen cells were suspended in RPMI 1640 medium (Flow Laboratories) supplemented with 2 mM glutamine, penicillin/streptomycin mixture (at 50 units/ml and 50 µg/ml, respectively), and 10% fetal calf serum (Flow Laboratories, Asnières).

Con A, Escherichia coli lipopolysaccharide (LPS), and 2-mercaptoethanol were purchased from Sigma. Phytohemagglutinin P (PHA-P) was purchased from Difco.

IL-1 was a supernatant from human monocytes stimulated with MDP (9) and was a kind gift of C. Damais (Pasteur Institute, Paris).

Con A Proliferation Assay. Spleen cells from infected or uninfected mice were adjusted to 2.5 × 10^6 cells per ml in culture medium and distributed in Falcon flat-bottomed microtiter plates (0.2 ml per well). Various concentrations of Con A were added and cultures were incubated at 37°C in a 7.5% CO_2/92.5% air humidified atmosphere (standard conditions) for 3 days. On day 2 of culture, 1 µCi (1 Ci = 3.7 × 10^10 Bq) of [3H]thymidine (Amersham, France, 2 Ci/µmol) was added to each well. Cultures were harvested 16–20 hr later on filter pads with a cell harvester (Titertek) and radioactivity was measured in scintillation fluid. Determination of [3H]thymidine incorporation was carried out in triplicate.

For Thy-1 depletion of spleen cells, 10^6 cells from infected mice were treated at 23°C for 45 min with anti-Thy-1.2 monoclonal antibody (F7D5, Olac, dilution 1:2,000), washed, and incubated for 45 min at 37°C with guinea pig complement (BioMérieux, France) diluted 1:2 in RPMI 1640. This was sufficient to completely abolish Con A-stimulated proliferation by splenocytes from uninfected animals.

IL-2 Production and Titration. Spleen cells from infected or uninfected mice were adjusted to 4 × 10^6 cells per ml in culture medium supplemented with 50 µM 2-mercaptoethanol and 5 µg of Con A per ml. After 24 hr of incubation (standard conditions), cultures were filtered on 0.22-µm Millex filters (Mil-

Abbreviations: IL-2, interleukin 2; IL-1, interleukin 1; Con A, concanavalin A; LPS, lipopolysaccharide; PHA, phytohemagglutinin.

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RESULTS

Responses of Spleen Cells from Infected Mice to Con A. In order to examine the responses of splenocytes from *T. cruzi*-infected mice, animals were inoculated with blood forms of Y and CL strain parasites. At various times the parasitemias were measured according to Brener (12) and the spleens were removed. The T-lymphocyte contents of the spleens were analyzed by indirect immunofluorescent staining with anti-Thy-1 antibodies. The cells were then examined for their ability to proliferate and produce IL-2 in the presence of varying amounts of Con A. The response to Con A of cells from animals infected 7 days previously with Y strain parasites was 10–20% of that of uninfected controls (Fig. 1). The response of cells from CL-infected mice (day 12) was <5% of the control values. Spleen cells from chronically infected mice showed a partially restored re-

![Fig. 1.](image1)  
**Fig. 1.** Con A-stimulated proliferation of spleen cells from normal and infected mice. After 2 days of stimulation with the indicated doses of Con A, cultures were pulsed overnight and [3H]thymidine incorporation was measured. (A) Strain Y of *T. cruzi*. (B) Strain CL of *T. cruzi*. ×, Control mice; ○, mice in acute phase of infection (strain Y, day 7; strain CL, day 10); □, mice in chronic phase of infection (>60 days).

![Fig. 2.](image2)  
**Fig. 2.** Titration curves of IL-2 produced by spleen cells from normal and infected mice. After 24 hr of stimulation with Con A, culture supernatants were assayed for IL-2 content on IL-2-dependent CTL-L2 cell. (A) Y strain of *T. cruzi*. (B) CL strain of *T. cruzi*. ×, Control mice; ○, mice in acute phase of infection; □, mice in chronic phase of infection.

response to Con A; cells from Y- and CL-infected animals gave 40–60% of the control values.

IL-2 production was measured in the supernatants of cultures incubated for 24 hr with Con A (Fig. 2). The IL-2 production by splenocytes from mice acutely infected Y strain was <10% of the control value; that of cells from animals acutely infected with CL strain was not detectable. Supernatants of *T. cruzi*-infected spleen cell cultures did not inhibit the activity of IL-2 produced by normal spleen cells (not shown), showing that there is no inhibitor for IL-2 activity in these supernatants. As was seen for the proliferative response, spleen cells from chronically infected mice showed partial restoration of IL-2 production.

![Fig. 3.](image3)  
**Fig. 3.** Time course of depression of Con A-stimulated proliferation and IL-2 production (for details, see legend to Figs. 1 and 2). (A) Y strain. (B) CL strain. △ Parasitemia; ○, Con A-stimulated proliferation; □, IL-2 production.
(infected cells to normal cells) (Fig. 4), suggesting that there are suppressive factors or cells in the infected spleen populations. In the case of the effect of infected spleen cells on the production of IL-2 by normal spleen cells, the suppression by infected cells was less marked: even at the highest ratio (1:1) the inhibition of IL-2 production was about 50%.

To explore the suppression further, spleen cells from infected mice were treated with anti-Thy-1 antibodies in the presence of complement. The surviving Thy-1+ cells were tested for their ability to suppress the Con A-stimulated proliferation of cells from uninfected animals. The suppressive activity of the Thy-1+ cells was greater than that of the untreated population (Fig. 4).

Response of T. cruzi-Infected Cells to Added IL-2. Because spleen cells from infected mice do not produce IL-2, the failure to proliferate in response to Con A could simply reflect the lack of IL-2 production. In order to test this possibility, exogenous IL-2 was added to spleen cells from infected animals. Although added IL-2 did not restore the Con A-stimulated proliferative response of cells from Y strain-infected mice, there was some stimulation, suggesting that the nonsuppressed cells retained their sensitivity to the growth factor (Table 1). Added IL-2 had little or no effect on the proliferation of cells from CL strain-infected mice.

Response of T. cruzi-Infected Spleen Cells to Added IL-1. IL-1 is produced by monocytes and macrophages and is necessary for the production of IL-2 by T cells (5, 6). Because T. cruzi grows in macrophages during the acute phase of infection, it was possible that IL-1 production was blocked by the intracellular parasites. This was not the case (Fig. 5). Addition of exogenous human IL-1 to spleen cultures from infected animals did not restore production of IL-2. Also, addition of normal peritoneal exudate cells (10% of spleen cells) did not restore IL-2 production (data not shown). Furthermore, peritoneal exudate macrophages from infected animals produced normal or increased levels of IL-1 (data not shown).

**DISCUSSION**

Acute infection of mice by *T. cruzi* results in severe immunodepression. It had been demonstrated previously that the immunodepression is accompanied by a low proliferative response of spleen T cells from infected animals to Con A stimulation. This low response has been shown to be due, at least in part, to the suppressive action of adherent cells from the infected spleen (2–4).

We have further analyzed the defects of T-cell regulation in mice infected with two strains of *T. cruzi*. In the acute phase, both strains (Y and CL) provoke a severe inhibition of Con A-

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**Table 1. Lack of restoration of Con A-stimulated proliferation with IL-2**

<table>
<thead>
<tr>
<th>Source of spleen cells</th>
<th>Final dilution of IL-2-containing supernatant</th>
<th>[3H]Thymidine incorporation, cpm*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice</td>
<td>0</td>
<td>202,430 ± 10,200</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>230,700 ± 30,220</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>260,600 ± 15,260</td>
</tr>
<tr>
<td>Infected mice¹</td>
<td>Strain Y²</td>
<td>38,000 ± 7,310</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>57,000 ± 961</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>80,300 ± 2,110</td>
</tr>
<tr>
<td></td>
<td>Strain CL⁴</td>
<td>15,500 ± 1,920</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>17,530 ± 690</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>19,380 ± 1,860</td>
</tr>
</tbody>
</table>

*Mean (=SD) of triplicate cultures.

¹The mice were in the acute phase of infection (day 7 for Y strain, day 10 for CL strain).

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**Fig. 5. Lack of restoration of IL-2 production by exogenous IL-1.** Spleen cells were incubated for 4 hr in the presence of both Con A and different doses of human IL-1 (giving a maximal effect in the thymocyte assay). The IL-2 content of supernatants was then titrated on CTLL2 cells. ×, Control supernatant; ○, supernatant of spleen cells from infected mice (Y strain, acute phase) alone; ◊, ▲, supernatant of spleen cells from infected mice (Y strain, acute phase) in the presence of human IL-1 diluted 1:10 or 1:5.
induced IL-2 production as well as the proliferative response to Con A stimulation. The IL-2 production defect did not result from a lack of IL-1 because it was not restored by the addition of exogenous IL-1. Also, the Con A proliferative response defect did not result exclusively from a lack of IL-2 because it was not restored by the addition of exogenous IL-2.

In our experiments, spleen cells of T. cruzi infected-mice inhibited the proliferation of cells from uninfected mice. The cells responsible for this effect were not removed by treatment with anti-Thy-1 antibodies and complement. The suppression of IL-2 production was less marked than that of proliferation.

However, this could be due to the differences in the two assays: IL-2 was measured on 24-hr culture supernatants whereas proliferation was assayed after 3 days of culture.

The immune dysfunctions of mice acutely infected with T. cruzi are remarkably similar to those of autoimmune mice homozygous for the lpr mutation (14, 15). As with lpr/lpr mice, T. cruzi-infected spleen cells neither produce nor respond to IL-2. Also, T. cruzi-infected mice have been shown to overproduce immunoglobulins of the IgG class. We have found 10- to 100-fold increases in levels of IgG-secreting B lymphocytes in the spleens of infected animals (unpublished data). Finally, Chagas disease is characterized by autoimmune symptoms.

Recently, Wood et al. (16) reported that T. cruzi contains antigens that crossreact with antigens of the mammalian tissues that frequently are affected during Chagas disease and suggested that they might play a role in the pathology of the disease. It is possible that the syndrome provoked by T. cruzi also could be important in the pathology of Chagas disease by creating optimal conditions for the development of autoantibodies in the presence of the crossreacting antigens.

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