NOTES

Development of Resistance to *Trypanosoma cruzi* in Mice Depends on a Viable Population of L3T4+ (CD4+) T Lymphocytes

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Mice injected with monoclonal antibody to the L3T4+ (CD4+) cell membrane surface glycoprotein of T lymphocytes and immunized with antigens of *Trypanosoma cruzi* had reduced antibody and cell-mediated immune responses to the organism. Mortality in these mice was 100% following challenge with 1.5 × 10^5 trypomastigotes, whereas controls had a 50% survival rate, indicating that T-cell activation and viable L3T4+ (CD4+) T lymphocytes are essential for the development of resistance.

Immune mechanisms responsible for resistance to *Trypanosoma cruzi* and for pathogenesis of the tissue lesions of Chagas’ disease are poorly understood (9, 11). A strong antibody response is essential (12), and thymectomy (17) and use of antilymphocyte serum (16) enhance the severity of the infection. Immunization with killed (14), chemically inactivated (1) *T. cruzi*, as well as with a purified glycoprotein of the organism (18), induces a partial protection against infection. To examine the role of L3T4+ (CD4+) T lymphocytes in the mechanism of resistance to *T. cruzi*, we have used the ability of mice to mount a partial resistance to *T. cruzi* following immunization with antigens of the organism mixed with adjuvants.

*T. cruzi* Tulahuen epimastigotes (3) were washed in phosphate-buffered saline (PBS) with 2% glucose, suspended in distilled water, and sonicated in an ice bath. The sonically treated material was centrifuged at 10,000 × g for 10 min, the pellet was discarded, and the supernatant was centrifuged again at 34,000 × g for 2 h in a refrigerated centrifuge. The supernatant of the last centrifugation was discarded, and the pellet (epimastigote antigen preparation [Epi-Ag]) was suspended in PBS, divided into aliquots, and kept frozen at −70°C until used. Epi-Ag was used at 100 μg of protein per intraperitoneal injection either alone or mixed with 30 μg of saponin (Sigma Chemical Co., St. Louis, Mo.) in a total volume of 0.2 ml. Each mouse received one weekly injection for 5 weeks. Monoclonal antibody (MAb) GK 1.5 was purified from ascites fluid by precipitation with 45% saturated ammonium sulfate and dialysis with PBS. Reactivity with L3T4+ T lymphocytes was determined by flow cytometry (7) and by immunofluorescent-antibody assay (6). MAb GK 1.5 was used at 300 μg of antibody per intraperitoneal injection 3 days before each injection of Epi-Ag. Experimental mice (BALB/c, females, 20 g) received MAb GK 1.5 and Epi-Ag (group A1, 6 mice), Epi-Ag alone (group A2, 10 mice), MAb GK 1.5 and Epi-Ag with saponin (group B1, 7 mice), Epi-Ag with saponin (group B2, 9 mice), and a nonrelevant MAb (group C, 10 mice). Five days after the last immunization, mice from each group were individually tested for antibodies (3). Immunoblots to determine antigens of *T. cruzi* recognized by antibodies in the sera were prepared as previously described (2). Delayed-type hypersensitivity (DTH) was examined by injecting mice in the right hind footpad with 20 μl of PBS containing 10^7 Formalin-killed and washed epimastigotes and in the left hind footpad with 20 μl of PBS only. The test was read 24 and 48 h later. One week after the last immunization, each mouse was challenged with 10^5 bloodstream-form trypanosomes of the Tulahuen strain.

Treatment with MAb GK 1.5 had a profound effect on the antibody response to Epi-Ag. Immunization with Epi-Ag alone (Table 1, group A2) or in combination with saponin (Table 1, group B2) resulted in a strong antibody response, particularly when saponin was used. In contrast, antibodies to Epi-Ag were not detected in mice treated with MAb GK 1.5 (group A1) and immunized with Epi-Ag or was very low when Epi-Ag was used with saponin (group B1). Immunoglobulin G immunoblots (Fig. 1) revealed that mice injected with MAb GK 1.5 and immunized with Epi-Ag (Fig. 1, lane 1) did not form antibodies to Epi-Ag; this aspect of the immunoblot is like that of noninfected controls (Fig. 1, lane 5). Mice immunized with Epi-Ag only (Fig. 1, lane 2) or with Epi-Ag and saponin (Fig. 1, lane 4) formed antibodies which reacted with a number of polypeptides of different Mr’s. These immunoblots are similar to those of mice infected with *T. cruzi* for 4 weeks (Fig. 1, lane 7) or for 3 months (Fig. 1, lane 6). Of interest was the immunoblot with serum from mice injected with MAb GK 1.5 and immunized with Epi-Ag in combination with saponin (Fig. 1, lane 3). Antibodies in the sera of these mice strongly recognized an antigen of *T. cruzi* with an approximate Mr of 35,000, suggesting that this antigen is T cell independent. Immunoglobulin M immunoblots were similar to immunoglobulin G immunoblots (data not shown). DTH was almost completely abrogated by administration of MAb GK 1.5. Mice immunized with Epi-Ag alone developed a minor DTH reaction which was completely absent in mice injected with MAb GK 1.5 and immunized similarly (Table 2, groups A1 and A2). However, mice immunized with Epi-Ag in combination with the adjuvant saponin developed a strong DTH reaction (Table 2, group B2), whereas in mice injected with MAb GK 1.5 and immunized similarly (Table 1, group B1), the DTH reaction was considerably less evident. The differences in thickness of the footpad swelling between the two experimental groups

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TABLE 1. Antibody response in mice treated with MAb GK 1.5 and immunized with T. cruzi antigen

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<th>Experimental group</th>
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| A1                 | 0.024 ± 0.003
| A2                 | 0.178 ± 0.010
| B1                 | 0.045 ± 0.009
| B2                 | 0.354 ± 0.069
| C                  | 0.023 ± 0.009
| Negative control   | 0.267 ± 0.015

* Mean absorbance of sera from three mice ± standard deviation.

of mice were statistically significant ($P < 0.001$, Student’s t test). Challenge of the immunized mice (Fig. 2) revealed that Epi-Ag only (group A2) did not induce any resistance, and their mortality rate was similar to that of controls (group C). In contrast, immunization with Epi-Ag in combination with saponin (group B2) induced a remarkable resistance which resulted in 50% survival rate for the animals. The development of this resistance, however, was completely abrogated by injection of MAb GK 1.5 (group B1). A delay in time to death, although not statistically significant, was noted for both groups injected with MAb GK 1.5 (groups A1 and B1), particularly the group receiving saponin.

Previous reports have suggested that L3T4* (CD4*) T lymphocytes are active participants in the pathogenesis of Chagas’ disease (4, 8, 13). Our results indicate that although L3T4* (CD4*) T lymphocytes may participate in the pathogenesis of T. cruzi infection, a viable population of these cells appears to be essential for the development of resistance to the organism. An observation similar to ours has been made in cases of murine schistosomiasis, in which depletion of Lyt-1* and L3T4* T lymphocytes decreased the development of resistance, antibody, and DTH directed against schistosome antigens. Morbidity due to the infection was increased (15). Functional analysis of cloned T lymphocytes has indicated that CD4* cells may be grouped into two categories: those which help specific as well as polyclonal B-cell responses but fail to mediate killing, suppression, and DTH; and those which help polyclonal but not specific B-cell responses, mediate DTH, and kill appropriate target cells (5). We have not defined the type of CD4* T lymphocytes depleted. The fact that specific antibody and DTH responses

![FIG. 1. Antigens of T. cruzi recognized by immunoglobulin G antibodies. The nitrocellulose paper strips containing T. cruzi antigens were treated with sera from mice injected with MAb GK 1.5 and immunized with Epi-Ag (lane 1; group A1); Epi-Ag only (lane 2; group A2); MAb GK 1.5, Epi-Ag, and saponin (lane 3; group B1); Epi-Ag and saponin (lane 4; group B2); or MAb from a nonrelevant hybridoma (lane 5). Lanes 6 and 7, respectively, were treated with sera from mice chronically or acutely infected with T. cruzi. Numbers to the left indicate molecular weight markers.](http://iai.asm.org/)

![FIG. 2. Percent mortality of immunized mice challenged intraperitoneally with $1.5 \times 10^5$ bloodstream trypanosomes. The experiment was terminated 30 days after challenge, at which time survivors of group B2 were healthy. Symbols: Δ, group C (10 mice); ●, group B2 (10 mice treated with Epi-Ag and saponin); ○, group B1 (7 mice treated with MAb GK 1.5, Epi-Ag, and saponin); ■, group A2 (10 mice treated with Epi-Ag); □, group A1 (6 mice treated with MAb GK 1.5 and Epi-Ag).](http://iai.asm.org/)
were almost completely inhibited suggests inactivation of both types of CD4+ cells. The slight delay in time to death noted in mice injected with MAb GK 1.5 may have been caused by an inhibitory activity on the CD4+ inflammatory cells. In this context, we have noted that treatment with MAb GK 1.5 significantly reduced the inflammatory response in the brains of mice infected with *Toxoplasma gondii* (10). The present observation and previous reports suggest that L3T4+ (CD4+) T lymphocytes have a dual role as modulators in the pathogenesis of Chagas’ disease and as essential participants in development of resistance to *T. cruzi*.

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LITERATURE CITED


