Thymus-Dependent Control of Host Defense Mechanisms Against Trypanosoma cruzi Infection

FELIPE KIERSZENBAUM* and MAREK M. PIENKOWSKI²

Departments of Microbiology and Public Health¹ and Anatomy,² Michigan State University, East Lansing, Michigan 48824

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Congenitally athymic homozygous (nu/nu) mice were shown to be significantly more susceptible to Trypanosoma cruzi infection than their thymus-bearing heterozygous (nu/+) littermates, as measured by increased parasitemia, mortality rate, and shortened survival time. In addition, transplantation of neonatal thymus into athymic mice reestablished normal levels of resistance to T. cruzi, i.e., comparable to those of normal littermates. These results constitute conclusive evidence that host defense mechanisms active in experimental Chagas’ disease are under thymic control.

MATERIALS AND METHODS

Congenitally athymic homozygous (nude, nu/nu) mice and their littermates, heterozygous thymus-bearing (nu/+) mice on BALB/cJ background at the ninth level of backcrossing, used in this work, were from a barrier-sustained colony maintained in the Department of Anatomy at Michigan State University (20). T-cell deficiency of nu/nu mice of this colony was evidenced by their capacity to accept xenogeneic grafts (18), by lack of response to stimulation with concanavalin A (19), and by the insignificant proportion (<1%) of Thy-1-bearing cells present in their lymphoid organs. Animals were 2 to 3 months old when infected with T. cruzi.

Bloodstream forms of Tulahuen strain of T. cruzi maintained by serial passage in mice were used in all experiments. Dilution as necessary was performed with a sterile phosphate-buffered saline solution (pH 7.2). The 50% lethal dose of the parasite for Swiss albino mice was 500 organisms and was determined by the method of Reed and Muench (21). Parasitemias, measured by a standardized microscopic procedure (11), were expressed as numbers of T. cruzi per milliliter of blood. Differences between parasitemia mean values were analyzed by Student’s t test and considered to be significant if P < 0.05. Mortality was recorded daily. The Mann-Whitney U test, two-tailed, was used to analyze these data, and statistical significance was assumed when P < 0.05.

Syngeneic thymus transplants during the neonatal period were performed using thymus glands from 1- to 2-day-old female nu/ + mice. One-half of an isolated organ was implanted subcutaneously into each nu/nu animal through a small dorsal skin incision. The remaining half of the organ was processed by a standard histological technique to confirm the identity of the isolate, and in all cases it was found to be thymus. The thymus-reconstituted mice were returned to nursing mothers for an additional 3-week period prior to wean-
ing. An aseptic technique was carefully observed throughout all procedures. The neonatal syngeneic thymus transplant, as performed here, is known to restore T-cell functions in congenitally athymic mice (12, 13, 29, 33).

RESULTS

The results of our experiments demonstrated that athymic mice have greater susceptibility to *T. cruzi* infection than their heterozygous, thymus-bearing littermates. This was in part expressed in terms of considerably increased parasitemia levels. A statistically significant (at *P* < 0.05) difference in the parasitemia level was observed as early as 6 days postinfection. On day 15 postinfection, surviving athymic mice harbored over 10 times more parasites than normal littermates (Table 1). The higher parasitemias accompanied shortened average survival times and increased mortality rates of athymic mice (Fig. 1). On day 16 postinfection, when the cumulative mortality of athymic mice reached 100%, only 50% mortality was noted with normal littermates. Similar results were obtained in two other experiments conducted under identical conditions, except that in those cases there were no survivors (including normal littermates) in any of the groups. With increased infective doses of parasites (up to 27,000), the mortality rate increased in both groups of animals, yet the mortality of athymic mice anticipated significantly that of normal littermates (compare Fig. 1 and 2).

That hypersusceptibility to *T. cruzi* infection was due to lack of thymus, and not to other genetic deficiencies of the athymic nu/nu mice, was indicated by the normal susceptibility of these mice after they were neonatally reconstituted with thymus (Table 2, Fig. 2). A comparable level of parasitemia was achieved by these two groups of animals through the course of infection. Also, a very similar rate of mortality was observed (Fig. 2). A group of nongrafted athymic nu/nu mice was included in this experiment, all of which were dead by day 11 postinfection; their average survival time was 7.4 days.

![Figure 1](http://iai.asm.org/) Comparison of mortality rates of athymic (nu/nu) mice and thymus-bearing littermates infected with *T. cruzi*. The curves correspond to the same groups whose parasitemias are shown in Table 1. *P* < 0.05.

![Figure 2](http://iai.asm.org/) Comparison of mortality rates of athymic (nu/nu) mice, thymus-reconstituted (T-R) nu/nu mice, and thymus-bearing (nu/+) littermates infected with *T. cruzi*. The curves correspond to the same groups whose parasitemias are shown in Table 2. *P* < 0.05.

| Table 1. Hypersusceptibility of athymic (nu/nu) mice to *T. cruzi* infection* |
|---|---|---|---|---|---|
| Mice | Mean parasitemia ± standard error on day: | --- | --- | --- | --- |
| &nbsp; | 6 | 8 | 11 | 13 | 15 |
| nu/+ | 0.5 ± 0.2c | 1.2 ± 0.3 | 10.5 ± 3.0 | 20.9 ± 5.4 | 16.0 ± 5.5 | 17.8d | 6/8 |
| nu/nu | 0.1 ± 0.05 | 0.5 ± 0.2 | 20.2 ± 5.5 | 107.1 ± 24.7 | 187.2 ± 43 | 14.7 | 8/8 |
| *P* | <0.001 | <0.02 | <0.02 | <0.01 | <0.001 | <0.05 |

* A total of 20,000 *T. cruzi* trypomastigotes were injected intraperitoneally.
* "s*, Mean survival time.
* "m" Million parasites per milliliter of blood.
* "This figure represents the average survival time of the six dead mice. The harmonic survival mean, considering the two survivors, was 21.9 days.
This faster course of the disease with respect to that in athymic animals used in previous experiments (with 20,000 T. cruzi), in which mortality was observed between 13 and 16 days postinfection, is probably due to the use of a higher infective dose (27,000 T. cruzi). The difference, 7,000 organisms, represents 14 50% lethal doses for Swiss mice and probably many more for the highly susceptible athymic mice. Due to this early mortality, parasitemias were not measured in the nu/nu animals.

DISCUSSION

The results demonstrated the thymus-dependent nature of host defense mechanisms against T. cruzi infection. Thus, athymic animals were hypersusceptible to this infection, and their hypersusceptibility was readily corrected, i.e., brought to normal levels, by neonatal thymus transplants, which led to reconstitution of thymus-dependent responses. These observations are consistent with the finding of Roberson et al. (22), who noted that treatment with anti-thymocyte serum to remove T cells exacerbated T. cruzi infection in mice and that neonatal thymectomy had similar consequences in rats.

The importance of specific circulating antibodies has been highlighted by a series of experimental findings, including demonstration of protection against challenge by passive antibody transfer (3, 6, 9, 17), opsonization of T. cruzi (27), and sensitivity of the parasite to immune lysis in vitro (1, 7). More recently, cultured T. cruzi epimastigotes, which share antigenic determinants with circulating forms of the parasites (5, 8), have been reported to be sensitive to antibody-dependent, cell-mediated cytotoxicity, probably effected by eosinophils (25). However, to develop protective humoral immunity in Chagas' disease, T-cell function in terms of helper activity is likely to be essential. It is premature to try to infer from the present results specific functions of T cells in host defense against T. cruzi. It is also inconceivable that destruction of T. cruzi by macrophages, shown to occur both in vivo and in vitro (4, 10, 32), could be regulated by soluble T-cell products. Furthermore, the possibility of a direct toxic effect on T. cruzi caused by lymphocytes and/or other types of cells remains to be explored.

We should also stress that although these findings clearly involve the thymus in host defense against T. cruzi they do not rule out its hypothetical participation in, or control of, T-cell-dependent reactions which might lead to production of pathology in Chagas' disease itself (26).

Interestingly, it is the athymic mice that display better resistance to infection with Trypanosoma rhodesiense (2), the extracellular parasite causing sleeping sickness. The opposite behavior of the nu/nu mice in the two trypanosomal infections supports the concept that pathogenicity and host defense mechanisms involved in experimental T. cruzi and T. rhodesiense infections are likely to follow dissimilar pathways.

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LITERATURE CITED

5. González-Cappa, S. M., G. A. Schmunis, O. C. Traversa, J. F. Yanovsky, and A. S. Parodi. 1968. Complement fixation tests, skin tests, and experimental immunization with antigens of Trypanosoma cruzi pre-

TABLE 2. Correction of the hypersusceptibility of athymic (nu/nu) mice to T. cruzi infection* by thymus reconstitution

<table>
<thead>
<tr>
<th>Mice</th>
<th>Mean parasitemia ± standard error on day:</th>
<th>5± (days)</th>
<th>Dead/tot al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus-reconstituted nu/nu</td>
<td>0.5 ± 0.1, 1.7 ± 0.6, 5.4 ± 2.2, 8.9 ± 0.3</td>
<td>13.2, 5/5</td>
<td></td>
</tr>
<tr>
<td>nu/+</td>
<td>0.3 ± 0.1, 1.0 ± 0.4, 2.7 ± 0.7, 5.8 ± 1.7</td>
<td>14.1, 5/5</td>
<td></td>
</tr>
</tbody>
</table>

* A total of 27,000 T. cruzi trypomastigotes were injected intraperitoneally.
+ Mean survival time.
Million parasites per milliliter of blood.


