Autoimmune myocarditis induced by
Trypanosoma cruzi

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ABSTRACT Antiheart immune reactions have been reported in patients with Chagas’ disease, and we have postulated that the observed cardiac lesions are mediated by autoimmune antihert reactions elicited by the etiologic agent Trypanosoma cruzi. In this report, BALB/c mice infected with a low inoculum of T. cruzi developed splenic lymphocyte cytotoxicity against normal syngeneic neonatal cardiac myofibers in vitro 150 days after infection, whereas splenic lymphocytes obtained from mice at 15, 45, 90, or 120 days after infection or from matched controls did not. No antihert antibody or antibody-directed cellular cytotoxicity was observed, nor was there an increase in natural killer cell activity. Hearts from mice studied at 150 days after infection showed mononuclear cell myocarditis with myocytolysis in the absence of intracellular T. cruzi forms. Hearts from the other mice did not exhibit any histologic changes. Other reports from our laboratory have identified a cross-reacting antigen (SRA) shared by T. cruzi and striated muscle. Immunization of BALB/c mice with SRA produced immunopathogenic dynamics similar to those seen with long-term T. cruzi infection. Collectively these data indicate that the cardiac lesions seen in patients with Chagas’ disease may be attributed to autoimmune reactions elicited by cross-reacting antigens of T. cruzi and striated muscle.


CHAGAS’ DISEASE or American trypanosomiasis (etiologic agent: Trypanosoma cruzi) is the most common cause of congestive heart failure and sudden death in the world, affecting an estimated 20 million individuals in the western hemisphere. One of the fundamental paradoxes of Chagas’ disease is the severe involvement of the heart in the absence of any intracellular T. cruzi forms there. Typically focal but widely distributed chronic mononuclear cell myocarditis is present with myocytolysis and fibrous replacement of the myocardium. The presence of intracellular T. cruzi forms is very difficult, if not impossible, to detect. Thus, although an etiologic agent has been described, the pathogenesis of Chagas’ disease has yet to be clearly determined.

Antiheart immune reactions have been described in both human and experimental Chagas’ disease. Lymphocytes from patients with Chagas’ disease have been shown to interact with and destroy normal heart cells in vitro. These patients have positive test results for leukocyte migration inhibition factor (MIF) with both T. cruzi and heart antigens. Similarly, lymphocytes from rabbits infected with T. cruzi or immunized with the microsomal fraction of T. cruzi destroyed normal allogeneic cardiac cells in vitro and exhibited positive MIF test results with either T. cruzi or heart antigens. These data have led us to postulate an autoimmune pathogenesis for the cardiac lesions of Chagas’ disease. In other reports from our laboratory, we have isolated and characterized a cross-reacting antigen (SRA) shared by T. cruzi and striated muscle. We believe that SRA is implicated in eliciting autoimmune antiheart reactions that result in the cardiac damage seen in chagasic hearts. The experiments reported here were performed to test the following hypotheses: (1) that T. cruzi infection gives rise to antiheart autoimmune reactions, (2) that the autoimmune antiheart reactions seen in those with T. cruzi infections are elicited by SRA, and (3) that the autoimmune antiheart reactions elicited by SRA produce the cardiac lesions seen in Chagas’ disease.

Methods

T. cruzi strain. The Colombia strain of T. cruzi was originally isolated from a patient with Chagas’ disease by xenodiagnosis, and maintained in liver infusion–tryptose cell-free medium. Trypomastigote forms were produced in cultures of African green monkey renal cells (M.A. Biproducts, McLean, VA).
SRA. The cross-reacting antigen SRA is present in the small membranes of *T. cruzi* and also in purified preparations of the calcium-sequestering ATPase of striated muscle sarcoplasmic reticulum. Cross-reactivity was determined by several serologic techniques, including indirect immunofluorescence, Ouchterlonely double diffusion in gels, hemagglutination, and enzyme-linked immunosorbent assay. Cross-reactivity was also demonstrated by the footpad reaction, which is a measure of the delayed type of hypersensitivity. SRA was found in all nine *T. cruzi* strains tested and has been found in the striated muscle of animals representative of the evolutionary scale ranging from nonhuman primate to fish. In the present experiments, SRA was isolated from African green monkey skeletal muscle (Pel-Freez Biologicals, Inc., Rogers, AR) and BALB/c-BYJ mice (Jackson Laboratories, Bar Harbor, ME) according to the method of MacLennan.

Mice. Five groups of young adult male BALB/c-BYJ mice (Jackson), those to be studied 15 (n = 6), 45 (n = 6), 90 (n = 6), 120 (n = 12), and 150 days (n = 9) after infection, were infected with 50 trypomastigotes per mouse intraperitoneally. An additional four mice per group were injected with suspension buffer and served as controls. Two groups of 10 young adult male BALB/c-BYJ mice were immunized with 50 μg of either heterologous monkey SRA or syngeneic BALB/c-BYJ SRA emulsified in Freund's complete adjuvant (FCA) (GIBCO Laboratories, Inc., Grand Island, NY) intraperitoneally biweekly for 8 weeks. An additional six mice were immunized with suspension buffer emulsified in FCA according to similar schedule and served as controls.

Syngeneic neonatal tissue cultures. Myocardial cell cultures were prepared from less than 48-hr-old neonatal BALB/c-BYJ mice according to the method of Boltot et al., as modified by Huber et al. Over 90% of the cells were identified as muscle fibers by phosphtungstitic acid–hematoxylin staining of sarcomeres. Endothelial cell tissue cultures were prepared from similar neonatal mice according to the method of Huber et al.

Splenitic mononuclear cell isolation. Splenic mononuclear cells were isolated by Hypaque-Ficoll density gradient centrifugation and adherent cells were removed. Cell viability was at least 90%, as determined by trypan blue dye exclusion tests.

Lymphocyte cytotoxicity assay. Myocardial and endothelial cell cultures were radiolabeled with 51Cr (New England Nuclear, Boston, MA) and incubated with splenic mononuclear cells at a 1:10 effector-to-target cell (E:T) ratio, as described by Gardner et al.

Antibody-directed cellular cytotoxicity (ADCC) assay. A 1:10 dilution of each serum sample from the 150 day group and splenic mononuclear cells from several normal BALB/c-BYJ mice were incubated with 51Cr-labeled myocardial cell cultures at an E:T ratio of 100:1 according to the method of Katz et al.

Cytotoxic antibody assay. A 1:10 dilution of each serum sample from the mice in the 150 day group was incubated with 51Cr-labeled myocardial cell cultures by the technique of Romano. Guinea pig serum (GIBCO) served as the source of complement.

Natural killer cell assay. 51Cr-labeled YAC-1 tumor cells were incubated with splenic mononuclear cells from the 150 day group at a 100:1 E:T ratio according to the method of Katz et al.

Enzyme-linked immunosorbent assay. Anti-*T. cruzi* immunoglobulin G antibody titers were determined with the use of *T. cruzi* cytosolic antigens. Serial twofold dilutions of serum, beginning at 1:20, were performed as previously described. Titers of all sera were done in duplicate and were reproducible within one twofold serial dilution.

Parasitemia. Blood from infected mice was placed in liver infusion–tryptose medium and checked weekly for the presence of *T. cruzi* flagellates for an 8 week period.

Histopathologic studies. Representative sections of heart, skeletal muscle, and colon were examined by conventional histologic techniques.

Calculations and statistical analysis. All 51Cr-release assays were performed in quadruplicate. Percentage 51Cr release was calculated by the formulas of Huber et al. Spontaneous release represents the amount 51Cr released into the supernatant in the absence of effector cells. Cytotoxicity was expressed as the percentage of 51Cr release by lymphocytes minus the percentage of spontaneous 51Cr release. Percent specific lysis represents the percentage lysis by sensitized lymphocytes minus the percentage lysis by normal and/or control lymphocytes. Values for ADCC, antibody and complement cytotoxicity, and natural killer cell assays were determined in an analogous manner. Statistical significance was determined by the Student t test and analysis of variance.

Results

No antiheart lymphocyte cytotoxicity was evident during the early or midterm phases of infection (table 1). Mice infected for 15, 45, 90, and 120 days did not exhibit splenic lymphocyte cytotoxicity to normal syngeneic cardiac myofibers (percentage specific lysis 1.7%, 2.4%, 1.4%, and 5.7%, respectively; not a statistically significant difference when compared with matched controls). No lesions or pseudocysts of *T. cruzi* amastigotes were found in the heart, skeletal muscle, or colon. In sharp contrast, mice infected for 150 days showed a significant (41.7%) specific lysis of syngeneic heart cells as compared with age- and sex-matched controls (p < .01). This value was also significantly different when compared with values obtained in the early and midterm phases of infection (p < .01). The observed antiheart lymphocyte cytotoxicity was specific since no cytotoxicity was observed against syngeneic endothelial cells (table 2). Importantly, this antiheart reaction was correlated with the

**TABLE 1**

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Parasi-temia</th>
<th>IgG anti-<em>T. cruzi</em></th>
<th>Anti-syngeneic heart cell lymphocyte cytotoxicity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Myocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>6</td>
<td>6/6</td>
<td>1.7 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Neg</td>
</tr>
<tr>
<td>45</td>
<td>6</td>
<td>6/6</td>
<td>2.4 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Neg</td>
</tr>
<tr>
<td>90</td>
<td>6</td>
<td>6/6</td>
<td>1.4 ± 1.4</td>
<td>Neg</td>
</tr>
<tr>
<td>120</td>
<td>12</td>
<td>10/12</td>
<td>5.7 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Neg</td>
</tr>
<tr>
<td>150</td>
<td>9</td>
<td>7/9</td>
<td>41.7 ± 4.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pos</td>
</tr>
</tbody>
</table>

IG = immunoglobulin.

<sup>a</sup>Percentage specific lysis ± SEM.

<sup>b</sup>For comparison with matched controls, p = NS.

<sup>c</sup>For comparison with matched controls or with other groups, p < .01.
presence of focal but widely distributed mononuclear cell myocarditis with myocardial cell necrosis in the absence of intracellular pseudocysts of *T. cruzi* (figures 1 and 2). Myocytolysis was attended by intermediate-sized lymphocytes. This mirrors the situation observed in human *T. cruzi* infection. No involvement of the ganglioneurons of the heart was observed, nor were any lesions found in sections of skeletal muscle or colon. Mice from the 150 day group did not exhibit any antiheart ADCC or cytolitic antibody activity, nor did they show any increase in natural killer cell activity as compared with matched controls (table 2).

Parasitemias were demonstrable throughout the entire course of infection (table 1). Anti-*T. cruzi* antibody titers were evident in the 90, 120, and 150 day groups, but not in the 15 or 45 day group (table 1). No concurrence between elevated anti-*T. cruzi* antibody titers and myocarditis was seen in the 90 and 120 day groups.

Mice immunized with SRA from either a syngeneic (BALB/c-BYJ) or a heterogeneic (monkey) source developed splenic lymphocyte cytototoxicity to normal syngeneic heart cells (specific lysis 23.2% and 17.2%, respectively; table 3). This was a significant difference in the comparison with FCA-injected controls (*p* < .01). SRA-immunized mice developed mononuclear

### TABLE 2

| Cytotoxic reactivity of BALB/c-BYJ mice 150 days after *T. cruzi* infection |  |
|---|---|---|---|---|
| Antisyngeneic heart cell lymphocyte cytotoxicity | Antisyngeneic endothelial cell lymphocyte cytotoxicity | Antisyngeneic heart cell ADCC cytotoxicity | Antisyngeneic heart cell antibody cytotoxicity | NK cell activity |
| 41.7 ± 4.4 | −2.51 ± 4.05^A | −0.6 ± 2.0^A | −0.1 ± 2.7^A | 1.2 ± 2.8^A |

^AFor comparison with matched controls, *p* = NS.

Percentage specific lysis ± SEM.

FIGURE 1. Low-power magnification of the ventricular wall of a BALB/c-BYJ mouse infected with *T. cruzi* for 150 days. A focal myocarditis with mononuclear cell infiltrates in the absence of intracellular *T. cruzi* amastigotes is evident. Hematoxylin and eosin stain.
cell myocarditis identical to that seen after long-term T. cruzi infection (figures 3 and 4). Focal areas of myocardial cell necrosis attended by intermediate-sized lymphocytes were evident throughout the heart. No lesions were found in sections of skeletal muscle or colon, nor were any lesions found in sections of heart, skeletal muscle, or colon in the FCA-injected controls.

**Discussion**

The data reported here indicate that long-term infection of mice with a low inoculum of T. cruzi produces autoimmune antiheart lymphocyte cytotoxicity in vitro. The autoimmune reactivity is apparently specific for myocardial cells since no destruction of syngeneic endothelial cells was observed. The cell-mediated antiheart response concurred with the development of mononuclear cell myocarditis. Significantly, myocardial damage and chronic inflammatory lesions of the heart were only evident when cell-mediated antiheart cytotoxicity was present. No cardiac lesions were demonstrable in the early or midterm phases of infection when antiheart cell-mediated cytotoxicity was absent. These results suggest that specific autoimmune antiheart cell-mediated reactions play a role in the production of the cardiac lesions seen in Chagas' disease. Furthermore, although T. cruzi parasitemias were demonstrated throughout the entire course of the infection, myocardial lesions were only evident when autoimmune antiheart reactivity was observed. No evidence of intracellular T. cruzi parasitosis was found in the heart at any time. These data imply that the cardiac damage cannot be simply attributed to parasitosis of heart cells by T. cruzi organisms. Cardiac damage is strongly associated with the concurrence of autoimmune cell–mediated antiheart reactions and T. cruzi parasitemia.

The autoimmune antiheart cytotoxicity reported in

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**FIGURE 2.** High-power magnification of the ventricular wall of a BALB/c-BYJ mouse infected with T. cruzi for 150 days. Myocardial necrosis attended by a mononuclear infiltrate is present. Hematoxylin and eosin stain.

**TABLE 3**

SRA immunization of BALB/c-BYJ mice

<table>
<thead>
<tr>
<th>SRA source</th>
<th>n</th>
<th>Antisynthetic heart cell lymphocyte cytotoxicity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Myocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous (BALB/c)</td>
<td>10</td>
<td>23.2 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pos</td>
</tr>
<tr>
<td>Heterologous (monkey)</td>
<td>10</td>
<td>17.2 ± 3.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pos</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percentage specific lysis ± SEM.

<sup>b</sup>For comparison with matched controls, p < .01.
these experiments is mediated by both muscle SRA- and T. cruzi–sensitized lymphocytes. Preliminary experiments from our laboratory show that treatment of either SRA- or T. cruzi–sensitized lymphocytes with antitheta antisera and complement abolishes all anti-heart cytotoxicity (data not shown). This indicates that the antiheart response is mediated by cytotoxic T lymphocytes. Our data show no evidence of complement-mediated antiheart antibody cytotoxicity, antibody-directed cellular cytotoxicity, or natural killer cell activity in T. cruzi–infected mice. Other published investigations have documented destruction of normal allogeneic myocardial cells in vitro by lymphocytes obtained from humans or from experimental animals infected with T. cruzi. These other observations similarly support a cell-mediated mechanism for the destruction of heart cells seen in Chagas’ disease. There are reports of antibodies reacting with endothelial, vascular, and interstitial (EVI) structures of the heart in individuals with T. cruzi infections and these have been postulated to play a role in the immunopathogenesis of Chagas’ disease. However, recent publications show that the EVI reactivity is due to heterophil antibodies nonspecifically interacting with heart structures. Thus, it is unlikely that EVI antibodies contribute to the heart damage seen in the experiments reported here. The absence of documented antibody-mediated antiheart cytotoxicity in our studies suggests that other circulating antibodies are not involved in the production of the myocardial lesions described. Present evidence indicates that the cardiac lesions observed may be primarily the result of autoimmune antiheart cell-mediated reactions that occur with T. cruzi infection.

Other investigators suggest that the cardiac damage seen in Chagas’ disease may be due to direct cell damage produced by rupture of T. cruzi pseudocysts or by the acquisition of T. cruzi antigens on the surface of host cells, which renders them susceptible to immune attack. The low prevalence of T. cruzi pseudocysts detected in patients with Chagas’ cardiomyopathy and our results cast serious doubt on a role for intracellular parasitosis in the production of the lesions seen in Chagas’ heart disease. Moreover, experimental myocarditis induced in Swiss mice by homologous heart immunization resembles chronic experimental Chagas’ heart disease. Furthermore, in other experiments from our laboratory we have shown that adoptive
transfer of lymphocytes obtained from mice with long-
term *T. cruzi* infections produces autoimmune anti-
heart lymphocyte cytotoxicity in syngeneic recipients. 
The antihart cell-mediated reactivity of adoptive 
transfer experiments is abolished by glucocorticoid 
immunosuppressive therapy of naive recipients (data 
not shown). These findings suggest that myocardial 
cell cytotoxicity can be produced by sensitized lym-
phocytes in the absence of *T. cruzi* parasitosis.

The mechanisms by which autoimmune antihart 
reactions are brought about in long-term *T. cruzi* infection may involve cross-reacting antigens shared by *T. cruzi* and heart muscle. The shared *T. cruzi*–striated muscle antigen SRA is present in purified preparations of the calcium-sequestering ATPase of sarcoplasmic reticulum. Unlike that of skeletal muscle fibers, the sarcolemma of myocardial cells has calcium transport properties and a calcium-sequestering ATPase of cardiac sarcolemma has been described. Antisera to ei-
ther *T. cruzi* membrane or to SRA has been shown by 
immunofluorescence tests to localize antibody on the 
sarcolemma of cardiac myofibers. Furthermore, as 
shown by gel immunoprecipitation, antiserum to heart 
sarcolemma preparations and to *T. cruzi* membranes formed two lines of identity with purified SRA. These reports indicate that SRA is present on the surface of cardiac myofibers. In the experiments reported here, immunization of mice with SRA produced au-
toimmune cell-mediated antihart reactions and myo-
cardial lesions identical to those found in individuals with long-term *T. cruzi* infections. These results sug-
gest that SRA is an immunopathogenic antigen. Pre-
viously published studies have shown that muscle 
SRA–immunized mice develop a dose-related delayed-type hypersensitivity to the small membranes of 
*T. cruzi*, whereas immunized athymic nude mice do 
not. It is possible that long-term infection by *T. cruzi* serves to sensitize the host’s immune system to a 
shared muscle antigen. This sensitization may trigger 
the development of cell-mediated autoimmune anti-
heart reactions that give rise to the mononuclear myo-
carditis and cardiac damage observed in individuals 
with Chagas’ disease.

References
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