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Association of Elevated Anti-Sarcolemma, Anti-Idiotype Antibody Levels With the Clinical and Pathologic Expression of Chronic Chagas Myocarditis

Moyes Sadigursky, MD, Betsy F. von Kreuter, PhD, Pei-Ying Ling, BS, and Charles A. Santos-Buch, MD

Antibody F(ab')_2 fragments derived from the sera of four patients with histology-proven chronic Chagas myocarditis [cF(ab')_2]-complexed antibody F(ab')_2, fragments of children with acute Trypanosoma cruzi infection [aF(ab')_2] in significantly higher molar ratios than those measured with F(ab')_2 antibody fragments of normal subjects [nF(ab')_2] from nonendemic areas (p<0.05). Anti-idiotype [cF(ab')_2×aF(ab')_2] immune-complex formation was significantly blunted by preabsorption of F(ab')_2 with porcine heart atria sarcolemma (PAMs) immobilized on sepharose beads (inhibition, mean, 78.1±2.4%, n=4). [cF(ab')_2×nF(ab')_2] immune-complex formation was also inhibited by pretreatment of F(ab')_2 with PAMs (inhibition, mean, 48.7±7.5%, n=4). The sera of three groups of subjects from a geographic zone endemic for T. cruzi infection in the northeast of Brazil were assayed for free and immune-complexed IgG anti-acute T. cruzi infection F(ab')_2. The indexed levels of free IgG anti-idiotype antibody activity and levels of IgG anti-idiotype immune complexes (IC') were markedly elevated in hospitalized patients with severe, decompenated chronic Chagas myocarditis (n=23), and their IC' indexes were significantly higher than those measured in asymptomatic seropositive subjects from a nearby endemic village of the northeast of Brazil (Moniz Ferreira, n=92, p<0.001) and in healthy seronegative villagers (n=84, p<0.001). There exists a strong correlation between elevated IgG anti-sarcolemmal, anti-idiotype activity levels and the clinical and pathologic expression of chronic Chagas myocarditis. (Circulation 1989;80:1269–1276)

Chronic Chagas myocarditis is the most frequent cause of congestive heart failure and sudden death in endemic areas of Central and South America. The etiologic agent of Chagas disease is the hemoflagellate, Trypanosoma cruzi, an obligatory intracellular protozoan. The autopsy of 35 mummies exhumed in the Chilean desert, dating between 470 BC and 600 AD, revealed evidence of chronic Chagas disease, and this study gave a strong indication that adaptation of the blood-sucking Triatome (Reduviidae) insect vector to dwellings had occurred since pre-Columbian times, documenting their persistent aggressiveness for human hosts. After the Triatome attack, metacyclic trypomastigotes deposited with the feces after the blood meal may contaminate the wound or mucosal membranes, and a subsequent parasitemia may ensue in human hosts. In an undetermined number of T. cruzi seropositive, chronically infected subjects with varying degrees of immunosupression, a severe and progressive myocarditis may develop months or years later. Chronic Chagas myocarditis is frequently associated with increased levels of IgG antibody against myocardial sarcolemma epitopes. Moreover, an anti–T. cruzi membrane antibody that is cross reactive with a myocardial cell sarcoplasmic reticulum antigen and is also expressed in the sarcolemma has been identified. However, the pathogenic potential of this and other anti-heart and anti-laminin antibodies shown in subjects with chronic Chagas myocarditis is not fully understood.

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Experimental observations have shown that *T. cruzi* parasitemia is often followed by myotropic infection of host type I striated muscle fibers, including the heart. Recent investigations have shown that myotropic host-cell recognition is promoted by the chemoaffinity of *T. cruzi* attachment molecules to receptors of the muscle fiber and that these attachment sites are complementary and antigenic. In a recent report we showed that *T. cruzi* plasma-membrane attachment sites interacted with heart muscle sarcolemma muscarinic cholinergic and \(\beta\)-adrenergic receptors in the recognition and adhesion phenomenon. Furthermore, an IgG antibody from patients with Chagas disease has been shown to bind \(\beta\)-adrenergic receptors of the myocardium and to modulate their adenyl cyclase activity. Moreover, when monospecific F(ab')\(_2\) antibody against *T. cruzi* surface antigens was allowed to react with monospecific F(ab')\(_2\) antibody against muscle surface antigens, immune complexes were formed in a concentration-dependent fashion similar to that seen with anti-idiotype immune reactions.

The hypothesis that anti-idiotype antibodies may be pathogenic has been raised in connection with idiopathic thrombocytopenia purpura, insulin-dependent diabetes mellitus, myasthenia gravis, polymyositis-dermatomyositis, and Kawasaki’s disease. Moreover, there are several observations suggesting a link between viral infection, anti-idiotype antibodies, and autoimmune diseases, including postviral myocarditis. The data in hand, therefore, suggested that an IgG anti-idiotype antibody against the primary anti-*T. cruzi* antibody may also be reactive to the antigenic, complementary recognition receptors of the sarcolemma, thereby inflicting myocarditis.

We sought to investigate the possible association of IgG anti-idiotype antibody activity (anti-Id) and severe chronic Chagas myocarditis. To accomplish this we showed first that the antigen-binding fragment of the IgG molecule [F(ab')\(_2\)] of four highly selected, *T. cruzi*-seropositive adult patients with histology-proven myocarditis complexed to the antigen-binding site of IgG antibodies obtained from children with acute *T. cruzi* parasitemia, a pooled source of primary antibody. We also compared serum levels of anti-Id and anti-Id–immune complexes in 92 seropositive but asymptomatic subjects of the village of Moniz Ferreira of the State of Bahia in the northeast of Brazil, which was endemic for *T. cruzi* infection, with 23 seropositive patients with severe chronic myocarditis and congestive heart failure hospitalized nearby in the capital city of Salvador. A group of 84 seronegative, apparently healthy villagers served as controls.

**Methods**

### T. Cruzi Serology

Seropositive sera were defined when hemagglutination, complement fixation, and immunofluorescent tests against *T. cruzi* antigens proved positive on two subsequent 1-month bleedings. Seronegative sera were within the normal limits of all three tests.

**T. Cruzi Parasitemia**

This was determined by direct examination of the peripheral blood of infected children on Giemsa-stained smears.

**Antigens**

*Acute T. cruzi infection F(ab')\(_2\) [aF(ab')\(_2\)].* One half- to 1-ml aliquots of sera from 29 children, 10 months to 8 years old, with acute *T. cruzi* parasitemia were pooled. The clinical history indicated that the duration of infection was not more than 60 days since onset. The F(ab')\(_2\) fragment was prepared from pepsin digests of IgG from the filtered, dilipidated serum by published technique. After chromatography, undigested IgG and Fc fragment contaminants were removed by protein A Sepharose 4B (Pharmacia, Piscataway, New Jersey) treatment and stored frozen at \(-20^\circ\) C until used.

*Nonendemic, normal F(ab')\(_2\) [nF(ab')\(_2\)].* Nonendemic, normal, healthy nontransfused, US male donors were the source of affinity-purified F(ab')\(_2\) fragments, purchased from Pel-Freeze (Rogers, Arkansas) and stored frozen at \(-20^\circ\) C until used.

**Test Antibodies**

F(ab')\(_2\) of histology-proven chronic Chagas myocarditis patients [cF(ab')\(_2\)]. The sera of three male patients, 32–45 years old, with endomyocardial biopsy-proven chronic Chagas myocarditis were obtained from the Cardiology Service of the Ramos Mejia Hospital, Buenos Aires, Argentina (Dr. Mauro M. Meijia). The serum of one patient from the borough of Queens, New York, a 24-year-old male immigrant from Honduras, who received a cardiac transplant for chronic Chagas myocarditis, was also drawn for the study. The clinical diagnosis of chronic Chagas myocarditis was based on 1) seropositive tests for *T. cruzi* infection; 2) cardiomegaly by chest radiograph; and 3) electrocardiographic studies showing bradycardia, right bundle branch block, or both, plus or without left anterior hemiblock, with severe intraventricular conduction abnormalities, SS-T changes, or both, plus or without varying degrees of heart block. The histopathologic diagnosis was based on the identification of a florid lymphoreticular interstitial exudate, occasional myofiber necrosis, and varying degrees of connective tissue replacement. Pseudocysts with *T. cruzi* amastigotes were not found. The F(ab')\(_2\) fragments of the IgG of each of the four patients were prepared as described for the pool of acute *T. cruzi* infection F(ab')\(_2\).

Reactive IgG of sera from the ambulatory villagers of Moniz Ferreira (Bahia, Brazil). Moniz Ferreira is a village of 2,500 inhabitants of the state of Bahia, about 80 km west of the capital city, Salvador, in northeastern Brazil. Public health seroepi-
demiologic surveys had shown that the village was endemic for *T. cruzi* infection and that many homes were infested by the vector, the Triatoma (*Reduviid*) insect. The sera of 84 apparently healthy, seronegative subjects, 38 men and 46 women, 16–44 years old (mean age, 29 years) were obtained for the study. Sera of 92, age- and sex-matched seropositive asymptomatic villagers were drawn. All samples were coded and stored frozen at −20°C until used.

**Reactive IgG of chronic Chagas myocarditis sera from hospitalized patients diagnosed by clinical criteria.** The sera of 23 patients, residents of nearby endemic zones who were hospitalized in the Edgar dos Santos Hospital in Salvador for congestive heart failure due to severe chronic Chagas myocarditis, were coded and stored frozen (−20°C) before use. The criteria for the clinical diagnosis of chronic Chagas myocarditis were based on the same criteria as those of the histology-proven myocarditis group. Fourteen men and nine women, 27–52 years old (mean age, 42 years) were culled for the study.

**1271**-acute *T. cruzi* infection F(ab′)2; chronic Chagas myocarditis F(ab′)2; complex formation. Private specificity was measured by complexing acute *T. cruzi* infection (1271)-F(ab′)2 [aF(ab′)2] and cF(ab′)2 fragments from patients with histology-proven chronic Chagas myocarditis in duplicate in 1.5-ml Eppendorf microfuge tubes (VWR, South Plainfield, New Jersey). Measurement of public specificity consisted of complexes formed by nonendemic, normal (1271)-F(ab′)2 [nF(ab′)2] obtained from Calbiochem, La Jolla, California. F(ab′)2 iodination was done by solid-phase glucose oxidase and lactoperoxidase treatment (Enzymobeads radioiodination reagent, BioRad, Rockville Centre, New York), followed with Na125I (ICN, Irvine, California). Unbound Na125I was removed by exhaustive dialysis at 4°C.13 Care was taken to maintain specific radioactivity at levels of 5,500–8,000 cpm/pmol F(ab′)2. Immune-complex formation was done by the addition of exactly 50 pmol of the radiolabeled aF(ab′)2 to 100–122 pmol of the test cF(ab′)2, bringing the final reaction volume to 500 μl in phosphate-buffered saline (PBS). The tubes were mixed and incubated at 37°C for 2 hours, followed by an overnight incubation at 4°C. The immune complexes were precipitated by polyethylene–glycol-6000 (PEG) treatment.13 The background radioactivity produced by the radiolabeled aF(ab′)2 fragments alone, which remained after completion of the procedure, consistently ranged 3–5% of the total applied (mean, 4.2%).

**Inhibition of immune complex formation by preabsorption of cF(ab′)2 with porcine heart atria sarcodlemma.** This was done by preincubation of 100–122 pmol of cF(ab′)2 antibody fragments with porcine heart atria sarcodlemma (PAMs) immobilized on sepharose and previously prepared from fresh tissue by our published technique.14 Sepharose CNBr-coupled PAMs were prepared according to the technique recommended by the manufacturer (Pharmacia). Two hundred μl of a 50% (vol/vol) PAMs on beads in a PBS suspension was used for absorption in each tube. Incubation for absorption was 2 hours at 37°C followed by overnight treatment at 4°C. After centrifugation at 15,000g for 1 hour at 4°C, immune-complex formation with radiolabeled aF(ab′)2 and cF(ab′)2 or nF(ab′)2 was measured in an aliquot of the supernatant.

**Total specific IgG anti-acute T. cruzi infection F(ab′)2.** All tests with population sera and with sera of patients with the clinical diagnosis of chronic Chagas myocarditis were done in duplicate without previous knowledge of their origin. The enzyme-linked immunosorbent assay (ELISA) was used to identify bound IgG.25 The sera were previously acidified with 0.15 M NaCl in 0.01 M glycine-HCL buffer (pH 2.5) in a final 1:40 dilution to dissolve immune complexes. The acidified (pH 3) test sera were next diluted in microtiter wells (Falcon, Oxnard, California) previously coated with F(ab′)2, antigens with an equal volume (100 μl) of 0.15 M NaCl in 0.06 M phosphate buffer, pH 7.4, with 5% (vol/vol) fetal bovine serum (FBS) (Hyclone, Logan, Utah) and 0.05% (vol/vol) Tween 20 (Sigma Chemical, St. Louis, Missouri). This resulted in conversion of the pH of the reaction mixture to 7.3 and allowed the nascent antibodies to react with the excess solid phase aF(ab′)2 antigens (1 hour at 37°C). The wells were coated with 0.5 μg aF(ab′)2 fragments in carbonate buffer pH 9.6 and 0.02% (wt/vol) NaCN according to published technique.28 Bound IgG was indexed with alkaline phosphatase-conjugated affinity-purified rabbit F(ab′)2 anti-human Fc fragment (Pel-Freeze, Rogers, Arkansas) at an appropriate dilution. The subsequent washes and substrate incubations followed published technique.24 The plates were read in a Titertek Multiscan Plus spectrophotometer (Flow Laboratories, McLean, Virginia), at an optical density (OD) of 405 nm. The OD obtained with nonendemic normal F(ab′)2 ranged 14–19% in all tests (n=199). The corrected specific total antibody activity was tabulated after subtraction of the OD obtained with nonendemic, normal human F(ab′)2 in duplicate parallel tests.

**Free IgG anti-idiotype activity and calculation of anti-idiotype immune complexes.** This was done in a similar procedure at physiologic pH (7.4) by omitting the acid treatment. Levels of anti-idiotype immune complexes (IC′) were indexed by calculating the difference between the optical density of total and free specific IgG anti-*T. cruzi* infection F(ab′)2.

**Metacyclic T. cruzi trypomastigotes.** Brazil-strain metacyclic trypomastigotes were harvested in the log phase of growth after Giemsa-stained counts showed that more than 90% of the flagellates were in the tissue-infective metacyclic form.26
TABLE 1. Myocarditis Immune-Complex Formation by Antigen-Binding Fragments of Primary Antibody and Inhibition of Immune-
Complex Formation by Sarcolemma

| Myocarditis immune-complex formation | Complexed acute parasitemia—
<p>| Acute parasitemia— | F(ab′)2; added | (125I)-aF(ab′)2 | Correction | Molar ratio |</p>
<table>
<thead>
<tr>
<th>(125I)-aF(ab′)2 (pmol)</th>
<th>(pmol)</th>
<th>(pmol)</th>
<th>(pmol)</th>
<th>(d/b×10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>none</td>
<td>Background</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>0.101±0.006</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Normal | 120 | 1.794±0.120 | 1.693±0.12 | 14.1±1.0
| 50 | Chronic myocarditis | | |
| 6.54±0.29 | 4.25±0.05 | 35.4±0.45 | 38.4±1.00 |
| 38.9±2.84 |
| Inhibition of immune-complex formation | Complexed acute parasitemia—
<p>| Chronic myocarditis—cF(ab′)2; absorbed with | (125I)-aF(ab′)2 | Molar ratio |</p>
<table>
<thead>
<tr>
<th>sarcolemma (pmol before absorption)</th>
<th>(pmol)</th>
<th>absorbed</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM</td>
<td>120</td>
<td>0.79±0.04</td>
<td>6.54±0.29*</td>
</tr>
<tr>
<td>PP3</td>
<td>122</td>
<td>0.99±0.04</td>
<td>8.11±0.32*</td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>0.90±0.01</td>
<td>8.99±0.12*</td>
</tr>
<tr>
<td>8.02±0.43</td>
<td>78.1±2.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aF(ab′)2, acute T. cruzi parasitemia antibody fragment; cF(ab′)2, chronic Chagas myocarditis antibody fragment; %
Inhibition=[100−(Molar ratio absorbed/Molar ratio not absorbed)×100].
*p<0.05 vs. not absorbed.
*Private specificity calculated by subtracting background and public specificity.

Statistical analysis. The results were compared by Student’s t test and Fisher’s exact test. A p
value of less than 0.05 was assigned significance.

Results
When the F(ab′)2 fragments of four histology-

proven myocarditis, T. cruzi seropositive patients were

allowed to react with the radiolabeled F(ab′)2 fragments of a pool of sera of children with acute T. cruzi parasitemia, 33.8–38.9 mM radiolabeled acute T. cruzi infection F(ab′)2/mol chronic Chagas myocarditis F(ab′)2, were specifically complexed [mean, 36.6±1.4 mM aF(ab′)2/M cF(ab′)2, n=4] (Table 1). These molar ratios were 2.6-fold higher than those obtained when normal, nonendemic antibody F(ab′)2, fragments were used against (125I)-aF(ab′)2, [(14.1±1.0

mM radiolabeled aF(ab′)2/mol normal F(ab′)2; p<0.05)]. When cF(ab′)2, fragments of the IgG of the four histology-proven myocarditis subjects were allowed to react with the radiolabeled F(ab′)2 fragments of normal, nonendemic IgG, 5.85–7.99 mM radiolabeled normal F(ab′)2/mol chronic Chagas myocarditis F(ab′)2, were specifically complexed [mean, 7.35±0.43 mM F(ab′)2/mol cF(ab′)2] (public specificity) (Table 2). These molar ratios were 5.0-fold less than those obtained when radiolabeled acute T. cruzi infection F(ab′)2 fragments were used (p<0.05). These results showed that the antigen-binding site of IgG molecules of the sera of patients with chronic Chagas myocarditis specifically complexed the antigen-binding site of IgG molecules of the sera of children with acute T. cruzi parasitemia, the source of primary antibody. When the cF(ab′)2, of patients with histology-proven chronic myocarditis was absorbed against PAMs, immune-complex formation with (125I)-af(ab′)2 (public specificity) was blunted (37.3–57.3% inhibition) (Table 2). When the chronic myocarditis cF(ab′)2 was absorbed against PAMs, immune-complex formation with (125I)-af(ab′)2 (private specificity) was also significantly inhibited (75.1–81.5% inhibition) (Table 1). These results indicated to us that chronic Chagas myocarditis patients had circulating anti-idiotype antibody with private and public specificity against 1) antibodies raised in children with acute T. cruzi parasitemia and 2) antibodies of healthy, nontransfused male donors of a nonendemic zone of North America. Moreover, a large proportion of the chronic Chagas myocarditis anti-idiotype antibody was also reactive against epitopes of the myocardial cell plasma membranes (Tables 1 and 2). With these data in hand, what remained was to show whether there were significant differences in levels of anti-idiotype antibody activity in large numbers of subjects from an endemic zone for T. cruzi infections.
TABLE 2. Myocarditis Immune-Complex Formation by Antigen Binding Fragments of Normal Antibody and Inhibition of Immune-Complex Formation by Sarcolemma

<table>
<thead>
<tr>
<th>Normal fragments—(125I)-nF(ab')2 (pmol)</th>
<th>F(ab')2 added (pmol)</th>
<th>Complexed normal—(125I)-nF(ab')2 (pmol)</th>
<th>Correction (pmol)</th>
<th>Molar ratio (d/b×10^-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>0.143±0.008</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Chronic myocarditis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UM</td>
<td>0.85±0.04</td>
<td>0.70±0.03</td>
<td>5.85±0.29</td>
</tr>
<tr>
<td></td>
<td>PP3</td>
<td>1.11±0.00</td>
<td>0.97±0.00</td>
<td>7.91±0.03</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.10±0.10</td>
<td>0.96±0.09</td>
<td>7.99±0.74</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.91±0.08</td>
<td>0.77±0.07</td>
<td>7.66±0.67</td>
</tr>
</tbody>
</table>

Inhibition of immune-complex formation

<table>
<thead>
<tr>
<th>Chronic myocarditis—cF(ab')2 absorbed with sarcolemma (pmol before absorption)</th>
<th>Complexed normal—(125I)-nF(ab')2 (pmol)</th>
<th>Correction (pmol)</th>
<th>Molar ratio (c/a×10^-6)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM</td>
<td>0.58±0.02</td>
<td>0.44±0.01</td>
<td>3.67±0.11</td>
<td>37.3</td>
</tr>
<tr>
<td>PP3</td>
<td>0.58±0.00</td>
<td>0.41±0.00</td>
<td>3.38±0.03</td>
<td>57.3</td>
</tr>
<tr>
<td>10</td>
<td>0.59±0.02</td>
<td>0.45±0.01</td>
<td>3.74±0.10</td>
<td>53.2</td>
</tr>
<tr>
<td>14</td>
<td>0.55±0.03</td>
<td>0.41±0.02</td>
<td>4.06±0.23</td>
<td>47.0</td>
</tr>
</tbody>
</table>

nF(ab')2, antibody fragment from normal sera; cF(ab')2, antibody fragment from chronic Chagas myocarditis sera; % Inhibition=100−(Molar ratio absorbed/Molar ratio not absorbed)×100.

The conditions for assay of Moniz Ferreira villager anti-idiotype antibody activity described in this report allowed for the indexing of total and free IgG anti-acute T. cruzi infection F(ab')2 activity of large numbers of test sera. The specificity of the assay for anti-idiotype antibody was further established by showing that preabsorption of the normal sera with live, tissue-infective, metacyclic try-ptomastigotes (5×10^6/ml) failed to inhibit the reaction of IgG anti-acute T. cruzi F(ab')2 by more than 15% (Table 3).

When the cutoff normal value was calculated from the median optical density plus 2 SEM of seronegative Moniz Ferreira villagers, 20 of 23 (87.0%) hospitalized patients with severe chronic Chagas myocarditis showed abnormal elevated levels of IgG anti-acute T. cruzi infection F(ab')2. In contrast, only four of 84 (4.8%) apparently healthy, seronegative Moniz Ferreira villagers had values above the normal calculated cutoff, and this difference was statistically significant by two-tailed Fisher’s exact test (p<0.001). Thirty of 92 (32.6%) seropositive but asymptomatic Moniz Ferreira villagers showed abnormal, elevated IgG anti-acute T. cruzi infection F(ab')2 activity (Table 3). We believe that subclinical cases are probably included in the latter group because no sophisticated cardiology was done to identify symptomless myocarditis in the seroepidemiologic survey conducted by the state of Bahia. Nevertheless, when Fisher’s exact test was used, statistically significant differences were again found between the hospitalized group of patients with congestive heart failure and severe chronic Chagas myocarditis and the asymptomatic group of seropositive villagers (0.02>p>0.01).

The distribution of free- and complexed-IgG anti-acute T. cruzi infection F(ab')2 was compared among seronegative and seropositive asymptomatic Moniz Ferreira villagers and hospitalized chronic Chagas myocarditis patients with congestive heart failure. Figure 1 plots the free IgG anti-Id and immune-complexed IgG anti-Id (IC') for each subject. When the $t$ distribution of IC' of the three groups is compared, each is significantly statistically different from the other (p<0.001). It can be appreciated from Figure 1 that the median values of free and

TABLE 3. Total Specific IgG Anti-Acute T. cruzi Infection F(ab')2 Activity

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Abnormal* OD$_{380}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;0.360 (%)</td>
</tr>
<tr>
<td>Chronic Chagas myocarditis†</td>
<td>20 (87.0)</td>
</tr>
<tr>
<td>Seropositive and asymptomatic</td>
<td>30 (32.6)</td>
</tr>
<tr>
<td>Seronegative and healthy</td>
<td>4 (4.8)‡</td>
</tr>
</tbody>
</table>

$p<0.001$ by Fisher’s exact test.

*The median optical density value plus two times the SEM of seronegative Moniz Ferreira subjects was used as the cutoff normal value (>0.360).
†After treatment with 5×10^6 flagellates/ml of live metacyclic tryptomastigotes to absorb primary IgG anti-T. cruzi surface antigen, the optical density was reduced 10–15%.
‡These four subjects remain seronegative and asymptomatic to date.
complexed-IgG anti-Id measured for the hospitalized patients with chronic Chagas myocarditis are markedly elevated above the seronegative villagers of Moniz Ferreira. It is particularly noteworthy that, even though values overlap among the seropositive subjects, IgG anti-Id immune complexes were highest among some of the hospitalized patients with severe clinical myocarditis (Figure 1).

**Discussion**

In recent experiments we showed that the F(ab')\textsubscript{2} fragments of the IgG of a patient with chronic Chagas myocarditis complexed the antigen-binding IgG fragment F(ab')\textsubscript{2} obtained from infants with documented acute *T. cruzi* parasitemia, and immune-complex formation was specifically inhibited by sarcolemma membranes derived from L6 clones of striated rat skeletal muscle.\textsuperscript{13}

We have demonstrated that *T. cruzi* hemoflagellates bear antigenic complementary surface-attachment molecules to receptors on L6 myoblast host cells.\textsuperscript{13} In a recent publication we have further clarified that *T. cruzi* plasma membrane, striated muscle sarcolemma recognition involves the specific interaction of paired muscarinic cholinergic and \(\beta\)-adrenergic receptors of heart muscle cells with parasite attachment sites.\textsuperscript{14} We now propose, therefore, a working hypothesis to explain the interrelation between the formation of anti-sarcolemma, anti-idiotype antibodies and the development of chronic Chagas myocarditis (Figure 2). In the initial infection, parasite-attachment molecules interact through molded tertiary structures of epitopes with complementary receptor sites on the muscle sarcolemma to initiate recognition and parasitosis. Later in the infection, anti-idiotype antibodies down-regulate the primary antibody response against the parasite-attachment molecules.\textsuperscript{28,29} In a subset of susceptible subjects, anti-idiotype antibodies are raised in excess, and some react with the mirror-imaged structures of sarcolemma muscarinic cholinergic and \(\beta\)-adrenergic receptors, thereby inducing myocarditis.

In the investigation reported here, we sought the association of elevated levels of IgG anti-acute *T. cruzi* F(ab')\textsubscript{2} with anti-sarcolemma activity in chronic Chagas myocarditis. Indeed, the data reported in this investigation showed that hospitalized patients with severe chronic Chagas myocarditis and congestive heart failure have 1) abnormal elevated levels of total IgG anti-acute *T. cruzi* infection F(ab')\textsubscript{2} (87.0%, \(n=23\)) and 2) abnormal levels of complexed anti-idiotype antibodies, which were significantly higher than those measured in asymptomatic *T. cruzi* seropositive subjects of an endemic village (\(p<0.001\)). Moreover, the investigation reported here showed that the cF(ab')\textsubscript{2} antibody fragments of 4 histology-proven, chronic Chagas myocarditis patients specifically complexed aF(ab')\textsubscript{2} and that this anti-idiotype reaction was significantly blunted by preabsorption of cF(ab')\textsubscript{2} with PAMs.

A slight rise of levels of IgG anti-acute *T. cruzi* F(ab')\textsubscript{2} is an expected result from normal anti-idiotype network immunoregulation of asymptomatic, chronically infected subjects, however, when
markedly elevated IgG anti-idiotype immune-complex activity levels were shown among hospitalized patients with chronic Chagas myocarditis, the association to lesion production was made (t distribution, p<0.001). This correlative statistic suggests but does not prove that a pathogenic mechanism may exist between elevated IgG anti-idiotype activity levels and the clinical and pathologic expression of myocarditis. In support of the data reported here is a recent investigation showing that peripheral blood T-cells from patients with the cardiac form of Chagas disease are stimulated to proliferate by monospecific anti-flagellate antibody raising the possibility that these patients have auto-anti-idiotyptic T-cells.

Two observations merit further discussion. In the study reported here, the median age of patients with chronic Chagas myocarditis was higher by 13 years than the age range of asymptomatic seropositive subjects of the Moniz Ferreira survey study, and it may be argued that the rate of reinfection was different between the two groups of _T. cruzi_-infected subjects. Against this proposition is the reasonable likelihood that the hospitalized patients with chronic Chagas myocarditis and the asymptomatic seropositive Moniz Ferreira village subjects had a similar exposure rate to infected Triatoma (Reduviid) insects because both groups probably lived in similar socioeconomic endemic zones near the referral hospital. Furthermore, it is generally accepted that seropositive patients with chronic _T. cruzi_ infection have a life-time low-load parasitosis because intracellular pseudocysts of amastigotes are seldom or sparsely found after careful histopathologic examination of hearts with myocarditis and of other target organs and because parasitemia may be established in only 40% of seropositive chronically infected patients by the sensitive test of xenodiagnosis. Therefore, an important pathogenic factor to consider is the interaction of the duration of the infection with a possible genetic predisposition to an abnormal immunoregulatory response to the _T. cruzi_ parasitosis.

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**References**


**KEY WORDS** *Trypanosoma cruzi* antibodies Chagas disease myocarditis