Chagasic Cardiopathy

Demonstration of a Serum Gamma Globulin Factor Which Reacts with Endocardium and Vascular Structures

By Patricio M. Cossio, M.D., Carlos Diez, M.D., Ana Szarfman, M.D., Eduardo Kreutzer, M.D., Bartolome Candiolo, M.D., and Roberto M. Arana, M.D.

SUMMARY
Twenty-four out of 25 patients with Chagas' heart disease have circulating immunoglobulins which react by indirect immunofluorescence technique with endocardium, interstitium and blood vessels of the heart. With skeletal muscle the reaction was observed in interstitium and vascular structures, but with other organs it was limited to vascular structures. This endocardial-vascular-interstitial factor (EVI) fixed complement. Some evidence indicated that this reaction could be obtained using the serum and tissues from the same patient; for instance, in one positive case a right atrium biopsy was performed. When this substrate was used for indirect immunofluorescence, employing the patient's own serum, positive results were obtained. Specificity is not related to AB blood group systems, or to Forssman or Wassermann antigens. The reacting factor was effectively absorbed from sera with organ homogenates, and with guinea pig red blood cells although it was independent of heterophil antibodies. In almost all cases studied, the EVI factor of the serum, when absorbed with epimastigotes of T. cruzi, results in a negative reaction, suggesting that the genesis of the reacting gamma globulin is related to antigens of T. cruzi.

The EVI factor was also observed in 19 of 47 asymptomatic controls from an endemic area with positive serology for T. cruzi and in 3 of 27 with negative serology. These 3 cases had anti-T. cruzi antibodies in titers just below those considered of clinical value. The EVI factor was not observed in 119 normal individuals and 286 patients with selected cardiovascular diseases or another pathology from a nonendemic area. These findings and those mentioned above were statistically significant (P < 0.001). These results indicate the possibility of a more accurate diagnosis of chagasic myocardopathy based on the study of the EVI factor, because in an individual case the diagnosis of chronic chagasic cardiopathy can be considered with a low probability in the absence of this factor.

Additional Indexing Words:
American trypanosomiasis
Parasitic heart disease
Immunity and the heart
Antibodies and vascular structures

Chagas' Disease (American trypanosomiasis) is a major health problem in many areas of Latin America. It is caused by a protozoan parasite (T. cruzi) and is transmitted by a reduvid bug. Human and experimental observations suggest that the pathogenesis of acute cardiac involvement is in some form related to the parasitic invasion of the myocardium; however, the pathogenesis of the chronic cardiac disease, which appears many years later, remains unexplained. It has been suggested that chronic chagasic cardiopathy may involve an immunological mechanism. Some experimental findings exist to support this possibility, although there is no reported evidence that a similar mechanism occurs in human beings.

From the Laboratory of Rheumatology and Immunology and the Cardiology Units, Centro de Educacion Medica e Investigaciones Clinicas (CEMIC), Buenos Aires, and Hospital Ingenio Ledesma, Provincia de Jujuy; the Department of Microbiology and Parasitology and Comision para el Estudio Integral de la Enfermedad de Chagas, Facultad de Medicina, Universidad de Buenos Aires; and the Escuela Municipal de Cirugia Cardiovascular, Hospital Durand, Buenos Aires, Argentina.

Dr. Szarfman is a fellow and Dr. Arana is a member of the Research Worker Career, Consejo Nacional de Investigaciones Cientificas y Tecnicas, Buenos Aires, Argentina.

Supported in part by grants from Fundacion Cossio, ADAR and Comision para el Estudio Integral de la Enfermedad de Chagas.

Address for reprints: Roberto M. Arana, M.D., CEMIC, Sanchez de Bustamante 2560, Buenos Aires, Argentina.

Received October 2, 1972; revision accepted for publication August 22, 1973.
In this investigation, a serum gamma globulin factor which reacts with endocardium and vascular structures in patients with Chagas' heart disease is described.

**Material and Methods**

**Patients**

Twenty-five patients with chagasic cardiopathy were studied, 21 of them chronic cases in whom symptoms appeared before or during the fourth decade of life, and four acute cases in childhood. The clinical diagnosis of Chagas' cardiopathy according to previous reports was based on the following criteria: 1) patients inhabited an endemic zone (Province of Jujuy, Northwest Argentina) and at least during infancy and adolescence lived in rudimentary houses where the insect vector was known to exist; 2) they had cardiac enlargement according to the radiological criteria of Ungerleider and Gubner; 3) they presented severe cardiac rhythm abnormalities, including marked sinus bradycardia and A-V block, and/or intraventricular conduction disturbances (right bundle branch block plus left anterior hemiblock), as well as ectopic impulse formation with frequent premature ventricular beats; 4) they had positive serology for Chagas' disease; and 5) congenital, atherosclerotic, rheumatic, hypertensive, metabolic, toxic, familial or obstructive heart diseases were ruled out as well as systemic diseases like obesity and malnutrition which could involve the myocardium.

The four patients with acute disease had clinical, radiological, electrocardiographic and enzymatic signs of cardiac involvement. They showed signs of portal of entry and systemic manifestations of the disease. The parasite was observed by direct microscopic examinations of the blood, and infective strains of T. cruzi were isolated from two cases by means of mouse inoculation. In acute patients two samples of blood were obtained: the first in the early stage of the disease before or in the third day of evolution since the appearance of symptoms, and the second 30 days later. In one acute case another blood sample was obtained one year later.

**Controls**

(1) A total of 74 individuals from the same endemic area, of the same age and sex and showing no cardiac abnormalities, were selected.

(2) One hundred nineteen normal individuals and 286 patients with selected cardiovascular diseases or another pathology, all from a nonendemic area, were also used as controls. Among the 286 patients, 120 were cases of acute rheumatic fever, (78 with acute carditis), 19 were cases of subacute rheumatic fever with cardiac involvement (1 to 6 months of evolution since the onset of the disease), and 25 were patients with residual valvular disease due to rheumatic fever. The diagnosis and number of the remaining controls were the following: "primary" cardiomyopathies (18 cases); idiopathic hypertrophic subaortic stenosis (IHSS) (17 cases); pericarditis (16 cases); bacterial endocarditis (11 cases); postcardiotomy and postinfarction syndromes (10 cases); myasthenia gravis (30 cases); and connective tissue diseases (20 cases: systemic lupus, 6 juvenile rheumatoid arthritis, 2 dermatomyositis, 3 scleroderma). Serum samples of patients and controls were coded and examined without knowledge of the clinical diagnosis.

**Indirect Immunofluorescence Technique with Tissue Antigens**

Heart muscle was obtained from the left ventricle of human and bovine species, and from ventricles of albino mice and guinea pigs immediately postmortem. The following were also used as substrates: skeletal muscle from human abdominal wall and human placenta, bovine psoas muscle, mouse abdominal wall, and mouse liver, kidney and stomach. The method of Coons and Kaplan was used employing 2 μ thick unfixed sections. In all cases a double dilution of the sera was performed starting from 1/10 and the period of washing was extended to 30 min each. Anti-human gamma globulin (IgG, IgA, and IgM) was prepared as described by Nairn. Protein/fluorescein ratio was 1:513 and it was used at 1/8 dilution.

The distribution of antibodies in the three major classes of immunoglobulins was studied using rabbits' antisera to IgG, IgA and IgM labeled with fluorescein. The antisera were obtained by immunization with pooled normal human IgG purified by chromatography in DEAE cellulose, and with IgA and IgM purified by gel filtration and/or ion exchange chromatography from patients with myeloma and macroglobulinemia. The antisera were made monospecific for gamma, alpha and mu chains absorbing them with the other purified immunoglobulins. By means of immunoelectrophoresis and double diffusion in agar, they reacted giving one precipitin line against normal human serum. Protein/fluorescein ratio was 1:3 for anti-IgG and anti-IgA, and 1:5 for anti-IgM. Complement (C) fixing capacity of antibodies was performed by indirect immunofluorescence as in a previous report; however, as mentioned above washes with PBS were extended to 30 min each. Goat anti-human C3 (β 1 C-A) labeled serum was obtained commercially (Hyland Division Travenol, Los Angeles, California) and was used in a 1/6 dilution. All labeled antisera were absorbed with mouse liver and bovine skeletal and heart muscle powder before being used. "Blocking" experiments with the same anti-immunoglobulins unlabeled serum were performed during 40 min prior to applying the labeled antiserum. A Leitz Ortholux microscope with a dark field condenser and an Osram lamp HBO 200 with UG 1 filter were used, and an ultraviolet excluding filter was placed in the eyepiece of the microscope.

For this statistical analysis, the geometrical mean of the titers of each group was calculated. For comparing these means a t-test was applied to the logarithm of the titers.

**Direct Immunofluorescence Techniques with Striated Muscle Biopsies**

In 10 chagasic patients skeletal muscle biopsies (biceps) were performed as a source of autologous antigen. In one additional case (not included as a
patient or a control) with a congenital heart disease and infected with *T. cruzi*, a right atrium appendage biopsy was obtained during heart surgery. The cryostat sections were washed in PBS and directly treated with the goat anti-human gamma globulin labeled serum, followed by three washes in PBS. Sections of eight of the skeletal muscle biopsies were also treated with an anti-human fibrinogen rabbit serum, labeled with fluorescein. Antiserum was prepared by immunizing with plasma and absorbing with serum. Its specificity was controlled by immunoelectrophoresis. The protein/fluorescein ratio was 1:3 and it was used at a 1/10 dilution.

**Absorption Experiments**

Some (high titer) sera were absorbed with human placenta and with human and bovine myocardium, skeletal muscle, kidney and liver homogenates. Homog- enates were prepared with a teflon tissue homogenizer in 0.15 M NaCl (five volumes/g tissue). Some sera were also absorbed with pooled human (A-B-Rh+) and guinea pig red blood cells (RBC), and with repeatedly washed, frozen and thawed and lyophilized epimastigotes of *T. cruzi*. Epimastigotes were obtained from the Tulahuen strain, maintained in our laboratory by successive passages (over two hundred) in a diphasic medium. Base phase was formed by 19 g agar Difco (Difco Laboratories, Detroit, Michigan), 38.5 g brain-heart infusion (Difco) and 1000 cm³ distilled water. Sheep blood was not added to the base phase. Overlay was formed by 28 g brain-heart infusion, 10 g glucose, 10 ml liver extract (10%) and 1000 ml distilled water. Sterilization was performed in an autoclave for 20 min at 15 per square inch. Epimastigotes obtained from the overlay of the diphasic medium after six days of growth, were washed ten times in saline solution, frozen and lyophilized.

Absorptions were also carried out with the lyophilized overlay of the diphasic medium (autoclaved) containing all the organic compounds of the culture medium. The overlay employed for the absorption was not previously used for culturing *T. cruzi*. Absorptions were performed in the following manners: 1) homogenates: 1.5 g tissue/ml serum; 2) washed RBC: 2 ml packed cells/ml serum (supernatants of absorptions with human RBC were tested against A+ and B+ erythrocytes, by direct agglutination and by indirect Coombs test, to demonstrate the effectiveness of the absorption); 3) epimastigotes: 45 mg powder/ml serum; 4) lyophilized overlay of the culture medium: 45 mg/ml serum. Mixtures were incubated at 37°C for one hour, left overnight at 4°C, and then centrifuged in the cold at 10,000 G. Absorptions with homogenates were planned to yield a final dilution of 1/10 of the original serum.

**Serological Determinations for Chagas' Disease**

*T. cruzi* were obtained from the Tulahuen strain prepared in a diphasic medium as described above. The following techniques were used: 1) direct agglutination by the microtiter method employing epimastigotes treated with trypsin and formal; 19 2) indirect hemagglutination by microtiter method using Maekell's antigens; 16 and 3) indirect immunofluorescence technique using epimastigotes as antigen. For these three tests titers of 1/64 were considered positive.

**Heterophil Antibodies**

The presence of heterophil antibodies against guinea pig erythrocytes was determined by the microtiter method in 47 sera using a 2% suspension of washed RBC. Titers ≥ 1/16 were considered positive. Sera were also tested after being treated with an equal volume of 0.1 M 2-mercaptopethanol. 17

**Results**

Four out of four patients with acute chagasic cardiopathy, and 20 out of 21 patients with chronic chagasic cardiopathy had positive reactions by the indirect immunofluorescence technique with structures present in heart sections (table 1). The immunofluorescence staining was located on the endocardium, the endothelium and the immediate area surrounding the light of small and medium sized coronary blood vessels, and on the interstitium (figs. 1-3). This endocardial-vascular-interstitial pattern will be referred to as the EVI pattern.

With skeletal muscle the reaction was observed with interstitium and vascular structures, but with other organs (liver, kidney, stomach and placenta) it was limited to vascular structures. The reaction was positive with organs of different animal species: human, bovine, guinea pig and mouse; however, it was much stronger with mouse tissues. Striated muscle from human blood group O Rh negative was also an adequate substrate. Forty-seven out of the 74 asymptomatic controls from the endemic area had positive serology for *T. cruzi* and 27 had negative serology (table 1); 19 out of the 47 infected individuals presented the EVI pattern. Only 3 controls from the endemic area showed the EVI pattern with a negative serology for *T. cruzi* (table 1). These 3 cases had anti-trypanosoma antibody titers just below those considered in this study to be of clinical significance. The difference in prevalence of EVI factor between the infected individuals without heart disease and the chagasic

---

**Table 1**

**Prevalence of EVI Factor in Chagasic Cardiopathy and in Asymptomatic Controls from the Endemic Area**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Chagasic serology</th>
<th>EVI positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute cardiopathy</td>
<td>4</td>
<td>4 (+)</td>
<td>4</td>
</tr>
<tr>
<td>Chronic cardiopathy</td>
<td>21</td>
<td>21 (+)</td>
<td>20</td>
</tr>
<tr>
<td>Asymptomatic controls</td>
<td>74</td>
<td>47 (+)</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 (--)</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 1
Fluorescence photomicrograph of unfixed mouse heart section treated with serum (diluted 1/10) of a patient with chronic Chagas' heart disease followed by fluoresceinated anti-human gamma globulin antiserum. Positive staining in the endocardium and interstitium is observed. Brilliant fluorescence of the endocardium follows a ventricular anfractuosity. The staining of interstitium is observed as irregular patches between myofibers. (× 400)

Figure 2
Fluorescence photomicrograph of unfixed mouse heart section treated as in figure 1. Small round brilliant fluorescence strongly suggests the staining of small coronary blood vessels and capillaries. Positive staining of the interstitium is also observed. (× 250)

Figure 3
Fluorescence photomicrograph of unfixed mouse heart section treated as in figure 1. Positive staining in the inner part of a coronary blood vessel is observed. Staining of the interstitium is also observed. (× 400)

Circulation, Volume XLIX, January 1974
cardiopathy group is statistically significant. \( x^2 = 20.94; P < 0.001 \).

Titers of the EVI pattern in patients and in asymptomatic controls from the endemic area are presented in table 2. In both groups considerable overlapping of titers is observed. Differences in distribution of the EVI titers in patients with chronic Chagas' heart disease and in asymptomatic controls from the endemic area are not statistically significant. With each serum, the end-titer of staining of endocardium, blood vessels or interstitium was similar.

EVI fluorescence was not observed in any of the 405 healthy or pathological controls from the nonendemic area. In the pathological control group, patients with myasthenia gravis and those with IHSS, rheumatic fever, postinfarction and postcardiotomy syndrome had the sarcomemmal or intrafibrillar pattern described\(^ {18-21} \) and known to occur in these diseases. In patients with rheumatic fever intermyofibrillar staining was also noticed.\(^ {21-22} \) As expected, in the connective tissue disease (CTD) group, antinuclear factors reacting with nuclei of striated muscle were observed (especially in systemic lupus); other positive patterns were not observed with these tissues and the CTD sera.

The staining of the EVI factor could be blocked by incubation of heart sections exposed to positive sera with unlabeled anti-human gamma globulin prior to the use of the fluorescein conjugated.

In the skeletal muscle biopsies of the 10 EVI positive patients in which the direct immunofluorescence technique was performed for investigating the possible in vivo fixation of the circulating EVI factor, immunoglobulins were found in the interstitium (fig. 4). This gamma globulin was removed by washing the sections for two hours with citrate buffered saline pH 3.2.\(^ {23} \) Washes with PBS for two hours had no effect. Staining could be blocked by incubation of biopsy sections with unlabeled anti-human gamma globulin prior to the use of the fluorescein conjugated. Studies with the labeled anti-fibrinogen antiserum showed that this protein was not present.

As controls, direct immunofluorescence technique was performed in skeletal muscle biopsies of 15 patients with juvenile rheumatoid arthritis and of eight asymptomatic controls from the endemic area with positive serology for the \( T. \) cruzi and a negative test for the EVI factor. None of these 23 muscle biopsies showed in vivo bound gamma globulin. The in vivo demonstration of bound gamma globulin in the interstitium of skeletal muscle of individuals with circulating EVI factor makes this tissue unsuitable for the indirect immunofluorescence technique and for the demonstration of its autoimmune nature. However, in the right atrium biopsy performed in an EVI positive patient no in vivo bound gamma globulin could be demonstrated by the direct immunofluorescence technique. Therefore this tissue was useful for the detection of the EVI factor by indirect immunofluorescence: when the patient's own serum was used, positive results were obtained.

Absorptions of ten EVI positive sera with organ homogenates (heart, skeletal muscle, kidney and liver from human and bovine sources and human placenta) resulted in negative reactions. On the contrary, pooled human RBC of ARh+ and BRh+ groups were ineffective.

In the absorption experiments with 13 sera using lyophilized epimastigotes, progressive addition of material diminished the EVI titer in 11 cases until it rendered the sera negative with 45 mg epimastigotes powder/ml serum. In the other two cases, the EVI titers decreased from 1/160 to 1/20, but they were still positive even after adding 150 mg epimastigotes/ml serum. The three sera with positive EVI pattern and negative serology for \( T. \) cruzi (table 1) had anti-trypanosoma antibody titers just below those considered in this study to be of clinical significance; after absorption with epimastigotes, the three sera became EVI negative. Absorptions with the lyophilized overlay of the culture medium carried out in 9 sera were

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titers of EVI Factor in Chagasic Cardiopathy and in Asymptomatic Controls from the Endemic Area</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of EVI positive cases</th>
<th>Titer</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \geq 1/10 )</td>
<td>( \geq 1/20 )</td>
</tr>
<tr>
<td>Acute cardiopathy</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic cardiopathy</td>
<td>20</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Asymptomatic controls</td>
<td>22</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Circulation, Volume XLIX, January 1974
ineffective in abolishing the EVI pattern or in modifying its titers. In 18 EVI positive cases the sera were absorbed with heart and skeletal muscle homogenates and tested for antibody activity against *T. cruzi*; reactions remained positive and titers unchanged.

Packed guinea pig RBC were effective in abolishing the EVI factor from positive sera. In 47 cases with positive serology for Chagas' disease heterophil antibodies for guinea pig RBC were investigated and then correlated with the EVI pattern: 16 EVI positive sera which were effectively absorbed by guinea pig erythrocytes did not have agglutinating heterophil antibodies. On the contrary, 13 individuals had heterophil activity in the absence of the EVI pattern. The inverse relationship between the presence of the EVI pattern and heterophil antibodies is shown in table 3. Mercaptoethanol treatment removed agglutinating activity against guinea pig erythrocytes.

The EVI pattern could also be observed in 10/10 sera when examined by the C fixation indirect immunofluorescence technique. The intermyofibrillar pattern of 19 sera from patients with rheumatic fever did not fix C.

Labeled monospecific antisera for immunoglobulins demonstrated that in ten chronic cases staining was related to IgG, and in one case also to IgM. In the acute cases, IgM was the only immunoglobulin carrying EVI specificity in the first 72 hours of evolution of the disease. After one month both IgM and IgG were involved. The blood sample of the patient studied one year later showed only the presence of IgG with EVI activity.

**Discussion**

This report demonstrates in the sera of patients with chagasic cardiopathy the presence of immunoglobulins that react with heart and vascular structures. In addition, their ability to interact with

| Heterophil Antibody and EVI Factor in 47 Individuals with Positive Serology for *T. Cruzi* |
|----------------------------------|-----------------|
| Heterophil (+)                  | EVI (+)         |
| Heterophil (−)                  | EVI (−)         |
| 17                               | 13              |
| 16                               | 1               |
| *P* <0.01.                       |                 |

Circulation, Volume XLIX, January 1974
complement is also demonstrated. The reaction with vascular structures of organs of different mammals showed the lack of both organ and species specificity. Absorption experiments with organ homogenates confirmed this conclusion.

There was no difference in the end-titers of the three patterns observed in each single serum which suggests that similar immunological systems are involved. Although the pattern distribution in the heart is similar to that of antigens of the AB system, the failure of pooled human red cells in absorbing the reacting gamma globulin and the demonstration of the pattern using organs from an O-Rh negative individual suggest that the EVI pattern is not related to this system. Moreover, Kaplan, Meyerson and Kushner showed the very low probability of observing isimmune reactions by immunofluorescence technique related to the AB system using heart as substrate. Forssman antigens were found to be unrelated because we showed that Forssman negative species were useful as a source of antigens, and we failed to absorb the EVI factor with human A red cells which are Forssman positive.

The histological location of the EVI pattern in the heart is different from that described in relation to the Wassermann antigen. In addition, serology for syphilis was negative in all the patients with Chagas’ heart disease reported here. The EVI pattern differs from other patterns observed with circulating immunoglobulins reacting with muscle structures known to occur in diseases involving the skeletal muscle and the cardiovascular system. However, in a previous report a gamma globulin in the walls of arteries and veins as well as in the interstitium of the myocardium was described in immunopathological studies carried out with heart tissue of five children who died from severe rheumatic fever.

The intermyofibrillar pattern observed in rheumatic fever can also be differentiated from the EVI pattern by its lack of C fixation by the immunofluorescence technique. The lack of C fixing ability of the intermyofibrillar antibody was demonstrated by tube C fixation test in another report. Recently, capillary reactivity has been observed frequently in immunofluorescence in the sera of patients with scleroderma, dermatopolymyositis, polymyalgia and temporal arteritis, using mouse kidney sections as substrate. The specificity of this factor is not elucidated, and it reacts with a lesser intensity with blood vessels of mouse spleen and testis. However, it fails to give positive results with tissues of other mammals, which distinguish it from the EVI factor. In addition, an attempt to demonstrate circulating antibodies which react with human skeletal muscle and with the patient’s own muscle tissue in myositis has failed.

It is interesting that guinea pig erythrocytes were able to absorb the EVI factor. The existence of high titers of agglutinating heterophil antibodies in Chagas’ disease is well known. Nevertheless, in our patients their presence was not related to the EVI factor, and in chronic cases the latter are almost always in IgG, and experiments with 2-mercaptoethanol suggest that the former are mainly in IgM. However, in other systems it has been demonstrated that IgG may have heterophil activity after a secondary challenge. Recent work has shown the existence of common antigens in human tissues and animal erythrocytes, and these cross reactions may account for the effectiveness of absorption. The present study reveals evidence of a cross reaction of the EVI factor with antigenic determinants present in T. cruzi. In almost all cases studied, absorption with epimastigotes was able to remove the EVI positivity of sera. The relatively high concentrations of epimastigotes necessary to absorb sera might indicate the presence of small amounts of common antigenic determinants in this stage of the T. cruzi’s cycle. Failure to absorb the antibodies against T. cruzi with organ homogenates might also be related to small amounts of cross antigenic determinants.

Failure in absorbing the EVI factor with culture medium rules out the possibility that the absorption is due to the presence of contaminants from diphasic medium in the lyophilized epimastigotes. Since biological components comprise part of the culture medium, their relative concentration or their antigenic alteration during preparation may account for these results. However, the possibility that the T. cruzi had incorporated in its genome antigenic information of the animal from which the Tulahuen strain has been isolated, or antigens from the laboratory mammals in which the strain has been passaged, could not be ruled out.

The recognition of IgM EVI factor in the early stage of acute Chagas’ heart disease shifting to IgG afterwards, such as in chronic cases, further relates its appearance to infection by T. cruzi. Moreover, asymptomatic individuals of the endemic area showing Chagas’ positive serology and the EVI factor also associate it with infection by T. cruzi, and might be representative of those individuals studied during the period of years between the moment of infection and the development of heart
disease. A close follow-up of this group would seem to be of great significance.

The autoimmune nature of an antibody is established when it reacts with self constituents. Direct immunofluorescence studies of skeletal muscle biopsies of EVI positive individuals showed the presence of in vivo bound immunoglobulins with a pattern similar to that of the EVI factor. The possibility of removing the deposited immunoglobulins by acid buffer treatment suggests their immunological nature. Furthermore, in one case it was demonstrated that the circulating EVI factor reacted with the patient’s own atrial appendage. The former and the latter experiments give support to the autoimmune nature of the immunoglobulins carrying EVI specificity. The demonstration of a cross reactivity between antigens present in human tissues and in T. cruzi suggests that the EVI factor may be the result of a response to the constituents of T. cruzi antigenically cross-reactive with human tissues. Chronic infection and/or the continuous liberation of self-antigens from injured tissue may be the stimulus for perpetuation of the autoimmune response. Experimental evidence supports this possibility. A similar mechanism has been extensively considered for explaining the appearance of autoimmune phenomena in rheumatic fever by cross-reacting antigens of group A streptococci and the heart, and in postcardiotomy and postinfarction syndromes by the liberation of myocardial antigens. Clinical and experimental data support these possibilities.

Different authors have stressed the high prevalence in chronic Chagas’ heart disease of an inflammatory and a fibrous process in the endocardium and interstitium with vascular involvement, not related to the location of the parasite. Although the EVI factor could be related to the pathogenesis of the heart disease, its reactivity is not specifically directed toward heart structures, and the hypothetical reasons for the presence of lesions in this restricted organ are unclear. Clinical diagnosis of chronic Chagas’ heart disease is difficult to prove because of the lack of a specific element. It is based upon the presence of significant myocardial damage associated to a positive serology, in the absence of other etiological factors. Positive serology alone has limited diagnostic value because of its high prevalence among asymptomatic people in endemic areas, and at best is only indicative of chronic infection with or without heart disease. These considerations are also valid for xeno-diagnosis. Thus it is possible that in endemic zones chronic Chagas’ heart disease will be over-diagnosed on account of nonobstructive cardiomyopathies, considered in other regions to be of undetermined etiology. The results of the present report are of clinical interest, because in individual cases the diagnosis of chronic chagasic cardiopathy can be considered with a low probability in the absence of the circulating EVI factor.

Acknowledgement

We are grateful to Miss Elisa T. Meyer, Dr. Teresa Mercado and Dr. Irving Kagan for the revision of the English manuscript, to Miss M. B. Casanova for the valuable technical assistance, and to Dr. Pedro Cossio and Dr. Mauricio Rosenbaum for their suggestions in the preparation of the manuscript.

We are also grateful to Dr. O. García Morteo and Dr. G. Schmunis for their support throughout this study, and to Marta García Ben from the Centro de Computo de la Facultad de Medicina-UBA, for performing some of the statistical analysis.

References

6. MARGARINO TORRES C: Patogenia de la miocarditis crónica en la enfermedad de Chagas. Quinta reunion de la Sociedad Argentina de Patología Regional del Norte 2: 902, 1929
Chagasic Cardiopathy: Demonstration of a Serum Gamma Globulin Factor Which Reacts with Endocardium and Vascular Structures

PATRICIO M. COSSIO, CARLOS DIEZ, ANA SZARFMAN, EDUARDO KREUTZER, BARTOLOMÉ CANDIOLO and ROBERTO M. ARANA

_Circulation_. 1974;49:13-21
doi: 10.1161/01.CIR.49.1.13

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1974 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/49/1/13

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/