POLYMERASE CHAIN REACTION AMPLIFICATION OF THREE DIFFERENT TRYPANOSOMA CRUZI DNA SEQUENCES FROM HUMAN CHAGASIC CARDIAC TISSUE

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Abstract. Chagas’ disease is caused by the hemoflagellate protozoan Trypanosoma cruzi. The most common, serious manifestation of Chagas’ disease is a progressive inflammatory cardiomyopathy, which occurs decades after primary infection. The inability to consistently demonstrate T. cruzi by histologic techniques in inflammatory cardiac lesions has suggested that the parasites’ persistence may not be required for the pathology of the chronic phase. In this report we further analyze the persistence and localization of T. cruzi DNA in the hearts of seven patients with chronic chagasic cardiomyopathy, along with four indeterminate patients and seven control patients seronegative for T. cruzi infection. In the seven patients with chronic chagasic cardiomyopathy, we extracted DNA from selected inflammatory foci-positive (IFP) and inflammatory foci-negative (IFN) areas of hematoxylin and eosin-stained cardiac tissue. We then used polymerase chain reaction methodology to amplify three different T. cruzi sequences (a minicircle sequence [MCS], a satellite repetitive sequence [RS], and a low copy number sequence within the gene coding for a flagellar protein [FPS]). The MCS was detected in ~100% of both the IFP and IFN areas analyzed. The RS was detected in 37.5% and 23% of the IFP and IFN areas, respectively (difference not statistically significant; P > 0.10, degrees of freedom = 1, G test of independence = 1.9522). The FPS was rarely detected (2%), and was only present in DNA extracted from IFP areas. The MCS was also detected in most indeterminate cases (none of whom had inflammatory lesions) although with a markedly diminished amplification signal relative to cardiomyopathy cases. The MCS was not amplified from the cardiac tissues from seronegative controls. These results suggest that the quantity of T. cruzi DNA persisting in hearts of patients with Chagas’ disease correlates with cardiomyopathy, but may not be preferentially associated with inflammatory foci.

Chagas’ disease is caused by the flagellate protozoan Trypanosoma cruzi. Ten to twenty million people in South and Central America are infected with the parasite, and it is estimated that 35 million people are at risk of infection.1,2 Infection occurs when metacyclic trypomastigotes in triatomid feces enter the vector host via a break in the skin, through contact with mucous membranes, or by scratching at the site of the vector bite. Although most of the human cases worldwide of T. cruzi infection are through contact with triatomid feces, transfusion of contaminated blood also represents a serious problem in some parts of the Western hemisphere. The migration of infected individuals from endemic rural areas has led to an increase in transfusion associated cases in nonendemic areas such as cities in endemic countries and in Europe and the United States.3

Patient morbidity of the acute phase of Chagas’ disease is directly associated with the level of parasitemia that occurs. The pathology of this phase includes inflammatory foci in cardiac muscle, which may become diffuse with interstitial edema. At this stage, parasites can easily be visualized in infected myocardial cells.4 Other focal inflammatory lesions may be seen in skeletal and smooth muscle, and in the central nervous system. Although organ failure in the acute phase may cause severe morbidity and occasional deaths, in most patients recovery of function is usually complete when parasitemia decreases.5

After the acute phase of the disease, the patients enter into an asymptomatic chronic stage (indeterminate form) in which high levels of anti-T. cruzi antibodies are detected and the parasitemia is difficult to detect. This asymptomatic stage can persist during the life span of the person. However, after 5–20 years patients may develop clinical manifestations of cardiac or digestive disease.6

The cardiac form of the disease is the most common serious manifestation of the chronic phase (30–40% of infected patients). Several autopsy and biopsy studies of chronic human chagasic cardiopathy cases and of experimental canine Chagas’ myocarditis have provided considerable insight into the pathology associated with this phase.5,7,8 Chagasic patients’ hearts with chronic myocardiopathy often show left ventricular dilatation and hypokinesis with characteristic aneurysm formation in the left ventricular apex. Light and electron microscopic analyses show foci of infiltrating mononuclear cells (in which lymphocytes are closely associated with myocardial cells), increased fibrosis, and significant atrophy of the contractile apparatus. However, without resorting to exhaustive means, such as serial sectioning and immunohistochemical staining for T. cruzi proteins, only a small minority of all chronic cases studied have identifiable organisms within the lesions. This has suggested that the parasite’s persistence may not be involved in the development of cardiomyopathy.1,2,9

The long period in which patients remain asymptomatic along with the almost undetectable levels of parasitemia, has led to the hypothesis that autoimmune processes are involved in the pathology of the disease. These theories have been bolstered by findings of autoantibodies directed against endocardium, vasculature, and interstitium of striated muscle in patients with chronic disease.5 Other investigators have suggested that molecular mimicry between T. cruzi surface antigens and mammalian nerves may trigger an autoimmune response.10,11 In murine models, the persistence of T. cruzi...
in affected cardiac tissue, but it did not establish what form of parasite persistence and localization of *T. cruzi* DNA was present or whether it was only localized to areas of hematoxylin and eosin-stained tissue sections as previously described.10,11 The DNA extracted was amplified by PCR technology to detect *T. cruzi* DNA sequences in blood from chronic chagasic patients. Our earlier study demonstrated the presence of a repetitive *T. cruzi* DNA sequence in affected cardiac tissue, but it did not establish what form of parasite DNA was present or whether it was only localized within inflammatory lesions in cardiac tissues.

In the present study, we have further examined the question of parasite persistence and localization of *T. cruzi* DNA sequences within human chagasic cardiac lesions. For this purpose, we extracted DNA from microscopically selected areas of hematoxylin and eosin-stained tissue sections as previously described.21,22 The DNA extracted was amplified by PCR technology. Three different *T. cruzi* sequences were amplified: 1) a 122-basepair (bp) sequence localized within the minirepeat of the kinetoplast minicircles (MCS);23,24 2) a 188-bp sequence within a 195 tandemly repeated sequence (RS);25 and 3) a sequence within the open reading frame of a gene coding for a 160-kD protein found on the surface of the parasite overlaying the flagellum (FPS).26 The RS and MCS represent a large amount of the total DNA content in *T. cruzi* (~100,000 copies/cell for RS and ~120,000 copies/cell for MCS), whereas FPS is a low copy number gene that represents a very small fraction of the parasite’s total DNA content.

### MATERIALS AND METHODS

**Parasites.** *Trypanosoma cruzi* (Y strain) epimastigotes were maintained at log phase in liver infusion tryptose (LIT) at 28°C with gentle agitation.26

**Primer and probe sequences.** All primers and probes were synthesized by Genosys Biotechnologies, Inc. (The Woodlands, TX) (Table 1). Oligonucleotide probes were labeled with ^32P as described below.

**Extraction of DNA from cultured *T. cruzi* epimastigotes.** Total DNA from approximately 8.8 × 10^8 LIT-cultured epimastigotes/ml was extracted using a standard protocol described previously.27 After extraction, both DNA concentration and purity were estimated by measuring the 260/280 optical density ratio, and the DNA was stored at −20°C until further use.

**Patient population and tissue collection.** The patient population, which we have previously described, consisted of individuals who based on their anti-*T. cruzi* serologies had chronic infection with *T. cruzi*, including cardiac (seven cases) and indeterminate (four cases) patients. Cardiac tissue collected on autopsy from each patient was analyzed, along with internal controls consisting of tissue samples from unaffected organs such as lymph nodes, ovaries/testicles, adrenal glands, and the gastrointestinal tract for each cardiac case. As negative controls, tissue from age- and sex-matched seronegative patients (seven cases) were also processed using the same conditions as applied to the seropositive population. These tissue specimens were the same as those collected and processed as previously reported. The study was conducted following National Institutes of Health (Bethesda, MD) guidelines and was approved by both the Internal Review Board of Vanderbilt University and the Ethics Committee of the Fundação Oswaldo Cruz.

**Collection of tissue sections for PCR.** Each sample was processed using disposable instruments (e.g., scalpels, microtome blades, and gloves) to avoid cross-contamination among tissue samples. The microtome and bench top were thoroughly cleaned with a solution of 0.2 M NaOH or DNA-Away (Molecular Bio-Products, Inc., San Diego, CA.) prior to sectioning each individual block. Sectioning of samples, as well as other pre-PCR methodology were carried out in a different room away from post-PCR analysis. For most cases with chronic disease, four 10-μm sections (first series) were obtained. Then 50 μm of tissue were discarded, and four 10-μm sections (second series) were obtained. The eight sections obtained from each block were stained with hematoxylin and eosin using disposable Copling jars (Evergreen Scientific, Los Angeles, CA).

At least four 10-μm sections were obtained from internal control samples (ovaries or testicles, adrenal gland, lymph nodes, gastrointestinal tract, indeterminate cases, and seronegative cases). Every case with chronic diseases processed was accompanied by seronegative samples. Internal controls and indeterminate cases were randomly analyzed along with cases with chronic disease.

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide</th>
<th>Sequence</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCS</td>
<td>S34 primer</td>
<td>5'–TAT ATT ACA CCA ACC CCA ATG GAA CC–3'</td>
<td>122</td>
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<tr>
<td></td>
<td>S67 primer</td>
<td>5'–TGG TTT TGG GAG GGG (C/G) (G/C) (T/G) TCA A (A/C) T TT–3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S33a probe</td>
<td>5'–TCA TGC ATC TC (C/A) CCC GTA CAT TAT TTG GCAA ATT TG–3'</td>
<td></td>
</tr>
<tr>
<td>RS</td>
<td>T CZI primer</td>
<td>5'–CGA GCT CTT GCC CCC AGC AGT GGT GCT–3'</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>T CZII primer</td>
<td>5'–CTT CCA AGC AGC GGA TAG TTC AGG–3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T CZ probe</td>
<td>5'–CAA TAT CTG TTT GCG TCT TCA CAC AC–3'</td>
<td></td>
</tr>
<tr>
<td>FPS</td>
<td>611 primer</td>
<td>5'–GGG GCG GTT G(T/G) (T/G) T C T C/G T G–3'</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>761 primer</td>
<td>5'–CAG GGC ATG TTT GGG A (A/T) T T (C/G) G T–3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FLP probe</td>
<td>5'–CGT CCC ATT CCG CAC TGC TCA CGC ACG AA–3'</td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>C16B primer</td>
<td>5'–GGT TTC CTC GAT CTT TAG GCC TGC CAC–3'</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>C16D primer</td>
<td>5'–GGT GCC ATG CTT TGA TGA GCN AA–3'</td>
<td></td>
</tr>
</tbody>
</table>

* bp = basepair; MCS = minicircle sequence; RS = satellite repetitive sequence; FPS = flagellar protein sequence; CF = cystic fibrosis transmembrane conductance regulator.
DETECTION OF T. CRUZI DNA IN HUMAN CARDIAC TISSUE

FIGURE 1. Hematoxylin and eosin-stained sections from case A12. A, typical inflammatory focus within the central portion of the field (magnification × 200). B, inflammatory foci-negative area (magnification × 100).

Selection of inflammatory and noninflammatory areas for the PCR. Stained slides were immersed in xylene to remove coverslips, and the areas of interest were scraped using a standard surgical scalpel. Inflammatory foci-positive (IFP, Figure 1A) and inflammatory foci-negative areas (IFN, Figure 1B) were selected and marked (~5 mm in diameter). An inflammatory positive area is a collection of 50 or more contiguous leukocytes. Areas with or without inflammatory foci were scraped separately. Up to five IFP or five IFN areas from a single section were collected in 1.5-ml microcentrifuge tubes. Because IFN areas were scarce and small within lesions, and did not yield a sufficient amount of DNA when extracted individually, the areas from two different serial sections were collected and treated as one IFN area.

Extraction of DNA for the PCR. Special precautions, such as using new scalpels, gloves, and bench-top paper after scraping each individual slide, were used. Microcentrifuges, pipettes, test tube racks, and other instruments were cleaned thoroughly with DNA-away before and after samples were analyzed. Also, a mask was used to prevent aerosolization of material by exhalation.

Extraction of DNA from tissue was carried out as previously described without modifications.21,22 Briefly, the scraped tissue was deparaffinized with 1 ml of xylene, and mixed at room temperature for 10 min. Samples were then washed twice with 1 ml of 100% ethanol, mixed at room temperature, and dried at 50°C using a drop of acetone. The DNA was extracted overnight at 55°C in 8 μl of digestion buffer (50 mM Tris, pH 8.5, 1 mM EDTA, and 0.5% Tween 20) and 2 ml of proteinase K (200 μg/ml). After digestion, the protease was inactivated by boiling for 6–8 min and residual debris was removed by centrifugation using a benchtop microcentrifuge at 15,000 × g for 5 min. Samples were stored at −20°C until further use.

Amplification by the PCR. The PCR was performed as previously reported.21,22 Exon 10 of the cystic fibrosis transmembrane conductance regulator (CF) was amplified after DNA extraction as a standard control to ensure the quality and roughly determine the quantity of DNA extracted. The amplified CF product was a 98-bp sequence that could be visualized after electrophoresis on an agarose gel by staining with ethidium bromide.28 Samples that did not amplify this CF product were discarded. All PCR experiments were run in duplicate using 100-μl reactions. Each reaction mixture contained 20 mM Tris·HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 200 mM each of the four deoxynucleotide triphosphates (Pharmacia, Uppsala, Sweden), 5 mM each of the primer pairs (Table 1), 1.25 units of Taq DNA polymerase (Gibco-BRL, Gaithersburg, MD), and 10 μl of template DNA and was overlaid with four drops of mineral oil. Reactions were denatured at 94°C for 6 min., and then subjected to 35 cycles in a programmable thermal cycler (480; Perkin-Elmer, Norwalk, CT). Each cycle included 1 min at 94°C for denaturation, 2.5 min at 55°C for annealing, and 3 min at 72°C for extension. Upon completion of the cycles, a final extension period for 10 min at 72°C was performed. The PCR products were stored at −20°C prior to further analysis. As positive controls for each reaction, 3 ng of DNA extracted and purified from cultured T. cruzi epimastigotes were used. For negative controls, sterile double-distilled water was added to the reaction instead of template DNA.

Gel electrophoresis, Southern blotting, labeling of probe, and hybridization. Five microliters (one-fifth of the total reaction volume) of the CF PCR products were electrophoresed using a 2% agarose gel, visualized by staining with ethidium bromide (1 μg/ml), and destained for 1 hr in double-distilled water. Other PCR products were separated by electrophoresis on a 1.1% agarose gel and visualized by staining with ethidium bromide. Amplified DNA was denatured by soaking the gel for 45 min in 1.5 M NaCl and 0.5 M NaOH. After rinsing the gel for 5 min in double-distilled water, the DNA was neutralized with two washes each of 1.5 M NaCl and 1 M Tris (pH 7.4) for 30 and 15 min, respectively. The DNA was transferred to a Zeta-Probe
membranes were washed twice again (30 min each) in 0.2 M dithiothreitol, 1 mM EDTA, 2×3 (3 min/wash) in 1×SSPE/0.5% SDS for 30 min at 45°C. After hybridization, the membranes were washed twice with the same solution. The autoradiography was performed for 24 hr at −70°C using an intensifying screen.

**Statistical methods.** To test whether the proportion of samples in which PCR amplification was successful differed between IFP and IFN areas, we used a G Test of Independence.²⁹ The null hypothesis for this statistic is that any observed difference between treatments in the proportion of successful amplification is due to chance alone.

**RESULTS**

**Analysis of cardiac tissue from human chronic chagasic cases.** Seven human chagasic cardiomyopathy cases were analyzed (Table 2). From each case, four 10-μm sections (first series) were obtained, 50 μm of tissue was then discarded, and four additional 10-μm sections (second series) were obtained for analysis. From each case several IFP areas were examined, along with a minimum of two IFN areas. All samples analyzed were determined to have a sufficient amount of DNA for PCR amplification and the reaction was not hindered by possible inhibitors present in the extracted material as revealed by CF amplification. These results indicate that small areas of hematoxylin and eosin-
DETECTION OF T. CRUZI DNA IN HUMAN CARDIAC TISSUE

FIGURE 3. Frequency (in percent) of a minicircle sequence (MCS), a satellite repetitive sequence (RS), and a flagellar protein sequence (FPS) in inflammatory foci-positive (IFP) and inflammatory foci-negative (IFN) areas within human chronic cardiac tissue. Closed bars = IFP areas; open bars = IFN areas. No significant difference was detected in RS amplification among positive and negative areas (G test of independence = 1.9522).

stained tissue (~5 mm in diameter) can be used for this type of study.

As shown in Table 2, amplification of MCS was detected in almost all samples from chagasic cardiomyopathy cases, both in areas of inflammation and in noninflammatory areas. The relative frequency of this sequence in all IFP and IFN areas studied was extremely high (99%), and the amplification signal was very intense (Figures 2 and 3). The only exception to these data was observed in case A4, in which the second series of sections of one IFP did not amplify for MCS. This may have been related to the fact that this case had extensive fibrosis with only a few, small inflammatory foci within the positive areas of the tissue examined.

Amplification of RS was detected in cardiomyopathy cases A4, A6, A10, A12, and A15, but the intensity of the signals was variable and in some cases (A4 and A6) quite weak (Figure 2). The RS did not amplify from the sections evaluated from cases A7 and A21 (Table 2). Within tissue from A10, A12, and A15, RS was detected only in some sections of the cardiac tissue. For example, in case A10, RS was not detected until the third section of the first series of sections in the IFP area, and then the amplification signal remained present through the fourth section of the second series. However, since a gap of 50 μm exists between these two series of sections, it is not possible to assess whether RS detection was constant through the entire lesion. In case A12, RS was amplified from the IFP through the first three sections of the first series. However, it was undetectable in the first two sections of the second series of sections and then detected again in the last two sections of the second series. A similar pattern is also observed in case A15. Overall, RS was detected in 37.5% of all inflammatory areas, and in 23% of all noninflammatory areas analyzed (difference not statistically significant; P > 0.10, degrees of freedom = 1, G test of independence = 1.9522) (Figure 3).

The FPS was only detected in the first series of sections of one IFP from case A15 (Figure 3). Also, the intensity of the amplification signal obtained for FPS was very weak.

Analysis of internal control tissues, indeterminate cases and tissue from seronegative cases. Detection of MCS within internal control tissues varied from case to case (Table 2). Sections of gonadal tissue from cases A4 and A12, and from A15-GI consistently amplified with MCS, while sections from cases A6-Adn, A7-O/T and A7-LN only sporadically amplified for MCS. Gonadal tissue from case A10 and adrenal tissue from case A21 did not amplify for MCS. In contrast, RS was only detected in A7-O/T-section 1, while FPS was not detected from any internal control.

Tissues from four indeterminate cases were analyzed. For each case, a minimum of three cardiac sections were examined for the ability to amplify DNA encoding MCS, RS, and FPS. As shown in Table 3, MCS was inconsistently detected in three of four cases, and the intensity of the signal was much weaker than that observed in cardiac cases (Figures 2 and 4). The RS and FPS were not detectable in any of the tissue sections examined from indeterminate cases.

Cardiac tissue from seven age- and sex-matched seronegative cases were also analyzed. No amplification of DNA for MCS, RS, or FPS was detected in any of these cases (Table 3 and Figure 4).

DISCUSSION

In this study we examined the presence of three T. cruzi DNA sequences (MCS, RS, and FPS) in hematoxylin and eosin–stained tissue sections from patients with chagasic cardiomyopathy, indeterminate patients (seropositive for T. cruzi infection without cardiac lesions), and sex- and age-matched seronegative individuals as controls. We also used a modified scrapping technique to study the presence of
TABLE 3
Results of polymerase chain reaction analysis for the indeterminate and seronegative cases in this study*

<table>
<thead>
<tr>
<th>Indeterminate cases</th>
<th>Total sections analyzed</th>
<th>MCS</th>
<th>RS</th>
<th>FPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A11</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A18</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A20</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Seronegative cases</th>
<th>Total sections analyzed</th>
<th>MCS</th>
<th>RS</th>
<th>FPS</th>
</tr>
</thead>
<tbody>
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<td>A1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>A13</td>
<td>6</td>
<td>0</td>
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<td>A14</td>
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<td>A19</td>
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<tr>
<td>A22</td>
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<td>0</td>
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</table>

* For definitions of abbreviations, see Table 1. Values for MCS, RS, and FPS are the number of sections that amplified for a particular sequence.

these parasite DNA sequences in relation to inflammatory lesions in the seven cases of chagasic cardiomyopathy. In our earlier studies we had established that DNA amplification of MCS, RS, and FPS was sufficiently sensitive to detect single amastigotes in infected murine cardiac tissue scrapings.

As we have previously reported, we consistently found T. cruzi DNA in the hearts of patients with chagasic cardiomyopathy. As shown in Table 2 and Figure 4, MCS was amplified in all cardiomyopathy cases analyzed. DNA was never amplified in seronegative cases, establishing the specificity of this technique (Table 3). Interestingly, MCS was also amplified in noncardiac tissues from chagasic patients, indicating that this T. cruzi DNA sequence is also present in tissues not containing lesions. Moreover, we also detected MCS (although weakly and sporadically) in cardiac tissue from indeterminate cases (Figure 4). These results indicate that T. cruzi DNA can often be detected in indeterminate cases, as well as cardiac patients, and suggests that the amount of DNA (perhaps as an indicator of parasite load) correlates with and may play a critical role in pathogenesis.

It is worth mentioning that in all the microscopic sections from hearts of chagasic patients analyzed in this study only one amastigote nest of \( \sim 80 \) parasites was observed. This observation correlates with our finding that the low copy number gene (FPS) was amplified from only one case on one occasion (Table 2). These findings suggest that the number of T. cruzi organisms in these cases of chagasic cardiomyopathy was extremely low.

Our earlier results demonstrated the persistence of RS-specific parasite DNA in cardiac tissue from patients with severe Chagas disease and suggested a role for parasite DNA persistence in the production of cardiac inflammation. However, since bulk DNA extraction from tissue was used in the previous report, the location of parasite’s DNA in chagasic hearts was not determined and because the presence of only a single parasite DNA sequence was evaluated, it was not possible to conclude whether this represented whole organisms or residual or integrated DNA. Therefore, we now report on the presence of T. cruzi-specific DNA sequences in microscopically selected areas with or without inflammation. We consistently detected MCS in inflammatory foci (IFP), which suggests the presence of T. cruzi DNA within these areas. However, MCS was also frequently detected in noninflammatory (IFN) areas in the seven cardiomyopathy cases of this study (Table 2 and Figure 3), as well as in noncardiac tissues, and was often found in cardiac tissue from indeterminate patients. This suggests that this particular sequence is widespread and indicates that the kinetoplast sequence does not correlate specifically with inflammatory lesions. On the other hand, we observed a tendency to detect RS more frequently in IFP as opposed to IFN areas, although this difference was not statistically significant (G test of in-
DETECTION OF T. CRUZI DNA IN HUMAN CARDIAC TISSUE

569
dependence = 1.9522). These findings fail to establish preferential localization of some parasite DNA sequences in inflammatory foci.

Since intact amastigotes were rarely observed, the parasite form and exact location of these sequences in the tissue is uncertain. It is possible that intact organisms are present but that their number is extremely low. On the other hand, it is also possible that intact organisms are not present in every instance that T. cruzi DNA is found, and MCS and RS represent sequences that remain in the host either as remnants of the initial parasite infection, or as integrated elements in the host’s genome. The latter hypothesis has been supported by at least one published paper suggesting possible integration of parasite DNA in the host genome. Another possibility is that T. cruzi organisms present in remote organs may shed their DNA into the blood stream, which is then taken up by the cardiac tissue. The central vein of the adrenal gland has been proposed as one potential site of parasite persistence.

Our results have confirmed that specific T. cruzi DNA sequences are present not only in cardiac tissue in chagasic cardiomyopathy but also in cardiac tissue from indeterminate cases. This suggests that the quantity of parasite DNA present in tissue may contribute to or correlate with the development and/or maintenance of chagasic pathology. We have shown that at least two T. cruzi DNA sequences can be frequently detected in heart lesions. Further approaches such as in situ PCR will be necessary to precisely determine the origin of the parasite’s DNA.

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