Absence of left ventricular dysfunction during acute chagasic myocarditis in the rhesus monkey

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ABSTRACT Recent studies suggest that intracellular Trypanosoma cruzi invasion with release of intracellular myocardial antigens during T. cruzi infection is crucial to the pathogenesis of chronic chagasic myocarditis. However, in areas endemic for Chagas’ disease, the incidence of clinical acute chagasic myocarditis has been reported to be low among infected individuals, while the incidence of chronic chagasic myocarditis is relatively high. Thus, either acute chagasic myocarditis rarely complicates T. cruzi infection and is not important to the pathogenesis of chronic chagasic myocarditis, or acute chagasic myocarditis rarely impairs left ventricular function and therefore causes no symptoms. To investigate this question we inoculated T. cruzi from a human patient with Chagas’ disease into the subconjunctivae of six rhesus monkeys (7.5 × 10^3 parasites each). Parasitemia was monitored and weekly two-dimensional echocardiograms (for determination of end-diastolic and fractional change in area, EDA and FCA) were obtained to quantify global left ventricular function for 10 weeks. Regional left ventricular function was assessed by visual analysis of two-dimensional echocardiograms. Extent of acute myocarditis was established at autopsy. All monkeys had the Romaña sign and detectable parasitemia in the second week. Parasitemia rose in all by the ninth week (mean = 1.8 × 10^4 parasites/ml); four monkeys lost weight (mean = −12%), three died, and three were killed. Two-dimensional echocardiographic EDA and FCA remained unchanged from control to the last study within 12 hr of death (EDA = 2.6 ± 0.9 to 2.7 ± 1.0 cm², FCA = 80 ± 6.8 to 74 ± 7.6%, NS). Furthermore, regional left ventricular function remained unchanged throughout the period of study. At autopsy two monkeys had severe acute myocarditis (one had been killed with no weight loss) with abundant intracellular T. cruzi (one nest of T. cruzi/cm² of myocardium sectioned). Two had moderate and two mild acute myocarditis. In conclusion, acute chagasic myocarditis may be severe after T. cruzi infection, yet cause no impairment in resting left ventricular function despite intense intracellular T. cruzi invasion.


CHAGAS’ DISEASE is caused by the parasite Trypanosoma cruzi. In endemic areas, individuals come in contact with and are infected by this organism, usually in childhood. In a small number of cases an acute illness ensues, characterized in some by an acute myocarditis. Although the vast majority of individuals remain asymptomatic for many years, some develop a chronic, frequently fatal, cardiomyopathy after a long latent period.

The pathophysiology of Chagas’ cardiomyopathy is for the most part unknown. Although the concept of a sequential pathogenetic link between acute and chronic Chagas’ disease is attractive, it remains to be proven. Against such a concept are observations including the one made first by Carlos Chagas himself, and later by other investigators, indicating that in areas endemic for Chagas’ disease the incidence of clinical acute form of the disease is low among infected individuals, while the incidence of chronic disease is high. Such observations suggest that either acute chagasic myocarditis is uncommon, rarely complicates T. cruzi infection, and is not important to the pathogenesis of chronic myocarditis, or acute chagasic myocarditis,
although common, rarely impairs left ventricular function and is therefore subclinical.

To aid in making this important distinction we studied resting left ventricular function by echocardiography in a nonhuman primate, the rhesus monkey, during acute myocarditis caused by T. cruzi.

Methods

Inoculation procedures. Six young female rhesus monkeys (Macaca mulatta, age 3.5 to 5 years, weight 11.1 ± 1.0 pounds) were inoculated into the subconjunctival sac with approximately 7500 metacyclic trypomastigotes. Parasites (Barbosa strain from Goiania, Brazil) were obtained from the cryopreserved feces of the insect Dipetalogaster maximus infected previously with blood from a human patient with acute Chagas’ disease. The insects’ feces were diluted in saline solution, and the size of the inoculum was calculated from microscopic counts of the solution samples. Subconjunctival inoculation was made under anesthesia (10 to 15 mg/kg intramuscular ketamine HCl). During the 4 weeks before and the 11 weeks after inoculation, the animals were kept in specially designed stainless steel, environment-isolated cages (Hazleton Systems, Inc., Aberdeen, MD, supplied by the Naval Medical Research Institute, Bethesda, MD). The monkeys remained in Constant environment-isolated cages (Hazleton Systems, Inc., Aberdeen, MD, supplied by the Naval Medical Research Institute, Bethesda, MD). The monkeys remained in the vivarium (at the Johns Hopkins Medical Institutions). Animal care and all research procedures were performed by properly dressed staff members of the vivarium and/or investigators. The dress code included head-to-foot plastic suits, face shields and masks, and two pairs of gloves. All staff members and investigators having any contact with the infected animals had serologic tests for Chagas’ disease performed before inoculation and after all animals had died or had been killed (sodium pentothal overdose). None of the tests were positive for T. cruzi infection at any time.

Experimental protocol. Before inoculation (control parameters), and every week for 11 weeks after inoculation, the following protocol was repeated: After intramuscular ketamine injection, the monkeys were weighed and their chests shaved. Blood was drawn for parasitemia assessment and a two-dimensional echocardiographic examination was made (see below).

Lastly, a 12-lead electrocardiogram was obtained with a standard Hewlett-Packard electrocardiograph. Occasionally, a second intramuscular ketamine injection was necessary to complete the protocol. Echocardiograms and electrocardiograms were also obtained from every animal within the 12 hr before death.

Parasitemia was measured by direct microscopic counts and biphasic blood agar culture.7 The animals were checked daily for changes in health and/or behavior.

Echocardiographic examination and data analysis. We used an ATL Model 300 IC two-dimensional echocardiograph that was entirely covered by transparent plastic except for a lateral hole through which the transducer cable passed. The transducer cable and the transducer (5 MHz) itself were enclosed in a plastic sheath containing conductive gel and the transducer was applied directly to the animal’s shaved chest. High-quality parasternal short-axis, long-axis, and apical four-chamber views were obtained for each monkey and images were recorded on ½ inch videotape (Sony Betamax).

The left ventricular cross-sectional cavity area at end-diastole (EDA) and the fractional change in cross-sectional cavity area (end-diastolic area minus end-systolic area divided by end-diastolic area, FCA) were used as indexes of global left ventricular size and function, respectively. All frames of selected beats were transferred to a contouring system (Microsonics EVII, In-

dianapolis), and the cavity area corresponding to the onset of the q wave of the electrocardiogram was taken as end-diastole. The end-systolic cavity area was chosen as the smallest area throughout the cardiac cycle. All echocardiograms were contourd by one of the investigators who was blinded to the echo sequence, clinical course, and autopsy findings. All values represent the average from three selected beats.

Regional left ventricular function was assessed by a semi-quantitative visual scoring system described previously. Briefly, the left and right ventricles were divided into segments that received numerical scores based on regional performance: 3 = normal segmental wall motion; 2 = regional hypokinesia; and 1 = akinesia or dyskinesia. Two-dimensional echocardiograms were analyzed visually by two independent observers and major discrepancies (observers differing by a numerical score greater than 1 were resolved by a “vote” of Minerva” from a third independent observer. Minor discrepancies (observers differing by a score of 1) were resolved by consensus. There were few major discrepancies between observers (less than 2% of all echocardiographic segments analyzed).

Autopsy and histologic examination. All autopsies were performed by the same pathologist (A. C.). After macroscopic examination, samples for histologic studies were taken from the heart and other viscera, including the central nervous system. Sections were stained with hematoxylin and eosin. Myocarditis was considered severe when it was diffuse and involved the entire thickness of the heart wall, with frequent intramyocardial nests of T. cruzi. Mild myocarditis was mainly focal, without intracellular T. cruzi, and moderate myocarditis represented a degree of intermediate involvement between mild and severe.

Statistical analysis. The significance of differences in echocardiographic EDA and FCA during the course of T. cruzi infection was calculated by repeated-measures analysis of variance (ANOVA). All data are presented as mean ± SD.

Results

All six monkeys developed the characteristic celluli-
tis of the eyelid (Romanya’s sign10) and had parasitemia by the second week after inoculation with T. cruzi. Peak parasitemia for the overall group occurred 6 weeks after inoculation (mean = 1.8 × 107 parasites/ml), declining thereafter (table 1). By the eighth week, four monkeys had lost weight (mean = −12% body weight) and had become apathetic. The remaining two monkeys neither lost weight nor exhibited changes in behavior (table 1). Between the ninth and tenth weeks, three of the four monkeys that had lost weight were found dead in their cages, and the remaining one developed nystagmus and drowsiness and was killed during the eleventh week after inoculation. The two animals suffering no weight loss were killed at the end of the eleventh week.

The size of the left ventricle, indexed by the EDA, did not change from control (2.6 ± 0.7 cm²) throughout the entire course of infection (2.6 ± 0.8 and 2.5 ± 0.3 cm² at the tenth and eleventh weeks, respectively; figure 1). Similarly, global left ventricular function, indexed by the FCA, remained unchanged from control (80.7 ± 5.3%) at all weekly testing times through-
TABLE 1
Clinical and pathologic findings

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Parasites/ml (6th week)</th>
<th>% weight loss, behavioral changes (8th week)</th>
<th>ECG changes (within 12 hr before death)</th>
<th>Mode of death (9-11th week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.9 x 10^3</td>
<td>11%, apathy</td>
<td>Right-axis shift with symmetrically inverted T waves in leads II, III, aVF, and V_1-6, none</td>
<td>Found dead</td>
</tr>
<tr>
<td>2</td>
<td>4.6</td>
<td>Neither</td>
<td>None</td>
<td>Killed</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>14%, apathy</td>
<td>PR prolongation left axis deviation, diffuse ST-T changes, and poor R-progression</td>
<td>Killed (found dead)</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>5%, apathy</td>
<td>None</td>
<td>Found dead</td>
</tr>
<tr>
<td>5</td>
<td>2.6</td>
<td>18%, apathy</td>
<td>None</td>
<td>Found dead</td>
</tr>
<tr>
<td>6</td>
<td>0.3</td>
<td>Neither</td>
<td>None</td>
<td>Killed</td>
</tr>
</tbody>
</table>

IC T. cruzi = intracellular nests of T. cruzi; ECG = electrocardiographic.

*Monkey 3 developed nystagmus and drowsiness at the eleventh week before death.

out the entire experiment (74.2 ± 3.3% and 72.7 ± 2.3% at the tenth and eleventh weeks, respectively; p = NS [ANOVA]; figure 2). Furthermore, no regional dysfunction was detectable in any segment at any time during the experiment by the use of the visual scoring system (see Methods) in any animal.

By contrast, autopsy of all six animals revealed varying degrees of inflammatory myocardial involvement (table 1). Two animals (one of which was killed with no weight loss) had severe pancarditis with intense interstitial lymphocytic infiltrate and no fibrosis. In these two, one nest of T. cruzi could be found per square centimeter of myocardium sectioned (figure 3). This picture is typical of acute chagasic myocarditis. In two of the other animals, myocarditis was moderate with easily detectable T. cruzi nests in one of them. The remaining two monkeys demonstrated mild myocarditis. Other autopsy findings included associated esophageal lymphocytic infiltrates in three monkeys and severe meningoencephalitis in the monkey (No. 3) that developed nystagmus and drowsiness during the 11th week after inoculation. In addition, nests of parasites could be found in the skeletal muscle and gut in two animals.

The last electrocardiogram, obtained within 12 hr of death, showed nonspecific changes in two monkeys (table 1). Monkey 1 (mild myocarditis) developed symmetrically inverted T waves in leads II, III, aVF, and V_1-6, with a slight rightward axial shift on the last electrocardiogram (figure 4). Monkey 3, with moderate myocarditis at autopsy, developed PR prolongation from 90 to 120 msec as the heart rate increased from 150 beats/min during control to 200 beats/min at the time of the final electrocardiogram (obtained a few minutes before the animal was killed). This monkey also developed left-axis deviation and deep S waves in leads I, aVL, and V_5-6, compatible with incomplete right bundle branch block with left anterior hemiblock. In addition, the ST segment was shifted upwards 1 mm in leads V_2-4. The electrocardiogram remained normal in the other four animals.

Discussion

In endemic areas, acute Chagas' disease is relatively rare when compared with chronic Chagas' disease. Most of the seropositive individuals living in such areas do not recall any prior episodes of acute illness resembling acute Chagas' disease. Moreover, asymptomatic infection by T. cruzi has been documented in humans. Among the few who do develop the acute disease — mostly children or young adults — overt clinical signs of impaired left ventricular function, e.g., congestive heart failure, are infrequent. When present, this presages an unfavorable outcome.
However, electrocardiogram alterations and/or cardiac enlargement on chest x-ray are more common than clinical signs.\textsuperscript{1,6} Autopsy in the approximately 10\% of patients who die during the acute stage of Chagas' disease\textsuperscript{1,6} frequently reveals myocarditis that in some cases is far more severe than previously suspected on the basis of clinical data, x-rays, and/or electrocardiograms.\textsuperscript{6} These discrepancies have been attributed to incomplete assessment of left ventricular function. Our data, however, support another explanation: Left
FIGURE 3. Left, Low-power histologic section of the left ventricle of monkey 2. This animal was killed at 11 weeks, having lost no weight. Pancarditis, a diffuse and intense interstitial inflammatory infiltrate of mononuclear cells involving the entire myocardium from endocardium to pericardium, is seen. Right, High-power view of another histologic section from the same heart, showing a nest of abundant intracellular T. cruzi surrounded by an intense mononuclear inflammatory infiltrate.

Ventricular dysfunction is not an early consequence of severe myocardial involvement by T. cruzi.

Monkeys have been used as models for Chagas' disease since its discovery. The acute disease has been studied extensively in the rhesus monkey and is very similar to the human disease. Electrocardiographic changes and overt heart failure have been reproduced in both primates and dogs, although in some studies heart failure followed infection with a much larger inoculum than would be expected to occur in nature. Rather than implying that myocarditis by T. cruzi cannot cause impairment of left ventricular function or heart failure, our data indicate that the absence of clinical or echocardiographic signs does not exclude the presence of severe myocardial involvement in this condition. These findings are in keeping with recent preliminary observations of other types of human myocarditides that suggest myocardial involvement out of proportion to clinical symptoms, signs, or noninvasive measures of left ventricular function.

The study of dynamic left ventricular function with two-dimensional echocardiography during acute Chagas' disease represents a more sensitive methodology in assessing cardiac performance than physical examination or chest x-rays, and has not been used previously in the functional assessment of the acute process. By this technique, Acquatella et al. have successfully detected regional myocardial damage due to chronic chagasic myocarditis in asymptomatic individuals with normal chest x-rays. Our study employs two-dimensional echocardiography to assess left ventricular function during acute chagasic myocarditis, which is pathologically different from the chronic disease process. Invasive techniques commonly used to study
FIGURE 4. For legend see opposite page.
regional left ventricular function in experimental animals could perhaps have elicited functional abnormalities that might have escaped detection by our methodology. Exercise studies of left ventricular function, whether by echocardiographic or radionuclide techniques, might likewise have elicited abnormalities not present at rest, but such studies would have been impractical in this experimental setting. Nevertheless, overall resting function was assessed quantitatively and regional abnormalities, such as wall thinning or aneurysms, would certainly have been detected echocardiographically.\(^6\)

We found electrocardiographic abnormalities in two animals on the last tracing obtained, just a few minutes before death in one, and 8 hr before the other was found dead in its cage. Such abnormalities have been reported during both experimental\(^{14, 16-19}\) and human\(^{1, 6}\) acute Chagas’ disease. The fact that the electrocardiogram was abnormal in these two animals does not mean that their left ventricular function was impaired, as evidenced by the normal echocardiographic findings. Nevertheless, these findings do suggest that at least in this animal preparation the electrocardiogram may be more sensitive than the two-dimensional echocardiogram in detecting myocardial involvement during acute chagasic myocarditis. This could be due to electrocardiographic detection of local changes in electrical activity that are secondary to inflammation and not severe enough to cause functional impairment. In this regard, the two monkeys that developed electrocardiographic abnormalities had only mild or moderate myocarditis at autopsy, while paradoxically, those with severe myocarditis at postmortem examination showed no electrocardiographic changes. The sensitivity of the electrocardiogram suggested by our data needs confirmation by further studies involving a larger population.

The pathogenesis of Chagas’ heart disease remains obscure. Although progression of disease in the absence of circulating \(T. \text{cruzi}\) has not been shown in humans, the original hypothesis of direct damage by the parasite\(^{24}\) does not explain the typical finding of severe myocardial destruction in the absence of tissue forms of \(T. \text{cruzi}\).\(^{23, 25, 26}\) Santos-Buch and Teixeira\(^{27, 28}\) postulate that cell mediated immune mechanisms are involved in the pathogenesis of Chagas’ heart disease is supported by several studies.\(^3, 4, 29, 30\) Circulating autoantibodies have also been demonstrated in Chagas’ disease.\(^{31, 32}\) However, the mode of induction of such autoimmune mechanisms remains unclear. Acosta and Santos-Buch\(^{33}\) have recently reported that reactions elicited by cross-reacting antigens of \(T. \text{cruzi}\) and striated muscle\(^{34, 35}\) can induce experimental myocarditis. On the other hand, Ribeiro dos Santos and Hudson\(^{3, 4}\) have documented the destruction of nonparasitized cells coated with adsorbed \(T. \text{cruzi}\) antigens that were released to the extracellular space after the disruption of parasitized cells. They suggested a link between autoantibody production and infection by \(T. \text{cruzi}\). In this regard, the reported differences in the incidence of clinically acute and chronic myocardial involvement in endemic areas have been held as evidence of the lack of a pathogenetic link between the two conditions. We hypothesize that the low incidence of acute clinical chagasic myocarditis in areas endemic for Chagas’ disease does not reflect the true incidence of cardiac involvement after \(T. \text{cruzi}\) infection.

In conclusion, acute chagasic myocarditis may be severe, with intense intracellular \(T. \text{cruzi}\) invasion and associated inflammatory infiltrate, yet cause no impairment of global or regional left ventricular function or increase in end-diastolic left ventricular dimensions evident on the resting two-dimensional echocardiogram. In our study, even those animals with the most intense myocarditis showed no deterioration with respect to these indexes. Thus, left ventricular dysfunction assessed by these methods cannot be used as an indicator of acute myocarditis.

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