Pathogenesis of Chronic Chagas Heart Disease

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Background—Chagas disease remains a significant public health issue and a major cause of morbidity and mortality in Latin America. Despite nearly 1 century of research, the pathogenesis of chronic Chagas cardiomyopathy is incompletely understood, the most intriguing challenge of which is the complex host-parasite interaction.

Methods and Results—A systematic review of the literature found in MEDLINE, EMBASE, BIREME, LILACS, and SCIELO was performed to search for relevant references on pathogenesis and pathophysiology of Chagas disease. Evidence from studies in animal models and in anima nobile points to 4 main pathogenetic mechanisms to explain the development of chronic Chagas heart disease: autonomic nervous system derangements, microvascular disturbances, parasite-dependent myocardial aggression, and immune-mediated myocardial injury. Despite its prominent peculiarities, the role of autonomic derangements and microcirculatory disturbances is probably ancillary among causes of chronic myocardial damage. The pathogenesis of chronic Chagas heart disease is dependent on a low-grade but incessant systemic infection with documented immune-adverse reaction. Parasite persistence and immunological mechanisms are inextricably related in the myocardial aggression in the chronic phase of Chagas heart disease.

Conclusions—Most clinical studies have been performed in very small number of patients. Future research should explore the clinical potential implications and therapeutic opportunities of these 2 fundamental underlying pathogenetic mechanisms. (Circulation. 2007;115:1109-1123.)

Key Words: autonomic nervous system ■ cardiomyopathy ■ Chagas disease ■ immune system ■ inflammation ■ microcirculation ■ Valsalva maneuver

A unique accomplishment in the history of medicine was the discovery by the Brazilian physician Carlos Chagas at the beginning of the 20th century of a new morbid entity—one that is by no means uncommon; rather it afflicts millions of patients.1 Besides describing most clinical features of the disease, Chagas also characterized its causal agent, the Trypanosoma cruzi, and the main mechanism of disease transmission, transcutaneous inoculation of the parasite by infected excreta of hematophagous insects.

Although chagasic megaesophagus and megacolon produce typical clinical conditions in 5% to 10% of patients, Chagas cardiomyopathy is by far the most serious form of the disease.2 Despite nearly a century of research, the most intriguing challenge to understanding the pathophysiology of Chagas heart disease still lies in the complex host-parasite interrelationship.3 This interaction, through mechanisms that are still not fully understood, determines that the myocardial aggression remains controlled at low levels in many patients. These patients remain throughout life with the so-called indeterminate form.4 Conversely, in 30% to 40% of patients the development of full-blown chronic Chagas cardiomyopathy is triggered, which leads to sudden death, complex arrhythmias, ventricular aneurysms (Figures 1 and 2), heart failure, and thromboembolism.5

From the evidence gathered through studies in animal models and in humans, 4 main pathogenetic mechanisms have been advocated to explain the development of Chagas heart disease: cardiac dysautonomia, microvascular disturbances, parasite-dependent myocardial damage, and immune-mediated myocardial injury.

Dysautonomia

Evidence of autonomic derangements in Chagas disease can be traced back to the primordial studies of Carlos Chagas and the cardiologist Eurico Vilella, when they reported in 1922 blunted chronotropic responses to atropine in chagasic patients.6 Over the following 2 decades, however, the disease fell into virtual neglect by the medical community. Only in the late 1940s, through improved specific serological tests to detect antibodies against T cruzi, the clinical syndromes of megaesophagus, megacolon, and heart failure, by then of unknown cause, were ascribed to Chagas disease, and the first report of cardiac neuronal damage appeared in the literature.7
Pathological Evidence of Cardiac Denervation

Possibly aware of Carlos Chagas’s early studies, some of which were published in German, Fritz Köberle, an Austrian pathologist who moved to Ribeirão Preto, Brazil, in the 1950s, conducted systematic studies of the intrinsic nervous system in organs that exhibited the pathological alterations caused by Chagas disease. He standardized the technique of cardiac intramural neuronal counting in microscopic sections obtained from the intercaval atrial strip and described intense neuronal depopulation in chagasic patients.8,9 The ganglionic damage and absolute reduction in subepicardial intramural neuronal countings (Figure 3) were confirmed by other independent investigators who used the Köberle method and other less systematic approaches in autopsied patients.10,11 These findings have been corroborated by studies in animals experimentally infected with the 

Subsequent studies showed that parasympathetic neuronal depopulation is not specific for Chagas disease. However, in all autopsy direct comparisons of subepicardial atrial denervation in inflammatory (rheumatic) and noninflammatory heart disease (endomyocardial fibrosis, dilated cardiomyopathy), the absolute reduction in the parasympathetic neuronal countings was more conspicuous in chagasic patients.14–16 The neuronal loss in Chagas heart disease has been postulated to occur predominantly during the acute phase of the infection, with participation of 3 mechanisms: direct parasitism of neurons, degeneration caused by periganglionic inflammation, and antineuronal autoimmune reaction.17,18

Functional Evidence of Cardiac Denervation

Consistent with anatomic parasympathetic denervation shown by morphological studies, abnormal cardiac autonomic regulation has been conclusively demonstrated in chagasic patients. Various pharmacological and physiological stimuli were used to demonstrate impaired parasympathetic heart rate regulation: metaraminol, phenylephrine and atropine intravenous injections, facial immersion, Valsalva maneuver, head-up and head-down tilt tests, respiratory sinus arrhythmia, hand grip, graded dynamic exercise, and spectral analysis of Holter recordings.19–27 Overall, these studies showed that chagasic patients are usually deprived of the tonic inhibitory action normally exerted by the parasympathetic system on the sinus node, and chagasic patients lack the vagally mediated mechanism to respond with rapid bradycardia or tachycardia to transient changes in blood pressure or venous return28,29 (Figure 4).

The autonomic dysfunction in chagasic patients can be detected before the development of ventricular dysfunction and in all phases of the disease, even the indeterminate and digestive forms.25,30,31 Thus, it is distinct from the nonspecific autonomic impairment that occurs in heart failure of whatever etiology, which is related to neurohumoral activation and postsynaptic desensitization of β-adrenergic receptor pathways32,33 and can be at least partially reversible with clinical control.34

Neurogenic Hypothesis of Chagas Cardiomyopathy

So striking was the intramural neuronal depopulation in some chagasic patients that Köberle postulated it should constitute the main mechanism of cardiomegaly and enteromegaly in Chagas disease. In fact, neuronal depopulation invariably occurs in patients with megasophagus and megalocol15,36 and the aperistalsis thus caused is generally considered the essential pathogenetic mechanism of chagasic digestive organic involvement.37 Because the intramural cardiac ganglia are mostly parasympathetic, the Köberle neurogenic theory of Chagas disease pathogenesis proposed that, similar to digestive organic involvement, Chagas cardiomyopathy was “parasympathicopriva”: in essence, it postulated that a long-lasting autonomic imbalance would eventually lead to a catecholamine-induced cardiomyopathy.8,38 However, pathogenesis of Chagas heart disease is definitely more complex, and the neurogenic theory for Chagas cardiomyopathy met several unsolvable conceptual obstacles, which are detailed in Table 1.

Detection of cardiac dysautonomia in all those studies was based solely on heart rate assessment and was caused by
damage in the neuronal supply to the sinoatrial structures. Hence, nerve damage that occurred at the ventricular myocardial level may have been overlooked. A more detailed study focused on iodine-123 metaiodobenzylguanidine (123I-MIBG) scintigraphy for evaluation of myocardial sympathetic nerve inervation; segmental sympathetic denervation was detected even in patients with the indeterminate form of Chagas heart disease before left ventricular (LV) wall motion abnormalities. An additional intriguing finding from that study was the increased 123I-MIBG washout rate observed in patients with normal ventricular function. This could be the result of increased cardiac sympathetic activity at the early stage of Chagas disease, which thus lends support to the neurogenic theory stated above. This abnormality could instead be caused by competition between the radiotracer and endogenous substances for the neurotransmitter receptors at the sympathetic nerve terminals, however. In fact, recent reports documented the existence of circulating antibodies that bind to myocardial adrenergic and cholinergic receptors in patients with Chagas disease. These antibodies have been shown to trigger physiological, morphological, enzymatic, and molecular alterations, and could potentially cause myocardial damage, desensitization, or downregulation of both muscarinic and adrenergic receptors.

Pathophysiological Impact of Sinoatrial Denervation

It is unlikely that autonomic dysfunction plays an essential pathogenic role for the development of chronic Chagas cardiomyopathy. Conversely, it is naive to conclude that such a striking disturbance in many chagasic patients would have no deleterious consequences. It is conceivable that neurogenic disturbances may be a contributory factor to the complications of the chronic phase of Chagas disease in at least 3 pathophysiological ways:

A. Early parasympathetic impairment could be a mechanism that triggers sudden death. This possibility is indirectly supported by autopsy reports of small, highly denervated hearts in patients who died suddenly; although the parasympathetically denervated chagasic heart could indeed be more vulnerable to malignant arrhythmia that is potentially able to trigger sudden death, this hypothesis has never been tested in appropriately designed clinical studies.
B. Dyssynergic areas develop in both ventricles at early stages of Chagas cardiomyopathy. Although the autonomic impairment is not causally responsible for the contraction abnormalities, it may aggravate these disturbances and lead to depression of global ventricular function in a subtle way: The parasympathetically denervated chagasic heart is deprived of the physiological mechanism of homeometric self-regulation warranted by fast heart-rate responses to a variety of physiological stimuli. Without the vagal mechanism to respond with rapid chronotropic changes, the chagasic heart depends more on heterometric adjustments that require more conspicuous variations in ventricular volume and shape during transient increases of venous return and ventricular afterload. In support of this hypothesis, it has been shown that chagasic patients respond to blood flow demand imposed by physical exercise with a greater increase in stroke volume\textsuperscript{23}; this compensates for deficient chronotropic changes during exercise, but clearly occurs at the expense of a reserve mechanism that requires ventricular dilatation, more forceful contraction, or both. Also in contrast to normal individuals, chagasic patients that show deficient chronotropic parasympathetic control have more marked elevation of peripheral resistance in response to the orthostatic stress\textsuperscript{28} and less increase in cardiac output during isometric exercise.\textsuperscript{59} These hemodynamic studies illustrate how denervated chagasic patients constantly face more stressful adaptation to intrinsically physiological conditions, which potentially lead to increased afterload and chamber dilatation.

C. Autonomic derangements may be responsible to trigger microcirculatory vasospasm, as discussed below.

It is noteworthy that circulatory dysautonomia in Chagas disease differs from other syndromes that affect the autonomic nervous system (eg, diabetes mellitus, amyloidosis), because no significant adrenergic vascular abnormality that leads to symptoms or objective evidence of postural hypotension exists. Moreover, there has been no demonstration that autonomic impairment per se is prognostically relevant, and no therapeutic implications are devised. Finally, in many studies the cardiac autonomic impairment was very subtle, and minuscule differences in comparison to normal controls may have been over interpreted.

**Microvascular Disturbances**

Various lines of evidence from pathological, experimental, and clinical grounds suggest that microvascular abnormalities that lead to myocardial ischemia may contribute to the pathogenesis of Chagas heart disease.

**Histopathology Studies in Humans**

The first reports on the pathology of the acute phase of Chagas heart disease implicated vascular disturbances caused by perivascular inflammation as mechanisms for myocardial degeneration.\textsuperscript{51} The concept was revived in necroscopic studies of chronic patients, which described diffuse collapse of intramyocardial arterioles, with lumen constriction attributed to intimal proliferation.\textsuperscript{52} These microvascular abnormalities were deemed responsible for the focal diffuse myocardial necrosis observed in these necroscopic studies and also in biopsy specimens that showed extensive capillary basement membrane thickening.\textsuperscript{53} Several subsequent investigations reinforced the notion of abnormal vasodilatation and vaso-constriction at the microcirculatory level that cause myocardial damage in patients with Chagas disease.\textsuperscript{5,9,54,55} Also, on the basis of the focal distribution of cell necrosis and subsequent reparative interstitial fibrosis found in chagasic hearts, which was similar to what is seen in experimental models of ischemia and reperfusion, transient microvascular ischemic disturbances of low intensity and short duration have been postulated to be causative mechanisms of Chagas cardiomyopathy.\textsuperscript{56,57} Among factors that lead to the development of aneurysms in chagasic patients, coalescent microinfarctions have been postulated to occur in watershed coronary areas because of unopposed sympathetic overstimulation.\textsuperscript{58} This last notion has been considered a pathophysiological link between the microcirculatory hypothesis and the neurogenic theory of Chagas heart disease.

**Experimental and In Vitro Studies**

In experimental murine models of Chagas disease, microcirculatory derangements include the occurrence of occlusive platelet thrombi in small epicardial and intramural coronary arteries, which lead to ischemia detected in vivo by histochimical techniques.\textsuperscript{59,60} Focal vascular constriction and microvascular proliferation were also prominent structural abnormalities in this experimental model.\textsuperscript{61} To elucidate the mechanisms responsible for the occlusive platelet thrombosis
and microcirculatory spasm, a direct participation of *Trypanosoma cruzi* infection that causes endothelial cell damage and endothelial damage by immune effector cells have been reported. Also, in vitro *Trypanosoma cruzi* infection of human umbilical vein demonstrated increased production of endothelin, which mediates arteriolar spasm and inhibits cAMP, with consequent stimulation of platelet adhesion to the vascular wall. Trypomastigote forms of *Trypanosoma cruzi* also produce a neuraminidase that removes the sialic acid from the surface of mammalian myocardial and endothelial cells. The loss of this protective component of the endothelial surface may cause platelet aggregation and microvascular thrombosis. These abnormal findings are corroborated by the demonstration of increased levels of thromboxane-A<sub>2</sub> and enhanced platelet adherence and aggregation in *Trypanosoma cruzi*-infected mice. Local production of cytokines by the inflammatory infiltrate cells may also contribute to microvascular abnormal reactivity in the chagasic heart. This has been shown to be partially reversible by long-term administration of verapamil, a calcium channel blocker with vasodilating and anti-platelet–aggregating effects that lead to attenuation of myocardial lesions and increased survival in a murine model of Chagas disease. This hypothesis was further supported by a study that used in vivo visualization of the murine cremaster vascular bed. In comparison with control animals, animals infected with *Trypanosoma cruzi* had segmental arteriolar spasm and flow velocity reduction; these abnormalities were also reversed by verapamil.

### TABLE 1. Pitfalls of the Neurogenic Theory From Experimental and Clinical Studies in Chagasic Patients

<table>
<thead>
<tr>
<th>Pitfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No demonstrable denervation sensitivity (Cannon law) of cardiac structures to cholinergic agents</td>
</tr>
<tr>
<td>No evidence of sympathetic overstimulation upon the heart ever shown</td>
</tr>
<tr>
<td>Sympathetic denervation, although less intense, also occurs:</td>
</tr>
<tr>
<td>Anatomic evidence of sympathetic neuronal depopulation and fiber degeneration</td>
</tr>
<tr>
<td>Blunted adrenergically mediated chronotropic responses to head-up tilt</td>
</tr>
<tr>
<td>Concomitant attenuation of both high-frequency (parasympathetic) and low-frequency (adrenergic) components of spectral chronotropic variability</td>
</tr>
<tr>
<td>No potentiation of myocardial lesion by adrenergic agents in <em>Trypanosoma cruzi</em> infected animals</td>
</tr>
<tr>
<td>Parasympathetic involvement is highly variable at the individual level:</td>
</tr>
<tr>
<td>Marked variability in individual neuronal counts and functional parasympathetic abnormalities among patients</td>
</tr>
<tr>
<td>Chagas cardiomyopathy can be caused by parasite strains apparently devoid of neurotropism; in such cases, nonspecific parasympathetic impairment only appears after the development of ventricular dysfunction (eg, in Venezuela)</td>
</tr>
<tr>
<td>No correlation between denervation and type of death or pathological features of the chagasic heart</td>
</tr>
<tr>
<td>No correlation between parasympathetic impairment and either initial myocardial depression or more advanced left ventricular contractile dysfunction</td>
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</tbody>
</table>

### Clinical Studies

Various clinical aspects of Chagas heart disease suggest the participation of myocardial ischemia in its pathophysiology. A substantial proportion of chagasic patients (20% to 30%) complain of chest pain that resembles angina in location and character, but this pain has no consistent relationship to effort and is not relieved by nitrates. Many patients have concomitant ST-T changes and abnormal Q waves compatible with electrically inactive ventricular areas. Furthermore, similar to coronary artery disease, segmental LV dysfunction is common in chagasic patients, even those with dilated hearts. Despite these findings that suggest the occurrence of myocardial ischemia, coronary angiography in Chagas cardiomyopathy invariably demonstrated the absence of obstructive disease at the epicardial level. Nevertheless, con-
sistent with the experimental studies, impairment of endothelium-dependent coronary vasodilatation in response to acetylcholine, with preserved response to adenosine, has been reported in patients with Chagas heart disease.\textsuperscript{75} Also, chagasic patients with atypical angina that was severe enough to warrant coronary angiography were shown to have blunted vasomotor epicardial responses to hyperventilation and dinitrate isosorbide.\textsuperscript{76} Taken together, these findings show abnormal coronary flow regulation related to endothelial and nonendothelium dysfunction in chagasic patients with chest pain and angiographically normal epicardial coronary arteries.

Several independent investigations have shown striking myocardial perfusion abnormalities in chagasic patients with angiographically normal coronary arteries, and these studies lend support to the concept of abnormal myocardial flow regulation at the microvascular level\textsuperscript{74,77,78} (Figure 5). In one of these investigations that used \textsuperscript{201}thallium stress-redistribution myocardial perfusion scintigraphy, every type of perfusion defect was seen in all 23 chagasic patients.
studied; although fixed defects that denoted myocardial fibrosis were found in myocardial segments that exhibited akinesis or dyskinesis, stress-induced reversible myocardial ischemia was detected in LV segments with less severe wall motion impairment in 8 patients (35%).

Another study that used 201thallium single photon emission computed tomography myocardial scintigraphy focused on the topographical correlation between segmental myocardial perfusion abnormalities and regional LV dysfunction in 37 patients with various stages of Chagas heart disease; again, some kind of perfusion defects (fixed, paradoxical, and reversible) were seen in 78% of patients. Significant topographical correlation occurred between perfusion changes and wall motion abnormalities that predominated in the apical and inferior-posterior LV segments. Reversible stress-induced myocardial perfusion defects were detected in segments that exhibited normal wall motion in 5 of 12 chagasic patients with no other evidence of myocardial disease. Of note, these reversible ischemic defects were seen in the apical and inferior-posterior LV segments (ie, the same regions where regional contractile dysfunction prevails in later stages of Chagas cardiomyopathy).

The tracer used in the studies quoted above was 201thallium, whose accumulation and retention could be influenced by cardiomyocyte metabolic derangements related to the underlying inflammatory process. Hence, some of the scintigraphic defects described in the previous studies could be independent of real perfusion abnormalities. This alternative possibility is reinforced by the frequent occurrence in those studies of reverse redistribution defects that predominate in LV regions with normal or mildly impaired wall motion. However, myocardial perfusion was also evaluated in 18 chronic Chagas heart disease patients with recurrent chest pain severe enough to warrant coronary angiography with an exclusive perfusion tracer, 99mtechnetium-labeled microspheres injected in the LV cavity. None of the patients had angiographic evidence of apical coronary artery disease. Ten of the 18 patients exhibited extensive perfusion defects in 49 of 126 (39%) LV segments. Nineteen of these segments (40%) with perfusion defects had severely impaired wall motion, which probably corresponded to extensive fibrosis. The remaining 60% of the segments with perfusion defects had normal or mildly reduced wall motion. Thus, this investigation clearly showed marked resting myocardial perfusion abnormalities before the appearance of wall motion impairment.

Pathophysiological Consequences of Microvascular Derangements

On the basis of these clinical investigations and the background information provided by the experimental studies discussed above, it is a reasonable hypothesis that chronic myocardial hypoperfusion would contribute as a pathogenetic mechanism to the development of the characteristic regional LV dysfunction seen in Chagas heart disease. This would be similar to hibernating myocardium seen in chronic coronary artery disease, and this might have relevant clinical implications for the management of chagasic patients. This hypothesis is compatible with the results of studies that show improvement of LV function in Chagas heart disease patients who received short-term or long-term administration of dipyridamole and isosorbide dinitrate. In these studies, however, no clear relationship between the LV function improvement and the relief of myocardial ischemia was demonstrated. Moreover, changes in ventricular loading conditions associated with these modalities of treatment could also have contributed to the improvement in the ventricular performance. Thus, although attractive, the hypothesis of chronic myocardial ischemia in Chagas cardiomyopathy still awaits the conclusive support that should be derived from a prospective cohort study that shows a beneficial effect of long-term vasodilator or antiplatelet therapy on the clinical course of chagasic patients with angina-like symptoms.

It should be emphasized that the triad of chest pain, myocardial perfusion disturbances, and normal coronary arteries has also been described in patients with ventricular dysfunction caused by dilated cardiomyopathy of other causes. Therefore, this is not a specific feature of Chagas cardiomyopathy. Moreover, no prospective cohort studies have been conducted that correlate the presence and extent of myocardial perfusion disturbances with the temporal development of segmental LV wall motion abnormalities in the same chagasic patients.

In summary, despite these limitations, experimental and clinical studies strongly support the notion that functional and structural microvascular abnormalities occur in Chagas cardiomyopathy, possibly as a consequence of the underlying inflammatory process. Thus, it is possible that microvascular ischemia, even if it is not an independent and self-sustained pathogenetic mechanism, could at least constitute an ancillary factor to potentiate and amplify the chronic inflammatory aggression to myocardial tissue.

Myocardial Damage Directly Related to Parasite Persistence

Chagas cardiomyopathy is essentially a myocarditis, and the inflammatory process, although more conspicuous in the acute phase, is clinically silent but incessant in patients with the indeterminate and chronic phases of the disease, as shown by studies in experimental models and in necropsy (chagasic patients dead from other causes) and biopsy specimens. The prevalence of myocarditis correlates with the severity of clinical heart failure and a significant correlation between ventricular dilation, inflammatory changes, and fibrosis was found in a Syrian hamster model of Chagas cardiomyopathy. Moreover, clinical progression and survival were significantly worse in patients with Chagas heart disease as compared with patients with noninflammatory forms of dilated cardiomyopathy. In addition, although the molecular mechanisms that determine how the host–parasite interaction is regulated in regard to invasion location and parasite tissue retention, it has been suggested that variations in tissue tropism, dependent on genetic properties of parasite and host, could explain the different clinical forms of Chagas disease.

Since the initial pathological studies, the tissue damage in the heart and gastroenteric tract, which occurred in the acute phase, has been clearly related to the intense parasitemia and parasitism of target organs. Histopathological studies in the
chronic phase of the disease, however, showed very low-grade myocardial fiber parasitism and an intriguing lack of topographical correlation between inflammatory foci and amastigote T. cruzi nests.9,50 On the basis of this evidence, the role of the parasite in the genesis of cardiac damage during the chronic phase of Chagas disease has been questioned by proponents of alternative pathogenetic hypotheses.9,91 However, the evidence of typical chronic focal diffuse lymphocytic myocarditis, with myocytolytic necrosis that occur in areas where no parasite elements could be seen, was largely based on studies that used traditional histological techniques now considered highly insensitive to identify parasites in the infected host.92 Thus, first in animal models of chagasic cardiomyopathy,93,94 and later in humans,95–98 several independent investigators employed immunohistochemistry, polymerase chain reaction, and in situ hybridization methods to identify tissue T. cruzi antigens or its genomic material in the inflammatory foci. These studies have completely reversed that concept and renewed the focus on a pathogenetic role for myocardial aggression directly dependent on parasite persistence in host tissues (Figure 6). Although a clear-cut correlation between the tissue parasite burden and the intensity of the inflammatory process has been described in experimental animals,99 these findings were not replicated in heart samples from patients.100,101 It is relevant that infected mice, in spite of the development of chronic myocarditis, do not evolve to heart failure and death as occurs in human Chagas disease.

That the persistence of T. cruzi is directly implicated in the pathology of the chronic phase is also suggested by experimental models of Chagas disease that show parasite load reduction by trypanocidal treatment and concomitant attenuation of cardiomyopathy.102,103 Conversely, in these models, enhancement of parasite burden results in exacerbation of the cardiomyopathy course.104–106 Moreover, during the chronic phase the intact amastigote parasites may coexist within the host tissue,98 even after trypanocidal treatment,107,108 and they may undergo active multiplication during favorable conditions (see below).

Also supportive of the concept of tissue parasitism in the pathogenesis of chronic Chagas disease is the persuasive evidence of organ specificity of the parasite infection that translates into clinical expression of disease. Thus, T. cruzi genetic material was not detectable in the heart from seropositive autopsied patients who had died without signs of cardiac involvement, but this material is consistently found in heart specimens from patients with chronic Chagas cardiomyopathy.100 Subsequent studies showed the same proportion of T. cruzi DNA among patients with Chagas cardiomyopathy and the indeterminate form of disease.99,100 In addition, T. cruzi DNA was detected in esophageal tissue from patients with clinical involvement of this organ, but not from patients dying of isolated cardiac disease.109 Finally, there is evidence that T. cruzi strain, its load during the acute phase of infection, parasitemia levels assessed by polymerase chain reaction, and reinfection could influence the chronic phase course.110–114

The exact mechanism whereby parasitism causes tissue damage in the chronic phase is unclear. Although direct myocyte aggression by the parasite is as yet an unproven possibility, and apoptosis may be seen in advanced stages of heart failure,115 the stimulation of immune responses that are targeted at the parasite and self tissues is the most likely cause of inflammation and myocytolysis.

**Immunologic Mechanisms**

Several independent investigations have shown that diffuse myocarditis with myocytolysis and reparative fibrosis, the hallmark of chronic Chagas heart disease, has the characteristics of a delayed hypersensitivity reaction, with the inflammatory infiltrates composed mainly of mononuclear cells.52,116–119 Together with the demonstration of immunoglobulin and complement deposition in myocardial tissue, these findings constitute first-line evidence for the involvement of immunologic factors in the pathogenesis of Chagas cardiomyopathy.120

**Parasite Antigen-Driven Immunopathology and Autoimmunity in Cardiac Inflammatory Lesions**

Despite clear evidence for the participation of immune responses in myocardial damage, the nature of the essential antigen or antigens that elicit destructive immune responses remains elusive. As discussed above, T. cruzi antigen and DNA have been identified in cardiac tissue from chronic Chagas disease patients, and T. cruzi–specific CD8+ T cells were isolated in endomyocardial biopsy material from patients with Chagas heart disease.121 This provides evidence for the recruitment and expansion of T. cruzi–specific T cells to the myocardium, possibly related to parasite persistence in the chronic phase of Chagas heart disease. The T. cruzi materials found in the myocardium of chronic chagasic patients are definitely scarce, however, in comparison with
TABLE 2. Autoreactivity After *T cruzi* Infection

<table>
<thead>
<tr>
<th>Host Component</th>
<th>Host</th>
<th>Molecular Definition</th>
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<tbody>
<tr>
<td>Neurons</td>
<td>H</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>Sciatic nerve homogenate</td>
<td>H</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>Heart homogenate</td>
<td>H</td>
<td>T cells</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>H, Rb</td>
<td>T cells</td>
</tr>
<tr>
<td>Heart homogenate</td>
<td>M</td>
<td>T cells</td>
</tr>
<tr>
<td>Cardiac myosin</td>
<td>M</td>
<td>CD4+ T cells, serum IgG</td>
</tr>
<tr>
<td>43-kDa Muscle glycoprotein</td>
<td>M</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>Nervous tissue, heart and skeletal muscle</td>
<td>M</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>Second extracellular loop, M2 cholinergic receptor</td>
<td>H,M</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>Second extracellular loop, β1 adrenergic receptor</td>
<td>M</td>
<td>Serum IgG</td>
</tr>
<tr>
<td>Small nuclear ribonucleoprotein</td>
<td>H</td>
<td>Serum IgG</td>
</tr>
</tbody>
</table>

M indicates mouse; H, human; Rb, rabbit; and IgG, immunoglobulin G.

Adapted from Cunha-Neto E et al,121 with permission from Elsevier. Copyright 2004.

...the intensity of the myocarditis. Moreover, the parasite that infests the myocardium in the acute phase of disease does not immediately lead to the lesions that later are typically found in the chronic phase. This constitutes the prominent time-scale dissociation between the acute infection and the characteristic morbidity that occurs in the late stage. Furthermore, *T cruzi* DNA—but not intact *T cruzi*—has been detected in cardiac tissue from both the indeterminate and overt cardiomyopathic forms.120 Finally, sometimes the foci of amastigote-parasitized myocardial cells remain free of inflammation despite intense parasitism of myocardial tissue. Together, these findings indicate that the topographical and temporal relation between the parasite presence and the development of myocardial pathology is far from straightforward. Similarly, the intensity of parasitism is not directly related to the clinical evolution of patients in the chronic phase of Chagas disease. Thus, some other factor must operate along with parasite persistence to lead a subgroup of *T cruzi*-infected individuals to progressive and incessant myocardial damage. Early investigators reported that inoculation of *T cruzi* homogenates induced inflammatory lesions in the myocardium of rhesus monkeys, which established the autoimmune/antigenic mimicry hypothesis of pathogenesis.116 It was then hypothesized that the breakdown of immunologic tolerance and consequent cardiac damage could be secondary to an originally protective response directed to a given *T cruzi* antigen that bore structural similarities to specific myocardial components. Several mechanisms have been postulated to trigger autoimmunity after *T cruzi* infection:20 antigen exposure secondary to tissue damage, followed by sensitization in the inflammatory environment (ie, bystander activation); molecular mimicry, whereby T and B cells recognize parasite antigens that share structurally similar epitopes with host antigens, which generates cross-reactive autoimmune responses (Table 2); and polyclonal activation that leads to autoantibody production. Autoimmune and *T cruzi*-specific responses secondary to parasite persistence are not incompatible or mutually exclusive in Chagas disease, and a combination of these types of immune responses may be involved in the establishment of myocardial damage.

Humoral Immune Phenomena in Acute and Chronic Human Chagas Disease

Shortly after the acute infection starts, the high-grade tissue parasitism elicits a strong cellular and humoral immune response against *T cruzi*, which leads to biological control but not elimination of the parasite. Macrophages and dendritic cells promote endocytosis of the parasites, and with the ensuing expression of interleukin (IL)-12 and costimulatory molecules they prime interferon γ (IFN-γ)—producing *T cruzi*-specific T cells that migrate in response to chemokines that are locally released and participate in the immune response against the parasite.122

In chronic human *T cruzi* infection, the cytokine profile remains shifted toward Th1 cytokines such as IFN-γ with suppression of Th2 cytokines such as IL-4123,124 and elevated plasma levels of tumor necrosis factor-α,125. In addition, CD4+ peripheral blood mononuclear cells from chronic Chagas cardiomyopathy patients produce more IFN-γ and less IL-10 than chagasic patients in the asymptomatic/indeterminate phase,124,126 which thus supports the notion that patients who evolve to chronic Chagas cardiomyopathy develop an exacerbated Th1 immune response. Plasma tumor necrosis factor-α levels are further increased in patients with more severe cardiac involvement.120,125 Moreover, chagasic patients with more severe cardiomyopathy who carry tumor necrosis factor-α alleles associated with high cytokine production have significantly shorter survival as compared with carriers of other alleles.127 Polymorphisms in several inflammatory genes (lymphotixin-α, BAT1, membrane cofactor protein-1) have also been implicated in the development of Chagas cardiomyopathy.128,129 In addition, immunogenetic differences probably determine differential susceptibility to factors that lead to the development of Chagas heart disease by modulation of the intensity of both protective and tissue-damaging immune responses.

The inflammatory infiltrate in Chagas heart disease includes macrophages, CD8+ T cells that express granzyme, and CD4+ T cells.120 There is also evidence for augmented local expression of adhesion molecules; human leukocyte antigen class I and II molecules; the chemokines membrane cofactor protein-1, IL-10, and measles immunoglobulin; the CCR2 and CXCR3 chemokine receptors; and the cytokines IFN-γ, tumor necrosis factor-α, IL-6, and IL-4130. Significant signaling by IFN-γ has also been observed in the myocardium of patients with Chagas cardiomyopathy, and IFN-γ and membrane cofactor protein-1 increased expression of atrial natriuretic factor, which is a marker of cardiomyocyte hypertrophy and heart failure in neonatal murine cardiomyocytes.130 Taken together, these observations suggest that IFN-γ-mediated chronic myocardial inflammation could contribute to the pathogenesis of chronic Chagas cardiomyopathy.
Autoimmunity in Experimental Chagas Disease

Experimentally, *T. cruzi*-infected mice display autoantibodies specific for various autoantigens contained in cardiac, nervous, and other tissues (Table 3); structural proteins such as cardiac myosin, desmin, and actin are common targets, and infected mice also develop autoantibodies against the β1-adrenergic and M2-muscarinic cholinergic receptors. Such mice seldom develop end-stage heart failure, however.

Cellular autoimmunity directed against heart autoantigens has also been observed in experimental Chagas disease. Passive transfer of CD4+ T cells or T cell lines from chronically *T. cruzi*-infected mice to noninfected syngeneic mice cause myocardial tissue inflammation. Also, CD4+ T cells from *T. cruzi*-infected mice recognize cardiac myosin but not actin. Tolerance induction with a myosin-enriched cardiac homogenate resulted in less myocarditis and fibrosis. Given the fact that cardiac myosin is still the single heart-specific–defined T cell autoantigen, these findings support the concept that cardiac myosin is a major autoantigen in chronic murine *T. cruzi* infection. Importantly, immunization with cardiac myosin induces severe T cell–dependent myocarditis in genetically susceptible mice. Moreover, *T. cruzi* cross-reactive antigens with both cardiac and noncardiac specificity have been identified, some of them with functional activity (Table 3). Immunization of mice with *T. cruzi* protein cruzipain induced autoantibodies to skeletal and cardiac myosin and against the cardiac muscarinic cholinergic receptor, which led to muscle damage and heart conduction abnormalities. Another notable finding was the development of autoreactive anti-heart antibodies and heart functional alterations after the immunization of BALB/c mice with *T. cruzi* ribosomal P1 and P2 protein synthetic peptide.

**Autoimmunity in Human Chagas Cardiomyopathy**

Sera from Chagas disease patients contain autoantibodies specific for various autoantigens expressed in cardiac, nervous, and other tissues (Table 3). Sera from >80% of patients contained anti-neuron autoantibodies, and functional antibodies against adrenergic and muscarinic receptors found in sera from arrhythmic chagasic patients induced arrhythmia in explanted rabbit hearts (Tables 2 and 3). Such functionally active antibodies have also been implicated in the production of autonomic impairment.

Sera from chronic chagasic patients also contain cross-reactive antibodies between human and *T. cruzi* proteins, many of which are evolutionarily conserved (Table 3). Thus, cross-reactive antibodies between *T. cruzi* and human ribosomal P proteins or the β1-adrenergic receptor have been detected in chagasic patients. Moreover, antibodies cross-reactive between cardiac myosin heavy chain and the *T. cruzi* protein B13 are more frequently found in sera from patients with definite Chagas heart disease than in patients with the indeterminate form of the disease. Also, CD4+ T cell clones derived from an endomyocardial biopsy sample from a patient with Chagas cardiomyopathy cross-reactively recognized cardiac myosin and *T. cruzi* protein.

### Autoimmunity in Human Chagas Cardiomyopathy

**TABLE 3. Molecular Mimicry After *T. cruzi* Infection**

<table>
<thead>
<tr>
<th>Host Component</th>
<th><em>T. cruzi</em> Antigen</th>
<th>Host</th>
<th>Molecular Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons, liver, kidney, testis</td>
<td>unknown</td>
<td>M, R</td>
<td>Mab</td>
</tr>
<tr>
<td>Neurons</td>
<td>sulphated glycolipids</td>
<td>H, R</td>
<td>Mab, sera</td>
</tr>
<tr>
<td>47-kDa Neuron protein</td>
<td>FL-160</td>
<td>H</td>
<td>rDNA, AS</td>
</tr>
<tr>
<td>Heart and skeletal muscle</td>
<td>microsomal fraction</td>
<td>H, M</td>
<td>Mab, serum IgG</td>
</tr>
<tr>
<td>Smooth and striated muscle</td>
<td>150-kDa protein</td>
<td>H, M</td>
<td>serum IgG</td>
</tr>
<tr>
<td>Human cardiac myosin heavy chain</td>
<td>B13 protein</td>
<td>H</td>
<td>rDNA, Ab</td>
</tr>
<tr>
<td>Human cardiac myosin heavy chain</td>
<td>cruzipain</td>
<td>M</td>
<td>Ab</td>
</tr>
<tr>
<td>95-kDa Myosin tail</td>
<td><em>T. cruzi</em> cytoskeleton</td>
<td>M</td>
<td>Mab</td>
</tr>
<tr>
<td>Skeletal muscle Ca++ dependent SRA</td>
<td>SRA</td>
<td>H, Rb</td>
<td>AS, serum IgG</td>
</tr>
<tr>
<td>Glycosphingolipids</td>
<td>glycosphingolipids</td>
<td>H, M</td>
<td>serum IgG</td>
</tr>
<tr>
<td>MAP (brain)</td>
<td>MAP</td>
<td>H, M</td>
<td>rDNA, AS</td>
</tr>
<tr>
<td>Myelin basic protein</td>
<td><em>T. cruzi</em> soluble extract</td>
<td>M</td>
<td>serum IgG, T cells</td>
</tr>
<tr>
<td>28-kDa Lymphocyte membrane protein</td>
<td>55-kDa membrane protein</td>
<td>H, M</td>
<td>Mab</td>
</tr>
<tr>
<td>23-kDa Ribosomal protein</td>
<td>23-kDa ribosomal protein</td>
<td>H</td>
<td>Ab</td>
</tr>
<tr>
<td>Ribosomal P protein</td>
<td>ribosomal P protein</td>
<td>H</td>
<td>rDNA, Ab, SP</td>
</tr>
<tr>
<td>38-kDa heart antigen</td>
<td>R13 peptide from ribosomal protein P1, P2</td>
<td>M</td>
<td>IgG1, IgG2</td>
</tr>
<tr>
<td>β1 Adrenoreceptor M2 muscarinic receptor</td>
<td>ribosomal P0 and P2β proteins</td>
<td>H</td>
<td>rDNA, Ab, SP</td>
</tr>
<tr>
<td>β1 Adrenoreceptor M2 cholinergic receptor</td>
<td>150-kDa protein</td>
<td>H, M</td>
<td>Mab</td>
</tr>
<tr>
<td>Cardiac muscarinic acetylcholine receptors (mACHR)</td>
<td>unknown</td>
<td>H</td>
<td>Ab</td>
</tr>
<tr>
<td>Cardiac muscarinic acetylcholine receptors (mACHR)</td>
<td>cruzipain</td>
<td>M</td>
<td>immunization with cruzipain</td>
</tr>
<tr>
<td>Cha antigen</td>
<td>SAPA, 36kDa TENU2845</td>
<td>M</td>
<td>Ab, T cells</td>
</tr>
</tbody>
</table>

R indicates rat; AS, antisera; Ab, patient antibody; Mab, monoclonal antibody; rDNA, recombinant DNA; IgG, immunoglobulin G; and SP, synthetic peptides.

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Determinination of whether autoimmunity and molecular mimicry, rather than parasite-driven immunopathology, are causes or consequences of cardiac tissue damage in human and experimental models of Chagas heart disease is key to understanding the essential nature of Chagas cardiomyopathy pathogenesis. Although the recent identification of persistent virus infection in multiple sclerosis and insulin-dependent diabetes mellitus may indicate that infection is a common theme in diseases classically dependent on immunologic mechanism, experimental and clinical data in Chagas heart disease fulfill several of the criteria required for an autoimmune pathogenesis. Thus, the identification of heart–*T cruzi* cell cross-reactive T-cell antigens (Table 3) with reproduction of pathobiological changes by passive transfer of immune cells in murine models, the amelioration of inflammation as a consequence of tolerance induction to myocardial antigens, and the induction of autoimmune disease after immunization with cardiac myosin have all been demonstrated. In addition, the isolation of cardiac myosin-autoreactive T cells in molecular mimicry with *T cruzi* B13 protein from affected tissue can be considered important indirect evidence. Together with the demonstration that in vitro immunization with B13 protein elicits T cell clones cross-reactive with cardiac myosin, these results suggest a role for autoimmunity targeted to heart autoantigens in the pathogenesis of Chagas heart disease. The finding that immunization with *T cruzi* ribosomal antigens induced cross-reactive antibodies and heart conduction abnormalities, and that similar cross-reactive autoantibodies present in Chagas heart disease sera induce arrhythmia in explanted hearts, suggest that these disturbances play a pathogenic role in some clinical manifestations of Chagas cardiomyopathy. The transfer of tissue lesions by T cell clones cross-reactive to known host and parasite epitopes, or a successful trial of tolerance to relevant heart-specific antigens among patients, would definitively establish how relevant autoimmunity is as a pathogenic mechanism in Chagas heart disease. A direct feasible test of the relevance of molecular mimicry between a *T cruzi* antigen and intrinsic heart proteins might be achieved through experimental infection with parasites devoid of the pertinent heart cross-reactive antigen (through genetic manipulation). The failure of this parasite line to induce myosin autoimmunity and/or myocarditis would directly imply molecular mimicry in myosin autoimmunity and Chagas disease pathogenesis. Finally, immunologic intervention (eg, pharmacological cytokine blockade) could be immediately available to millions of patients with Chagas disease in the Americas.

Removal of parasite stimulus has been attempted in experimental models of chronic myocarditis by treatment with trypanocidal drugs after acute infection. However, because the effectiveness of trypanocidal treatment is only partial and the currently available assays for parasitological cure are less than ideal, it has been impossible to prove with 100% certainty that the parasite has indeed been removed after treatment. At any rate, the dichotomy between tissue-damaging autoimmunity and systemic parasite persistence may indeed be false in Chagas disease. Even if heart autoantigens were the primary targets for immune responses that produce tissue damage in Chagas heart disease, persistent

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**Figure 7.** Schematic view of main pathogenetic mechanisms in chronic Chagas cardiomyopathy.

B13. In vitro sensitization of human peripheral lymphocytes with B13 protein peptides elicited cardiac myosin–cross-reactive T cell clones. The finding of molecular mimicry between cardiac myosin and *T cruzi* protein B13 indicates that it is antigen receptor crossreactivity, rather than simple antigen exposure bystander activation, that causes antimyosin autoimmunity in human Chagas heart disease.

**Overview of the Pathogenesis of Chagas Heart Disease**

Although autonomic derangements and microcirculatory disturbances constitute prominent peculiarities in Chagas cardiomyopathy, their role in the pathogenesis of chronic myocardial lesions is probably ancillary rather than fundamental in the mechanism of disease. Also, similar to what happens in every disease that affects the cardiac shape and dimension, neurohormonal activation per se can lead to a vicious cycle of progressive remodeling in chronic Chagas heart disease. Nevertheless, the pathogenesis of chronic Chagas heart disease is inexorably dependent on a low-grade but incessant systemic infection with documented autoimmune reaction. In fact, parasite persistence and immunologic mechanism seem inextricably related in myocardial aggression in the chronic phase of Chagas heart disease (Figure 7). Thus, future research should appropriately focus on the exploration of the logical consequences of both underlying pathogenetic mechanisms and their clinical potential implications and therapeutic opportunities.
T cruzi infection supplies a continuous source of cross-reactive antigen presentation and innate immune response stimuli, which triggers and maintains a powerful Th1 cytokine response that may activate and exacerbate the pathogenic potential of T cruzi–specific heart–cross-reactive T and B cell clones.

The notion that the immunologic system acts to control parasitism at low levels is supported by the evidence that reactivation of infection consistently occurs under immunosuppression treatment to prevent transplant rejection or in patients with virally acquired immunodeficiency syndromes. Recent cross-sectional studies in chronic chagasic patients that correlate severity of heart disease to the maturation and migratory properties of the memory CD8+ T cell compartment are consistent with the hypothesis of a gradual clonal exhaustion in the CD8+ T cell population, most likely as a result of continuous antigenic stimulation by persistent parasites. There is also emerging experimental evidence that genetic immunization (ie, injection of DNA that encodes T cruzi antigen genes that can be expressed in the host) can elicit antigen-specific protective immune responses, which decreases disease severity in the murine model of Chagas disease.

These findings also suggest that effective vaccine-mediated control of T cruzi infection may prevent the clinical development of Chagas disease. Another clinical implication is that effective measures to control the parasite burden should be welcome and probably would reduce tissue damage. In fact, trypanocidal therapy has been shown to improve parasite–host related outcomes (eg, benzimidazole significantly reduced the proportion of positive xenodiagnosis in both children and adults and increased the rate of negative seroconversion in asymptomatic chagasic patients). Systematic reviews of published controlled trials on both asymptomatic individuals and patients with overt Chagas cardiomyopathy, however, showed that there are insufficient data to provide evidence that currently available trypanocidal therapy can alter clinical outcomes in chronic Chagas disease. The BENEFIT Trial (Benznidazole Evaluation for Interrupting Trypanosomiasis): Evaluation of the Use of an Antiparasital Drug (Benznidazole) in the Treatment of Chronic Chagas Heart Disease, a large, randomized, double-blind, controlled study of benzimidazole versus placebo in patients with chronic Chagas heart disease, is currently being performed to overcome this therapeutic dilemma.

Disclosures

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Pathogenesis of Chagas Heart Disease


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