Achievements and challenges in controlling Chagas disease
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ABSTRACT

American trypanosomiasis or Chagas disease continues to endanger the lives of many million people in Latin America, and through travel and population migration there is a risk of congenital cases in nonendemic settings. Substantial improvements in the transmission of the disease have been achieved through vector control and blood-bank screening. However, vector-borne transmission remains the main mode of acquisition of infection in many settings coupled with congenital transmission and food-borne and accidental exposure through transplantation or laboratory exposure. The main sites of affection include the heart and gastrointestinal tract. Antiparasitic treatment of indeterminate forms is successful in many cases by delaying the risk of progression of cardiomyopathy, but treatment of chronic chagasic cardiomyopathy remains mainly supportive. The BENEFIT trial that will be completed by late 2011 or early 2012 will provide evidence for or against treating chronic symptomatic forms. Control or eliminating Chagas disease transmission coupled with decreasing the associated burden of disease in Latin America will promote better health and social and economic development among the most impoverished populations in the region.

Key words: Chagas disease, Trypanosoma cruzi, cardiomyopathy, Latin America

INTRODUCTION

Despite major successes in controlling Chagas disease transmission during the last few decades, this disease continues to endanger a significant number of people in the Americas, and its chronic manifestations are a very real concern to the lives of millions of people. Chagas disease, also known as American trypanosomiasis, is a zoonotic tropical disease caused by the parasite Trypanosoma cruzi and mainly transmitted by the Triatominae insect vector. The T. cruzi protozoan is a member of the order Kineto-plastida within the same genus as parasites causing other major tropical diseases such as African trypanosomiasis and leishmaniasis. Multiple strains of T. cruzi have been identified, which display strain-specific variation in clinical parameters.1 Two principal groups of strains have been categorized as T. cruzi I and T. cruzi II. T. cruzi II is mainly associated with the domestic environment and is the most common group found in human infections.2,3 Further research on the parasite, including the Trypanosoma cruzi Genome Initiative and proteome analyses, continue to add to the current knowledge of the biological characteristics and metabolism of the parasite and suggest targets for drug therapies and vaccine development.4,5

Transmission of the parasite is by insects of the Reduviidae family and subfamily Triatominae (“kissing bugs”) to humans or other mammals within either a domestic, sylvatic, or peridomestic cycle. Although a large number of species exist, the most important vectors to humans are Triatoma infestans and Rhodnius prolixus as well as Triatoma dimidiata, Panstrongylus megistus, and Triatoma brasiliensis.1 Most Triatominae live in the tropics between 45°S and 40°N with climatic variations. The main chagasic vectors tend to inhabit distinct areas: those in the Southern Cone countries are domiciliated, whereas those in Central America, Mexico, Andean countries, and the Amazon basin infest both human homes and uninhabited areas.6

The life cycle of T. cruzi involves different parasitic stages and both invertebrate and vertebrate hosts.5 Within the insect gut, the parasite replicates in epimastigote form and then develops into a metacyclic trypomastigote. This
infective stage is transmitted to humans when the bug ingests a blood-meal, simultaneously excreting the parasite with its feces and inoculating the human via breaks in the skin or mucosa. Once in the blood, the metacyclic trypomastigote infects host cells where it transforms to an aflagellate amastigote in the cytoplasm, replicates via binary fission, and converts to a flagellated trypomastigote, which is then released from the cell to circulate and infect other cells. The cycle is completed when a triatomine takes up circulating parasites during a blood-meal.

Clinical variations based on parasite strains have been reported, interrelated with other epidemiological factors. For instance, the incidence of chronic digestive Chagas disease varies according to location. Patients in Panama and Venezuela do not exhibit the digestive form of chronic Chagas, whereas 2–8.8% of patients with chronic Chagas in central Brazil have megaesophagus. Another example is the incidence of congenital transmission, which varies geographically from 1% or less in Brazil to 7% or more in Bolivia, Chile, and Paraguay.

**Epidemiology**

Longitudinal studies indicate that roughly half of infected individuals display clinical manifestations of the disease and 25% of infected individuals die as a direct or indirect result of the infection. The most recent estimates of the disease burden of Chagas disease show significant impact despite improvements since previous assessments in the 1980s. Estimated total infection prevalence is 8–11 million. A 2000 WHO report highlighted that 5–6 million people are infected in Andean and Central American countries and 25 million are at risk of infection. Nevertheless, significant decreases in infection and mortality have been achieved through vector control initiatives in Central and South America. For example, in the Southern Cone countries between 1990 and 2000, the number of new cases per year decreased from 700,000 to 200,000 and number of deaths decreased from more than 45,000 to 21,000.

Chagas disease is interrelated with socioeconomic factors. The insect vectors tend to infest the nooks and crannies of poorly constructed homes; therefore, it predominantly affects the poor in rural areas. In addition, healthcare access for diagnosis and treatment is limited in impoverished areas, further skewing the burden of disease.

The main route of transmission is directly via triatomine insect. However, the infection can also be transmitted through blood transfusion, congenital transmission, organ transplantation, laboratory accident, and oral transmission. Blood transfusion is the second most common way of acquiring the disease. This mode of transmission expands the problem from rural areas to urban centers and from endemic to nonendemic countries that receive infected immigrants. Although many endemic countries have implemented blood donor screening, others have not and transfusion-associated transmission of the parasite continues to be documented. Meanwhile, seven cases of transfusion-transmitted *T. cruzi* have been documented in the U.S. and Canada and 1/25,000 donors in the U.S. is infected with the parasite, with increased prevalence of up to 1/5,400 in areas of at-risk populations; it is expected that Canada and Europe face similar concerns. Congenital infection is related to prevalence of vectorial-transmitted infection in women of childbearing age. Risk varies geographically in addition to other epidemiological parameters, ranging from 1-7%. An emerging mode of transmission is that of organ transplantation from seropositive donors, and the recipient’s clinical response to infection is aggravated by the induced immunosuppression. Reports of *T. cruzi* transmission via renal transplants have previously been reported from endemic areas and, to date, five cases of transmission have been reported in the U.S. A much less frequent mode of transmission is via laboratory accidents. There have also been reports of epidemics of acute Chagas disease secondary to oral transmission via infection of contaminated fruit juices and sugar cane juice, causing acute myocarditis and a high rate of death.

Chagas disease is also an emerging opportunistic infection among immunosuppressed populations, particularly with HIV infection. Reactivation of Chagas disease is a well-known occurrence among immunosuppressed patients. A growing number of cases have been reported among HIV+ patients, typically among those with CD4 counts <200 cells/mL (four have been reported in the U.S. to date; many more have been reported in endemic areas). The clinical presentation of the reactivation differs distinctly from the disease in immunocompetent patients, as described below.
Clinical Manifestations

Chagas disease manifests in two phases: acute and chronic. The acute phase, preceded by an incubation period of 7 to 15 days,\textsuperscript{10} may be heralded by a chagoma, an area of inflammation at the site of inoculation (the triad of conjunctivitis, periorbital edema, and preauricular lymphadenopathy appearing after conjunctival inoculation is known as the Romaña sign).\textsuperscript{19} Systemic dispersion of multiplying parasites during the acute phase may be asymptomatic or may manifest as fevers, tachycardia, malaise, lymphadenopathy, hepatosplenomegaly, edema, vomiting, diarrhea, anorexia, or rash.\textsuperscript{1,19,20} Lack of symptoms and lack of access to health care lead to only 1−2% of acute cases actually being recognized.\textsuperscript{1} Whereas the acute phase is typically mild, parasitization of cardiac muscle and brain may cause acute myocarditis and meningoencephalitis, respectively,\textsuperscript{21} and fatality ranges from 5−10% if untreated.\textsuperscript{29} However, most cases are self-limited, with symptoms resolving in 3−8 weeks.\textsuperscript{22}

The clinical latency that follows the acute phase may last for years or for a lifetime, but patients continue to carry antibodies and a low parasitemia. Whereas this time is referred to as the “indeterminate” phase and considered an asymptomatic period, the existence of a discrete intermediate phase is questionable because of studies suggesting that chronic tissue damage is a continuous progressive process despite lack of overt clinical manifestations,\textsuperscript{23} and most patients in this phase have subclinical functional cardiac involvement.\textsuperscript{24}

The percentage of infected patients is ~10−40% in the indeterminate phase progressing to the chronic phase of Chagas disease,\textsuperscript{10} whereas the rest of the patients remain asymptomatic indefinitely. Chronic Chagas disease generally occurs 10−20 years after acute illness\textsuperscript{25} and mainly impacts the heart and digestive tract with significant morbidity and mortality. Cardiac abnormalities are the most common manifestations of chronic disease and about 2% of indeterminate-phase patients progress to chronic cardiac disease every year.\textsuperscript{19} Chronic tissue changes—inflammatory infiltration and myocardial fibrosis\textsuperscript{25}—disrupt the cardiac conduction system and cause structural alterations that lead to serious cardiac morbidities including arrhythmias, congestive heart failure, and sudden cardiac death, in addition to systemic and pulmonary thromboembolism. Cardiac symptoms include atypical chest pain, dyspnea, palpitations, light-headedness, dizziness, and syncope.\textsuperscript{26} Cardiomyopathy, typically characterized by biventricular dilatation and an apical aneurysm, is the most common cardiac problem and carries a poorer prognosis than other forms of heart failure.\textsuperscript{27} Sudden death comprises 55−65% of deaths in Chagas disease\textsuperscript{28} and typically occurs in patients with severe cardiac involvement.\textsuperscript{26}

The second most common manifestation of chronic illness is in the digestive tract, commonly referred to as “megadisease.” Destruction of autonomic neurons in the viscera leads to gastrointestinal immotility, most frequently in the esophagus and colon, producing megasphagus and megacolon. The most common symptoms are from megasphagus and include dysphagia, pain, and regurgitation, leading to coughing, aspiration bronchitis and pneumonia, and malnutrition.\textsuperscript{22} Importantly, there is a greater incidence of gastric and esophageal cancer in patients with this condition.\textsuperscript{29} Megacolon is the next most common digestive effect and typically affects the sigmoid colon with dilatation and thickening of the colonic wall. This causes symptoms of constipation and pain, with serious complications such as fecal impaction, toxic megacolon, and volvulus.\textsuperscript{22} Other digestive manifestations of chronic illness are gastric hypertrophy with delayed emptying and decreased acid secretion,\textsuperscript{22} enteropathy from involvement of the small intestine,\textsuperscript{30} and hypertrophy of the salivary glands.\textsuperscript{22}

It is important to note that the clinical manifestations of the disease can differ in immunocompromised hosts experiencing reactivation of the disease. In HIV patients in particular, the presence of disease in the central nervous system (CNS) dominates the clinical picture, and cardiac involvement is only the second most common manifestation.\textsuperscript{31} CNS disease manifests typically as an acute meningoencephalitis or brain mass with symptoms and signs of headache, fever, cognitive changes, seizures, hemiparesis, and aphasia all being reported.\textsuperscript{32} Neuroimaging reveals single or multiple hypodense, subcortical lesions with or without enhancement and mainly in the white matter.\textsuperscript{33} These lesions can be confused with those of \textit{Toxoplasma gondii}, especially in an HIV+ patient, and must be distinguished by further testing (see below) if previous exposure to \textit{T. cruzi} is suspected.\textsuperscript{17}

Pathogenesis

The pathogenesis of both acute and chronic phases has been under intense examination in recent years. One focus
of research has been the origin of tissue damage in chronic illness—a critical question because it is highly pertinent to treatment strategies for chronic disease. Another area of inquiry has been the investigation of the immune response to parasite infection with the hope of developing novel therapeutic targets within the inflammatory cascade.

An accurate description of the pathogenetic mechanisms leading to chronic chagasic disease is currently quite controversial. The question of chronic disease pathogenesis is more than academic; it has pressing significance for effective treatment strategies by shedding light on whether there is a need for etiological treatment, more focus on immunomodulatory interventions, or even the use of neurohormonal antagonists. Much of the research has focused on chronic cardiomyopathy, the most common and most serious manifestation of chronic illness. In the past, lack of evidence of parasite persistence in chronic chagasic patients suggested an autoimmune etiology for tissue damage and although more sensitive tests demonstrating parasite presence in both blood and tissue in chronic patients now implicate parasite presence as a direct component of pathological processes, the contribution of autoimmune processes continues to be considered. Autoimmune hypotheses include the “molecular mimicry” and the “bystander” scenarios; these propose that autoantibodies and autoreactive cellular response to host cell components elicited either by similar host and parasite epitopes or to cell contents released by host cell lysis, respectively, are responsible for cardiac tissue damage, a [self-perpetuating] process that eventually leads to cardiac dysfunction. Antibodies to multiple cardiac tissue components have been found in the sera of chagasic patients, cross-reactive antibodies to host and parasite epitopes have been demonstrated, and evidence linking autoantibodies to conductive abnormalities has been shown. However, no experiments have shown production of Chagas-like disease via passive transfer of candidate antibodies and evidence that immunosuppressed patients have more severe clinical manifestations challenges a purely autoimmune theory. In addition, experiments have shown T-cells mediating cardiac transplant rejection and production of cross-reactive autoantibodies, but there is some controversy over whether these results occurred exclusive of parasite presence. Overall, current evidence does not indicate that autoimmune processes are the primary mediator of chronic tissue damage, but immunological mechanisms likely do

play a role in the pathogenesis of chronic chagasic cardiopathy. As mentioned above, discovery of parasite persistence in chronic disease argues against a purely autoimmune hypothesis and suggests a parasite-dependent mechanism of disease development, with the corollary that etiological treatment may be effective for chronic disease. The high prevalence of parasites in the blood and tissue of chronic chagasic patients, the association of *T. cruzi* antigens with cardiac inflammation, and correlation of parasite presence with disease severity points to a primary role of the parasite in the pathogenesis of chronic Chagas cardiomyopathy. Importantly, a recent study showed etiological treatment of chronic and indeterminate phase patients improved clinical cardiac outcomes, reinforcing the centrality of parasite persistence in chronic disease. In this model, parasites in myocardium either directly or indirectly, through host immune response, induce tissue damage causing an insidious progression to severe cardiac dysfunction. Several unified theories have been proposed; adequacy of immune response to the parasite may determine degree of parasite persistence and subsequent inflammatory response or parasite presence may act as a trigger for autoimmune activation. Still another unified hypothesis proposes that initial localized damage to myocardium induces cardiac remodeling and then neurohormonal activation and autoimmune processes without the requirement of parasite presence. As the previous paragraph reveals, the pathogenesis of chronic Chagas disease remains controversial and still has not been clearly defined. Unfortunately, this has implications for treatment as discussed below.

Despite the controversy over the primary pathogenetic mechanism, acute and chronic inflammation mediated by the host immune system is undoubtedly important in development of tissue damage. An understanding of the delicate balance between parasite control and inflammatory tissue damage could lead to new therapeutic targets to spare tissue injury. Various studies have outlined the progression of immunologic events. A strong innate response and a polyclonal activation of B- and T-cells follow the initial encounter with the parasite, with the induction of cytokines, particularly IFN-γ and TNF-α, and expression of adhesion molecules that promote CD8+ dominant leukocyte recruitment. This inflammatory response leads to an acute myocarditis, and the sustained production of IFN-γ-inducible cytokines establishes a
facilitative environment for continued inflammation. Interestingly, the immunological profile in chronic chagasic patients seems to correlate with the severity of clinical manifestations; differential expression of various cytokines, chemokine receptors, and T-cell receptor components have all been demonstrated in groups of patients categorized by the phase of the disease and severity of clinical symptoms, although not all studies agree in their findings. These descriptions imply that novel therapeutic strategies targeting components of immunological pathways such as chemokine receptors and adhesion molecules could ameliorate chronic inflammation and tissue damage.

Transmission Control
The main routes of disease transmission are by triatomine vector and blood transfusion, so the main focus of control so far has been in these two areas. Great success has been seen so far, but maintenance and control must be continued. Although vector control programs date back to the 1950s in South America, a major step in this area was accomplished with the Southern Cone Initiative in 1991. This was a collaboration between the ministries of health in six South American countries targeting both interruption of vector and blood bank transmission. It has been remarkably successful both in controlling disease and in encouraging similar efforts in other endemic countries; the Andean Countries Initiative and the Central American Countries Initiative in 1997 expanded transmission control agendas to a total of 17 countries in Central and South America. Interruption of vectorial transmission has been achieved in many endemic areas of the Southern Cone—Uruguay, Chile, four (of 18) Argentinian provinces, and nine (of 11) Brazilian states—with continued progress in Bolivia and Paraguay. Screening of blood donors has improved secondary to increased frequency and better serological tests so that now 13/17 countries screen at least 90% of donors with seven countries screening 100%; however, four countries screen <25%.

The success of these transmission control initiatives should not be allowed to diminish the importance of continued work in these areas. Current achievement must be maintained and continued effort is needed to extend vector control to the nondomiciliated triatomine vectors as well as to expand blood donor screening in both endemic and at-risk nonendemic areas.

Diagnosis
Diagnosis of acute disease rests on demonstrating parasites in the blood of the patient. This can usually be accomplished by direct microscopy either of fresh blood or its buffy coat or Giemsa-stained thick and thin smears. Further microscopy techniques use centrifuged samples. In case of low parasitemia that yields negative microscopy results, the parasite can also be isolated by xenodiagnosis (in which uninfected triatomines feed on the patient’s blood and are then evaluated for parasite presence in the gut a month later) or hemoculture in an appropriate medium. Molecular techniques such as PCR are not used routinely in clinical settings.

Because parasitemia is low or transient in chronic disease, standard diagnosis in this stage relies on identifying the immune response of the patient to the parasite, by serological testing. Conventional serology relies on specifically indirect immunofluorescent antibody test (IIF), indirect hemagglutination test (IHA), and enzyme-linked immunosorbent assay (ELISA), are widely used. A single immunodiagnostic test is not adequate due to lack of specificity, and diagnosis is reserved for positive results on two tests. To increase specificity and avoid potential cross-reactivity with sympatric parasitoses such as leishmaniasis, serological tests using recombinant antigens and synthetic peptides have been developed in recent years, and those that are commercially available can be used in conjunction with the conventional tests but have not supplanted them. In addition, with the advent of molecular techniques, direct detection of parasitemia is now possible in chronic patients and is especially important in patients with uncertain serology. PCR assay of parasite DNA has been shown to have a high specificity, especially when compared to other parasitological techniques and, in fact, has been shown to detect parasitemia in seronegative patients, raising questions about future standards for diagnostic testing, therapeutic decision-making, and blood-bank screening. At present, however, PCR is mainly used for research and not widely used in clinical settings.

Of note, in HIV patients suspected of chagasic reactivation, diagnosis can be made by direct examination of blood, but repeated examinations may be necessary to visualize the trypomastigote form. However, blood culture and xenodiagnosis are not considered diagnostic of reactivation in this situation. Examination of cerebrospinal fluid
(CSF) may show trypomastigotes as well as lymphocytic pleocytosis and increased protein.\textsuperscript{79} As mentioned above, CT scanning of the brain often reveals cerebral masses, and brain biopsy can be done, showing amastigote presence in glial cells with accompanying inflammation.\textsuperscript{33}

Diagnostic tests are not only important in untreated patients but also for evaluation of cure in treated patients. Etiological treatment aims to eliminate the parasite; thus, assessment of treatment efficacy requires verification of parasite absence. This is a difficult task. The current criteria for cure require serial serological tests to verify absence of antibodies to the parasite, but disappearance of immune response can take many years.\textsuperscript{79} However, parasitological tests have low sensitivities, and negative results cannot be taken as proof of elimination of the parasite. PCR has been suggested as a potential test for cure,\textsuperscript{77,80} but its availability and optimization is limited in endemic countries. At this time, parasitological tests are best used for detection of treatment failure in order to change treatment regimens if necessary.\textsuperscript{81} In this context, PCR is more sensitive than other parasitological tests (hemoculture, xenodiagnosis) and is a useful tool in therapeutic decision-making.

**Treatment**

Treatment is absolutely indicated for acute phase infection, congenital infection, accidental infection, indeterminate cases in those 18–50 years of age, and reactivation of disease in immunosuppressed patients. The two mainstays of etiological treatment are the nitroheterocyclic drugs benznidazole and nifurtimox. For acute disease, these drugs are moderately effective, producing cure in an estimated 60% of cases, but their effectiveness varies with geography and both have severe side effects. Benznidazole, the most available and widely used drug, can cause hypersensitivity reactions, bone marrow depression, thrombocytopenic purpura, agranulocytosis, and neuropathies\textsuperscript{82} (the recommended adult benznidazole dosage is 5-7 mg/kg/day in two divided doses for 30 to 60 days, or nifurtimox can be given at 8-10 mg/kg/day in 3-4 doses for 90-120 days).\textsuperscript{83}

Secondary prophylaxis for chronic patients co-infected with HIV is recommended with either benznidazole or nifurtimox, 5 mg/kg, three times per week.\textsuperscript{32}

One of the most important questions regarding treatment is also one of the most controversial—specifically, should patients with late chronic infection be treated with etiological agents? In the past, attribution of chronic disease pathogenesis to autoimmune processes and concern about significant side effects prevented use of trypanocidal treatments. However, the growing body of evidence that parasite persistence is integral to chronic disease challenged this standard and has provoked further consideration of etiological treatment in the chronic phase. No definitive studies have answered this question thus far but the results of the BENEFIT trial will be unveiled in 2012 to address this question in a clinical trial in more than 3000 patients.

Based on the limited efficacy of the current standard drugs and their side effect profiles, and in light of the important public health consequences of inadequately treated acute and chronic disease, there is a pressing need for better drugs. Despite a long hiatus in research on new drugs, there has been increased research activity recently into new targets and approaches to \textit{T. cruzi} chemotherapy in light of increased knowledge of parasite metabolism and current focus on parasitologic treatments for chronic disease. The following are highlights of drug development research organized by \textit{T. cruzi} metabolic targets.\textsuperscript{82,84-86}

Finally, as to prevention of disease, there are currently no active vaccine development programs,\textsuperscript{87} although several potential vaccine targets have been investigated. Murine models have shown some success with purified parasite proteins\textsuperscript{88} and DNA vaccines.\textsuperscript{89,90} Future vaccine development may be enhanced by information from genome and proteomic analyses with strategies such as reverse vaccinology.

**Next Steps**

As the preceding overview reveals, Chagas disease is a widely dispersed vector-borne disease carrying an enormous burden of morbidity and the potential to affect millions of people, with the additional challenges of emergence into nonendemic areas. Vector control efforts have achieved laudable success, but sustainability remains precarious and peri-domesticated vectors pose another transmission control frontier. Critical gaps remain in our knowledge base regarding pathogenesis, diagnosis, and treatment. Unacceptably limited drug treatment options are available. The current situation calls for continuation and intensification of research in these areas with the aim of eradicating this neglected disease.\textsuperscript{10,91} A particular area of interest is the development of preventive and/or therapeutic vaccines against \textit{T. cruzi}. 

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