Review Article

New insights into Chagas disease: a neglected disease in Latin America

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Abstract
Like other neglected diseases, Chagas disease affects the daily lives of millions of people in Latin America and thereby hinders development in that region. A vector-borne disease that generates significant morbidity and mortality, Chagas disease is a major public health threat. In recent years, the changing epidemiological pattern of the disease and discoveries in its pathogenesis have renewed interest in this disease and have led to new approaches to its therapeutic management. In this article, we review these breakthroughs and suggest a more comprehensive approach to this neglected disease.
Key Words: Chagas disease, *Trypanosoma cruzi*, Development, Latin America, neglected diseases.

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Introduction

This decade marks the one hundredth anniversary of Carlos Chagas' discovery of this parasitic disease. Despite major successes in controlling disease transmission since that time, Chagas disease continues to endanger the lives of a significant number of people in the Americas.

In addition, its chronic manifestations profoundly affect millions of previously infected people. Worse still, potential and emerging vectors and means of transmission threaten to expand the scope of this disease, even beyond its usual ecoepidemiologial setting in the Latin American region.

The Parasite and its Vector

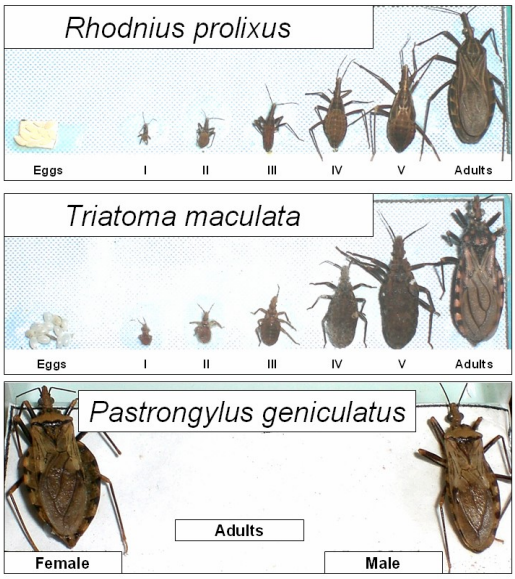
Chagas disease, also known as American trypanosomiasis, is a zoonotic tropical disease caused by the parasite *Trypanosoma cruzi*. This protozoan is a member of the order *Kinetoplastida* and is within the same family, *Trypanosomatidae*, as the causal parasites of African trypanosomiasis and Leishmaniasis. Multiple strains of *T. cruzi* have been identified and are categorized into two principal groups, *T. cruzi* I and *T. cruzi* II. Interestingly, there are strain-specific variations in the clinical patterns of the disease [1]: *T. cruzi* II is the most common group found in human infections and is more closely associated with domestic areas [2,3]. Research including the *Trypanosoma cruzi* Genome Initiative and proteome analyses continue to add to our current knowledge of the biological characteristics and metabolism of this parasite and suggest targets for drug therapies and vaccine development [4,5].

In the majority of cases, transmission of the parasite occurs within a domestic, sylvatic, or peridomestic cycle and arises when insects of the *Reduviidae* family, subfamily *Triatominae* ("kissing bugs") bite humans or other mammals.

While a large number of species exist, the most important vectors to humans are *Triatoma infestans*, *Rhodnius prolixus*, *Triatoma dimidiata*, *Panstrongylus megistus*, *Triatoma brasiliensis*, *Triatoma maculata* and *Panstrogylus geniculatus* (Figure 1) [1].

Most *Triatominae* live in the tropics between 45° S and 40° N with variations based on climate. The main Chagasic vectors tend to inhabit distinct regions of Latin America; those in the countries of the Southern Cone are domesticated while those in Central America, Mexico, Andean countries, and the Amazon basin infest both domestic and uninhabited, wild areas (Figure 2). Accordingly, different approaches to elimination of vectors are employed in these regions (Figure 2).

Figure 1. Chagas disease vectors (Triatominae) (selected).



(Photo: A. J. Rodriguez-Morales),

The life cycle of *T. cruzi* involves different parasitic stages and both invertebrate and vertebrate hosts [5]. Within the *Triatomine* gut, the parasite replicates in epimastigote form and then develops into the infectious metacyclic trypomastigote. While the insect ingests a blood meal, inoculation occurs as the parasite is excreted via feces into breaks in the skin or mucosa of the victim. Once in the blood, the metacyclic trypomastigote infects host cells and transforms to an aflagellate amastigote in the cytoplasm. Replication then occurs via binary fission and a flagellated trypomastigote, which is released from the cell to circulate and infect other cells, is produced. The cycle is completed when a *Triatomine* takes up circulating parasites during a subsequent blood meal.

As mentioned above, clinical variations based on parasite strains have been reported, and appear to be interrelated with other epidemiological factors. For example, the incidence of chronic digestive Chagas disease varies according to location. Patients in Panama and Venezuela do not generally exhibit the digestive manifestations of chronic Chagas disease whereas 2-8.8% of patients in Central Brazil have megaesophagus [7]. Additionally, the incidence of congenital transmission varies geographically from approximately 1% in Brazil to more than 7% in Bolivia, Chile, and Paraguay [1].

Figure 2. Regional Initiatives to Eliminate Chagas disease in Latin America.

Modified from TDR, Regional Initiatives to Eliminate Chagas disease (http://www.who.int/tdr/research/progress/chag_afr/map.htm) and Control of Chagas Disease: Second Report of the WHO Expert Committee, 2000. p.44.

Epidemiology

Longitudinal studies indicate that while only half of infected individuals display clinical manifestations of the disease, approximately 25% of infected individuals die as a direct or indirect result of the infection [8]. Recent estimates indicate that although there has been significant improvement in the disease burden of Chagas since the 1980s, a heavy impact still remains. Estimated annual incidence of Chagas disease is 200,000 and prevalence is 13 million worldwide, 3 to 3.3 million cases of which are symptomatic. A 2000 World Health Organization (WHO) report suggests that 5 to 6 million people are infected in Andean and Central American countries and 25 million are at risk [1]. 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 was decreased from 700,000 to 200,000 and the number of deaths from more than 45,000 to 21,000 [1].

As is the case with all neglected diseases, Chagas disease is interrelated with socioeconomic factors. The insect vectors tend to infest the crevices of poorly constructed homes and therefore often disproportionately affect the poor in

rural areas (Figure 3). In addition, health care access for diagnosis and treatment is limited in impoverished areas, further skewing the burden of disease [6].

Figure 3. Endemic zone for Chagas Disease in Venezuela (Trujillo, Western Region).

(Photo: A. J. Rodriguez-Morales)

The main route of transmission is directly via the *Triatomine* insect as discussed above. However, infection can also be transmitted through blood transfusion, congenital transmission, organ transplantation, laboratory accidents, and oral transmission. Blood transfusion is the second most common way of acquiring Chagas disease. This mode of transmission expands the problem from rural areas to urban centers and from endemic to non-endemic countries that receive infected immigrants. While many endemic countries have implemented blood donor screening [10], others have not, and transfusion-associated transmission of the parasite continues to be documented [11]. For example, seven cases of transfusion-transmitted *T. cruzi* have been documented in the United States and Canada, and 1 in 25,000 donors in the U.S. is infected with the parasite, with increased prevalence of up to 1 in 5,400 in areas of at-risk populations. It has been suggested that Canada and Europe face similar concerns [12,13].

Congenital infection is related to prevalence of vectorial-transmitted infection in women of child-bearing age [1]. An emerging mode of transmission is that of organ transplantation from seropositive donors. Reports of *T. cruzi* transmission via renal transplants have previously been reported from endemic areas [14], and to date, five cases of transmission have been reported in the United States [15]. The recipient's clinical course may be fulminant as the ability to fight infection is typically limited by the immunosuppression necessary with organ transplantation. There are currently no screening protocols for either blood or organ donation in the United States. A much less frequent mode of transmission is via laboratory accidents. Additionally, there have been reports of epidemics of acute Chagas disease secondary to oral transmission via infection of edible plants [16]. These cases are associated with acute myocarditis and a high death rate [16].

Finally, Chagas disease is also an emerging opportunistic infection among immuno-compromised populations, particularly those with HIV infection. Reactivation of Chagas disease is a well-known risk among the immuno-suppressed and a growing number of cases have been reported in HIV positive patients, typically among those with CD4 counts below 200 cells/ml [17,18]. The clinical presentation of reactivation disease differs distinctly from Chagas disease that is seen in immunocompetent patients, as described in the Clinical Manifestations section below.

Pathogenesis

The pathogeneses of both acute and chronic phases of Chagas disease have been under intense examination in recent years. One focus of research has been the origin of the tissue damage in chronic illness, a question critical to designing 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.

A full description of the mechanisms leading to chronic Chagas disease remains controversial. The question of chronic disease pathogenesis is more than academic; it has pressing significance for effective treatment strategies targeting specific etiologies or by means of immunomodulatory interventions or possibly neurohormonal antagonists. Much of the research has focused on chronic cardiomyopathy, the most common and serious manifestation of chronic Chagas disease.

Previously, there was a lack of evidence of parasite persistence in chronic Chagas disease

suggesting an autoimmune etiology for tissue damage. Although more sensitive tests demonstrating parasite presence in both blood and tissue in chronic Chagas now implicate parasites as a direct component of pathological processes, an autoimmune contribution remains likely. Autoimmune hypotheses include the "molecular mimicry" and the "bystander" scenarios; both propose that autoantibodies and autoreactive cellular response to host cell components are elicited either by similar host and parasite epitopes or to cell contents released by host cell lysis, respectively. According to these theories, it is the cellular response that is responsible for cardiac tissue damage, a self-perpetuating process that eventually leads to cardiac dysfunction. In support of autoimmune theories, antibodies to multiple cardiac tissue components have been found in the sera of Chagas disease patients [19-22] and cross-reactive antibodies to host and parasite epitopes have been demonstrated [22,23]. Further, evidence linking autoantibodies to conductive abnormalities has been shown [24,25]. However, no studies 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.

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 cardiomyopathy [27-29]. As mentioned above, discovery of parasite persistence in chronic disease argues against a purely autoimmune hypothesis. The high prevalence of parasites in the blood and tissue of chronic Chagas disease patients [7,30-32,58], the association of *T. cruzi* antigens with cardiac inflammation [33,34], and the correlation of parasite presence with disease severity [35,36], point to a primary role of the parasite in the pathogenesis of chronic Chagasic cardiomyopathy. If this is the case, it suggests that etiologic treatment may be extremely effective for chronic disease. In fact, a recent study showed such treatment improved clinical cardiac outcomes in chronic and indeterminate phase patients [37] 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. The adequacy of immune response to the parasite may determine the degree of parasite persistence and subsequent inflammatory response [27] or parasite presence may act as a trigger for autoimmune activation [28]. 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 [29]. As stated above, the pathogenesis of chronic Chagas disease remains controversial and still has not been clearly defined; unfortunately, this has implications for treatment as is discussed below.

Despite the controversy over the primary pathogenesis of Chagas disease, 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 [38,39]. A strong innate immune response and polyclonal activation of B and T cells follow the initial encounter with the parasite [40-43], with the induction of cytokines, particularly IFN-γ and TNF-a, and expression of adhesion molecules that promote CD8 dominant leukocyte recruitment [44]. This inflammatory response leads to an acute myocarditis, and the sustained production of IFN-γ-inducible cytokines establishes a facilitative environment for continued inflammation [45]. Interestingly, the immunological profile in chronic Chagasic patients seems to correlate with the severity of clinical manifestations [46]; differential expression of various cytokines, chemokine receptors, and T-cell receptor components, such as IFN-γ, TNF-a, IL-10, CCR5, CXCR4, and T-cell receptor V region repertoire, have all been demonstrated in groups of patients categorized by the phase of the disease and severity of clinical symptoms, though not all studies agree in their findings [47-52]. 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.

Clinical Manifestations

Chagas disease manifests in two phases, acute and chronic. The acute phase, preceded by an incubation period of 7 to 15 days [10], may be heralded by a chagoma, an area of inflammation at the site of inoculation. If conjunctival inoculation occurs, the triad of conjunctivitis, periorbital edema, and preauricular lymphadenopathy, known as the Romaña sign, may occur [53]. Systemic dispersion of multiplying parasites during the acute phase may be asymptomatic or may manifest as fever, tachycardia, malaise, lymphadenopathy, hepatosplenomegaly, edema, vomiting, diarrhea, anorexia, and/or rash [1,54,55]. Lack of symptoms and limited access to health care result in only 1-2% of acute cases being recognized [1]. During the acute phase, parasitization of cardiac muscle and brain may cause acute myocarditis and meningoencephalitis, respectively [56]. If left untreated, mortality ranges from 5-10% [54]. Most acute cases, however, are self-limited with symptoms resolving in four to eight weeks [56].

The acute phase is then followed by a period of clinical latency referred to as the indeterminate phase that may last for years (or even a lifetime) although patients continue to carry antibodies and possess a low level of parasitemia. There is question of a discrete subclinical intermediate phase because of studies that suggest that chronic tissue damage is a continuous progressive process despite lack of overt clinical manifestations [57] and most patients in this phase have subclinical functional cardiac involvement [58].

While the majority of patients in the indeterminate phase remain asymptomatic, a significant percentage of infected patients, 10-40%, progress to the chronic phase of Chagas disease [10]. Chronic Chagas disease generally occurs 10 to 20 years after acute illness [59] and mainly targets the heart and digestive tract, resulting in significant morbidity and mortality. Cardiac abnormalities are the most common manifestations of chronic disease and about 2% of indeterminate phase patients go on to chronic cardiac disease each year [53]. Chronic tissue changes, especially inflammatory infiltration and myocardial fibrosis [59], disrupt the cardiac conduction system and cause structural alterations which lead to serious cardiac sequelae including arrhythmias, congestive heart failure, and sudden cardiac death, in addition to systemic and pulmonary thromboembolism [60]. Cardiac symptoms include atypical chest pain, dyspnea, palpitations, light-headedness, dizziness, and syncope [60]. Cardiomyopathy, the most common manifestation, is typically characterized by biventricular dilatation and an apical aneurysm, and carries a poorer prognosis than other forms of heart failure [61]. Sudden death comprises 55-65% of deaths in Chagas disease [62] and typically occurs in patients with severe cardiac involvement [60].

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 producing megaesophagus and megacolon. The most frequent symptoms result from megaesophagus and include dysphagia, pain, and regurgitation that lead to coughing, malnutrition, and aspiration bronchitis or pneumonia [56]. Importantly, there is a greater incidence of gastric and esophageal cancer in patients with this condition [63]. Megacolon is the next most common digestive expression, and typically affects the sigmoid colon by causing dilatation and thickening of the colonic wall. This creates symptoms of constipation and pain, and serious complications such as fecal impaction, toxic megacolon, and volvulus can occur [56] Other digestive manifestations of chronic illness are gastric hypotonia with delayed emptying and decreased acid secretion [56], enteropathy from involvement of the small intestine [64], and hypertrophy of the salivary glands [56].

It is important to note that clinical manifestations can differ in reactivation Chagas disease in immunocompromised hosts. In HIV patients in particular, the presence of disease in the central nervous system dominates the clinical picture [65]. CNS disease typically manifests as an acute meningoencephalitis or brain mass with symptoms and signs of headache, fever, cognitive changes, seizures, hemiparesis, and aphasia reported [66]. Neuroimaging reveals single or multiple hypodense, subcortical lesions with or without enhancement, and occur mainly in the

white matter [67]. These lesions can be confused with those of *Toxoplasma gondii,* especially in an HIV positive patient, and must be distinguished by further testing (see below) if previous exposure to *T. cruzi* is suspected [17].

Diagnosis

Diagnosis of acute disease rests on demonstrating parasites in the blood of the patient [68,68]. 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 cases of low-level parasitemia that yield negative microscopy results, the parasite can also be isolated by xenodiagnosis, a technique in which uninfected *Triatomines* feed on the patient's blood and are subsequently evaluated for parasite presence in the gut a month later, or by hemoculture in an appropriate medium.

Since 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-serological testing. Conventional serology tests, specifically indirect immuno-fluorescent antibody test (IIF), indirect hemagglutination test (IHA), and enzyme-linked immunoabsorbent 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 [1,56]. 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 [70]. 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 [71,72], 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 [73-75]. 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. Blood culture and xenodiagnosis, however, are not considered diagnostic of reactivation in this situation [65]. Examination of CSF may show trypomastigotes as well as lymphocytic pleocytosis and increased protein [76]. 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 [67].

Diagnostic tests are not only important in untreated patients but also for evaluation of cure in treated patients. Etiologic treatment aims to eliminate the parasite and 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 [77]. 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 [75,78], but its availability and optimization is limited in endemic countries. At this time, parasitological tests (hemoculture, xenodiagnosis) are best used for detection of treatment failure in order to change treatment regimens if necessary [79]. In this context, PCR is more sensitive than other parasitological tests and is a useful tool in therapeutic decision-making.

Transmission Control

The main routes of disease transmission are by Triatomine vector and blood transfusion as discussed above, and consequently these have been the main focus of control [1,10,80]. These strategies have seen great success, but maintenance and control must be continued. Vector control programs date back to the 1950s in South America, but a major advance in this area was accomplished with the introduction of the Southern Cone Initiative in 1991. This collaboration between the Ministries of Health in six South American countries targeted 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. For example, the Andean Countries Initiative and the Central American Countries Initiative in 1997 expanded transmission control agendas to a total of seventeen 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 eighteen) Argentinian provinces, and nine (of eleven) Brazilian states, with continued progress in Bolivia and Paraguay (Figure 2).

Screening of blood donors has improved secondary to increased frequency and better serological tests. 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 non-domesticated *Triatomine* vectors as well as expand blood donor screening in both endemic areas and non-endemic areas at risk.

Treatment

Treatment is absolutely indicated for acute phase, congenital, accidental (e.g. laboratory related), transplant donor, and recipient infections. Additionally, treatment is recommended for chronic infection that was acquired less than ten years prior and for reactivation of disease in immuno-suppressed patients [81]. The two mainstays of etiologic treatment are the nitroheterocyclic drugs benznidazole and nifurtimox. For acute disease, these drugs are moderately effective, producing cure in an estimated 60% of cases [81]. Their effectiveness, however, 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, agranu-locytosis, and neuropathies [81]. The suggested adult benznidazole dosage is 5-7mg/kg/day in 2 divided doses for thirty to sixty days; nifurtimox can be given at 8 to 10 mg/kg/day in 3 to 4 doses for 90 to 120 days [82]. Secondary prophylaxis using either benznidazole or nifurtimox at a dose of 5 mg/kg three times per week is recommended for chronic patients co-infected with HIV [66].

These therapeutic schemes are relatively well tolerated by children. The reported side effects of anti-*Trypanosoma cruzi* treatment increase with patient's age.

A summary of the therapeutic schemes is shown in the Table 1.

Table 1. Therapeutic schemes in Chagas Disease.

Infection	Drug	Dosage
Acute phase infection*	Nifurtimox	> 40 kg: 10-12 mg/kg/day in 2-3 doses x 30-60 days
		< 40 kg: 8 mg/kg/day in 2-3 doses x 30-60 days
	Benznidazole	> 40 kg: 7.5 mg/kg/day in 2-3 doses x 30-60 days
		< 40 kg: 5 mg/kg/day in 2-3 doses x 30-60 days
Congenital infection*	Nifurtimox	10-15 mg/kg/day x 60 days
	Benznidazole	10 mg/kg/day x 60 days
Early Chronic infection†	Nifurtimox	8 mg/kg/day x 30-60 days
	Benznidazole	5 mg/kg/day x 30-60 days
Accidental infection*	Benznidazole	7 to 10 mg/kg/day for 10 days
Reactivation in HIV‡	Benznidazole	5 mg/kg/day BID for 60 days
	Nifurtimox	10 mg/kg/day, TID or QID for 120 days

*Drug dosing information from OPAS/OMS 1998, Tratamiento Etiologico de la Enfermedad de Chagas. Conclusiones de una consulta tecnica [83].
† Drug dosing information from Coura and de Castro, 2002 [81].
‡ Drug dosing information from Walker and Zunt, 2005.

One of the more important and controversial questions regarding treatment relates to the use of etiologic treatments in patients with late chronic infection. Previously, concern over significant side effects and theories of primary autoimmune pathogenesis in chronic disease discouraged the use of trypanocidal agents. However, the growing body of evidence that parasite presence is integral to chronic disease challenges this practice and has provoked further consideration of etiological treatment in the chronic phase.

Some studies have suggested that etiological treatment has a positive effect on patients' serology and clinical progression of the disease, but this evidence is most convincing in pediatric populations and in earlier stages of the chronic phase [84-86]. In addition, a recent study showed reduced clinical progression of cardiac disease in treated chronic Chagas patients [37]. Unfortunately, however, few well-designed, randomized control trials have been conducted in this area.

A 2005 systematic review of related research concluded that insufficient evidence is available to recommend treatment with nitro-derivatives and that caution should be exercised in light of side effects [87]. While the World Health Organization and the Pan American Health Organization have previously recommended treatment of chronic disease [1,83], experts at the Scientific Working Group on Chagas disease in 2005 emphasized the lack of evidence, the risk of side effects, and the need for future research [88].

Based on the limited efficacy of the current standard drugs and their poor 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 treatments. Additionally there are limitations in the formulations of anti-*Trypanosoma cruzi*, e.g. drugs for infants and children not available in syrups or solutions. Secondary to increased knowledge of parasite metabolism and the current focus on parasitologic treatments for chronic disease, there has been renewed interest in the search for new Chagas chemotherapies for chronic disease. The following are highlights of drug development research organized by *T. cruzi* metabolic targets [82,89,90,92].

New Therapeutic Targets for Chagas Disease Treatment

Sterol Synthesis: The main sterol in *T. cruzi*, unlike in humans, is ergosterol, making its synthesis a prime candidate for pharmacological inhibition. While antifungal sterol inhibitors such as ketoconazole are not effective anti-parasitics [91], some new triazole derivatives (eg. posoconazole, ravuconazole, TAK-187) have shown *in vivo* efficacy in murine acute and chronic disease and are in preclinical development [92-94]. Synergistic combination with amiodarone has recently been suggested [95].

*Cruzi*pain (Cysteine Protease): *Cruzi*pain is a protease essential for replication of the intracellular parasite, and its inhibition is trypanocidal [96]. Chemical modification of scaffold compounds have demonstrated increased potency [97]. These drugs are potentially limited by parasite development of resistance, but are in preclinical trials [98].

Purine Salvage: *T. cruzi* cannot synthesize purine nucleotides *de novo* and must salvage exogenous purines. Purine base analogs such as allopurinol or inhibitors of hypoxanthine-guanine phosphoribosyltransferase interfere with HGPRT-mediated purine salvage, thereby selectively targeting parasites. Allopurinol has shown some efficacy in chronic disease [99,100], and in reactivation after cardiac transplant [101]. Other analogs have shown *in vitro* activity [102].

Farnesylpyrophosphate Synthase: FPPS is an enzyme involved in the synthesis of sterols and polyisoprenoids. Bisphosphonates such as risedronate and pamidronate inhibit FPPS and result in reduced parasitemia in mice [103,104]. FPPS is found in all eukaryotic cells, but these drugs are appear to act as selective inhibitors, probably because of preferential uptake into acidocalcisomes, the condensed stores of phosphates in *T. cruzi*.

Trypanothione Synthesis and Metabolism: A parasite-specific molecule, trypanothione, participates in several important metabolic processes such as scavenging, DNA precursor synthesis, and in oxidative stress. Inhibitors of trypanothione reductase, essential for trypanothione function, show promise as trypanocidals. These include the nitrofurans (which include nifurtimox), naphthoquinones, and the tricyclic neuroleptic drug phenothiazine.

Other targets: Lysophospholipid analogs such as miltefosine are currently under investigation for use against leishmaniasis and show promise as low toxicity trypanocidal agents [105,106]. Inhibition of both class I and II DNA topoisomerases has shown *in vitro* trypanocidal activity [107,108]. Dihydrofolate reductase (DHFR) and trypanosomatid-specific pteridine reductase (PTR1) are targets for interference with parasite nucleotide synthesis [109,110]. Another possible direction is suppression of intracellular glycolytic energy production by inhibition of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) [111,112]. Inhibitors of inositol metabolism, polyamine synthesis, and the phosphotransferase arginine quinase have also been suggested as potential targets [90].

Finally, as to prevention of disease, there are currently no active vaccine development programs [113], though several potential vaccine targets have been investigated. Murine models have shown some success with purified parasite proteins [114] and DNA vaccines [115,116]. Future vaccine development may be enhanced by

information from genome and proteomic analyses with strategies such as reverse vaccinology.

Future

As the preceding overview reveals, Chagas disease is a widely dispersed vector-borne disease carrying an enormous burden of morbidity and mortality with the potential to affect millions of people. Vector control efforts have achieved laudable success, but with the additional challenge of peridomestic vectors and the emergence of disease into non-endemic areas, sustainability remains precarious. Many new challenges have been posed by the threat of this disease, as occurs with the migration of people from endemic countries and the risk of contaminated blood transfusion in non-endemic countries, as well the incidence and management of congenital Chagas disease. Further, critical gaps remain in our knowledge of Chagas pathogenesis, diagnosis, and treatment and few drug treatment options are available. The current situation calls for intensification and continuation of research in these areas with the aim of eradicating this neglected disease [10,116-122].

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