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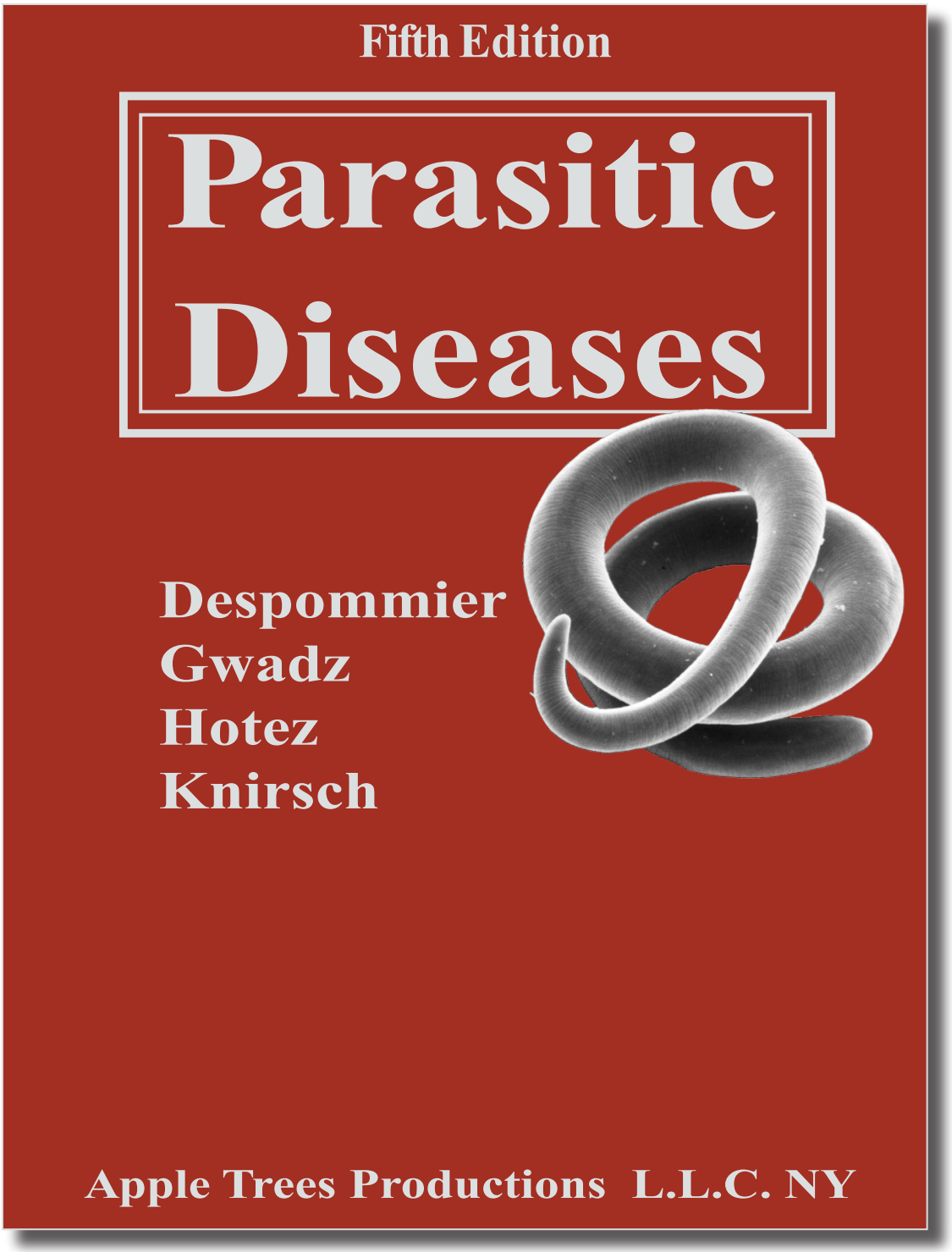


7. American Trypanosomiasis:

Trypanosoma cruzi
(Chagas 1909)

Introduction

Trypanosoma cruzi is the cause of American trypanosomiasis, also known as Chagas' Disease. It is an intracellular parasite for the majority of its life, in contrast to its relatives, the African trypanosomes, that live in the blood and lymph. *T. cruzi* infects nearly all species of mammals native to South and Central America and is vector-borne. Insects in the order Hemiptera (true bugs), called "kissing bugs," are the only known vectors (see Fig. 38.29). Chronic infection with this parasite often leads to life threatening diseases of the hollow organs. It is one of the world's leading causes of cardiomyopathy. *T. cruzi* is found throughout Central and South America, and occasionally in the Southwestern portion of the United States.[1, 2] In contrast to most of South America, the incidence rate in Brazil and Chile is now under 1% in children under the age of 10. Transmission has been essentially eliminated in those two countries.[3] The disease has a case fatality rate of about 5% in its acute phase.[4]

Infection can be transmitted through blood transfusion,[5, 6] bone marrow transplants,[7] and organ transplants.[8, 9] *T. cruzi* can also infect the fetus across the placenta.[10] An outbreak in Santa Catarina, Brazil, of a particularly virulent strain of *T. cruzi* was initiated by drinking sugar cane extract that apparently was contaminated with "extract" of kissing bug. Some 30 people were infected, with a high degree of mortality. Apparently, the infected bug got into the processing apparatus by accident. Sugar cane extract is a popular drink on many of the beaches of Brazil. This is the first recorded epidemic of Chagas' disease in which transmission was by the oral route (see ProMed report on 04/01/05).

Figure 7.1. Kissing bug nymph, feeding.

Figure 7.2. Thatched roofed hut. Ideal breeding sites for kissing bugs.

Rats, dogs, sloths, bats, and various non-human primates are important reservoir hosts, depending upon the region. Transmission takes place in both rural and urban settings. Incidence is highest in children, with the notable exceptions of Brazil and Chile.[11]

Historical Information

Carlos Chagas, in 1909,[12,13] observed the infective stage of *T. cruzi* by chance while conducting a survey for vectors of malaria. He inoculated many species of mammals with the new agent and showed that they all became infected. He correctly speculated that humans were likely to be infected as well. He identified infected people in rural areas of Brazil. Chagas also described the major clinical features of the disease and the morphology of the trypomastigote stage of the parasite. All this work was accomplished within months after his initial discovery. He named the organism after his beloved teacher and close friend, Oswaldo Cruz. Chagas went on to describe the essentials of the life cycle as well. Brumpt, in 1912,[14] completed the description of the life cycle of *T. cruzi*, while Vianna, in 1916,[15] published the details of the pathological consequences of infection with this important pathogenic protozoan.

Life Cycle

The biology, molecular biology, and epidemiology of American trypanosmiasis have been reviewed.[16–18] Organisms (metacyclic trypomastigote stage) are present in the fecal droppings of the infected reduviid bug. (Fig. 7.1) Transmission occurs usually by a person rubbing the organisms into a mucous membrane or bite wound. Triatomid bugs are large, robust insects, and characteristically feed at night, biting the victim near the mouth or eyes while they are asleep. The bite itself

is painless, hence the term "kissing bug." The vector ingests a large quantity of blood, and in order to make room for the new meal, it simultaneously defecates the remains of the last one, depositing it adjacent to the bite wound. The salivary secretions of the bug induce itching, causing the victim to rub the bug feces, laden with parasites, into the wound, or mucus membranes.

The infection can also occur without direct contact with the vector. Thatched roofs of rural houses can harbor large numbers of the bugs (Fig. 7.2), and their feces have the opportunity to fall onto people while they are sleeping. Individuals become infected simply by rubbing the parasites into their mucous membranes of the eye or oral cavity. The probability of infection by this route is high, because triatomids feed on many mammals, and rural peoples live in close proximity to their livestock and pets. Infection by transfusion, organ transplantation, or congenital transmission introduces the parasite directly into the host. For a somewhat gory account of what it's like to wake up covered with well-fed reduviid bugs, see Charles Darwin's description in his famous journal, *Voyage of The Beagle*. Because of this encounter, much speculation has centered around the possibility that Darwin actually contracted and suffered from chronic Chagas' disease. In fact, he most likely suffered from lactose intolerance masquerading as Chagas' disease![19]

Attachment to host cells is mediated through galectin-3 on the surface of host cells.[20] The parasite protein that binds to galactein-3 has yet to be identified. The trypomastigote can penetrate a wide variety of cells,[21] and the process is mediated by calcium ions and at least two parasite membrane proteins: a neuraminidase/trans-sialidase, which binds to sialic acid, and penetrin, which binds to heparin sulfate.[22] Another protein, gp82 might also be necessary for penetration into gastric epithelium if the metacyclic trypomastigote stage is swallowed,[23] as was the case in the recent outbreak in Santa Catarina, Brazil, involving the ingestion of sugar cane juice contaminated with at least one infected reduviid bug. Animals can become infected by ingesting infected reduviid bugs, and this might be the usual way for them to pick up the infection.[24]

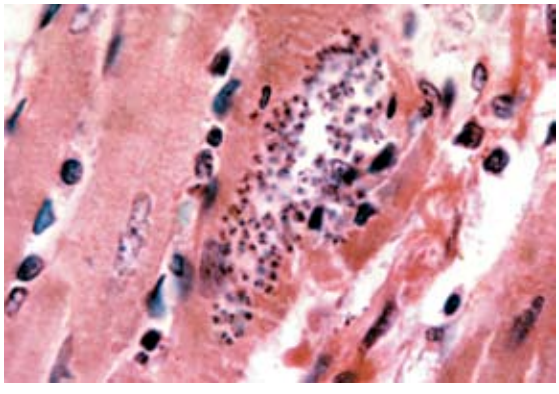


Figure 7.3. Histologic section of heart muscle infected with *Trypanosoma cruzi* amastigotes.

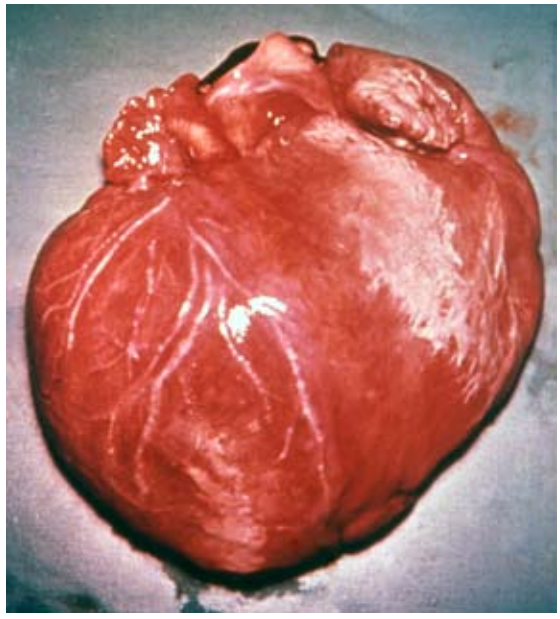
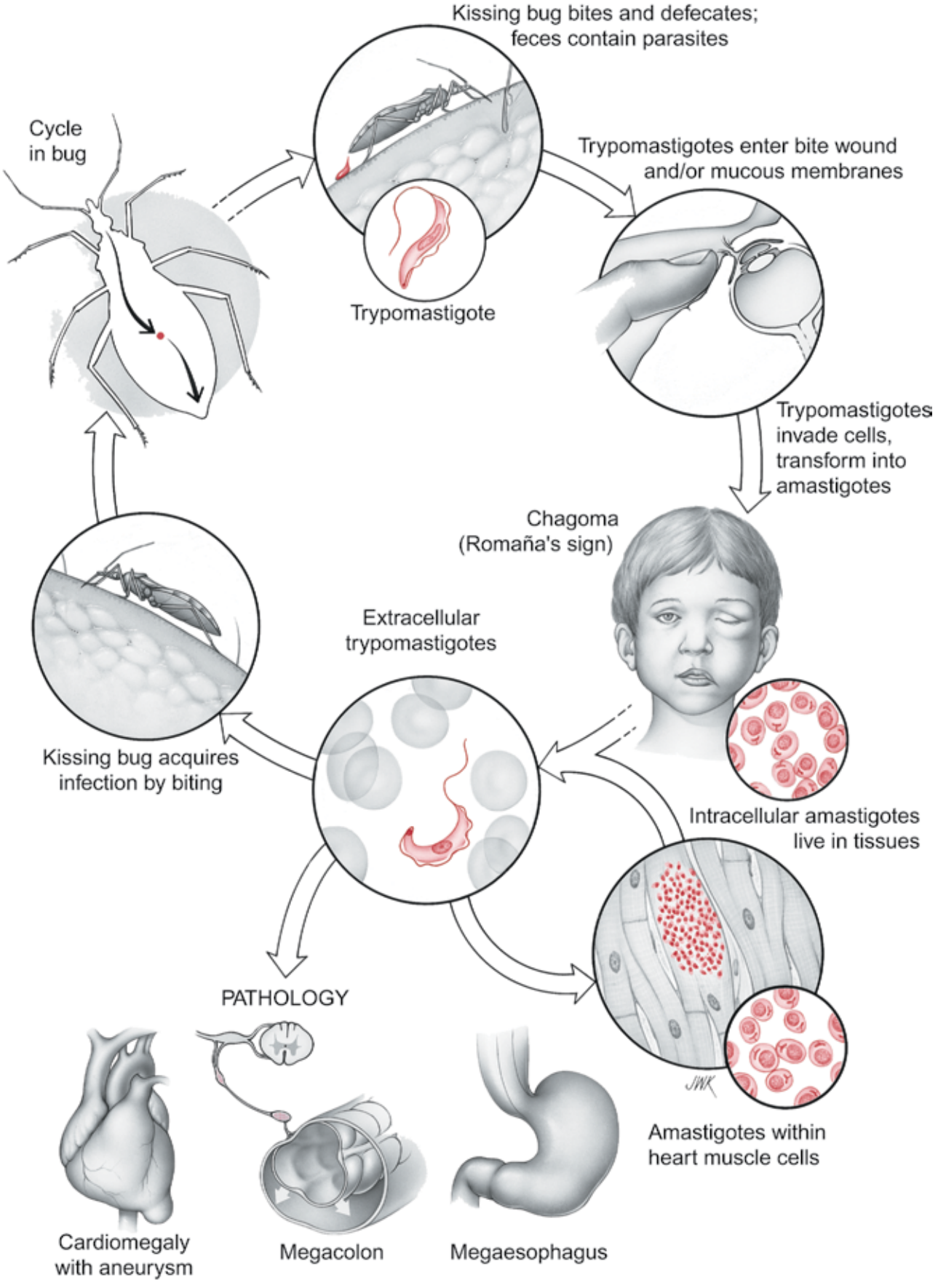


Figure 7.4. Enlarged heart of a patient who died of chronic Chagas' disease.

After entering the parasitophorous vacuole, the trypomastigote enlists several escape mechanisms to aid in its survival there. It begins by neutralizing the pH of that intracellular space, thereby escaping the potentially damaging effects of exposure to the active forms of lysosomal enzymes.[22] The organism also produces a number of proteins which offer it additional advantages once inside the host cell. Chagasin is a cysteine protease inhibitor and is apparently necessary for avoiding lysosomal-derived cysteine protease activity and insures that the parasite has the time needed to differentiate into the amastigote stage.[25] Cruzipain is thought to play a major role in helping the parasite avoid being digested once inside the parasitophorous vacuole. Cruzipain also induces the upregulation of host-derived arginase-2, a known inhibitor of apoptosis.[26] Thus, the parasite may be engineering the longevity of its host cell, while at the same time, avoiding the ravages of lysosomal digestion.

The parasite then rapidly penetrates into the cytosol and differentiates into the amastigote stage. This is the dividing form of *T. cruzi* and the one that inflicts cell damage on the host. After several division cycles, some of the parasites transform back into trypomastigotes. The affected cells die, releasing the parasites that can now enter the bloodstream and become distributed throughout the body. They infect cells in many types of tissues, including the central nervous system,

Trypanosoma cruzi



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heart muscle, the myenteric plexus, the urogenital tract, and the reticuloendothelial system.

Triatomids become infected by taking a blood meal from an infected individual.[27] The trypomastigote migrates to the midgut of the insect, where it transforms into the epimastigote, and then undergoes many divisions. Thousands of organisms are produced within one insect without apparently affecting it. The triatomids remain infected for life (i.e., 1-2 years). Epimastigotes maintain their place in the gut of the insect by specific receptor-ligand interactions involving at least one parasite surface glycoprotein and a carbohydrate lectin on the gut cells of the insect.[28] Ultimately, epimastigotes transform into metacyclic trypomastigotes and migrate to the hind gut, and from there they are excreted with feces following the taking of a blood meal.

Cellular and Molecular Pathogenesis

Infection with *Trypanosoma cruzi* results in partial immunosuppression[29, 30] that further aids the parasite in remaining inside the host cell for extended periods of time. For example, in vitro culture of human dendritic cells infected with *T. cruzi* resulted in a dramatic down regulation of synthesis of IL-6, IL-12, TNF-α, HLA-DR, and CD-40, and inhibited their maturation into antigen processing cells.[31] Parasite-derived calreticulin may also be important for amastigote survival in the intracellular environment, implicating a central role for calcium trafficking and storage in the life of the parasite.[32]

Release of trypomastigotes into the blood stream seemingly places them at risk for immune attack, since serum antibodies against them can be demonstrated at this time in the infected host. However, *T. cruzi* has an answer for this defense strategy. The surface coat of the free-swimming trypomastigote contains a specific compliment regulatory protein[33] that binds the C3b and C4b component, inhibiting the alternate pathway.

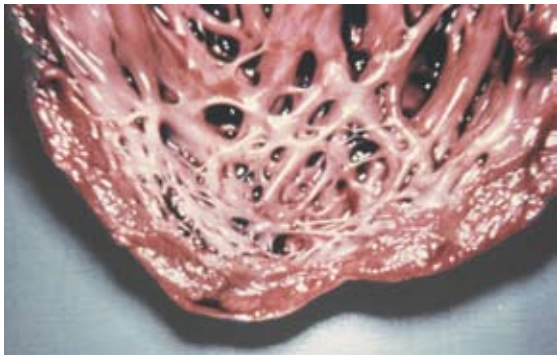

Figure 7.5. Portion of enlarged heart of a patient who died of chronic Chagas' disease. Note thin wall of ventricle.

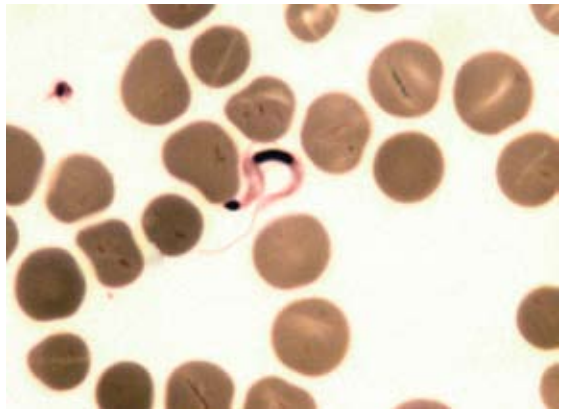

Figure 7.6. Trypomastigote of *T. cruzi*. 20µm x 3µm.

Host protection can develop, despite these highly evolved parasite evasion mechanisms. Immunity depends on CD1d antigen presentation and the upregulation of IL-12 for the production of natural killer cells, the protective arm of the immune system most effective against the amastigotes in the tissues.[34] Parasites are killed is by way of induction of nitric oxide synthase and the production of nitric oxide.[35] CD8+ T cells with specificities for parasite antigen are thought to be essential in maintaining some control of the infection throughout the chronic phase.[36]

Chagas' disease manifests in all hollow organs. Infected individuals remain so for life and most of the pathological consequences are those resulting from cell death (Fig. 7.3) Myenteric plexus damage results in loss of muscle tone and enlargement of the organ, particularly the digestive tract. Megacolon and megaesophagus are late onset sequelae to chronic infection. Heart damage is almost invariably associated with Chagas' disease in some regions of Central and South America,[37] and is detectable early on in infection.[38] Erosion of heart tissue is typical, and in many cases results in aneurysm and heart failure (Figs. 7.4, 7.5).

Current thinking regarding a dominant role for auto-antibodies inducing cardiomyopathy plays down this mechanism to account for heart damage during chronic infection.[38] This is because: 1. PCR has been able to demonstrate the presence of *T. cruzi* in heart tissue, even at times when biopsy material used in conventional histological mode could not reveal the presence of the parasite,[38] and: 2. disease progresses rapidly when parasites are abundant and not as fast when they are hard to demonstrate on biopsy.[39] Nonetheless, auto-antibodies have been detected in many individuals suffering from long term infection with *T. cruzi*.[40, 41] Meningoencephalitis occurs during the acute phase and is characterized by infiltrates of CD8+ T cells.[42]

Clinical Disease

Acute Chagas' Disease

A review of clinical aspects of Chagas' disease is available.[43] The incubation period for Chagas' disease is 4-12 days after introduction of the organisms. The acute stage is often asymptomatic, or the symptoms that develop are generalized and the disease is therefore misdiagnosed. A chagoma develops at the site of the bite within 2-4 days. If organisms are introduced into the body through mucous membranes by rubbing them into the eye, then the swelling associated with the chagoma is known as Romaña's sign. It occurs mostly as a unilateral swelling. The swollen eyelid is firm to the touch, and there may be associated conjunctivitis. If the bite occurs elsewhere, the adjacent area is erythematous, brawny, and firm to the touch. When the chagoma disappears after several weeks, it leaves an area of depigmentation. An associated neuropathy develops, and then disappears when the patient enters the chronic phase of the infection.

Systemic involvement includes fever, lymphadenopathy, hepatomegaly, splenomegaly, and myocarditis. The myocarditis of acute Chagas' disease presents with tachycardia, congestive heart failure, and cardiomegaly.

Chronic Chagas' Disease

Most patients survive the acute phase and become asymptomatic. The chronic phase can last the rest of their lives (i.e., 20-30 years). Infection persists, exacting its toll on all affected organs, particularly the heart. Cardiac involvement can be silent for some years after infection, and limited to ECG abnormalities consisting of conduction defects. A complete heart block with progressive destruction of the myocardium and conduction system leads to Chagas' cardiomyopathy. Ultrastructural studies showed that vinculin costameres in cardiomyocytes become disrupted during intracellular infection with the amastiogote stage, and this is thought to make a major contribution to the cardiomyopathy so typically seen in the chronic infection.[44]

Clinically, the patient experiences extrasystoles, right ventricular enlargement, and eventually heart failure. Right bundle branch block is typical, and eventually may lead to death.[45]

Gastrointestinal involvement includes development of megaesophagus, characterized by dysphagia and regurgitation, and megacolon,[46] leading to constipation and fecal retention.

Disease is not limited to the heart and gut. Rarely it leads also to megaureters, megabladder, megagallbladder, and bronchiectasis.

Patients suffering from HIV/AIDS exhibit signs and symptoms of the acute phase of infection, and if left untreated, usually die from overwhelming infection due to *T. cruzi*.[47]

Patients in the chronic phase of infection that acquire HIV can experience a reactivation of *T. cruzi* resembling the acute phase of the disease.[48]

Diagnosis

The use of PCR using primers based on kinetoplast DNA sequences[49, 50] has greatly facilitated definitive diagnosis in patients with chronic infection, and has essentially replaced ELISA-based immunological tests as the test of choice, next to microscopic identification (Fig. 7.3). Although PCR would also be considered practical for screening in blood banks, a chemiluminescent ELISA appears to offer more promise.[51] Parasites can also be identified microscopically from biopsy samples of infected tissue. Inoculating blood from suspected individuals into susceptible animals can reveal the organism, but this approach presents too many impracticalities for most diagnostic facilities. Xenodiagnosis, employing uninfected reduviid bugs, allowing them to feed on the patient, then dissecting the bugs some days later, can also reveal the presence of parasites in chronically infected individuals, but it is a special test requiring extensive laboratory infrastructure and technical assistance.

Treatment

The drugs of choice are nifurtimox[52] and benznidazole.[53] Neither drug is recommended without reservation; they are both associated with high toxicity and incomplete cure rates in adults, especially when they are used to treat the chronic phase of the infection.[53] Benznidazole is recommended for use in children who have either just acquired the disease (i.e., congenitally), or who are in the chronic phase of their infection.[54] Very little is known regarding the mode of action of either drug. Nifurtimox may exert its toxic effect by reacting with sulfhydryl compounds such as coenzyme A, glutathione, and cysteine.[55] Itraconizole and allopurinol[56] have also been tried with about a 40-50% cure rate. Newer drugs are being developed, some of which take advantage of known metabolic pathways.[57, 58]

Heart transplantation as a treatment modality for the cardiomyopathic aspects of Chagas' disease has been in vogue for as long as heart transplantation has been tried in humans.[59, 60] In fact, the fourth recipient ever to receive a heart transplant suffered from chronic *T. cruzi* infection. However, the use of cyclosporin-A for immunosuppression, in order to prevent rejection of

the transplanted heart, allows *T. cruzi* to reproduce uncontrolled, resulting in the death of the patient in most cases.

Prevention and Control

Control of Chagas' disease depends upon interfering with two major routes of transmission; vector-borne and transfusion. Control of vectors, by prudent use of insecticides (pyrethroids), has significantly reduced transmission of *T. cruzi* in Brazil and Chile.[61, 62] Unfortunately, this trend has been slow to spread to neighboring countries. Prevalence remains at about 15 million throughout Central and South America. Transfusion-induced infection is still on the rise,[63] especially in countries where *T. cruzi* is not vector-borne, complicating the control of disease. Blood bank screening for *T. cruzi* should be mandatory in all countries experiencing high rates of immigration from South and Central America. Paid blood donors should be outlawed in all countries in which Chagas' disease is endemic.[63] A more permanent solution, and one that interfaces well with the concepts of medical ecology, is building better housing for the poor.[64, 65]

Houses constructed without a thatched roof, the slat board wood siding, or rough textured wall surfaces inside the house are relatively safe from reduviid bug colonization. Keeping pet dogs and pigs out of the house further reduces the chances of acquiring Chagas' disease.[66]

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