

Myopathogenesis of Chagas' Disease

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Abstract

Chagas' disease, caused by *Trypanosoma cruzi*, is an important cause of cardiac disease in Latin America. The etiology of chagasic cardiomyopathy is unclear. However, in recent years compromised coronary microvasculature, neurogenic dysfunction and autoimmunity have all been proposed as important in the etiology of chagasic cardiomyopathy. In addition other myopathies and neurological manifestations have also been associated with this infection.

Key words: *Trypanosoma cruzi*, Chagas' disease, myopathy, cardiomyopathy.

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T*rypanosoma cruzi* is a hemoflagellate protozoan that causes Chagas' disease, an important cause of heart disease in Latin America [4]. Examination of autopsied Indian mummies suggest that Chagas' disease was present in South America in the pre-Colombian era [146]. Millions of people are infected with this parasite and approximately 50,000 people die annually [42]. *T. cruzi* has been found in insect vectors and mammals in the United States.

Natural transmission of *T. cruzi* to humans is associated with the bite of the reduviid bug. During the blood meal excreta containing infectious metacyclic trypomastigotes are deposited onto the bite and other mucosal surfaces. In endemic areas of Latin America, it is most common among the rural poor who live near feral reservoirs. Control programs directed at decreasing transmission of the parasite to humans have met with varying success and a reliable vaccine has not been developed [73].

Blood transfusion is another important mode of transmission [198] which is not only common in endemic areas but has also been reported in North America where there is an increase in the chronically infected Latin American population [54]. Congenital Chagas' disease has been reported and breast feeding, organ transplantation, and laboratory accidents are other occasional modes of transmission [16, 21, 23, 137].

Life Cycle

The life cycle of *T. cruzi* is complex. Four life stages exist and are differentiated by morphological and biochemical criteria. Trypomastigotes (extracellular nondividing forms) and amastigotes (intracellular replicative forms) are found in mammalian hosts. After the insect vector takes a blood meal containing trypomastigotes, the parasites transform into epimastigotes which multiply in the insect midgut. After 3 to 4 weeks, infective metacyclic trypomastigotes are present in the hindgut of the vector. These are then deposited in excreta during subsequent blood meals and may then enter new mammalian hosts. Trypomastigotes may be phagocytized or directly penetrate cells and undergo transformation to amastigotes [32, 154-157, 178]. Ortega-Barria et al [121] have demonstrated a 60 kDa *T. cruzi* surface protein, penetrin that binds to heparan sulfate receptors on non-phagocytic host cells, thereby, promoting adhesion and invasion. Within the parasitophorous vacuole the parasite may be killed by a variety of cytotoxic mechanisms [52, 82, 127, 168]. However, the intracellular amastigote synthesizes a hemolytic protein, active at acid pH, capable of lysing the vacuolar membrane. This allows the parasite to evade the cytotoxic mechanism of the host, lie free and replicate within the cytoplasm [11]. Parasitized host cells eventually rupture and release trypomastigotes and amastigotes which reinfect other cells. It has been

these agents in suppressing *T. cruzi* parasitemia. More recently, Viotti *et al* [197] have reported on the successful treatment of chronically infected patients with benzidazole. Data from our laboratory suggests that verapamil might also be useful as adjunctive therapy [112, 176]. However, the chemotherapy of chronic Chagas' diseases remains controversial and more clinical studies are required.

Immunosuppression

Immunosuppression of experimental animals and chronically infected patients can lead to recrudescence of the disease [9, 74, 80, 84, 140]. Patients at risk for whom immunosuppressive treatment is being planned, either as primary or post-transplantation therapy, should be required to undergo serological testing and if positive should be monitored closely for evidence of reactivation while immunosuppressed. In addition, there are several recent reports of reactivation of *T. cruzi* infection in HIV-infected patients [36, 142]. Of interest is that all of these patients developed cerebral lesions, which are rarely seen in immunocompetent patients.

Patients with end-stage Chagas' heart disease have undergone cardiac transplantation in Brazil and the United States [86, 164] and the immunosuppression administered after heart transplantation resulted in reactivation of *T. cruzi* infection in the majority of these patients.

Experimental Considerations

The etiology of Chagas' disease is multifactorial and is the result of the interaction between the parasite and host. In a number of *in vivo* and *in vitro* models some of the possible factors have been elucidated.

Parasite Biochemistry

The biochemistry of the parasite may be a contributing factor in the pathogenesis of cardiomyopathy. Trypanosomes contain a variety of enzymes (proteases, gelatinases and collagenases) which may degrade native type I collagen, heat denatured type I collagen (gelatin) and native type IV collagen [56, 174]. Proteolytic activities against laminin and fibronectin were also detected. These enzymes may play an important role in the degradation of extracellular matrix (ECM) and the subsequent tissue invasion by the parasite. It has been proposed that degradation of the collagen matrix, evident in acute murine Chagas' disease, may result in chronic pathology such as apical thinning [45]. In addition, supernatants obtained from cultures of infected fibroblasts, vascular smooth muscle cells, and myocardial cells were found to stimulate fibroblast DNA and protein synthesis as well as proliferation, suggesting a mechanism by which fibrosis may occur in chronic chagasic cardiomyopathy [199]. Trypomastigotes contain both sialic acid and a developmentally regulated trans-sialidase [174] that removes sialic acid from the surface of cardiac and endothelial cells [85]. The loss of sialic acid from cardiocytes may alter intracellular Ca^{2+} homeostasis

and ultimately myocardial function. This enzyme can transfer sialic acid from host glycoconjugates to compounds located on the surface of the parasite. The *T. cruzi* trans-sialidase promotes adhesion to host cells and is inhibited by antibodies to this enzyme and high-density lipoprotein (HDL) which enhance the infectivity of cells *in vitro* [132, 133]. It is noteworthy that mice with higher levels of HDL are more susceptible to *T. cruzi* infection [133]. The biochemistry and molecular biology of this unique enzyme has been extensively studied [101, 158].

Morphologic and Biochemical Studies

During the course of murine Chagas' disease structural and biochemical alterations of the myocardium have been reported and may be related to the development of cardiomyopathy. For example, the destruction of autonomic ganglia may have profound implications not only for the development of heart disease but also for gastrointestinal involvement. In addition, it has been shown that during acute infection endothelial cells of the coronary vasculature were parasitized and associated with focal alterations in the coronary microvasculature [170].

Factor *et al* [45] have extensively examined the ECM of the heart using a silver impregnation technique. The skeletal framework surrounds and interconnects myocytes to each other and to non-myocyte structures such as blood vessels. The ECM consists of collagen fibers (usually types I and III), proteoglycans, fibronectin and basement membrane components (i.e., collagen types IV, laminin). The function of the ECM is the enhancement and coordination of forceful muscle contraction. Loss of this ECM can lead to the development of a thin-walled, dilated and poorly contracting ventricles even in the absence of myocyte injury. It was found that the myocardium of infected mice had multiple areas that were devoid of ECM. The degradation of ECM was associated with parasites even in the absence of inflammation. Of interest was the observation that these changes also developed in areas of normal myocardium adjacent to infected and necrotic areas thereby enhancing the effect of parasitic infection *per se*. These changes may lead to remodelling of the ventricles resulting in cardiomyopathy. In addition, areas of the left ventricular apex showed marked loss of silver impregnated connective tissue which contributes to the development of apical aneurysm (Figure 2). It has been suggested that *T. cruzi* infection is associated with microvascular spasm resulting in transient ischemia without necrosis. This may lead to damage of the ECM in regions without overt pathology such as at the ventricular apex [45].

Biochemical alterations of the myocardium have been reported in *T. cruzi* infection which may lead to cardiomyopathy. For example, reductions in myocardial choline acetyltransferase (CAT) [171], acetylcholine (Ach) [90], norepinephrine (NE) [91] and adenylate cyclase [105, 110] have been reported in animal models. Of interest is that many of these biochemical perturbations antedate morphological alterations.

The ultimate function of the microvascular and cardiac myocyte involves the operation of an integrated system of complex, macromolecular signal transduction pathways [39]. Through these macromolecular complexes, physiologic and pharmacologic ligands interact with cell surface receptors and initiate a cascade of biochemical events that ultimately result in a physiological response. A number of studies suggest that in pathological states, abnormalities in the integrity of the components of the signal transduction complex participate in the dysfunctional myocardial activity. Accordingly, we have focused attention in evaluating the extent to which infection with *T. cruzi* influences the integrity of components in the signal transduction pathways affiliated with the microvasculature as well as the cardiac myocyte.

In one type of signal transduction pathway, a membrane receptor interacts with a heterotrimeric GTP-binding protein (G-protein), composed of an "active" α subunit linked to a membrane bound-hydrophobic $\beta\gamma$ subunit. Upon activation of the G-protein, the α subunit interacts with an effector-catalytic unit. Direct actions of the β subunits have also been reported. The activated α subunit in conjunction with a catalytic effector unit thus results in either the generation of cAMP, or the hydrolysis of P_1P_2 to produce IP_3 and diacylglycerol. Alternatively, activated G-protein α or β subunits may communicate more directly with plasma membrane ion channels, permitting the accumulation of intracellular cations such as Ca^{2+} , resulting in action potentials and other physiological responses. The signal transduction pathways facilitating normal myocardial contractility include β and α adrenergic receptors that are linked to adenylate cyclase, phospholipase C or Ca^{2+} -channels. Microvasculature endothelial and smooth muscle cells also possess signal transduction pathways, which respond to a number of stimuli including endothelin, bradykinin, catecholamines and nitric oxide, and include intermediary G-proteins linked to phospholipase C and/or adenylate cyclase [39].

Our earliest observations associated a compromise in the activity of the β -adrenergic receptor (β AR) complex of the myocardium with acute murine Chagas' disease [62, 63]. Moreover, the association of this decline in β AR activity and the microvasculature was strengthened by the observation that treatment of infected mice with verapamil, a Ca^{2+} channel blocker, reduced the mortality of acute murine Chagas' disease, modified the cardiac pathology and preserved β -adrenergic adenylate cyclase activity (ACA) [112]. Similarly, verapamil treatment also prevented the appearance of cardiomyopathy in the hypertensive Syrian hamster model and in viral-induced cardiomyopathy [40]; both models of myocardial dysfunction associated with microvascular pathology. The basis for the action of verapamil remains to be determined, but is thought to involve protective actions at the level of the microvasculature as well as the cardiac myocyte *per se*. A direct relationship between Ca^{2+} channel blockade and

β AR complex activity has been well established. Moreover, Ca^{2+} plays a critical role in endothelial, smooth muscle and cardiac function, and abnormalities in Ca^{2+} homeostasis have been observed *in vitro* and *in vivo* under stressful conditions such as ischemia. Elevated intracellular Ca^{2+} was reported in cardiac myocytes from cardiomyopathic Syrian hamsters as well as in *T. cruzi*-infected cardiac myocytes and endothelial cells [19, 106, 108]. It should be noted, however, that increased intracellular Ca^{2+} in cardiac myocytes did not result in depressed myocardial β -adrenergic ACA [19].

Recently, we have extended our observations on the effect of *T. cruzi* infection on signal transduction pathways. Infection profoundly altered the G-proteins associated with myocardial β -adrenergic adenylate cyclase complex, specifically substrates for cholera toxin (CT)-dependent ADP-ribosylation, Gs, as well as Gi and Go, substrates for pertussis toxin (PT)-dependent ADP-ribosylation. Gs and Gi serve as the stimulatory and inhibitory, respectively, mediators of ACA. CT or PT-catalyzed ADP-ribosylations result in the covalent linkage of ADP-ribose to the α subunits of their respective G-protein substrates [66]. In the intact membrane, accessibility and state of activation of the G-proteins, as well as other factors, ultimately dictate the magnitude of the reaction. Thus, these reactions performed with cell membranes provide much information about the functional state of the G-protein substrate [109]. In contrast, identification of the individual G-proteins by Western blot analysis using antisera directed against unique sequences of the α subunits provides more quantitative information about these proteins, since the reaction conditions destroy membrane architecture and remove barriers to identification.

Acute *T. cruzi* infection altered both the magnitude (decreased) and the kinetics (accelerated) of ADP-ribosylation of the PT-substrates in cardiac tissue [63]. The decline in PT-dependent ADP-ribosylation was not simply due to a general decrease in levels of the Gi and Go protein substrates; immunochemical studies revealed an infection-associated decrease in α_{i1} , and α_o , but no change in α_{i3} and a marked increase in α_{i2} [63]. To account for the differences in results in the analysis of Gi and Go proteins by PT-dependent ADP-ribosylation and immunochemical analysis, it appeared that infection altered the organization as well as the amount of these G-protein substrates within the plasma membrane. Interestingly, we found that infection-associated changes in the PT substrates were evident independent of verapamil treatment. Therefore, although verapamil-treated infected mice were clinically normal and had normal β -adrenergic ACA the changes in properties of the G-proteins persisted. This suggested that verapamil treatment fostered an alternative pathway through which the cell could overcome the alterations resulting from infection.

In murine Chagas' disease there are alterations in the myocardium that are both directly attributable to the presence of the parasite, as well as the systemic response

to the parasite *per se*. Therefore, to focus on the parasite-tissue interaction directly, similar studies were performed using infected neonatal rat cardiac myocytes. In these studies, identical changes in G-proteins were observed [63]. However, unlike the mouse myocardium, β -adrenergic ACA was unaffected by infection, in contrast to the marked influence on α -adrenergic-mediated contractility and intracellular Ca^{2+} homeostasis [19]. This suggested that other factors, such as compromised microvasculature, influenced myocardial function.

More recent studies delineated the functional consequences of the infection-associated changes in G-proteins [62]. These studies employed as a tool the so-called "release phenomenon". The "release phenomenon" occurs when membranes are incubated with GTP γ S resulting in the dissociation of heterotrimeric G-protein into the membrane bound $\beta\gamma$ and soluble α subunits. The release of the α subunit into the cytosol, free to associate with other subcellular organelles, is thought to be an additional pathway for G-protein modulation of cellular physiology [165]. In a study of the influence of infection on GTP γ S-dependent release, we monitored GTP γ S-dependent release of α subunits by two different methods: [^{32}P]-ADP-ribose labelled proteins or by direct identification using specific antibodies. For reasons stated above, $\text{G}\alpha$ substrates that are toxin radiolabelled are a small part of the pool of total G-proteins. Infection decreased the magnitude of [^{32}P]ADP-ribose free α substrates labelled by PT released in response to GTP γ S; paradoxically, the relative amounts of immunochemical $\alpha_{i,o}$ subunits released in response to GTP γ S, were markedly increased post-infection. This suggested that in the infected animal, the inhibitory G-proteins are far more likely to be activated i.e. released in any given concentration of GTP, when compared to uninfected membranes. This may contribute, in part, to the infection-associated decrease in ACA.

Host Cell Signal Transduction Alterations

Additional studies of the influence of infection on individual cells has provided confirmatory evidence that the parasite *per se* can directly interfere with host cell biochemistry, specifically components of the signal transduction pathway. We have reported perturbations in the generation of cAMP in myoblasts and endothelial cells [107, 111]. In contrast to an influence of infection on components of the signal transduction pathway, the generation of endothelial cell cAMP in response to hormonal stimulation in infected cells is significantly reduced by virtue of an increase in endogenous phosphodiesterase. There are infection-associated changes in the production of prostacyclin [170] and in the mobilization of intracellular Ca^{2+} [106, 108]. These perturbations in endothelial-cell signal transduction mechanisms may contribute to focal pathology, which may include coronary microvascular spasm [43].

Another important aspect of perturbations of host cell signal transduction pathways in *T. cruzi* infection occurred as the result of modifying the extent to which receptor function changed in response to infection. Infected neonatal rat cardiac myocytes were studied with regard to chronotropic responses and intracellular Ca^{2+} mobilization following addition of adrenergic agonists. Spontaneous beats of control myocytes were more rapid than in infected cells [19, 27]. The beat rate responded to isoproterenol (ISO) and norepi nephine (NE). The effect of ISO on infected and uninfected cells were similar, and ACA was similar in control and infected cells. However, NE produced a more marked chronotropic response on infected cardiac myocytes and altered intracellular Ca^{2+} mobilization as determined by single-cell analysis [19]. In control and infected cells, NE increased intracellular Ca^{2+} levels in the presence and absence of external Ca^{2+} . However, basal and α -adrenergic-stimulated intracellular Ca^{2+} levels were higher in infected cells. Nevertheless, the fractional increase in infected cardiac myocytes of intracellular Ca^{2+} following NE exposure was lower than that observed in controls. Therefore, both chronotropic and intracellular Ca^{2+} mobilization responses to NE, an α -adrenergic agonist, were altered in *T. cruzi*-infected rat neonatal cardiac myocytes; the chronotropic responses to ISO, a β -adrenergic agonist, were unaffected. In embryonic mouse cardiac myocytes, Aprigliano *et al* [13] found that infection increased the beat rate and that the response to NE was blunted. The basis for these apparent differences are currently being investigated. More recently, Tardieux *et al* [180] reported on the induction of intracellular Ca^{2+} transients by trypomastigotes in NRK fibroblasts. The effect of infection on intracellular Ca^{2+} continues to be explored by several laboratories.

Finally, in discussing the ramifications of infection on host cell signal transduction pathways it is also important to note that similar pathways have been described in the parasite [12, 41, 123, 196]. In this regard, putative *T. cruzi* G-proteins have been described which may be developmentally regulated. However, we have been unable to demonstrate a function for these G-proteins in the adenylylate cyclase signal transduction system [122]. Their presence suggests that they may function in other signal transduction pathways yet to be elucidated. How the signal transduction pathways of the host cell and the parasite interact to modulate the host-parasite interaction is yet another aspect of further investigations.

Skeletal Muscle Involvement in Human and Experimental Chagas' disease

Although the effect of *T. cruzi* on the myocardium is well-described, its effect on peripheral skeletal muscle has been of less interest to investigators. During acute murine infection myositis, myonecrosis and marked tissue parasitism (Figure 3) were evident and was associated with expected increases in muscle enzymes such as LDH and CPK in the serum [98, 99]. In addition there was an increase in

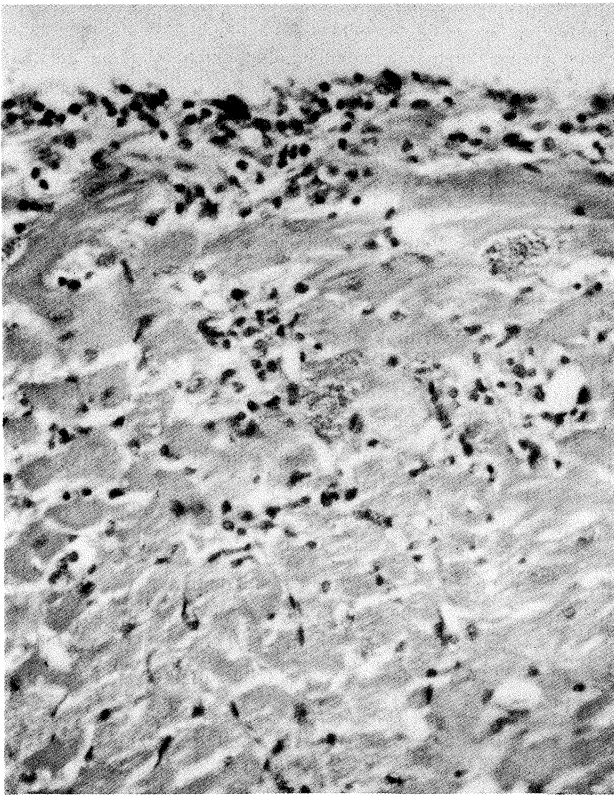


Figure 1. Myocardium of a *T. cruzi* infected mouse. Note the presence of myonecrosis, inflammation and parasite pseudocysts.

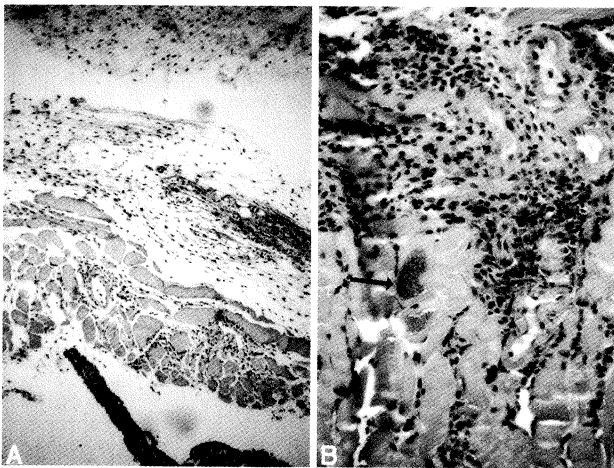


Figure 3. Cremaster muscle of CD1 male mouse infected with the Brazil strain of *Trypanosoma cruzi*. (A). Note the massive inflammatory response and myonecrosis. (B). High power photomicrograph showing nests of amastigotes (arrow).

skeletal muscle nicotinic acetylcholine receptors [172]] and a decrease in acetylcholinesterase [24]; consistent with denervation hypersensitivity.

In acute murine infection Molina *et al* [102] studied the neuromuscular junctions and found degeneration of intra-

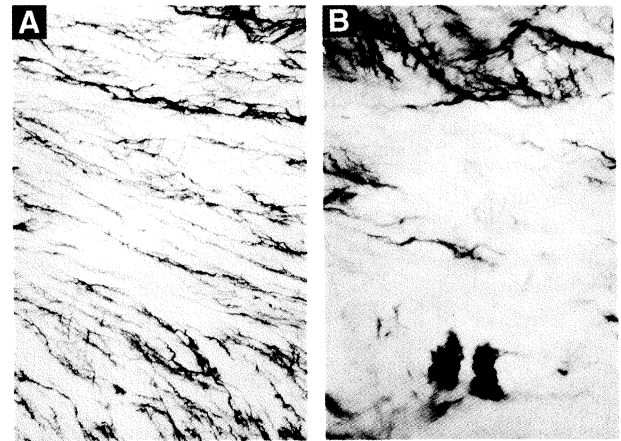


Figure 2. (A). Normal silver-impregnated connective tissue matrix in the left ventricle of a control mouse. (B). Left ventricular apex of an infected mouse revealing a marked loss of silver-impregnated connective tissue.

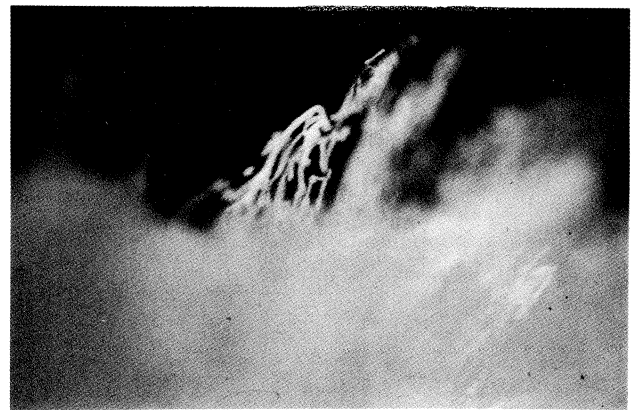


Figure 4. Microfil injection studies of the coronary microcirculation reveals numerous saccular microaneurysms and focal vascular constriction. See reference 43 for details.

muscular nerve fibers with swelling and distortion of nerve endings. Inflammatory neuropathy associated with demyelination and axonal regeneration were observed. Similarly, Gonzales-Cappa *et al* [53] performed EMG studies on chronically infected mice and found evidence of active