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Chagas Heart Disease Pathogenesis: One Mechanism or Many?

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Abstract

Chagas heart disease (CHD), caused by the protozoan parasite *Trypanosoma cruzi*, is the leading cause of infectious myocarditis in the world. The etiology of CHD is unclear and multiple mechanisms have been proposed to explain the pathogenesis of the disease. This review describes the proposed mechanisms of CHD pathogenesis and evaluates the historical significance and evidence supporting each. Although the majority of CHD-related pathologies are currently attributed to parasite persistence in the myocardium and autoimmunity, there is strong evidence that CHD develops as a result of additive and even synergistic effects of several distinct mechanisms rather than one factor.

Chagas Heart Disease

Chagas heart disease (CHD), along with African sleeping sickness and leishmaniasis, is one of a triumvirate of diseases caused by parasites of the protozoan family *Trypanosomatidae*. Resulting from infection with *Trypanosoma cruzi*, CHD is endemic throughout Central and South America, where it poses a tremendous public health burden due to high morbidity and mortality and the expense and controversy of treatment for chronically infected patients. CHD is the leading cause of infectious myocarditis in the world, resulting in 50,000 deaths per year [1]. Despite a drastic reduction in the incidence of CHD over the past two decades due to far-reaching efforts of Latin American public health organizations to control vectorial transmission, the World Health Organization reported that approximately 8 million cases of CHD were extant in 2007 (www.who.int/mediacentre/news/releases/2007/pr36/en/index.html). Of the estimated 100,000 infected individuals currently residing in the United States, most acquired the disease while traveling or living in endemic areas [2]. In non-endemic areas, such as the United States, transmission usually occurs via transfusion of blood products rather than via insect vector. The disease may also be acquired through laboratory accidents, organ transplantation, congenital transmission, and even orally [3].

Two antiparasitic drugs, benznidazole and nifurtimox, have proven effective at treating Chagas disease; neither of these drugs is approved in the United States, but both can be obtained from the Centers for Disease Control and administered under investigational protocols. However, frequent gastrointestinal and neurological side effects are associated with the use of both of these drugs, especially in adults. Currently there is no cure for CHD and the most effective form of prevention is vector eradication.

T. cruzi has a complex life cycle involving two intermediate hosts, the triatomine insect vector (the reduviid bug) and virtually any vertebrate, and three distinct morphological and functional developmental stages: epimastigotes, trypomastigotes and amastigotes. The

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epimastigote form replicates in the midgut of the reduviid bug and develops into nonreplicative metacyclic trypomastigotes. *T. cruzi* is transmitted to humans when the bite wound from a reduviid bug or a mucosal surface such as the conjunctiva is contaminated with parasites present in the triatomine excreta [4]. After transmission, the bloodform trypomastigote form of *T. cruzi* is able to enter a variety of host cells where it differentiates into the replicative amastigote and multiples in the cytoplasm [4]. Eventually the parasitized cell ruptures, releasing trypomastigotes, which may infect adjacent cells or be disseminated through the blood and infect cells at other locations in the body [5].

CHD is a complex disease with two clinically distinct phases: acute Chagas disease which appears shortly after infection and last 4 to 8 weeks, and chronic Chagas disease which develops from an asymptomatic indeterminate form to a chronic symptomatic phase 10 to 30 years later in about one-third of cases. Acute Chagas disease is largely undiagnosed because symptoms are absent or mild and nonspecific, even though *T. cruzi* trypomastigotes can be readily isolated from the blood [6]. Mild inflammation and enlargement of the heart may occur, accompanied by mononuclear cell infiltration of cardiac tissue [4]. Anorexia, fatigue, fever, hepatosplenomegaly, high parasitemia, inflammation around the bite wound, lymphadenopathy, and nausea may develop during acute infection [4]. The three possible outcomes of the acute phase of disease are (i) death, which occurs in less than 5% of cases, and is usually due to heart failure or meningitis and encephalitis (ii) entrance into an indeterminate phase characterized by subpatent parasitemia and nearly complete reduction in inflammation modulated by host immunity and suppressor factors which may last for the life of the individual or be followed by (iii) entrance into the chronic symptomatic phase of disease [5,7]. Chronic CHD is a progressive, fibrosing inflammatory cardiomyopathy that results in permanent heart damage. The inflammatory infiltrate characteristic of CHD consists mainly of lymphocytes, with T cells predominating in a 3:1 ratio of $CD8^+$ to $CD4^+$ cells [8]. Macrophages, eosinophils, plasma cells, neutrophils and mast cells are also present to a lesser extent [4,9-11]. *T. cruzi* infection influences the cytokine milieu by eliciting prolific production of type I cytokines such as IFN-γ, IL-12, and TNF-α and by regulatory type II cytokines such as IL-4 and IL-10 [3]. Interestingly, parasites are rarely found in the hearts of chronic CHD patients, yet parasite DNA can be detected in some inflammatory lesions [12]. Cardiomyocyte necrosis continues throughout the course of disease, resulting in gradual coalescing of focal lesions, accumulation of extracellular matrix (fibrosis), thrombus formation in the dilated left ventricle or aneurysm, decreased contractility of muscle fibers, and destruction of the intrinsic innervation of the heart. This muscle damage leads to dilation and cardiac dysrhythmia, high-degree heart block, and ultimately to congestive heart failure, which is the leading cause of death in chronic CHD patients [13]. During chronic Chagas disease, some individuals also develop gastrointestinal tract disorders such as megaesophagus or megacolon, entities whose pathogenesis is not completely understood but generally involves damage to intramural neurons. Such non-cardiac disease manifestations will not be discussed in this review [4]. It should be emphasized that the majority (approximately two-thirds) of infected individuals develop none of these sequelae.

Possible Mechanisms of CHD Pathogenesis

The complex etiology of CHD pathogenesis is not clearly understood and has been a controversial topic for many years. Multiple explanations for the variable cardiac damage observed in individuals with CHD have been proposed, involving (i) parasite-specific immune responses to *T. cruzi* parasites or antigens persistent in the heart (ii) parasite-mediated myocytolysis (iii) primary neuronal damage (iv) damage to cardiac microvasculature (v) antibody-mediated cytotoxicity and non-specific damage caused by eosinophils and neutrophils (vi) toxin secretion by the parasite and (vii) parasite-induced autoimmunity Fig. (1).

Parasite-Specific Immunity

It is well documented that in humans and mice with CHD, cardiac histopathology is characterized by development of a diffuse cellular infiltrate comprised mainly of lymphocytes and mononuclear cells, but also of eosinophils and occasional polymorphonuclear leukocytes. This process may contribute to the genesis of CHD cardiac pathology due to destruction and displacement of cardiac myofibrils or disruption of the local vasculature. The mononuclear cell infiltrate is presumed to mainly contain parasite antigen-specific lymphocytes; this has not been proven. However, the finding of ovalbumin-specific T cells in inflammatory infiltrates produced from infection with transgenic trypanosomes expressing ovalbumin, strongly suggests that at least some of the cells comprising the infiltrate are parasite-specific [14].

Parasite-Induced Myocytolysis

One of the earliest and most obvious mechanisms to account for the myocarditis resulting from *T. cruzi* infection, based on the identification of parasites in inflamed tissue, posited that the chronic inflammation is a reparative process that arises to ameliorate the damage mechanically inflicted by the parasite [7]. Because host cell lysis occurs following differentiation of amastigotes into bloodform trypomastigotes, parasite-induced myocytolysis and subsequent inflammation is a predictable outcome of cellular infection. Although this process clearly contributes to pathogenesis and actually triggers additional mechanisms of damage (see below), many investigators concur that it is not sufficient to account for the advanced degree and variable nature of myocyte destruction present in cardiac and noncardiac tissues during chronic infection. The absence of parasite pseudocysts in tissue sections isolated from the majority of deceased CHD patients has historically detracted from the establishment of a direct correlation between *T. cruzi* infection and chronic CHD pathology. However, the employment of more sensitive detection methods has revealed that parasite antigen can persist in tissues even in the absence of intact parasites. Using PCR, Belotti *et al.* found evidence of *T. cruzi* in 69% of 16 chronic CHD patients and parasite antigen was detected in over 70% of heart sections showing moderate or severe myocarditis but in less than 17% of regions with mild or no myocarditis [15]. An alternative immunological assay for indirect detection of *T. cruzi* antigens and genetic markers which relied on *in situ* localization of parasite kinetoplastid DNA (kDNA) in tissues from CHD patients was developed in 1999 by Zhang and Tarleton. Using this highly sensitive method, the authors identified what they interpreted as an absolute correlation between parasite persistence and tissue inflammation [16]. Simões-Barbosa et al. later found evidence that the horizontal transfer of *T. cruzi* mitochondrial kDNA to the genomes of naturally infected humans via integration into LINE-1 retrotransposons may play an important role in the pathogenesis of CHD [17]. Although these findings have provided the strongest evidence to date that parasite persistence is crucial for the protracted progression of CHD pathology, no distinction could be made between antigens present on intact *T. cruzi* in the tissue and lingering *T. cruzi* antigens or DNA that had been shed from parasites no longer present in the proximate tissue. Indeed, evidence has been presented that only detection of nuclear DNA (nDNA) is reflective of active *T. cruzi* infection, since it is possible for the mitochondrial DNA of the parasite, released from killed parasites, to integrate into the host genome [7].

Primary Neuronal Damage

Dysautonomia in CHD was reported as early as 1922 when Chagas and Vilella described evidence of autonomic degeneration in *T. cruzi*-infected patients [18]. By the 1950s several studies had been published detailing severe cardiac neuronal damage in Chagas patients leading Köberle to propose the neurogenic hypothesis of Chagas cardiomyopathy [19]. Köberle postulated that intramural denervation constituted the primary mechanism of

cardiac pathogenesis in CHD based on analysis of cardiac sections from deceased chronic Chagas patients involving a standardized method of counting intramural neurons. Although multiple clinical and experimental studies by Köberle and others substantiated the neurogenic hypothesis, several discrepancies have continually detracted from the applicability of this theory [3]. These include the subtleness and variability of cardiac denervation in CHD patients and the lack of correlation between degree of denervation and type of death or pathological features exhibited in the heart. While some researchers still maintain a modified neurogenic hypothesis, insisting that impairment of parasympathetic innervations and overactivation of the sympathetic nervous system and other neurohormonal pathways is sufficient to produce lesions in the heart, the importance of this mechanism of CHD pathogenesis has been largely deemphasized in recent decades [3,20].

Damage to Cardiac Microvasculature

Based on clinical, experimental, and histopathological data from various sources, it has been postulated that microvascular disturbances leading to ischemia contribute to the pathogenesis of CHD. The hearts of chronic CHD patients exhibit focal lesions of myocyte necrosis accompanied by reparative interstitial fibrosis that is very similar to what is seen in experimental models of ischemia and reperfusion as a result of transient microvascular ischemic disturbances [21]. In experimental models of CHD, several microcirculatory malformations leading to ischemia were identified in infected mice, including occlusive thrombi and platelet aggregates found in small epicardial and intramural coronary arteries. Focal vascular constriction and other prominent structural abnormalities such as dilation, altered extracellular matrix deposition, and proliferation of microvessels were also observed [22]. These phenomena could be explained by vascular endothelial cell damage caused by *T. cruzi* or immune effector cells directly, or could result from effects of the inflammatory infiltrate. *T. cruzi* induce production of the potent vasoconstrictor endothelin-1 from infected endothelial cells which may aggravate myocardial ischemia due to decreased circulatory capacity [23]. Another possibility is that microvascular damage may be indirectly initiated by the parasite, as it has been shown that *T. cruzi* calreticulin, which helps the parasite evade the host immune system by modulating the complement system, also inhibits angiogenesis *in vivo* [24]. Additionally, *T. cruzi* produce several bioactive lipids such as thromboxane A and prostaglandin F2α which are potent vasoconstrictors that also promote vascular permeability, vascular smooth muscle cell proliferation, and platelet aggregation [25]. Further evidence of *T. cruzi*-induced microvasculature damage includes observations that the parasites subvert the bradykinin system, activating bradykinin B_2 receptors during invasion of endothelial cells, resulting in vasodilation and subsequent edema [26].

Antibody-mediated Cytotoxicity and Non-Specific Damage Caused by Eosinophils and Neutrophils

Inflammatory cell infiltration is characteristic of the cardiac pathology that develops during acute and chronic CHD. The leukocytic infiltrate consists mainly of lymphocytes, and to a lesser extent macrophages, eosinophils, plasma cells, neutrophils and mast cells [4,9-11]. Although the precise contribution of each of these cell types remains speculative, several lines of evidence suggest an important role for eosinophils and neutrophils in CHD pathogenesis. In the mid-1980s, Molina and Kierszenbaum noted that the presence of eosinophils and neutrophils in cardiac lesions correlates with disease severity, with maximal levels of infiltration occurring in necrotic, degenerative lesions [27]. The presence of activated eosinophils and deposition of eosinophil granule components in degenerative cardiac lesions was later demonstrated by immunohistochemistry [28]. When human eosinophils or neutrophils were cocultured with rat heart myoblasts and *T. cruzi* amastigotes, myoblast injury resulted as indicated by significant cell detachment and noticeable cell lysis [8]. Neutralization of the toxic granule components inhibited cell injury, suggesting the

myoblast destruction is mediated by products secreted by eosinophils and neutrophils into the cell supernatant [8]. This *in vitro* bystander cell damage was shown to not be caused by the *T. cruzi* amastigotes because none of the myoblasts were found to be infected and incubation of myoblasts with amastigotes alone did not result in significant cellular destruction. Interestingly, incubation of myoblasts with eosinophils or neutrophils alone did not cause cell injury either [8]. These observations are consistent with the supposition that degranulation of eosinophils and neutrophils, which are initially recruited to clear parasites and tissue debris, results in bystander tissue damage contributing to the severity of CHD lesions. This could conceivably result in a self-propagating cycle of recruitment of granulocytes to clear tissue debris only to cause more damage triggering the recruitment of additional granulocytes. This would be inconsistent with the aforementioned *in vitro* data which suggests that the presence of parasites is requisite for perpetuation of this response because live parasites are not consistently detected in the hearts of chronic CHD patients. However, as mentioned earlier, several PCR-based studies have indicated that parasites can indeed persist in cardiac tissues for a prolonged period of time. It is also possible that shed or secreted parasite antigens remain in the proximity of tissue lesions for some time after all live parasites are cleared.

Cardiac injury involving bystander cell damage may also involve antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC involving mononuclear cells, eosinophils, and neutrophils targeting *T. cruzi* bloodform trypomastigotes has been demonstrated in mice [29]. Through this or a similar mechanism, parasite-bound antibodies may indirectly recruit cytotoxic effector cells to cardiac lesions, increasing the possibility of tissue damage caused by granulocyte components. This is in addition to damage caused by autoreactive antibodies, which is discussed in a later section of this review. It is interesting to note that antibody-mediated damage has also been reported to cause non-cardiac pathologies in chronic Chagas patients, such as mesangial glomerulopathy resulting from type III hypersensitivity reactions involving deposition of *T. cruzi*-specific antibodies in the kidney [30].

Toxin Secretion by the Parasite

No conventional toxins have been described in the literature to date that are directly responsible for the clinical symptoms associated with CHD or with African sleeping sickness and leishmaniasis, which are caused by the closely related protozoan parasites *T. brucei* and *Leishmania spp.*, respectively. However, it is possible that some substances produced by *T. cruzi* have considerable toxic effects on cells *in vivo*. One example is an acid-active hemolysin secreted by *T. cruzi* named TC-Tox that is immunologically related to the human complement protein C9 [31]. Andrews *et al.* have proposed that this protein functions during cell invasion, perhaps mediating the lysis of the membrane of the phagosome in which the parasite resides at early times after invasion [31]. Another protein with similar hemolytic activity to TC-Tox was identified in 2001 by Manning-Cela *et al.* This protein, named LYT1, is of similar size to TC-Tox and also reactive to anti-C9 antibodies, suggesting structural similarity [32]. However, bioinformatics analysis has failed to identify any significant DNA or protein homology between the two. Biallelic LYT1 knockouts generated in the CL Brener strain of *T. cruzi* exhibited attenuated infectivity *in vitro* accompanied by reduced hemolytic activity at low pH, suggesting a function for LYT1 in cell invasion similar to that described previously for TC-Tox [32]. It is conceivable that these or related parasites protein may also damage some host cells, leading to tissue injury and repair.

Parasite-Induced Autoimmunity

Significance of autoimmunity in CHD—The autoimmunity hypothesis arose in the mid-1970s following observations by Santos-Buch and Teixeira that rejection of allogeneic

heart cells was accelerated in *T. cruzi*-infected rabbits and that target embryonic cardiomyocytes were destroyed within one hour of co-culture with lymphocytes from *T. cruzi*-infected but not uninfected rabbits [33]. Three decades of clinical and experimental investigation into the etiology of CHD have established that autoimmunity is one of the most important mechanisms of CHD pathogenesis; however, the exact role of autoimmunity in disease progression is still not completely understood. By inducing tolerance to myosin in myosin-immunized and *T. cruzi*-infected mice, we have shown that myosin-specific autoimmunity is not essential for the development of inflammation in acute CHD [34]. However, these results do not reflect whether myosin-autoimmunity makes a significant but non-essential contribution to inflammation, nor do they eliminate the possibility that autoimmunity to other cardiac antigens is pathogenic. Although the other mechanisms described in this paper are likely operative in the majority of CHD cases and could account for a large degree of the inflammation associated with tissue damage caused by the infection, there are multiple compelling lines of evidence for pathogenic autoimmunity.

The autoimmunity hypothesis suggests that cardiac damage, regardless of its initial cause, leads to a breakdown in self-tolerance resulting in an immune reaction against self-proteins. There is strong evidence that autoreactivity of T cells during *T. cruzi* infection is limited to the CD4+ compartment. Splenic CD4+ T cells isolated from chronically infected mice mediated syngeneic heart graft rejection when injected *in situ* whereas CD8+ T cells and non-T cells did not [35]. *In vivo* depletion of CD4+ T cells with monoclonal antibodies abrogates this rejection but depletion of CD8+ T cells does not [35]. Further support for this observation came from *in vitro* studies demonstrating that CD4+ but not CD8+ T cells proliferate in response to stimulation with cardiac autoantigens and peripheral tolerance induction to a myosin-enriched heart homogenate efficiently attenuated myocardial reactivities *in vitro* only when mice were given concurrent anti-CD4 treatment [35]. There is also evidence that *T. cruzi*-infected mice and humans undergo less robust intrathymic negative selection resulting in a T cell repertoire containing increased numbers of potentially autoreactive cells including those of the CD4+CD8+TCRVβ5+ and CD4+CD8+TCRVβ12+ phenotypes [36]. This may further contribute to the propensity for development of autoimmunity during CHD.

The presence of autoantibodies is characteristic of many autoimmune diseases and autoantibody titers often reflect disease severity in both humans and animal models. The contribution of autoantibodies to CHD pathogenesis is not fully understood, but several published reports indicate links to cardiac pathology. *T. cruzi*-induced autoantibodies, such as those against β adrenergic and muscarinic cholinergic receptors, alter the contraction and cell signaling of cardiomyocytes and lyse myocytes *ex vivo* through an antibody-dependent cytotoxicity mechanism [37,38]. Also, immunization of BALB/c mice with the *T. cruzi* antigen cruzipain induced autoantibodies to skeletal myosin, IgG deposits in heart sections, and cardiac conduction abnormalities [39]. The authors of this research suggested that the autoantibodies are pathogenic because of the apparent correlation between the presence of autoantibodies and conduction abnormalities. However, transfer of autoantibodies from an infected donor to naïve recipients did not induce disease.

Several mechanisms may play a role in the induction of autoimmunity following *T. cruzi* infection. All of these mechanisms are based on the observation that immunocompetent humans and animals maintain a population of circulating T cells and B cells that are potentially autoreactive, but are normally tolerant to self-antigens. Each mechanism discussed here may induce autoimmunity by causing direct activation of autoreactive T cells or via an antibody-dependent cytotoxicity mechanism.

Bystander activation—*T. cruzi* infection results in tissue destruction facilitating release of host antigens and inflammatory mediators. Release of large amounts of self antigens in an environment rich in inflammatory cytokines, chemokines, lymphotoxin, and nitric oxide may overcome self-tolerance by lowering the threshold of activation enough to activate potentially autoreactive T cells and initiate autoimmunity. Bystander activation can also be initiated by CD8+ T cells specific for *T. cruzi* antigens that are presented on the MHC molecule of infected cells. Once activated, autoreactive T cells can then proliferate in response to self-antigen presented by antigen-presenting cells. Inflammatory factors such as IFN-γ and nitric oxide present at increased concentrations during infection can promote the activation of autoreactive T cells encountering cognate antigen in the context of self MHC, which may be enhanced by increased processing and presentation of self-peptides following myocytolosis. In support of the bystander activation hypothesis, we recently showed that a reduction in parasitemia achieved via treatment with the antiparasitic drug benznidazole significantly decreases or eliminates myosin-specific autoimmunity and myocarditis during *T. cruzi* infection [40]. We hypothesize that reduction of parasite load subsequently reduces parasite-induced myocytolysis and release of host antigens while also dampening the infection-induced inflammatory environment, and ameliorating bystander activation.

Cryptic epitope—If *T. cruzi* infection leads to release of previously sequestered epitopes, or if the inflammatory environment generated during infection induces the processing and presentation of novel self-epitopes, then immunity against these cryptic epitopes may be rapidly induced due to lack of tolerance [41]. This hypothesis operates on the assumption that autoreactive T cells specific for peptides not normally presented on self-MHC molecules escape the central and peripheral tolerization mechanisms responsible for honing the T cell repertoire to avoid spontaneous autoimmunity. One well-characterized example of inflammatory mediators influencing the presentation of cryptic epitopes involves the effect of IFN-γ on proteases involved in antigen processing of the proteasome. *In vitro* IFN-γ treatment has been shown to alter the conformation and activity of cellular proteases, thereby increasing the rate of peptide processing and resulting in production of novel self-peptides which are subsequently presented on the MHC I molecules of those cells [42].

Polyclonal activation—Several strains of *T. cruzi* have been shown to stimulate proliferation of both T and B lymphocytes during mouse and human infections irrespective of antigen specificity, leading to extensive polyclonal immune responses [43]. Induction of polyclonal lymphocyte responses likely exacerbates CHD pathology through a combination of three factors: (i) extraneous production and subsequent deletion of a large number of parasite non-specific lymphocytes, reducing the efficacy of adaptive immunity, (ii) polyclonal activation of B cells leading to skewing of the T helper cell repertoire from Th1 to Th2, thereby reducing the effectiveness of T cell-mediated responses to clear parasites, and (iii) polyclonal activation leading to expansion of autoreactive lymphocytes responsible for the autoimmune pathology associated with CHD. As expected, attenuation of polyclonal lymphocyte activation has been shown to increase resistance to *T. cruzi* infection in mice [44]. In a review of parasite polyclonal activators, Minoprio outlined several immunomodulatory mechanisms linked to polycloncal activation that may contribute to the pathophysiology associated with chronic CHD. These include (i) preferential activation of lymphocyte subpopulations (CD5+ B cells and γδ T cells) that are associated with autoimmune disorders, (ii) induction of hypergammaglobulinemia, (iii) suppression of cellular and humoral immune responses to homologous and heterologous antigens, and (iv) expansion of autoreactive B cell clones that may be involved in late developing autoimmunity.

Molecular mimicry—Molecular mimicry occurs when antigenic determinants of a microorganism that evoke an immune response are immunologically similar, via structural similarity or secondary sequence identity, to a host antigen. An immune response to the parasite that is "cross-reactive" with self then develops. Molecular mimicry may contribute to pathology in several models of autoimmunity including rheumatic fever and rheumatic heart disease and experimental autoimmune encephalomyelitis (EAE) [45-47]. Cardiac myosin, which is the most abundant heart protein and also the major antigenic target in most cases of cardiac-specific autoimmunity including that associated with rheumatic fever and coxsackievirus B3 myocarditis, has also been identified as a target autoantigen in CHD [48-52]. Development of robust myosin-specific autoimmunity following immunization with *T. cruzi* protein extracts and parasite-specific immunity following immunization with myosin suggest that molecular mimicry is a likely mechanism of autoimmunity during experimental CHD [53]. Concordant with this idea is the finding that peripheral immune tolerization to myosin suppresses parasite-specific immunity and tolerization to parasite suppresses myosin autoimmunity [34]. Several groups have identified putative mimic antigens in *T. cruzi* that are cross-reactive to antibodies or T cell clones specific for mammalian proteins. Antibodies and stimulated T cell clones isolated from human CHD patients react against epitopes of both myosin and epitopes of B13, an abundant *T. cruzi* antigen [54]. The *T. cruzi* cysteine protease cruzipain was identified as another mimic antigen candidate after immunization of mice with an enzymatically inactive form of the protein triggered expansion of myosin autoantibodies and autoreactive T cells and caused formation of ultrastructural abnormalities in cardiac tissue [39]. Three regions of linear sequence homology between cruzipain and cardiac myosin were identified as potential mimic epitopes [39]. In addition to myosin, other autoantigens have been identified as crossreactive with *T. cruzi* proteins. The novel autoantigen Cha, isolated from the sera of infected individuals, contains both B cell and T cell epitopes crossreactive with *T. cruzi* proteins such as the shed acute-phase antigen (SAPA) [55]. Also, antibodies have been isolated from chronic CHD patients that react with ribosomal P proteins in *T. cruzi* and the β1 adrenoreceptor in cardiac tissue [56]. The observations that immunization with *T. cruzi* protein lysate or portions of *T. cruzi* ribosomal P1 and P2 proteins induces functional and structural alterations in the hearts of young and adult mice provide direct evidence that crossreactive *T. cruzi* proteins can induce autoimmunity [57]. However, in order to definitively "prove" molecular mimicry occurs, it must be demonstrated that a single antigen receptor (T cell or antibody) reacts with epitopes of both the parasite and the host and can promote tissue inflammation. The first element crossreactivity of a T cell clone with parasite and host peptides has been demonstrated, but the second has not [54,58].

Epitope spreading—Following bystander activation, the autoantigen that initiates autoimmunity may not be the same autoantigen involved during development of disease. Autoimmunity that develops against one epitope can cause tissue damage resulting in the release of additional self antigens, the processing and presenting of which induces the stimulation of non-cross-reactive autoimmunity against additional epitopes [59,60]. Intermolecular spreading, or antigen-specific autoimmune responses that spread between distinct antigens, likely occurs between cardiac myosin and other heart-abundant proteins (e.g. myosin binding protein C (MyBPc), Cha antigen, desmin, actin, myoglobin, tubulin, and the human β1 adrenergic receptor) as a result of *T. cruzi* infection ([61-64] and K. M. Bonney, unpublished results). Observations that autoimmune responses are also initiated against these antigens in susceptible mouse strains immunized with purified cardiac myosin or myosin peptides in various models of experimental autoimmune myocarditis (EAM) support this hypothesis [65,66]. Serological analysis of infected mice also indicates responsiveness to a multitude of other yet unidentified cardiac antigens [51]. Unpublished data from our lab suggests that intramolecular antigenic spreading, or the spreading of

autoimmune responses among different epitopes of the same antigen (e.g. cardiac myosin), might also contribute to the propagation of autoimmunity during CHD similar to models of virally-induced EAM [67].

Concluding Remarks

A number of distinct pathogenic mechanisms of CHD have been defined during decades of study involving human Chagas patients and experimental models of *T. cruzi* infection. Considerable variability in disease course is observed in both human CHD patients and in animal models of *T. cruzi* infections. The use of different model systems and experimental techniques has led different groups to widely varying conclusions regarding the precise contribution of each potential mechanism to disease progression. While some consider the variations in strain-strain combinations of mice and parasites a shortcoming of experimental CHD models, others maintain that this variation simply reflects the natural heterogeneity seen in human infections and that each model system is useful for providing insight into certain aspects of the disease. The caveat here is that no single strain-strain combination can be interpreted as representative of all aspects of CHD.

While the majority of cardiac damage in CHD might be attributed to just a few distinct mechanisms such as autoimmunity, parasite-specific immunity, and parasite-induced myocytolysis, there is compelling evidence that multiple other mechanisms are also involved in pathogenesis. Given the scenario that both host and parasite antigens are present in the myocardium either with or without live parasites for an unknown amount of time during the course of infection, it is very difficult to determine whether anti-parasite immunity or autoimmunity is responsible for observed tissue damage. One frequent point of contention arises from the mistaken assumption that all of the proposed disease mechanisms are mutually exclusive. As discussed here, numerous studies have been published validating each of the proposed mechanisms with at least some respectable degree of conclusiveness. The most recent findings of our lab support the idea that multiple mechanisms act coincidentally if not synergistically during the complex etiology of CHD. In addition to the mechanisms discussed in this review, other aspects of the immune system including various endocrine factors and regulatory T cell subtypes may be influential or even subverted during *T. cruzi* infection, leading to pathological consequences. For example, several studies have linked differential expression of corticosterioids with variations in susceptibility of different mouse strains to *T. cruzi* infection [68,69]. Although initial reports suggest traditional CD4+CD25+ regulatory T cells do not play a significant role in regulating acute phase responses to *T. cruzi* infection in mice, investigation into the role of regulatory T cells during *T. cruzi* infection has not been exhaustive [70]. Future goals of our lab include further investigation of molecular mimicry and the contribution of antigenic spreading to autoimmunity during *T. cruzi* infection.

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References

1. Fralish, BH.; Tarleton, RL. Vaccine. Vol. 21. 2003. p. 3070-3080.
2. Leiby, DA.; Herron, RM., Jr; Read, EJ.; Lenes, BA.; Stumpf, RJ. Transfusion (Paris). Vol. 42. 2002. p. 549-555.
3. Marin-Neto, JA.; Cunha-Neto, E.; Maciel, BC.; Simoes, MV. Circulation. Vol. 115. 2007. p. 1109-1123.

4. Tanowitz, HB.; Kirchhoff, LV.; Simon, D.; Morris, SA.; Weiss, LM.; Wittner, M. Clin Microbiol Rev. Vol. 5. 1992. p. 400-419.
5. Andrade, ZA. Mem Inst Oswaldo Cruz. Vol. 94. 1999. p. 71-80.
6. Miles, MA.; Feliciangeli, MD.; de Arias, AR. BMJ. Vol. 326. 2003. p. 1444-1448.
7. Teixeira, AR.; Nascimento, RJ.; Sturm, NR. Mem Inst Oswaldo Cruz. Vol. 101. 2006. p. 463-491.
8. Molina, HA.; Kierszenbaum, F. Immunology. Vol. 66. 1989. p. 289-295.
9. Melo, RC.; D'Avila, H.; Fabrino, DL.; Almeida, PE.; Bozza, PT. Tissue Cell. Vol. 35. 2003. p. 59-67.
10. Postan, M.; Correa, R.; Ferrans, VJ.; Tarleton, RL. Int Arch Allergy Immunol. Vol. 105. 1994. p. 251-257.
11. Elias, FE.; Vigliano, CA.; Laguens, RP.; Levin, MJ.; Berek, C. Am J Trop Med Hyg. Vol. 68. 2003. p. 242-247.
12. Tarleton, RL.; Zhang, L.; Downs, MO. Proc Natl Acad Sci U S A. Vol. 94. 1997. p. 3932-3937.
13. Rodriguez-Salas, LA.; Klein, E.; Acquatella, H.; Catalioti, F.; Davalos, VV.; Gomez-Mancebo, JR.; Gonzalez, H.; Bosch, F.; Puigbo, JJ. Echocardiography. Vol. 15. 1998. p. 271-278.
14. Kumar, S.; Tarleton, RL. J Immunol. Vol. 166. 2001. p. 4596-4603.
15. Bellotti, G.; Bocchi, EA.; de Moraes, AV.; Higuchi, ML.; Barbero-Marcial, M.; Sosa, E.; Esteves-Filho, A.; Kalil, R.; Weiss, R.; Jatene, A.; Pileggi, F. Am Heart J. Vol. 131. 1996. p. 301-307.
16. Zhang, L.; Tarleton, RL. J Infect Dis. Vol. 180. 1999. p. 480-486.
17. Simoes-Barbosa, A.; Arganaraz, ER.; Barros, AM.; Rosa Ade, C.; Alves, NP.; Louvandini, P.; D'Souza-Ault, MR.; Nitz, N.; Sturm, NR.; Nascimento, RJ.; Teixeira, AR. Mem Inst Oswaldo Cruz. Vol. 101. 2006. p. 833-843.
18. Chagas, C.; Vilella, E. Mem Inst Oswaldo Cruz. Vol. 1922. 1922. p. 5-61.
19. Koberle, F. Wien Klin Wochenschr. Vol. 68. 1956. p. 333-339.
20. Davila, DF.; Rossell, O.; de Bellabarba, GA. Int J Parasitol. Vol. 32. 2002. p. 107-109.
21. Morris, SA.; Tanowitz, HB.; Wittner, M.; Bilezikian, JP. Circulation. Vol. 82. 1990. p. 1900-1909.
22. Rossi, MA.; Goncalves, S.; Ribeiro-dos-Santos, R. Am J Pathol. Vol. 114. 1984. p. 209-216.
23. Wittner, M.; Christ, GJ.; Huang, H.; Weiss, LM.; Hatcher, VB.; Morris, SA.; Orr, GA.; Berman, JW.; Zeballos, GA.; Douglas, SA., et al. J Infect Dis. Vol. 171. 1995. p. 493-497.
24. Ferreira, V.; Molina, MC.; Schwaeble, W.; Lemus, D.; Ferreira, A. Trends Parasitol. Vol. 21. 2005. p. 169-174.
25. Ashton, AW.; Mukherjee, S.; Nagajyothi, FN.; Huang, H.; Braunstein, VL.; Desruisseaux, MS.; Factor, SM.; Lopez, L.; Berman, JW.; Wittner, M.; Scherer, PE.; Capra, V.; Coffman, TM.; Serhan, CN.; Gotlinger, K.; Wu, KK.; Weiss, LM.; Tanowitz, HB. J Exp Med. Vol. 204. 2007. p. 929-940.
26. Todorov, AG.; Andrade, D.; Pesquero, JB.; Araujo Rde, C.; Bader, M.; Stewart, J.; Gera, L.; Muller-Esterl, W.; Morandi, V.; Goldenberg, RC.; Neto, HC.; Scharfstein, J. FASEB J. Vol. 17. 2003. p. 73-75.
27. Molina, HA.; Kierszenbaum, F. Int J Parasitol. Vol. 17. 1987. p. 1297-1305.
28. Molina, HA.; Kierszenbaum, F. J Parasitol. Vol. 75. 1989. p. 129-133.
29. Kipnis, TL.; James, SL.; Sher, A.; David, JR. Am J Trop Med Hyg. Vol. 30. 1981. p. 47-53.
30. Costa, RS.; Monteiro, RC.; Lehuen, A.; Joskowicz, M.; Noel, LH.; Droz, D. Clin Immunol Immunopathol. Vol. 58. 1991. p. 102-114.
31. Andrews, NW.; Abrams, CK.; Slatin, SL.; Griffiths, G. Cell. Vol. 61. 1990. p. 1277-1287.
32. Manning-Cela, R.; Cortes, A.; Gonzalez-Rey, E.; Van Voorhis, WC.; Swindle, J.; Gonzalez, A. Infect Immun. Vol. 69. 2001. p. 3916-3923.
33. Santos-Buch, CA.; Teixeira, AR. J Exp Med. Vol. 140. 1974. p. 38-53.
34. Leon, JS.; Wang, K.; Engman, DM. J Immunol. Vol. 171. 2003. p. 4271-4277.
35. dos Santos, RR.; Rossi, MA.; Laus, JL.; Silva, JS.; Savino, W.; Mengel, J. J Exp Med. Vol. 175. 1992. p. 29-39.
36. Mendes-da-Cruz, DA.; de Meis, J.; Cotta-de-Almeida, V.; Savino, W. Microbes Infect. Vol. 5. 2003. p. 825-832.

37. Borda, ES.; Sterin-Borda, L. Int J Cardiol. Vol. 54. 1996. p. 149-156.
38. Laguens, RP.; Meckert, PC.; Chambo, JG. Infect Immun. Vol. 56. 1988. p. 993-997.
39. Giordanengo, L.; Maldonado, C.; Rivarola, HW.; Iosa, D.; Girones, N.; Fresno, M.; Gea, S. Eur J Immunol. Vol. 30. 2000. p. 3181-3189.
40. Hyland, KV.; Leon, JS.; Daniels, MD.; Giafis, N.; Woods, LM.; Bahk, TJ.; Wang, K.; Engman, DM. Infect Immun. 2007.
41. Lanzavecchia, A. J Exp Med. Vol. 181. 1995. p. 1945-1948.
42. York, IA.; Goldberg, AL.; Mo, XY.; Rock, KL. Immunol Rev. Vol. 172. 1999. p. 49-66.
43. Minoprio, P. Int J Parasitol. Vol. 31. 2001. p. 588-591.
44. Minoprio, P.; Eisen, H.; Joskowicz, M.; Pereira, P.; Coutinho, A. J Immunol. Vol. 139. 1987. p. 545-550.
45. Fae, KC.; da Silva, DD.; Oshiro, SE.; Tanaka, AC.; Pomerantzeff, PM.; Douay, C.; Charron, D.; Toubert, A.; Cunningham, MW.; Kalil, J.; Guilherme, L. J Immunol. Vol. 176. 2006. p. 5662-5670.
46. Cunningham, MW. Clin Microbiol Rev. Vol. 13. 2000. p. 470-511.
47. Mana, P.; Goodyear, M.; Bernard, C.; Tomioka, R.; Freire-Garabal, M.; Linares, D. Int Immunol. Vol. 16. 2004. p. 489-499.
48. Dale, JB.; Beachey, EH. J Exp Med. Vol. 164. 1986. p. 1785-1790.
49. Neu, N.; Beisel, KW.; Traystman, MD.; Rose, NR.; Craig, SW. J Immunol. Vol. 138. 1987. p. 2488-2492.
50. Cunha-Neto, E.; Duranti, M.; Gruber, A.; Zingales, B.; de Messias, I.; Stolf, N.; Bellotti, G.; Patarroyo, ME.; Pilleggi, F.; Kalil, J. Proc Natl Acad Sci USA. Vol. 92. 1995. p. 3541-3545.
51. Leon, JS.; Godsel, LM.; Wang, K.; Engman, DM. Infect Immun. Vol. 69. 2001. p. 5643-5649.
52. Tibbetts, RS.; McCormick, TS.; Rowland, EC.; Miller, SD.; Engman, DM. J Immunol. Vol. 152. 1994. p. 1493-1499.
53. Leon, JS.; Daniels, MD.; Toriello, KM.; Wang, K.; Engman, DM. Infect Immun. Vol. 72. 2004. p. 3410-3417.
54. Cunha-Neto, E.; Coelho, V.; Guilherme, L.; Fiorelli, A.; Stolf, N.; Kalil, J. J Clin Invest. Vol. 98. 1996. p. 1709-1712.
55. Girones, N.; Rodriguez, CI.; Carrasco-Marin, E.; Hernaez, RF.; de Rego, JL.; Fresno, M. J Clin Invest. Vol. 107. 2001. p. 985-993.
56. Kaplan, D.; Ferrari, I.; Bergami, PL.; Mahler, E.; Levitus, G.; Chiale, P.; Hoebeke, J.; Van Regenmortel, MH.; Levin, MJ. Proc Natl Acad Sci U S A. Vol. 94. 1997. p. 10301-10306.
57. Motran, CC.; Fretes, RE.; Cerban, FM.; Rivarola, HW.; Vottero de Cima, E. Clin Immunol. Vol. 97. 2000. p. 89-94.
58. Cunha-Neto, E.; Bilate, AM.; Hyland, KV.; Fonseca, SG.; Kalil, J.; Engman, DM. Autoimmunity. Vol. 39. 2006. p. 41-54.
59. Lehmann, PV.; Forsthuber, T.; Miller, A.; Sercarz, EE. Nature. Vol. 358. 1992. p. 155-157.
60. Vanderlugt, CJ.; Miller, SD. Curr Opin Immunol. Vol. 8. 1996. p. 831-836.
61. Kierszenbaum, F. Parasitol Today. Vol. 12. 1996. p. 414-415.
62. Girones, N.; Rodriguez, CI.; Basso, B.; Bellon, JM.; Resino, S.; Munoz-Fernandez, MA.; Gea, S.; Moretti, E.; Fresno, M. Clin Diagn Lab Immunol. Vol. 8. 2001. p. 1039-1043.
63. Ternynck, T.; Bleux, C.; Gregoire, J.; Avrameas, S.; Kanellopoulos-Langevin, C. J Immunol. Vol. 144. 1990. p. 1504-1511.
64. Smulski, C.; Labovsky, V.; Levy, G.; Hontebeyrie, M.; Hoebeke, J.; Levin, MJ. FASEB J. Vol. 20. 2006. p. 1396-1406.
65. Kasahara, H.; Itoh, M.; Sugiyama, T.; Kido, N.; Hayashi, H.; Saito, H.; Tsukita, S.; Kato, N. J Clin Invest. Vol. 94. 1994. p. 1026-1036.
66. Matsumoto, Y.; Tsukada, Y.; Miyakoshi, A.; Sakuma, H.; Kohyama, K. J Immunol. Vol. 173. 2004. p. 3535-3541.
67. Krebs, P.; Kurrer, MO.; Kremer, M.; De Giuli, R.; Sonderegger, I.; Henke, A.; Maier, R.; Ludewig, B. J Autoimmun. 2007.

68. Roggero, E.; Perez, AR.; Tamae-Kakazu, M.; Piazzon, I.; Nepomnaschy, I.; Besedovsky, HO.; Bottasso, OA.; del Rey, A. J Endocrinol. Vol. 190. 2006. p. 495-503.

69. Correa-de-Santana, E.; Paez-Pereda, M.; Theodoropoulou, M.; Kenji Nihei, O.; Gruebler, Y.; Bozza, M.; Arzt, E.; Villa-Verde, DM.; Renner, U.; Stalla, J.; Stalla, GK.; Savino, W. J Neuroimmunol. Vol. 173. 2006. p. 12-22.

70. Kotner, J.; Tarleton, R. Infect Immun. Vol. 75. 2007. p. 861-869.

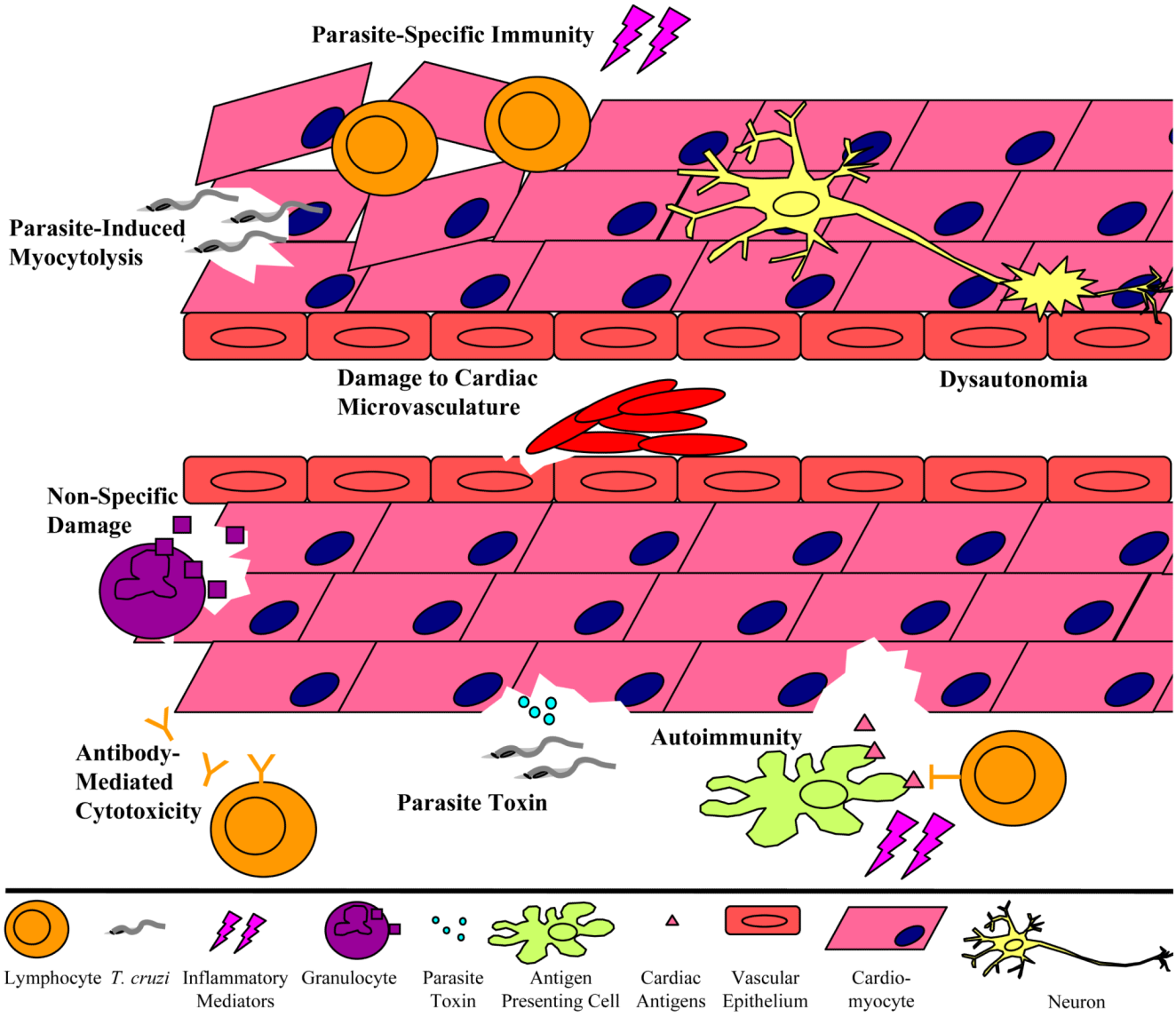

Figure 1.

Proposed mechanisms of CHD pathogenesis. (i) Parasite-specific immunity may contribute to cardiac pathology due to destruction and displacement of myocytes leading to disruption of contractility and microvasculature. (ii) Parasite-induced myocytolysis occurs following differentiation of intracellular amastigotes into bloodform trypomastigotes and this may result in significant cardiac damage. (iii) Dysautonomia consisting of parasympathetic impairment and overactivation of sympathetic and neurohormonal pathways as reported in many cases of CHD may contribute to disease pathology. (iv) Microcirculatory malformations leading to ischemia, including occlusive platelet aggregations is speculated to contribute to cardiac pathology. (v) Non-specific damage caused by eosinophil granule components and (vi) antibody-mediated cytotoxicity may cause significant bystander injury to cardiomyocytes. (vii) A toxic hemolysin secreted by *T. cruzi* may cause a minor degree of myocytolysis. (viii) Parasite-induced autoimmunity generated by a number of different mechanisms including molecular mimicry and epitope spreading is thought to be a major contributor to CHD pathology.