ABSTRACT
There are many potential mechanisms underlying the pathogenesis of chagasic heart disease. The frequent absence of parasites from the inflamed heart tissue of chronically infected individuals suggests that the disease may be, in part, autoimmune in nature. Mechanisms to explain the induction of *T. cruzi* induced autoimmunity include (i) polyclonal lymphocyte activation, induced by the parasite, (ii) bystander activation induced by tissue damage and stimulation of normally tolerant, self-reactive lymphocytes, (iii) altered antigen processing leading to the generation and presentation of “cryptic self epitopes,” and (iv) molecular mimicry, immunity to a parasite epitope that cross-reacts with a self epitope that “mimics” it. The genetics of host and parasite also determine susceptibility to *T. cruzi* induced autoimmunity. To date, there is little evidence that the *T. cruzi* induced autoimmunity directly causes pathology in human Chagas disease or even in mouse models of the disease. Therefore, public health interventions should focus on control of the insects that transmit the parasite, development of parasiticidal drugs and vaccines, and testing of blood products since they are important sources of potential new infections.

INTRODUCTION
Chagas disease encompasses three main, largely non-coincident, pathologies: inflammation of the heart, dilation of the esophagus or colon, and abnormalities of the central nervous system, affecting roughly 30%, 5%, and less than 5% of individuals infected with protozoan parasite *Trypanosoma cruzi*, respectively (Moncayo, 1999) (see Chapter 10). These varied diseases typically develop years to decades after infection. Well over half of the infected individuals develop none of these sequelae. After nearly a century of investigation, the mechanisms of Chagas disease pathogenesis are unclear and under debate. Why only some individuals develop disease, why there is such variability in disease manifestations, why it takes so long for the disease to manifest, and what triggers disease initially are some of the many questions clinicians and researchers have investigated during the past several decades.

One proposed hypothesis is that Chagas disease is an autoimmune disease (reviewed in Eisen and Kahn, 1991; Kierszenbaum, 1999), an immune reaction against self antigens causing tissue inflammation or cellular damage (Abbas et al., 2000). Autoimmunity results when mechanisms responsible for maintaining immunological self-tolerance fail. Autoimmune responses may be present and measurable in the host in the absence of tissue inflammation or overt disease. Furthermore, the presence of antibodies or T cells reactive with
host antigens may be a consequence and not a cause of tissue injury. To unequivocally prove that Chagas disease is an autoimmune disease, tissue inflammation or cellular damage must be shown to be directly caused by the autoimmune reactivity, whatever the initiating factor.

The clinical and public health significance of whether Chagas disease is an autoimmune disease is great. If Chagas disease is an autoimmune disease, then the therapies for the illness must address autoimmune mechanisms. Chemotherapies solely directed against the parasite might not prevent autoimmune tissue destruction; a two-pronged strategy to kill the parasite and reduce autoimmune tissue damage should be employed. In addition, potential anti-\textit{T. cruzi} vaccine candidates would have to be screened to make sure that they do not induce an autoimmune disease. Thus, anti-\textit{T. cruzi} chemotherapies and \textit{T. cruzi} vaccines must be pursued with the potential for autoimmune sequelae in mind.

The hypothesis that Chagas disease is an autoimmune disease arose from initial studies on Chagas cardiac pathology and the discovery of \textit{T. cruzi}-host cross-reactive antibodies. Histologic analysis of tissues from Chagas patients, particularly cardiac tissue, showed tissue inflammation and fibrosis occurring in the apparent absence of \textit{T. cruzi}, suggesting that these inflammatory lesions were not initiated by \textit{T. cruzi} or parasitized tissue as previously believed (Torres, 1941). Later, antibodies against host tissue were reported in Chagas patients (Cossio et al., 1974). This report was later retracted for methodological concerns (Khoury et al., 1983), but it encouraged other groups to search for and publish on autoantibodies in Chagas patients (reviewed in Kierszenbaum, 1986).

If inflammatory processes are not associated with \textit{T. cruzi} or infected cells, and the immune system is targeting host tissue, what could initiate these inflammatory processes? In other words, is Chagas disease an autoimmune disease? The concept of Chagas disease as an autoimmune disease was put forth by Santos-Buch and Teixeira (Santos-Buch and Teixeira, 1974). In support of this possibility, several groups have reported that autoimmunity is induced upon infection with \textit{T. cruzi} in humans and experimental animals and that this autoimmunity can directly contribute to pathology (discussed below and in Engman and Leon, 2002; Kierszenbaum, 1999). A second possibility is that inflammation is targeting "residual" \textit{T. cruzi} antigens and that autoimmunity is an epiphenomenon. With the advent of sensitive nucleic acid based technologies, such as PCR and in situ hybridization, several groups have shown the presence of \textit{T. cruzi} DNA in inflammatory foci and presumably protein antigen as well, even if intact amastigotes may not be present (reviewed by Tarleton, this volume). Therefore, evidence exists to support both hypotheses: (i) Chagas disease is an autoimmune disease and (ii) Chagas disease is caused by residual parasite and anti-parasite immunity. Several other potential mechanisms, including microvascular spasms, ischemia, and direct toxicity of the parasite will not be addressed here (Tanowitz et al., 1992, and this volume).
EVIDENCE THAT CHAGAS DISEASE IS AN AUTOIMMUNE DISEASE

The hypothesis that Chagas disease is an autoimmune disease should be considered as two separate issues: (i) autoimmunity is induced by infection with *T. cruzi*, and (ii) *T. cruzi* induced autoimmunity is pathogenic. The distinction between these two issues is, in our view, the most important source of confusion in the literature debating the autoimmune hypothesis for disease pathogenesis. Infection of humans with *T. cruzi* has been shown to induce autoantibodies against antigens in heart, skeletal muscle and nervous tissue (reviewed in Kierszenbaum, 1999). These include ribosomal P proteins (Levin et al., 1989), myosin (Cunha-Neto et al., 1995), β1 adrenoreceptor (Stern-Borda and Borda, 2000), cytoskeletal microtubule associated proteins (Kerner et al., 1991), LIST neuronal proteins (Petry, 1989; Van Voorhis et al., 1991), and a novel mammalian protein, Cha (Girones et al., 2001). Many of these target proteins have ubiquitous expression that does not support the observation of heart specific inflammation in Chagas disease. Furthermore, there is little evidence that these antibodies are more prevalent in patients with Chagas disease than in asymptomatic, *T. cruzi* infected individuals. The clinical significance of these autoantibodies is not yet clear. T cells that recognize cardiac antigens have also been identified in chronically infected humans and mice. In humans, T cell clones from Chagas disease patients proliferated when cultured with a human cardiac myosin peptide or a parasite antigen, B13 (Cunha-Neto et al., 1996). In mice, T cells proliferated *in vitro* to heart homogenate (Ribeiro dos Santos et al., 1992) or to myosin (Rizzo et al., 1989).

To address whether *T. cruzi* induced autoimmunity is pathogenic researchers have studied the contribution of autoantibodies to disease. To date, there is no evidence that *T. cruzi* induced autoantibodies can induce disease upon transfer into a naive host. However, *T. cruzi* induced antibodies do affect the contraction and cell signaling of cardiac myocytes *in vitro* (Borda and Sterin-Borda, 1996). In addition, sera from chronically infected mice lysed myocytes *in vitro* through an antibody dependent cytotoxicity mechanism (Laguens et al., 1988). Finally, immunization of mice with a *T. cruzi* antigen, cruzipain, induced autoantibodies to myosin, IgG deposits in heart sections, and conduction abnormalities in both the mother and pups (Giordanengo, 2000b). The authors of this research suggested that autoantibodies are pathogenic because of the association between the presence of autoantibodies and conduction abnormalities. Many of the targets of these autoantibodies are intracellular and thus it is difficult to understand how these autoantibodies cause disease if their target antigen(s) is inaccessible.

There is little evidence supporting the contribution of cellular autoimmunity to Chagas disease. One report demonstrated that splenocytes from a chronically infected mouse can lyse syngeneic myoblasts (Laguens et al., 1989). Transfer of splenocytes from infected mice stimulated *in vitro* with *T. cruzi* extract induced sciatic nerve (Hontebeyrie-Joskowicz et al., 1987) and cardiac inflammation (Laguens et al., 1989). The most compelling evidence supporting a role for cellular autoimmunity in disease pathogenesis was the finding that CD4⁺ T cells from chronically infected mice mediated the rejection of implanted syngeneic newborn hearts (Ribeiro dos Santos et al.,
However, if a different combination of parasite and mouse strains was used, this did not occur (Tarleton et al., 1997). The conflicting nature of these results may be explained by differences in the ability of individual *T. cruzi* strains to induce autoimmunity or differences in the susceptibility of particular mouse strains to develop autoimmunity (discussed below). Recently, blocking immunity to heart antigens (tolerizing to heart antigens), enriched for myosin, reduced inflammation and fibrosis in *T. cruzi* infected mice (Pontes-De-Carvalho et al., 2002). Though this report offers compelling evidence for the pathogenicity of anti-heart antigen immunity in *T. cruzi* infected mice, there are methodological concerns which detract from its impact including: small sample size, no evidence that the immune system was tolerized to heart antigens, and discordant results of their positive control with published results (Godsel et al., 2001). Finally, immunization of mice with specific parasite proteins can induce autoimmunity and cardiac disease. Specifically, immunization of mice with regions of the *T. cruzi* ribosomal P1 and P2 protein induced production of autoantibodies to the mouse ribosomal proteins as well as electrocardiographic alterations (Motran et al., 2000). Immunization with cruzipain induced autoantibodies and T cell responses to myosin, skeletal myositis (Giordanengo et al., 2000a), and cardiac conduction abnormalities (Giordanengo et al., 2000b). Since no live parasites were used, the autoimmunity is believed to be induced through a molecular mimicry mechanism.

In conclusion, there is a large body of evidence that infection with *T. cruzi* induces both humoral and cellular autoimmunity. However, the pathogenic potential of the autoimmunity has not been proven. *T. cruzi* induced autoantibodies affect cells *in vitro*, but have not been shown to have a direct role *in vivo*. Regarding cellular immunity, adoptive transfer and immunization experiments with parasite proteins do not necessarily recapitulate events in infected mice. As a result, no conclusion can be made about whether *T. cruzi*-induced autoimmunity directly contributes to tissue damage in human Chagas disease, or for that matter, in infected mice.

**CRITICISMS OF THE AUTOIMMUNE DISEASE HYPOTHESIS**

Two criticisms are levied against the autoimmune hypothesis of pathogenesis. One criticism is based on the fact that immunosuppressants, which generally relieve symptoms of autoimmune disease, exacerbate disease and mortality in Chagas patients. The best examples of this are Chagas heart transplant recipients receiving immunosuppressants, and Chagas patients infected with HIV. In both cases, the presence of the parasite confounds the question of whether autoimmunity contributes to disease, since suppressing host immunity results in an increased proliferation of parasites. For the record, the largest study on Chagas heart transplant recipients concluded that Chagas patients have no difference in mortality compared to heart transplant recipients suffering from idiopathic dilated cardiomyopathy or ischemic cardiomyopathy (Bocchi and Fiorelli, 2001). The second criticism posits that autoimmunity does not contribute to disease because *T. cruzi* chemotherapy alone reduces clinical disease in humans and experimental animals. However, there is no consensus on the efficacy of chemotherapy on human disease (Viotti et al., 1994; Parada et al., 1997; Bahia-Oliveira et al., 2000; Fabbro De
Suasnabar et al., 2000; Inglessis et al., 1998; Lauria-Pires et al., 2000). Unless chemotherapy completely eliminates disease, any residual disease can be explained by additional mechanisms. In experimental models of T. cruzi infection, chemotherapy given immediately after infection reduces and sometimes eliminates cardiac disease (Urbina, 1999). Because T. cruzi is the trigger for autoimmunity, elimination of this trigger in the acute disease phase could potentially eliminate the induction of autoimmunity, making the analysis of the contribution of autoimmunity irrelevant.

**POTENTIAL MECHANISMS OF T. CRUZI-INDUCED AUTOIMMUNITY**

How can T. cruzi induce autoimmunity in a host which is normally tolerant to its own antigens? Four possible explanations include the mechanisms of polyclonal activation, bystander activation, cryptic epitope, and molecular mimicry (reviewed in Leon and Engman, 2001).

**Polyclonal activation.** Autoimmunity may result from antigen-independent stimulation of self-reactive lymphocytes that are not deleted during lymph development. Polyclonal activators stimulate many T and B lymphocytes, irrespective of antigen specificity and, in some cases, by interacting with surface molecules other than antigen receptors. The most common example of a polyclonal activator is lipopolysaccharide, which induces a wide repertoire of acute autoantibodies in mice (Granholm and Cavallo, 1992). These autoantibodies have weak affinities and are often of the IgM isotype. Certain T. cruzi strains have been shown to possess polyclonal activators, suggesting that polyclonal activation may be a possible mechanism for the induction of T. cruzi-induced autoimmunity (Minoprio, 2001).

**Bystander activation.** In the mechanism of bystander activation, T. cruzi infection causes tissue destruction and release of host antigens. The excess levels of released host antigens in the presence of an environment rich in proinflammatory cytokines, nitric oxide and chemokines may overcome self-tolerance and initiate autoimmunity. Evidence for this hypothesis includes the observation that cardiac autoimmunity can result from many, varied types of insults to the heart, including transplantation, surgery, and infection (Neu et al., 1987; de Scheerder et al., 1989; Fedoseyeva et al., 1999).

**Cryptic epitope.** The third mechanism, cryptic epitope, suggests that either (i) T. cruzi infection releases previously sequestered epitopes or (ii) that the inflammatory environment induced by T. cruzi induces the processing and immune presentation of novel self epitopes. Immunity against these novel epitopes is rapidly induced because the immune system is not tolerant to these novel epitopes (Lanzavecchia, 1995). In support of this hypothesis, antigen processing and presentation is altered after in vitro treatment with IFN-γ (York et al., 1999). The mechanism of cryptic epitope has been used to explain the genesis of rheumatoid arthritis and systemic lupus erythematosus (Warnock and Goodacre, 1997).
Molecular mimicry. The last and arguably most popular mechanism is molecular mimicry. Molecular mimicry leads to autoimmunity as a result of a "misdirected" immune response. When a parasite antigen closely resembles a host antigen, the immune system may be induced first against the parasite antigen and then "cross-react" with a self antigen, causing autoimmunity. Evidence for this hypothesis includes the reports of autoimmunity upon immunization with a \( T. cruzi \) antigen (Giordanengo et al., 2000a; Motran et al., 2000) and the many reports of cross-reactive (\( T. cruzi \)-host) autoantibodies such as B13-myosin (Cunha-Neto et al., 1995), or cruzipain-myosin (Giordanengo et al., 2000b), \( T. cruzi \)-mammalian ribosomal P proteins (Motran et al., 2000) and \( T. cruzi \) shed acute phase antigen-Cha autoantigen (Girones et al., 2001).

Epitope spreading. As a final twist on the bystander activation mechanism, the autoantigen that initiates autoimmunity may not be the autoantigen involved during development of disease. This phenomenon is called "epitope spreading" and it describes how the primary epitope/antigen target of autoimmunity may change during the course of disease. At one point, autoimmunity against one epitope develops, causing damage to tissue(s) containing that epitope. This damage then results in the release of additional self antigens, the processing and presenting of which induces the stimulation of autoimmunity of additional epitope specificity(ies). Interestingly, the initial responses typically wane as immunoregulatory mechanisms kick in, leading to "waves" of autoimmunity targeted to different epitopes/antigens (Vanderlugt and Miller, 1996).

When is autoimmunity first induced by \( T. cruzi \)? Autoimmunity may be induced immediately after the initial contact of the parasite with the host, during the acute phase of disease (Ternynck et al., 1990; Grauert et al., 1993; Leon et al., 2001). This early autoimmunity likely results from tissue damage caused by the parasite and/or molecular mimicry. The polyclonal, polyspecific nature of the autoantibody response supports the former hypothesis. Autoimmunity may also develop later in the disease course (Acosta and Santos-Buch, 1985; Laguens et al., 1988). Persistent, chronic inflammation may be necessary to overcome the threshold of cardiac damage or produce the correct inflammatory environment for the stimulation and expansion of autoreactive cells. In closing, these mechanisms are not exclusive of each other and combinations of these mechanisms may play a role in the induction of autoimmunity during Chagas disease.

Host immunogenetics and parasite genetics in autoimmunity

Immunogenetic factors of the host and genetic characteristics of the parasite may also influence the induction of autoimmunity. Factors that may influence the induction of autoimmunity in the host include genetic background, gender, and age among others. To date, the role of host genetic background is the only factor examined in the induction of autoimmunity caused by \( T. cruzi \) infection. Certain strains of mice are susceptible while others are resistant. Acute infection of A/J mice with \( T. cruzi \) induces cellular and humoral autoimmunity against myosin while acute infection of C57BL/6
mice does not (Leon et al., 2001). There is evidence that *T. cruzi* genetics determine the induction of autoimmunity. Infection of mice with the Brazil strain of *T. cruzi* induces anti-heart antibodies whereas infection with the Guayas strain of *T. cruzi* does not (Tibbetts et al., 1994; Rowland et al., 1995).

**CONCLUSIONS**

In conclusion, there is clear evidence that autoimmunity can be induced by *T. cruzi* infection in humans and experimental animals (Figure 1). However, there is no proof that autoimmunity directly contributes to the pathogenesis of Chagas disease. It should also be emphasized that it is presumed by many (see Tarleton chapter) but not proven that *in vivo* anti-*T. cruzi* immunity is responsible for inflammation and damage present in the myocardium.

It may be that the cause of Chagas disease is autoimmunity, *T. cruzi* and its associated anti-*T. cruzi* responses, other mechanisms, or a combination. If both autoimmunity and anti-*T. cruzi* immune responses contribute to Chagas disease, then it is necessary to address the relative contributions of both to disease in human patients. The balance in certain subpopulations may be skewed more towards the parasite than autoimmunity and vice versa. If autoimmunity plays no part in disease, then how is it induced and will it disappear once the parasite is cleared? Various mechanisms may explain how an infectious organism can break immunologic self tolerance. To convince skeptics that *T. cruzi*-induced autoimmunity contributes to the pathology of Chagas disease additional evidence is required. Therefore, from the public health perspective, anti-*T. cruzi* chemotherapy and vaccine trials are worth pursuing until Chagas disease is proved to have an autoimmune component to pathogenesis.
Figure 1. Model of autoimmunity induction in Chagas heart disease. Host infection with *T. cruzi* may induce autoimmunity and/or disease depending on host immunogenetics and parasite genetics. Autoimmunity may be induced via a sole or a combination of mechanisms listed under Autoimmunity. Autoimmunity may progress to disease (Autoimmune Inflammation) and disease may induce autoimmunity through tissue damage (Tissue Damage).

References


