Pathogenesis of Chagas heart disease: role of autoimmunity

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Abstract

Chagas heart disease is caused by infection with the protozoan parasite Trypanosoma cruzi. The apparent absence of parasites from the hearts of most individuals who succumb to this illness has led some to propose an autoimmune basis for disease pathogenesis. This hypothesis has been extremely difficult to test, because other mechanisms of tissue inflammation may coexist in the setting of active infection. Here we review the proposed mechanisms of Chagas disease pathogenesis and present new evidence in support of an autoimmune contribution to cardiac inflammation in the context of these other mechanisms. While we do not yet have a definitive answer to the autoimmunity question, we hope that our views will provide those engaged in the debate fresh perspective on this challenging issue. © 2002 Elsevier Science B.V. All rights reserved.

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1. Trypanosoma cruzi and Chagas disease

Trypanosoma cruzi is a single-celled eukaryotic parasite with a complex life cycle involving several stages in both mammals and blood-sucking triatomine bugs. Transmission of T. cruzi to humans may occur when bug excreta, contaminated with the infective, flagellated trypomastigote form of the parasite and released by the bug while it takes a blood meal, contaminate mucous membranes or breaks in the skin. Once beyond the barrier of the skin, T. cruzi trypomastigotes are able to invade a wide variety of host cells where they subsequently differentiate into nonflagellated amastigotes and multiply in the host cell cytoplasm. At some point, the amastigotes differentiate into flagellated bloodform trypomastigotes, rupture out of the cell, and either penetrate adjacent cells or spread hematogenously to infect tissues at distant locations. Muscle cells, including those of the heart, are the most heavily infected. A few circulating bloodforms are always present and may be taken up by a new triatomine during a blood meal, completing the cycle. Individuals residing in mud huts in rural areas of Latin America are at highest
risk of infection, since the bugs live in these dwellings and feed on the inhabitants at night; however, transfusion with contaminated blood and congenital transmission also account for some new infections. Many infected individuals have entered the USA in recent years as legal and illegal immigrants. Therefore, transfusion-acquired Chagas disease may become a significant problem in this country, especially in areas where Latin American immigrants have settled in large numbers (Kirchhoff, 1989).

Chagas disease is a complex illness having both acute and chronic phases (Kirchhoff, 1993; Tanowitz et al., 1992). In acute Chagas disease a local inflammatory lesion appears at the site where the metacyclic trypanostigotes enter and undergo their first rounds of multiplication. After dissemination, symptoms of cardiac insufficiency develop in a small number of patients, reflecting the underlying severe myocarditis, and most deaths from acute Chagas disease are due to heart failure (Laranja et al., 1956). Meningoencephalitis may also occur, especially in the immunosuppressed (Hoff et al., 1978). By far the most common manifestations of the disease develop many years after the initial infection with *T. cruzi* (Kirchhoff, 1993). The heart is the organ most commonly involved, and sudden death due to cardiac dysrhythmias may occur. Cardiomyopathy frequently develops, and cardiomyopathic congestive heart failure is a common cause of death in these patients. In addition, individuals with Chagas heart disease often develop mural thrombi which embolize and cause cerebrovascular accidents. Finally, megadisease of the esophagus and/or colon may develop during chronic infection, which, in the most severe form, cause life-threatening malnutrition and intractable constipation. Approximately 18 million individuals are infected with *T. cruzi*, with 120 million at risk (Moncayo, 1999).

2. Possible mechanisms of cardiac pathogenesis

Gross examination of the hearts of patients who have died of heart failure secondary to Chagas disease reveals biventricular enlargement with occasional apical aneurysms and mural thrombi. Histologically, the tissue is characterized by diffuse interstitial fibrosis, lymphocytic infiltration and myocytolysis—all of which occur in the apparent absence of parasites. It should be stated at this point that parasite DNA is present in the inflammatory foci (Zhang and Tarleton, 1999), and presumably protein antigen as well, even if intact amastigotes may not be present. More recently, amastigotes were found in a high percentage of cardiac biopsies of chronically-infected individuals (Anez et al., 1999). Still, many investigators feel that the initial observations still hold true and many of the inflammatory lesions in Chagas heart disease are devoid of organisms. Fibrotic changes and chronic inflammation are also found in the conduction system of the heart, which may account for the high incidence of dysrhythmias among these patients. The pathogenesis of the cardiac lesions of chronic Chagas disease is not precisely understood and has been the subject of considerable controversy for several decades. At the present time, there are a number of basic hypotheses to explain how cardiac pathology develops in the absence of intact parasites.

(i) **Parasite-induced myocytolysis** is an obvious mechanism, since the host cell lyses after amastigotes differentiate into bloodform trypanostigotes.

(ii) **Primary neuronal damage** may occur during acute disease and lead to the development of chronic phase lesions (Koberle, 1970). (iii) A parasite-derived product may be secreted by the organism that is toxic for host tissues (Koberle and Nador, 1955). (iv) **Parasite-induced microvascular changes** may lead to cardiac hypoperfusion and finally to myocyte degeneration and chronic inflammation (Factor et al., 1985; Morris et al., 1990; Petkova et al., 2001). (v) **Polyclonal B cell activation** occurring during infection may disrupt normal immune regulatory mechanisms and can cause both immunosuppression and autoimmunity (Minoprio, 2001). (vi) **Persisting T. cruzi antigens** may act as foci for specific T-cell mediated delayed-type hypersensitivity (DTH) processes leading to damage to host tissues (Ben Younes-Chennoufi et al., 1988; Tarleton, 2001; Tarleton and Zhang, 1999). (vii) **Autoimmunity** may occur
either by so-called molecular mimicry, in which an immune response to parasite proteins cross-reacts with host tissues, eventually leading to the development of pathology (Cossio et al., 1974a,b; Santos-Buch and Teixeira, 1974; Wood et al., 1982) or simply from bystander activation resulting from significant release of self antigen by parasite-mediated myocytolysis (Cossio et al., 1984). In the latter case, it is necessary that the individual or mouse strain be immunogenetically ‘susceptible’ (Leon and Engman, 2001). An important point that is often ignored is that none of the seven mechanisms listed above is mutually exclusive. Without removing the parasite from the equation, it is impossible to attribute the inflammation to one and only one mechanism.

3. Key problems in understanding Chagas disease

One of the most challenging aspects of Chagas disease—which is in large part responsible for the disagreement among experts regarding the mechanism of pathogenesis—is the tremendous variation in the outcome of T. cruzi infection. Potential consequences of infection range from lifelong, asymptomatic infection in the majority of cases to acute myocarditis and sudden death in a very small minority. Development of chronic disease, cardiomyopathy and/or megadisease, occurs in approximately one-third of infected individuals and at a wide range of times after infection. This huge variation in the outcome of T. cruzi infection indicates that Chagas disease is a mixture of distinct clinical illnesses. There are likely many factors that contribute to this heterogeneity which, although grounded in the genetic variation of both parasite and host, are poorly understood even today. Moreover, since individuals may be infected with a mixture of parasite clones of differing pathogenic potential (see next), it is possible that the disease course in these cases is actually a composite of pathogenically distinct infections. These mixed infections may result either from primary infection with a mixed population or from reinfection.

Another problem is that individual research groups have developed their preferred animal models of Chagas disease, which employ different combinations of parasite and mouse strains and vary widely in virtually every aspect imaginable. Some combinations show little if any parasitemia or target organ damage and may be reflective of the majority of infected humans who do remain asymptomatic for life (see next). Others lead to death of the animal in a matter of days, requiring immunization or drug treatment to allow survival (Ribeiro dos Santos et al., 1992). Still others lead to target organ inflammation, either within weeks or months and with many, few or no parasite pseudocysts. The timing of disease development itself raises a complex issue. Some investigators consider an animal 3–4 months post infection to be reflective of ‘chronic’ human Chagas disease, but not an animal prior to this time, while others may view a 1–2 month animal to be ‘chronic.’ It is more useful in our opinion not to ascribe any disease model to human disease, but rather to carefully define the specific aspect of infection and disease for which a model may be relevant. Many different labs employ many different models to study a very complex disease. This is actually a good thing, which permits the discovery of new information about many aspects of T. cruzi infection and Chagas disease. The problem arises when individual investigators interpret their findings to be relevant to ‘Chagas disease’ instead of to the specific type of Chagas model employed. Another problem is that it is possible, indeed likely, that several of the mechanisms for inflammation described above coexist in T. cruzi-infected humans and experimental animals and that disease results from multiple coincident processes.

4. Autoimmunity in Chagas disease

After several decades of productive investigation on the subject, the etiology of Chagas heart disease, both in humans and in experimental animal models, is not completely understood. Many pathogenic mechanisms, including those described earlier, have been described for what is in essence a heterogeneous set of infections with highly varied outcomes. Among all the inflammatory mechanisms, it is our opinion that parasite-spe-
specific immunity is operative in virtually all cases and it seems logical that it accounts for a good deal of the tissue inflammation as well. However, for the remainder of this article, we will focus on the possibility that pathogenic autoimmunity, induced by molecular mimicry and/or bystander activation, contributes to tissue damage (reviewed in Kierszenbaum, 1986; Eisen and Kahn, 1991; Kierszenbaum, 1999). This view is supported by a large amount of circumstantial evidence (Acosta and Santos-Buch, 1985; McCormick and Rowland, 1989; Petry and Eisen, 1989; Takle and Hudson, 1989), including some of our own (Leon and Engman, 2001; Leon et al., 2001; Tibbetts et al., 1994).

Perhaps the most compelling evidence supporting a role for autoantigen-specific DTH responses in disease pathogenesis is the finding that CD4\(^+\) T cells from chronically-infected mice mediate the rejection of implanted syngeneic newborn hearts (Ribeiro dos Santos et al., 1992), although this does not happen when a different combination of parasite and mouse strains is employed (Tarleton et al., 1997). Of particular relevance to the new data presented below are reports of autoimmunity specific for cardiac myosin developing in T. cruzi-infected mice (Rizzo et al., 1988; Tibbetts et al., 1994) and humans (Cunha-Neto et al., 1995, 1996). It should be emphasized that not a single report published to date indicates that autoimmunity is pathogenic. This is true of parasite-specific immunity as well. In both cases, tissue inflammation is accompanied by readily measurable specific peripheral immunity to parasite and/or self antigens. However, in no case has the antigen specificity (parasite, self, nonspecific) of lymphocytes within inflamed tissue sections been determined.

The criteria put forth by Kierszenbaum (1986) as essential for the proof that heart disease is autoimmune in nature are based on the view that immunologic cross-reactivity between trypanosomes and heart tissue (molecular mimicry) must exist. We feel that this scenario is less likely to be true than myocytolysis/antigen release in the susceptible host leading to expansion of normally tolerant myosin-reactive T cells, particularly since myosin autoimmunity is seen in myocarditis associated with other insults, including viral infection, myocardial infarction and cardiotoxic chemotherapy. ‘Aberrant’ antigen exposure per se, is not even necessary, since peptides of cardiac myosin, a cytoplasmic protein, are found complexed with class II MHC molecules on antigen presenting cells (APC) in normal mouse myocardium (Smith and Allen, 1992).

When is autoimmunity first induced by T. cruzi? One possibility is that autoimmunity is induced immediately after the initial contact of the parasite with the host, during the acute phase of disease. In support of this hypothesis, autoantibodies against actin, laminin and myosin have been detected in acue human infection (Grauert et al., 1993). In addition, autoantibodies specific for tubulin, actin and myosin can be detected in acute murine infection (Ternynck et al., 1990; Leon et al., 2001). These results suggest that tissue damage caused by the parasite and/or cross-reactive immunity with T. cruzi antigens (molecular mimicry) are the initial trigger for autoimmunity. The polyclonal, polyspecific nature of the autoantibody response supports the former hypothesis.

Autoimmunity may also develop later in the disease course. There are reports that serum and splenocytes from chronically infected mice promote in vitro cell lysis while serum and splenocytes from acutely infected animals do not (Acosta and Santos-Buch, 1985; Laguens et al., 1988). The caveat to these reports is that only the lytic responses were assessed—not antigen-specific humoral and cellular autoimmunity. It is possible that non-pathogenic autoimmune responses were present in both acute and chronic disease yet became pathogenic (directly responsible for inflammation) only in chronic disease. Moreover, several mechanisms can be envisioned for the elaboration of cytolytic responses that do not involve antigen-specific autoimmunity. 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.
5. Mechanisms of induction of autoimmunity

There are several mechanisms to explain autoimmunity induced by infectious agents (Malkiel et al., 1996). All are based on the observation that an immunocompetent host possesses circulating, autoreactive T cells and B cells that are normally tolerant to self antigens (Dighiero and Rose, 1999). (i) Bystander activation may occur in the setting of a favorable proinflammatory environment induced by T. cruzi parasitization of host tissue (Talvani et al., 2000). This environment, rich in cytokines, nitric oxide and chemokines, may be sufficient to activate autoreactive T cells by lowering the threshold of activation (Fedoseyeva et al., 1999). These cells may then proliferate in response to self antigen presented on host APC. A contributing factor is myocardial cytolyis resulting from T. cruzi infection leading to the release of self antigen, which promotes increased presentation of self peptide and stimulation of autoreactive cells. In support of this mechanism, there are several examples of autoimmunity occurring after cardiac damage, including those which develop after cardiac surgery (de Scheerder et al., 1989), cardiac transplant rejection (Fedoseyeva et al., 1999), and infection with viruses (Neu et al., 1987). (ii) Cryptic epitopes found in intracellular proteins are not normally presented in the context of Class I MHC and are, therefore, not normally encountered by circulating lymphocytes. Upon tissue damage caused by T. cruzi infection, these internal proteins are released and available for processing and presentation to be presented by APC. Circulating T cells may become activated and initiate autoimmunity. Cryptic epitopes also may be produced when processing and presentation of peptides is altered (York et al., 1999). (iii) The hypothesis of molecular mimicry is popular in the T. cruzi field. As mentioned earlier, this posits that the immune response to a T. cruzi protein ‘crossreacts’ with a self protein sharing the target epitope. The anti-self response is initiated and tissue damage may result if the response is of sufficient intensity and/or if bystander activation occurs.

Two candidate molecular mimicry combinations are peptides of the T. cruzi B13 protein and human cardiac myosin (Cunha-Neto et al., 1995) and peptides of T. cruzi cruzipain and skeletal myosin (Giordanengo et al., 2000). One criticism often levied against these ‘crossreactive’ proteins is that, while indirect evidence suggests the presence of host responses against both the parasite protein and the putative self protein, there is no direct evidence demonstrating that the crossreactive T. cruzi protein can induce autoimmunity. Although not necessarily proving molecular mimicry, two reports that have addressed this criticism showed that immunization with T. cruzi lysate (Laguens et al., 1989) or T. cruzi ribosomal P protein induces functional changes in the heart (Motran et al., 1999). Some investigators equate autoimmunity with molecular mimicry, ignoring the bystander activation mechanism for the generation of anti-self responses. While we will present new evidence for molecular mimicry below, we feel that the bystander activation mechanism is probably more likely, particularly in an ‘autoimmunity-predisposed’ host. The temporal ‘redirection’ of autoimmunity to different epitopes is called epitope spreading and is observed in several other models of organ-specific inflammation (Vanderlugt et al., 1998).

6. Some new evidence for molecular mimicry

For our studies, we have employed a murine model of infection that exhibits (i) severe myocarditis, (ii) strong parasite-specific immunity and (iii) strong autoimmunity. A/J mice infected with the Brazil strain of T. cruzi develop all three essential disease properties within a mere 21-days of infection. The cardiac inflammation is characterized by massive mononuclear cell infiltration, myofibrillar edema, fibrosis and occasional parasite pseudocysts (Fig. 1). Of particular importance is the fact that the quality, magnitude and kinetics of the autoimmune response in these animals are similar to those induced by immunization with cardiac myosin in complete Freund’s adjuvant (CFA)—a model in which autoimmune process is clearly pathogenic, and both disease and autoimmunity in the two models show the same host genetic susceptibility (Leon et al., 2001). How...
autoimmunity develops as a result of *T. cruzi* infection and how it contributes to tissue inflammation are questions of ongoing study in our laboratory.

A/J mice infected with the Brazil strain of *T. cruzi* develop strong myosin-specific DTH and production of myosin-specific autoantibodies (Leon et al., 2001). To determine whether this might result from molecular mimicry rather than by bystander activation, we immunized A/J mice with an acetone (protein) extract of *T. cruzi* Brazil strain epimastigotes emulsified in CFA. Mice were also immunized with phosphate-buffered saline (PBS)/CFA and myosin/CFA as negative and positive (Godsel et al., 2001) controls, respectively. An additional group was infected with *T. cruzi* trypomastigotes as another positive control (Leon et al., 2001). Only the *T. cruzi* infected and myosin/CFA immunized groups developed myocarditis (data not shown; see ref. (Leon et al., 2001) for histopathology). However, three of the groups developed both myosin-specific DTH and autoantibody responses (Fig. 2). In light of this, it is highly significant both that the magnitude of the myosin DTH in myosin/CFA-immunized, *T. cruzi*-infected, and *T. cruzi*/CFA-immunized are similar, and that the *T. cruzi*/CFA-immunized animals did not develop myocarditis. The finding of strong myosin DTH in the *T. cruzi*/CFA-immunized animals implies that there is indeed molecular mimicry between a *T. cruzi* protein and cardiac myosin. In light of the magnitude of the DTH, it is somewhat surprising that these mice did not develop disease, since the myosin/CFA-immunized mice have such severe myocarditis. However, it is probable that autoreactivity itself is not sufficient to give tissue inflammation, and that a proinflammatory environment, induced by infection (Talvani et al., 2000) or provided by another inflammatory stimulus (such as lipopolysaccharide) is also necessary. *T. cruzi*-specific DTH was high in the *T. cruzi*-infected and *T. cruzi*/CFA-immunized animals, as expected.

To investigate the possibility that the Brazil strain is comprised of multiple clones with individual pathogenic potential (see above), we generated a panel of clones by limiting dilution. Twelve clones were derived and, although each gives a slightly different outcome when used to infect A/J mice, none gives the same disease as that given by

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**Fig. 1.** Histopathology of Chagas heart disease. A/J mice were infected by intraperitoneal injection of 10,000 trypomastigotes of the Brazil strain of *T. cruzi* and their hearts were analyzed 21 days post infection. Serial sagittal sections were generated and stained with hematoxylin and eosin. Lesions in infected mouse hearts were found throughout the myocardium and typical sections from infected and uninfected animals are shown here. While hearts of uninfected mice are histologically normal, with well ordered myofibrils and scattered myocyte nuclei and interstitial cells, those of *T. cruzi*-infected mice show massive mononuclear infiltration, occasional parasite pseudocysts (not in this section) and myocyte swelling and necrosis.
Myosin Delayed Hypersensitivity

Fig. 2. Induction of myosin-specific cellular and humoral autoimmunity by *T. cruzi* infection or immunization with a *T. cruzi* protein extract. A/J mice were (i) immunized with PBS in CFA, (ii) immunized with myosin in CFA, (iii) immunized with *T. cruzi* protein (acetone) extract in CFA or (iv) infected with *T. cruzi*. (Left) 21 days post immunization/infection, myosin- or *T. cruzi*-specific DTH was measured using a standard 24-h ear swelling assay (Leon et al., 2001). Briefly, one ear of each mouse was injected with 10 μg test antigen (myosin or *T. cruzi* extract in PBS) and the other was injected with 10 μg control antigen (BSA in PBS). The net increase in ear thickness (increase in test minus increase in control) was measured after 24 h. (Right) Serum taken at the same time was analyzed by myosin-specific IgG ELISA to measure myosin autoantibodies, as described (Leon et al., 2001). Five mice per group were used for assessment of DTH responses and eight per group for serology. Error bars represent standard error of the mean.

the uncloned Brazil strain (data not shown). As an example, the Brazil 4 clone (Brc4), when used to infect A/J mice, caused no myocarditis and no myosin DTH (Fig. 3), although parasite-specific DTH and antibody production were identical to those of the parental strain (not shown). This provides specific and strong support for the notion that natural infection can involve multiple, unique parasite clones with differing pathogenic potential.

7. Final remarks

The study of animal models of Chagas disease has permitted the definition of a number of distinct mechanisms of pathogenesis, including parasite antigen-specific inflammation, parasite-induced myocardial cell necrosis and repair, microvascular spasm and autoimmunity. As is observed in human infections, there is a wide variety of outcomes of the animal infections; a single strain of mouse may develop many different types of disease depending on the parasite isolate used and a single parasite clone can cause very different disease in different strains of mice. Therefore, no single strain–strain combination or mechanism defined within is reflective of all Chagas disease. While some may view this as a shortcoming of the murine models, we believe that it is a strength, since the variation in outcome is precisely what is observed in humans. However, it is equally important that conclusions drawn from the study of one strain-strain combination not be interpreted as representing all of Chagas disease. Furthermore, the different pathogenetic mechanisms are not mutually exclusive, a point overlooked by some. Finally, and perhaps most important, when both parasite and host antigens are present in the inflamed myocardium, it is difficult to determine whether anti-parasite immunity, autoimmunity, both or neither are responsi-
Fig. 3. Clones of a pathogenic *T. cruzi* strain may be nonpathogenic. The pathogenic Brazil strain of *T. cruzi* was cloned by limiting dilution to produce the Brazil 4 clone (Brc4), among others. A/J mice were (i) injected with PBS, or infected with 10,000 trypomastigotes of (ii) the Brc4 clone or (iii) the parental Brazil strain. (Left) 21 days post infection, myosin-specific DTH was measured using a standard 24 h ear swelling assay (Leon et al., 2001). Briefly, one ear of each mouse was injected with 10 µg test antigen (myosin in PBS) and the other was injected with 10 µg control antigen (BSA in PBS). The net increase in ear thickness (increase in test minus increase in control) was measured after 24 h. Five mice per group were used for assessment of DTH responses. Error bars represent standard error of the mean. (Right) Serial sagittal sections were generated and stained with hematoxylin and eosin.

Ble for the tissue damage. It may be logical to presume that immunity to foreign antigen in the tissue is inflammatory and also that one need not invoke an autoimmune hypothesis to explain the damage. However, reasonableness and conclusive determination are not the same, even without considering the presence of additional, coexistent inflammatory mechanisms. Many groups have contributed substantially to our understanding of Chagas disease pathogenesis and it is logical that parasite persistence is a major factor in tissue inflammation. We would like to contribute to the issue by rigorously testing the autoimmunity hypothesis suggested by vast amounts of circumstantial evidence published by others and us in the past. The *T. cruzi* Brazil/A/J mouse combination offers an attractive model for study in this regard, since it possesses very strong autoimmune features. New approaches for the selective inhibition of antigen-specific cellular immunity may also help to determine the relative contributions of different types of immunity to Chagas disease pathogenesis.
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References


