[Frontiers in Bioscience 8, e315-322, May 1, 2003]

THE SIGNIFICANCE OF AUTOIMMUNITY IN THE PATHOGENESIS OF CHAGAS HEART DISEASE

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1. ABSTRACT

Chagas heart disease develops in approximately one-third of individuals infected with the protozoan parasite *Trypanosoma cruzi.* Among the many possible mechanisms responsible for this illness, an autoimmune mechanism has received much experimental support during the past several decades. Initial observations of the absence or near absence of parasites from the inflamed tissues suggested the autoimmunity hypothesis, and the finding of measurable autoimmune responses in humans and experimental animals has bolstered this idea. The rigorous testing of the hypothesis has been difficult, primarily because other mechanisms are likely to play a role during active infection, particularly immunity to parasite antigens that may persist in the infected animal. While the role autoimmunity plays in disease pathogenesis is not known, it is clear that autoimmune responses are induced during infection of some humans and animals. A number of mechanisms may explain the induction of autoimmunity during *T. cruzi* infection, including parasite-induced polyclonal lymphocyte activation, molecular mimicry, bystander activation, and presentation of cryptic self epitopes. The genetic makeup of both the parasite and host are also critical to the outcome of infection. The autoimmune hypothesis deserves further exploration, while 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, which have become an important threat of new infections.

2. INTRODUCTION

Chagas heart disease (CHD) is caused by the protozoan *Trypanosoma cruzi* and develops in roughly 30% of infected individuals (1). CHD encompasses both acute and chronic manifestations of myocarditis and may take months to decades to develop. After nearly a century of investigation, the exact mechanism of CHD is unclear and under debate. Why only some individuals are susceptible to CHD, why there is such variability in disease manifestations, why it takes so long for chronic CHD to develop, and what causes CHD are some of the many questions investigators and physicians have addressed. There are at least six proposed mechanisms for CHD pathogenesis. CHD may be caused by: (i) anti-*T. cruzi* immune responses to persistent parasites or parasite antigens; (ii) autoimmunity induced by *T. cruzi*; (iii) microvascular spasm; (iv) ischemia; (v) chronic eosinophilia; and (vi) direct toxicity of the parasite (reviewed in (2-4)). These mechanisms may be interrelated. This review summarizes our views on the first and second hypotheses – that CHD is caused by anti-*T. cruzi* immunity and persistent parasite or parasite antigens (reviewed in (5-8)) and that CHD is an autoimmune disease (reviewed in (3, 9-13)).

The hypothesis that Chagas disease is an autoimmune disease arose from early observations of cardiac pathology and the discovery of antibodies cross-reactive to *T. cruzi* and host in patients with chronic CHD. Histologic analysis indicated that chronic CHD patients had cardiac inflammation and fibrosis in the apparent absence of *T. cruzi* suggesting that these inflammatory lesions were not caused by parasitized tissue as previously believed (14). Later, antibodies specific for host proteins, which could be blocked by *T. cruzi* antigens, were found in chronic CHD patients (15). This report was later retracted for methodological concerns (16), but it encouraged other groups to research autoantibodies in patients with CHD (reviewed in (13)). If inflammation was not associated with parasitized tissue and the immune system was targeting host antigens, what caused these inflammatory processes? The concept of Chagas disease as an autoimmune disease was thus put forth by Santos-Buch and Teixeira in 1974 (17).

An autoimmune disease is defined as tissue inflammation or cellular damage caused by an immune reaction against self antigens (autoimmunity) that results

when mechanisms responsible for mantaining immunologic self-tolerance fail (18). Autoimmunity may be present in the host in the absence of tissue inflammation or disease. In fact, autoimmunity may be the effect and not the cause of tissue damage. To prove that CHD is an autoimmune disease, tissue inflammation must be shown to be directly caused by autoimmunity, regardless of the initiating agent.

The public health and clinical significance of whether CHD is an autoimmune disease are serious. If CHD is an autoimmune disease, then the treatments for the disease must address autoimmune mechanisms. Therapies solely directed against the parasite may not prevent autoimmune destruction and should be developed to eliminate the parasite and reduce autoimmune damage. In addition, vaccine candidates would have to be screened to confirm that they do not induce an autoimmune disease. Thus, anti-*T. cruzi* chemotherapy and *T. cruzi* vaccines would safeguard against the potential for autoimmune sequelae.

3. CHAGAS HEART DISEASE IS CAUSED BY PERSISTENT *T. CRUZI* AND *T. CRUZI*-SPECIFIC IMMUNITY

The hypothesis that chronic Chagas disease is due to residual parasites or antigens and accompanying anti-*T. cruzi* immunity has four supporting lines of evidence: (i) *T. cruzi* or *T. cruzi* DNA/antigens can be detected in chronic lesions; (ii) *T. cruzi* or *T. cruzi* DNA/antigens can sometimes colocalize with inflammation; (iii) inflammation is targeted against *T. cruzi* and has been shown to cause damage; and (iv) elimination of *T. cruzi* reduces disease severity. Each of these points is covered briefly here but are explored in depth in references (3, 6-8).

As discussed above, examination of cardiac tissue from chronic Chagas patients by early clinicians revealed tissue inflammation and fibrosis occuring in the apparent absence of *T. cruzi* (14). However, with the advent of more sensitive techniques, such as immunohistochemistry (19-23), whole tissue PCR (24-26), *in situ* hybridization (27), and *in situ* PCR (28), parasite antigens and DNA have been shown to persist in the tissues of infected humans and experimental animals. An important point to highlight is that these techniques cannot distinguish between live parasites and residual parasite antigens or DNA. One report using beta-galactosidase-expressing transgenic *T. cruzi* demonstrated that live parasites could be detected in mice 10 months post infection (29). Interestingly, the number of live parasites was far smaller than estimated by other techniques (23, 30), suggesting that antigens from destroyed *T. cruzi* may remain in tissues for a long time and provoke ongoing inflammatory processes (29).

The second line of evidence is that *T. cruzi* can sometimes colocalize with inflammation. Colocalization often means “present in the same section” because, to date, there are no reports on the correlation between localization of *T. cruzi* antigens and physical proximity to inflammatory lesions. Several reports showed that inflammation is often found in cardiac sections with *T. cruzi* detected by immunohistochemistry (19-23) or *in situ* PCR (28). Sections with high severity of myocarditis are significantly associated with the presence of the parasite (22). At the same time, there are many sections with mild or moderate inflammation in which *T. cruzi* antigens are not detected (21, 22, 29). There are also reports that state that the severity of inflammation is not associated with the presence of the parasite (19, 26). Interestingly, the degree of inflammation sometimes seems disproportionate to the quantity of *T. cruzi* in the tissue (23, 29). These observations could support either the parasite persistence hypothesis or the autoimmunity hypothesis. The inflammation could be due to (i) the presence of parasite antigens from nearby disintegrating *T. cruzi*; (ii) autoimmunity against neighboring cells; (iii) tissue ischemia and subsequent inflammation; (iv) an overly aggressive anti-parasite inflammatory response to the chronic presence of the parasite, among other hypotheses (29). In summary, *T. cruzi* antigens are associated with severely inflamed tissue but there is not a high association between *T. cruzi* antigens and the presence of inflammation, because of limitations in the specific assay or biologic reasons.

In support of the third line of evidence, immune responses have been shown to target *T. cruzi* and mediate destruction of infected cells *in vitro*. There is a wealth of reports in the literature on this topic (reviewed in (7, 31)), including destruction by antibody-dependent cellular cytotoxicity (32, 33), cytotoxic T lymphocytes (34-36), and Th1 $CD4^+$ lymphocytes (37), among others.

Lastly, elimination of *T. cruzi* by chemotherapy reduces disease in certain instances. Administration of anti-*T. cruzi* chemotherapy during acute disease generally protects mice from parasitosis, mortality and disease (reviewed in (38)). Administration of chemotherapy during the chronic phase has been reported to reverse lesion development in experimental animals (39). In human disease, there is no consensus on the efficacy of chemotherapy on cardiomyopathy (40-45) and so it is difficult to ask the question of whether elimination of *T. cruzi* eliminates disease.

Although it might seem obvious that the presence of parasite antigen in tissue would lead to the development of parasite-specific immunity and consequent tissue inflammation, this has not been proven. The mere coexistence of parasite antigen or DNA and infiltrating mononuclear cells does not address the antigen specificities of the lymphocytes and certainly does not speak to the inflammatory potential on a per cell basis. The one experiment that may address this in part was conducted by Kumar *et al.* (37) in which *T. cruzi* expressing ovalbumin was used for infection. Ovalbumin-specific $CD4^+$ lymphocytes, adoptively transferred into animals infected with the ovalbumin transgenic *T. cruzi*, accumulated in the parasitized tissue as determined by immunohistochemical staining with a clonotype-specific antibody. In our view,

this result strongly suggests that the transferred lymphocytes detected their cognate antigen expressed by the trypanosome. It will be important to follow up on this finding perhaps by testing for ovalbumin-specific cells that expand naturally upon infection with the transgenic parasite (*i.e.*, not via adoptive transfer) or by developing a number of *T. cruzi* antigen-specific T cell clones and clonotype specific antibodies that can be used to assess antigen-specific T cell infiltration and effector function using wildtype parasites.

In conclusion, there is strong evidence in support of the hypothesis that Chagas disease can be explained by persistent parasite and anti-parasite immunity. At the same time, this does not exclude a role for autoimmunity or other mechanisms in disease (8).

4. CHAGAS HEART DISEASE IS CAUSED BY AUTOIMMUNITY

The hypothesis that CHD is an autoimmune disease should be divided into two separate questions: (i) whether autoimmunity can be induced by infection with *T. cruzi*, and (ii) whether *T. cruzi* induced autoimmunity is pathogenic. The difference between these two questions is, in our view, the main source of confusion in the literature debating the hypothesis that CHD is an autoimmune disease.

The first hypothesis, whether autoimmunity can be induced by infection with *T. cruzi*, is supported and well documented. Infection with *T. cruzi* in humans and experimental animals induces both humoral and cellular autoimmunity to host antigens including cardiac, skeletal, and nervous antigens, among others (reviewed in (3)). Infection with *T. cruzi* induces autoantibodies to myosin (46, 47), beta adrenergic receptor (reviewed in (48)), cytoskeletal and microtubule associated proteins (49), nervous tissue proteins (50), ribosomal P proteins (51, 52), and a novel mammalian protein, Cha (53). On the cellular side, infection with *T. cruzi* induces T cells specific for heart homogenate (54, 55), neuronal antigens (56), cardiac (47, 57) and skeletal myosin (58, 59), and Cha antigen (53). Therefore, infection with *T. cruzi* induces both humoral and cellular autoimmunity.

The second hypothesis, whether *T. cruzi* induced autoimmunity is *pathogenic*, is not as well documented. To date, there is no direct evidence that *T. cruzi* induced autoantibodies cause disease. Many of these target proteins have ubiquitous expression which do not support the heart specific disease in CHD patients. In addition, many of the targets are intracellular so it is difficult to devise a mechanism by which autoantibodies cause disease if their targets are inaccessible. Furthermore, the evidence that these autoantibodies are more prevalent in patients with CHD than in asymptomatic, *T. cruzi* infected individuals, is scarce (46, 60; reviewed in (3)) and contested (3). On the other hand, antibodies from infected patients affected the cell signaling and contraction of cardiac myocytes (reviewed in (48)) and lysed myocytes through antibody dependent cytotoxicity *in vitro* (61). In addition, immunization with *T. cruzi* cruzipain induced autoantibodies to myosin, conduction abnormalities, and IgG deposition (62). The authors suggested that these autoantibodies are pathogenic because of the association with the observed conduction abnormalities in mice.

There is some direct evidence supporting a contribution of cellular autoimmunity to CHD. It has been reported that splenocytes from chronically infected mice can lyse syngeneic myoblasts *in vitro* (63, 64) or induce inflammation in sciatic nerves (56) and hearts when adoptively transferred to naïve recipient mice (63). Immunization of mice with *T. cruzi* ribosomal proteins (65) or cruzipain induced cardiac conduction abnormalities (58, 62). Cruzipain immunization also induced skeletal myositis accompanied by production of myosin autoantibodies and autoreactive T cells. Since no live parasites were used in the ribosomal or cruzipain protein immunization experiments, the induction of autoreactive cells and cardiac damage was thought to be due to a molecular mimicry mechanism. Transfer of $CD4^+$ T cells from chronically infected mice mediated the rejection of implanted syngeneic newborn hearts (54), but if a different combination of mouse and parasite strain was used, this did not occur (30). Perhaps the conflict in these results was due to the difference in susceptibility to autoimmunity of host strains infected with certain parasite strains (discussed below). Strikingly, suppressing autoimmunity to heart antigens enriched for myosin reduced cardiac inflammation, fibrosis and myosin autoantibody production in chronically infected mice (66). Though the autoimmune disease control used in this study does not agree with previously published results (67), the result still suggests that cardiac autoimmunity significantly contributes to chronic experimental CHD.

Two criticisms are often used against the hypothesis that CHD is an autoimmune disease. The first is that immunosuppressants, which generally relieve symptoms of autoimmune diseases, exacerbate mortality and disease in Chagas patients. The best examples of this are Chagas heart transplant recipients receiving immunosuppressants, and Chagas patients infected with HIV. For the record, the largest multicenter study on CHD heart transplant recipients concluded that CHD patients have no difference in mortality compared to heart transplant recipients suffering from idiopathic dilated cardiomyopathy or ischemic cardiomyopathy (68). In addition, 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 and therefore disease. The second criticism states that autoimmunity does not contribute to CHD 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 CHD (40-45). 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

(reviewed in (38)). 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.

In summary, infection with *T. cruzi* induces autoantibodies and autoreactive T cells to a variety of autoantigens. Though there are some reports on the pathogenic potential of this autoimmunity, CHD has not yet been shown to be an autoimmune disease. Adoptive transfer and immunization experiments with parasite proteins do not necessarily recapitulate events in infected mice and so no conclusion can yet be made about whether *T. cruzi*-induced autoimmunity directly contributes to tissue damage in human Chagas disease, or for that matter, in infected mice.

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

A number of mechanisms may explain how *T. cruzi* induces autoimmunity in a host which is normally tolerant to its own antigens. These are reviewed in some detail in ref. (10) and are briefly summarized here.

Polyclonal activation involves the antigen-independent stimulation of self-reactive lymphocytes. Polyclonal activators stimulate a large percentage of both T cells and B cells, 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 production of a wide repertoire of acute autoantibodies in mice (69). These autoantibodies have weak affinities and are often of the IgM isotype. Certain strains of *T. cruzi* have been shown to possess polyclonal activators, suggesting that this mechanism may possibly induce autoimmunity during infection (70).

In **molecular mimicry**, autoimmunity results from a "misdirected" immune response. The immune response is first directed to a parasite antigen and "cross-reacts" with a host antigen that resembles it, causing autoimmunity. Putative cross-reactive proteins of host and *T. cruzi* have been identified. These include *T. cruzi* ribosomal P proteins and their mammalian homologues (51, 52), *T. cruzi* shed acute phase antigen and the human Cha autoantigen (53), *T. cruzi* B13 and cardiac myosin (46) and *T. cruzi* cruzipain with skeletal myosin (58). Furthermore, autoimmunity can result from immunization with a *T. cruzi* antigen (58, 65).

The third mechanism, **cryptic epitope**, posits that *T. cruzi* infection leads to release of previously sequestered epitopes or, alternatively, that the inflammatory environment induced by *T. cruzi* alters antigen processing and presentation such that novel self epitopes are generated. Because the immune system is not tolerant to the novel epitopes, immunity develops rapidly (71). In support of this hypothesis, antigen processing and presentation was altered after *in vitro* treatment with interferon gamma (reviewed in (72)). Although not formally demonstrated in *T. cruzi* infection, this mechanism is thought to be involved in the pathogenesis of rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune diseases (73).

In the mechanism of **bystander activation**, *T. cruzi* infection causes tissue destruction and release of host antigens. The excess levels of host antigens released in a proinflammatory environment may stimulate autoreactive cells, initiating autoimmunity. Evidence for this hypothesis includes the observation that cardiac autoimmunity can result from many, varied types of insults to the heart, including infection with viruses and parasites, heart surgery and heart transplantation (74-76). In addition, the autoantigen that initiates bystander autoimmunity may not be the major target autoantigen throughout the course of disease. In this process, called "epitope spreading," 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 presentation of which induces the stimulation of autoimmunity of additional epitope specificity(ies). Interestingly, the initial responses may wane due to immunoregulation, leading to "waves" of autoimmunity targeted to different epitopes (77).

In addition, there is the question of the time at which *T. cruzi*-induced autoimmunity develops. It may be induced immediately after the initial contact of the parasite with the host, during the acute phase of disease (47, 78, 79). 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 (61, 64). Persistent, chronic inflammation may be necessary to overcome the threshold of cardiac damage or produce the proper inflammatory environment for the stimulation and expansion of autoreactive cells. These mechanisms are not exclusive of each other and combinations of these mechanisms may play a role in the induction of autoimmunity during *T. cruzi* infection.

Finaly, immunogenetic factors of the host and genetic characteristics of the parasite may also influence the induction of autoimmunity. Certain strains of mice are susceptible to *T. cruzi* induced autoimmunity, while others are resistant (47). There is also 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 (80, 81)

6. CONCLUSIONS

It is clear that autoimmunity can be induced by *T. cruzi* infection in humans and experimental animals (Figure 1), although, to date, there is no proof that autoimmunity directly contributes to disease pathogenesis. However, as discussed above, it is only presumed and not proven that *in*

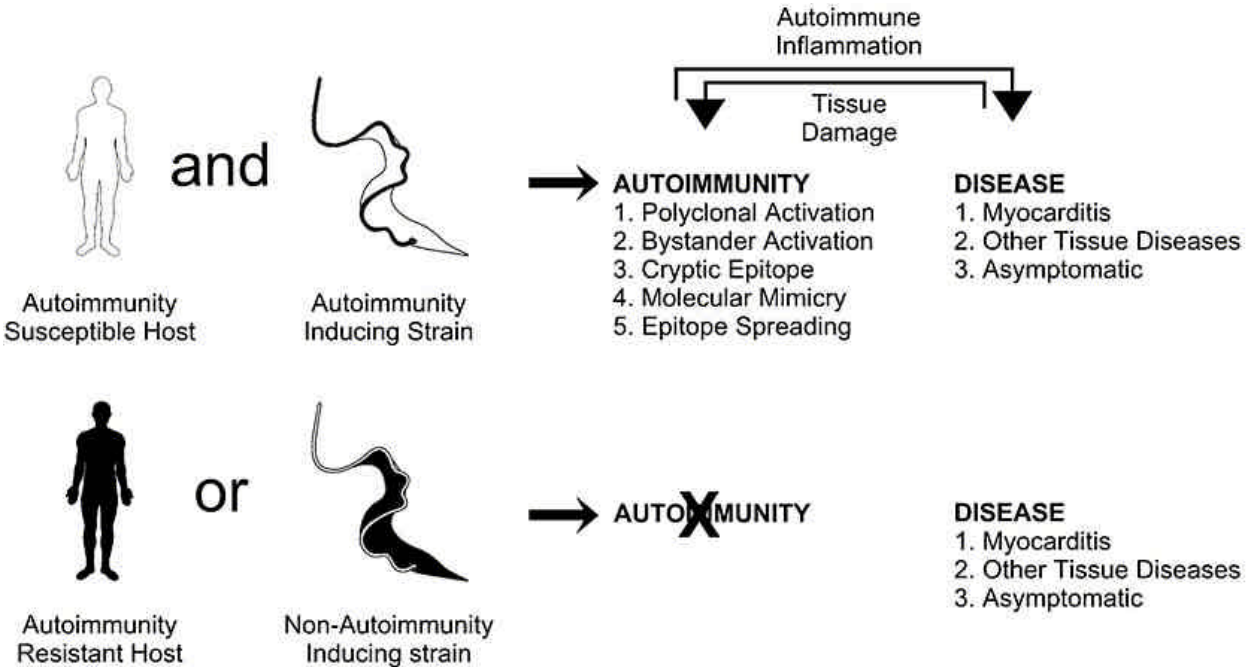

Figure 1. Model of autoimmunity induction in Chagas heart disease. Host infection with *T. cruzi* may induce autoimmunity and/or disease depending on host immunogenetics and parasite genetics. Infection involving an "autoimmunity susceptible" host and "autoimmunity virulent" parasite clone (open figures, top) may lead to pathology via direct or indirect (*i.e.*, bystander activation after tissue damage) mechanisms. Infection involving an "autoimmunity resistant" host or "autoimmunitiy avirulent" parasite (filled figures, bottom) may lead to disease only through mechanisms other than autoimmunity.

vivo anti-*T. cruzi* immunity is responsible for inflammation and damage present in the myocardium. In our opinion, the mechanism(s) that underlie the pathogenesis of Chagas disease are as varied as the outcome of human infection and the factors that determine which mechanisms may participate are only beginning to be understood. Why is it that less than half of all infected people develop clinical disease? In what fraction of individuals with Chagas heart disease does autoimmunity exist and in what fraction of those is the autoimmunity pathogenic and not merely phenomenologic? 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. Anti-*T. cruzi* chemotherapy and vaccine trials must be pursued regardless of whether CHD is proven to have an autoimmune component to pathogenesis.

7. ACKNOWLEDGEMENTS

We thank Drs. Kegiang Wang and Randal Tibbetts for their contributions to the research. Much of the work was supported by grants and fellowships from the National Institutes of Health and the American Heart Association.

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Key Words: Heart, Infection, Parasite, Myocarditis, Autoimmunity, Chagas Disease, *Trypanosoma cruzi*, Review

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