REVIEW

Autoimmune Pathogenesis of Chagas Heart Disease

Looking Back, Looking Ahead

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Chagas heart disease is an inflammatory cardiomyopathy that develops in approximately one-third of individuals infected with the protozoan parasite *Trypanosoma cruzi*. Since the discovery of *T. cruzi* by Carlos Chagas >100 years ago, much has been learned about Chagas disease pathogenesis; however, the outcome of *T. cruzi* infection is highly variable and difficult to predict. Many mechanisms have been proposed to promote tissue inflammation, but the determinants and the relative importance of each have yet to be fully elucidated. The notion that some factor other than the parasite significantly contributes to the development of myocarditis was hypothesized by the first physician-scientists who noted the conspicuous absence of parasites in the hearts of those who succumbed to Chagas disease. One of these factors—autoimmunity—has been extensively studied for more than half a century. Although questions regarding the functional role of autoimmunity in the pathogenesis of Chagas disease remain unanswered, the development of autoimmune responses during infection clearly occurs in some individuals, and the implications that this autoimmunity may be pathogenic are significant. In this review, we summarize what is known about the pathogenesis of Chagas heart disease and conclude with a view of the future of Chagas disease diagnosis, pathogenesis, therapy, and prevention, emphasizing recent advances in these areas that aid in the management of Chagas disease. (*Am J Pathol* 2015, 185: 1537–1547; <http://dx.doi.org/10.1016/j.ajpath.2014.12.023>)

Chagas disease, caused by infection with the protozoan parasite *Trypanosoma cruzi*, is a neglected tropical disease that affects up to 10 million people worldwide. The disease is endemic in vast areas of the western hemisphere from the United States to Argentina, and many thousands of *T. cruzi*-infected individuals also reside in Canada, Europe, Japan, and Australia.¹ Although most infected individuals will experience a lifelong subclinical infection, approximately one-third will develop a potentially fatal inflammatory cardiomyopathy and/or megadisease of the gastrointestinal tract. Since the discovery of the illness and its causative agent by Chagas in 1909,² there has been extensive basic, translational, and clinical research on this important disease, which has led to the development of several successful strategies for public health intervention and informed approaches to vaccine

development and drug discovery. Among the features of this disease, perhaps the most interesting is also the most understated—the tremendous variability in the outcome of infection, largely determined by genetic heterogeneity in both the virulence of specific *T. cruzi* strains and the susceptibility of a specific individual to infection. This unpredictable variability, compounded by differential effects of the physiological state of both parasite and host on the

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Figure 1 Clinical course of *Trypanosoma cruzi* infection. During the acute phase of infection, nearly all individuals are unaware of infection, having no signs or symptoms of infection; mild and/or non-specific signs and symptoms, such as anorexia, fever, fatigue, or lymphadenopathy; or more specific, if not pathognomonic, signs of recent infection, such as unilateral periorbital edema (Romaña sign). A few individuals (approximately 1%), usually children in endemic regions, develop clinically apparent myocarditis, which normally resolves within several weeks; a few of these children (0.01% to 0.05%) succumb to myocarditis, typically due to fatal dysrhythmias. After this acute phase of a few months' duration, virtually all infected individuals harbor subclinical (silent) disease for a variable time (several years). From that point forward, approximately 30% of infected individuals develop cardiomyopathy or megadisease during the next several decades, with approximately one-third of these (ie, 10%) with clinically apparent, symptomatic disease above the clinical threshold. Approximately 20% develop organ dysfunction that is detectable through clinical testing. Most (approximately 70%) have lifelong infection with no organ dysfunction. This figure was adapted from Rosenbaum,³ copyright 1964, with permission from Elsevier. The percentages given in this figure are rough estimates, and the outcome of infection may vary greatly depending on variables.

outcome of infection, has complicated efforts to treat and cure Chagas disease for decades.

Trypanosoma cruzi infection is often characterized as having distinct acute, indeterminate, and chronic phases. Although this convention is widely used, we believe that it is easier to understand the course of infection as conveyed by our modification of a scheme by Rosenbaum (Figure 1).³ After infection through contact with trypanosome-containing excreta from the reduviid bug vector that transmits *T. cruzi*, consumption of food or beverages contaminated with excreta, or transfusion/transplantation of blood/tissue from an infected donor, the parasite goes through several rounds of host cell invasion, division, and cell lysis for up to several weeks. During this time, parasites can often be detected in the blood. However, most infected individuals demonstrate little, if any, indication of being infected. These are included in the 99% subclinical group (Figure 1), even though some may develop non-specific symptoms, including anorexia, chills, diarrhea, drowsiness, fever, lethargy, lymphadenopathy, and swelling near the infection site.⁴ Approximately 1% of the infected individuals, mostly children, develop clinically apparent myocarditis; most recover, but a few (1% to 5%) develop fatal complications, including acute heart failure, fatal dysrhythmias, or fulminating meningoencephalitis.⁴ Morbidity and mortality during acute infection can be much higher in some cases. In two microepidemics in

northeastern Brazil caused by consumption of *T. cruzi*-contaminated food, 12 of 13 infected individuals developed fever and dyspnea, 5 developed congestive heart failure, and 2 died.⁵

In most immunocompetent individuals, parasite-specific adaptive immunity is generally sufficient to keep the parasite at low or undetectable levels. This is the indeterminate phase of Chagas disease. Beginning a variable time after infection (Figure 1), and extending for decades, approximately 30% will develop chronic Chagas disease, which primarily affects the heart and gastrointestinal tract, but may also involve the nervous and endocrine systems.^{6,7} Approximately one-third of these individuals will have clinically overt cardiomyopathy, typically manifested as congestive heart failure, whereas two-thirds will develop subclinical Chagas disease that is detectable via several diagnostic modalities. Death from chronic Chagas disease is often the result of congestive heart failure after gradual development of myocardial dysfunction due to damage caused by chronic inflammation.^{6,7}

In chronic Chagas heart disease (CHD), cardiac myocytes undergo necrosis and cytolysis via various mechanisms, and areas of myocellular hypertrophy and mononuclear cell infiltration develop in the cardiac tissue, with fewer macrophages, eosinophils, neutrophils, and mast cells.^{4,8–10} Fibrosis develops in response to myocardial damage. This remodeling

may disrupt the cardiac conduction system, leading to dysrhythmias as well as myocardial thinning and cardiac hypertrophy.⁴ Although parasite DNA or antigen can be detected in some inflammatory lesions, intact parasites are frequently absent from the heart in chronic CHD.¹¹ In addition to the histopathological changes mentioned earlier in this paragraph, development of occlusive thrombi, apical aneurysm of the dilated left ventricle, decreased contractility of muscle fibers, and destruction of the cardiac conduction system are also commonly found. The associated muscle damage leads to dilation and cardiac dysrhythmias, high-degree heart block, and, ultimately, congestive heart failure, which is the leading cause of death in chronic CHD patients.¹²

Diagnosis of acute *T. cruzi* infection may be made by microscopic analysis of whole blood or buffy coat for the presence of motile bloodform trypomastigotes released from host cells after initial rounds of replication. In chronic Chagas patients, enzyme-linked immunosorbent assay, indirect hemagglutination, or indirect immunofluorescence can be used to test for the presence of *T. cruzi*-specific IgG in sera.⁴ Large-scale screening of the blood supply is underway in many parts of the western hemisphere, with a variety of commercial/industrial immunoassays used; however, several areas with at-risk populations still do not routinely screen all blood products.¹³

To date, only two anti-*T. cruzi* drug treatments are available, nifurtimox and benznidazole, both of which oxidize the parasite's nucleotide pool, leading to lethal double-stranded DNA breaks. Proper administration of either of these drugs can successfully ameliorate the symptoms of Chagas disease in many individuals, especially those experiencing the acute form of the disease. However, existing treatments for Chagas disease have limited efficacy in certain populations because they require repeated administration over a prolonged time period, and often produce severe adverse effects that cannot be tolerated by all individuals. These characteristics likely suppress compliance with treatment, and subsequently delay resolution of this substantial public health burden. Although the treatment of chronically infected individuals is somewhat controversial, given that most will never develop organ dysfunction, the Latin American Network for Chagas Disease now recommends treatment for all chronically infected individuals.¹⁴ Heart transplantation may also be performed on patients with severe chronic CHD experiencing congestive heart failure; however, the reappearance of *T. cruzi* infection may occur with immunosuppressive therapy after the transplantation procedure. Currently, no effective vaccine is available for preventing *T. cruzi* infection, although many share the view that the outlook is more promising now than in the past.¹⁵

Mechanisms of Disease Pathogenesis

Several distinct mechanisms have been proposed to account for the widely varying cardiac pathology observed in

T. cruzi-infected individuals. These mechanisms include damage to cardiomyocytes that is directly mediated by live parasites, bystander damage resulting from exposure to parasite-specific immune responses, cellular damage due to the effects of non-specific immune responses in the local microenvironment, microvasculopathy leading to ischemia and inflammation, and autoimmunity, which may result from several pathogenic mechanisms (Figure 2). Most published research on CHD pathogenesis addresses two issues—parasite persistence and autoimmunity. This does not mean that the other mechanisms are not operative or important—just that they have not been as well investigated.

In any individual who develops disease, one or more of these mechanisms may contribute to pathogenesis. This issue is further complicated by the fact that infections commonly involve mixtures of parasite clones, each having unique pathogenic potential (ie, polyparasitism).¹⁶ *Trypanosoma cruzi* clonal variation may influence tissue tropism and the ability to establish a chronic infection, whereas host genetic polymorphisms that affect cytokine production may modulate disease severity and even the mechanism of pathogenesis.^{17–21} Integration of *T. cruzi* kinetoplast DNA into the genomic DNA of host cells may further alter the outcome of infection.^{6,22,23} Determining the contributions of one mechanism or another is also extremely difficult in the presence of live *T. cruzi* infection, in which multiple coincident mechanisms may be operative. However, the current body of evidence suggests that parasite is a key requirement for every mechanism.

Parasite Persistence

An obvious explanation for the myocarditis associated with *T. cruzi* infection was based on reports of parasites in the inflamed hearts of deceased Chagas patients and the presumption that the chronic inflammation and myocardial fibrosis associated with *T. cruzi* infection were primarily the result of a reparative process initiated to ameliorate the damage to myocytes that was mechanically inflicted by live parasites.⁶ A direct correlation between parasite persistence and inflammation has been proposed on the basis of detection of *T. cruzi* antigens and genetic markers via PCR in heart sections showing signs of myocarditis.^{24,25}

Lysis of host cells by *T. cruzi* is undoubtedly responsible for some degree of cardiac tissue damage (Figure 2A). Robust cardiac infiltration by leukocytes may also cause mechanical disruption to the myocardium and associated vasculature and innervation (Figure 2B), and inflammatory mediators released by lymphocytes, macrophages, eosinophils, neutrophils, and mast cells may cause further bystander damage to myocytes (Figure 2C).^{4,26} *Trypanosoma cruzi* is capable of subverting the host bradykinin system by activating bradykinin B2 receptors during endothelial cell invasion, leading to vasodilation and subsequent interstitial edema.²⁷ *Trypanosoma cruzi* also produces several bioactive lipids, including thromboxane A and

Figure 2 Mechanisms by which *T. cruzi* infection can cause myocarditis. **A:** Direct damage by parasites caused by cell lysis by trypomastigotes that have differentiated from intracellular amastigotes (**top panels**) or a likely toxic (lytic) product of trypomastigotes (**bottom panels**). **B:** Parasite-specific immunity may contribute to cardiac pathology due to the destruction and displacement of myocytes, followed by mononuclear infiltration and fibrosis. **C:** Non-specific damage caused by innate immune responses, granulocyte activation, and antibody-mediated cytotoxicity may cause bystander injury to cardiomyocytes. **D:** Microvasculopathy leading to ischemia, including occlusive platelet aggregation. **E:** Parasite-induced autoimmunity generated by molecular mimicry between parasite and self-antigens (**left panels**) or bystander activation of autoreactive T cells after cell lysis by *T. cruzi* (**right panels**).

prostaglandin F2 α , which promote vascular constriction, platelet aggregation, and vascular smooth muscle proliferation (Figure 2D).²⁸ Another mechanism through which live parasites may induce myocardial damage is via the induction of oxidative stress—induced injury. It has been shown that oxidative damage to mitochondrial respiratory complexes in *T. cruzi*—infected cells leads to reduced mitochondrial ATP generation and deficiencies in cellular antioxidant defense mechanisms, such as reduced levels of superoxide dismutase,²⁹ and the level of oxidative injury biomarkers in the myocardium positively correlates with chronic disease severity.²⁹ Most recently, it was determined that cardiac miRNAs are dysregulated during *T. cruzi* infection, and several of these regulate key signaling pathways that are likely involved in disease pathogenesis.³⁰

Others have hypothesized that production of some type of toxin may contribute to parasite-mediated damage (Figure 2A). Although no conventional toxins have been identified in *T. cruzi*, the parasite produces some substances that may have substantial toxic effects on cells *in vivo*. Two examples of such substances are TC-Tox and LYT1, both acid-active hemolysins presumably involved in escape of the parasite from the parasitophorous vacuole into the cytoplasm of infected cells.^{31,32} Interestingly, both of these proteins immunologically cross-react with human complement

protein C9, although considerable dissimilarity exists between them on both the DNA sequence and protein level. It is unlikely that these, or similar molecules if they exist, play a significant role in CHD pathogenesis, but it is conceivable that some amount of tissue injury may occur secondary to cellular damage inflicted by these pore-forming proteins.

Although dogma presumes that most infiltrating lymphocytes recognize specific parasite antigens, this has not been definitely proved. Notably, it has been widely documented that the degree of tissue parasitism does not correlate with the severity of Chagas cardiomyopathy, and even the application of highly sensitive techniques, such as PCR and immunohistochemistry, has variably found correlation²⁴ or lack of correlation^{27,33} between the presence of parasite antigen/DNA and tissue inflammation. In an extensive, highly detailed histopathological analysis of CHD, Laranja et al³³ found that parasites were rarely present in >20% of tissue sections containing significant inflammation. These findings, in conjunction with the extant evidence that other mechanisms, such as autoimmunity, are operative in *T. cruzi*—infected humans and experimental animals, indicate that the pathogenic immune response generated in individuals with CHD is not entirely parasite specific. Indeed, the variability in cardiac histopathology in CHD likely reflects the heterogeneity in the inflammatory mechanism involved. Different

Figure 3 Different histological outcomes of *Trypanosoma cruzi* infection in the heart. The cardiac pathological manifestations of infection range from no inflammation to focal carditis to pancarditis, each of which can develop with or without focal, regional, or massive tissue parasitosis. The complex interplay between parasite pathogenicity, host susceptibility, and physiological variables determines disease outcome. Shown here are four representative images of hematoxylin and eosin–stained cardiac sections. **A:** Normal histology, which can occur with a low virulence *T. cruzi* strain or a resistant host. **B:** Mononuclear cell infiltration, fibrosis, and edema, without parasites, which can occur during infection with certain *T. cruzi* strains and/or human or animal hosts. **C:** Inflammation with parasitosis. **D:** Higher magnification of the parasite pseudocyst marked by the arrow in C. The pseudocyst is filled with intracellular amastigotes. Original magnification: $\times 40$ (A–C); $\times 160$ (D).

combinations of parasite and host strains can be used for experimental infections having three basic cardiac histological outcomes: normal histology (Figure 3A), inflammation without parasitosis (Figure 3B), and inflammation with parasitosis (Figure 3, C and D). The degree of inflammation can vary from extremely mild to extremely severe (Figure 3, B and C).

Autoimmunity

The autoimmunity hypothesis suggests that cardiac damage, regardless of initial cause, leads to a breakdown of self-tolerance, resulting in an immune reaction against self-proteins. Cardiac autoimmunity may be initiated by parasite-induced damage to cardiomyocytes^{34,35} and/or by molecular mimicry between immunologically similar epitopes of parasite and host proteins (Figure 2E).^{36–38} During bystander activation, mechanical damage caused directly by infective *T. cruzi*, followed by subsequent parasite-specific and non-specific immune responses, would result in the release of copious amounts of self-antigens in an environment particularly rich in inflammatory mediators, including cytokines and chemokines, lymphotoxin, nitric oxide, and granule components from eosinophils and polymorphonuclear cells.³⁹ This milieu of potent immune stimuli may overcome the threshold of activation needed to breach self-tolerance, and stimulate cytotoxic T cells without directly triggering the T-cell

receptor, resulting in autoimmunity targeted against multiple antigens. Bystander activation involving CD8⁺ T cells may also occur in response to *T. cruzi* antigens that are presented on the major histocompatibility complex I molecules of infected cells. Inflammatory factors present in the local environment, such as interferon- γ and nitric oxide, are known to promote the activation of potentially autoreactive T cells encountering major histocompatibility complex–bound cognate antigen. Once activated, autoreactive lymphocytes may proliferate in response to self-antigen presented on host antigen-presenting cells. Molecular mimicry between parasite and host antigens may also contribute to Chagas pathogenesis by driving a deleterious autoimmune response, as has been demonstrated for experimental autoimmune encephalomyelitis (EAE), a better understood model of autoimmune pathology induced by molecular mimicry that is a surrogate for studying aspects of multiple sclerosis pathogenesis in humans.⁴⁰ The potential to extend the knowledge of autoimmunity in Chagas disease to understand other infectious diseases with autoimmune components and to develop potential treatments further supports continued study of *T. cruzi*–induced autoimmunity.

History and Future of Autoimmunity in CHD

The earliest discussions of autoimmunity in the literature did not use this term, but rather refer to *allergic* or *hyperergic* mechanisms of pathogenesis, perhaps induced by infection but ultimately independent of the parasite.⁴¹ This view was reinforced by the finding that cardiac autoantibodies could induce myocarditis in rats⁴² and the establishment of the first experimental autoimmune myocarditis models.⁴³ Autoimmunity has been considered an important contributor to the complex immune response that develops in *T. cruzi*–infected individuals since the mid-1970s, when it was observed by Santos-Buch and Teixeira⁴⁴ that *T. cruzi* infection promoted rejection of allogeneic heart cell transplants, and that lymphocytes from *T. cruzi*–infected animals rapidly destroyed embryonic cardiomyocytes in culture. This work, together with those of Cossio et al⁴⁵ on the endocardial-vascular-interstitial antibody and of Ribeiro dos Santos et al⁴⁶ on immunoglobulin and complement deposition on neurons, was highlighted in a commentary in the *British Medical Journal* in 1977.⁴⁷

By the late 1970s, some prominent researchers were cautioning those working on the development of *T. cruzi*–specific vaccines and other immunomodulatory therapies because of the poorly gauged possibility of inadvertently inducing potentially fatal autoimmunity.⁴⁸ However, reports that protective *T. cruzi*–specific immunity could be induced without eliciting pathogenic autoimmunity were soon published, providing some assurance that parasite-specific therapeutics could be used without the concern that exposure to *T. cruzi* antigens in the presence of immune-activating

adjuvants might induce harmful autoreactive responses.^{49,50} The debate about a role for autoimmunity in Chagas disease, and the quest for clues to the etiology of this phenomenon, continued to unfold in a series of articles published in the 1980s.^{51–54} Autoreactive T cells and antibodies were identified in individuals with chronic Chagas disease, and several specific antigens abundant in the myocardium were identified as targets of autoreactive responses.^{53–57}

By the 1990s, it had been proposed by several groups that molecular mimicry between *T. cruzi* antigens and host antigens was involved in the induction of autoimmunity.^{58–60} Several *T. cruzi* proteins, including ribosomal P protein and B13, were identified as cross-reactive to host proteins, such as cardiac myosin,^{61–63} lymphocyte antigens,⁶⁴ neuronal tissues,⁶⁵ human ribosomal P proteins,^{36,66} muscle antigens,⁶⁷ and small nuclear ribonucleoprotein.⁶⁸ Antibodies targeting β 1- and β 2-adrenergic receptors in human Chagas patients were then linked to the development of atrial arrhythmias and dilated cardiomyopathy,⁶⁹ and antibodies specific for human m2 muscarinic acetylcholine receptors were identified in patients with Chagas disease as a potential contributor to Chagas' cardioneuromyopathy.⁷⁰ Antibody-dependent cytotoxicity, whereby antibodies present in the sera of chronically *T. cruzi*-infected mice bind to and mediate destruction of myocytes, was identified as another potential mechanism of autoimmune pathology.⁷¹

A series of reports describing robust autoreactive T-cell and antibody responses specific for cardiac myosin, combined with findings that molecular mimicry and breach of immunological tolerance to cardiac autoantigens trigger potentially pathogenic autoimmunity, were published in the early 2000s.^{72–75} Potential targets for cross-reactive antibodies in sera from Chagas patients and experimentally infected animals were identified as ribosomal proteins, G-protein-coupled receptors, neuronal proteins, and at least one novel host protein named Cha.^{46,57,62,63,76–78} Interestingly, immunization of mice with the *T. cruzi* cysteine protease cruzipain induced autoantibodies that pathologically inhibit the function of cardiac muscarinic acetylcholine receptors, whereas a DNA vaccine containing the genes that encode several *T. cruzi* proteins, including cruzipain, was later found to provide protective immunity against *T. cruzi* infection without causing cardiac abnormalities.⁷⁹ The ability of cruzipain to modulate antigen expression by macrophages and induce a proinflammatory cytokine response likely contributes to both scenarios.⁸⁰

Exposure to *T. cruzi* antigens in the absence of infection with live parasites was shown to be sufficient to induce both autoimmunity and a modest, but detectable, level of cardiomyocyte damage, as evidenced by production of polyantigenic humoral and cell-mediated autoimmunity and release of cardiac troponin I into the serum, respectively.^{73,81} The idea behind the latter finding was that the autoimmune response to *T. cruzi* infection would exhibit epitope spreading,⁸² the stepwise temporal development of

peptide-specific autoimmune responses, as it does in mouse models of multiple sclerosis.⁸³ Rather, *T. cruzi* infection leads to an explosion of autoimmune responses to many cardiac antigens simultaneously.⁸² We found that treatment of *T. cruzi*-infected mice with benznidazole to eliminate the parasite also resulted in a resolution of cardiac pathology and humoral and cell-mediated autoimmunity.³⁴ Reinfection caused reestablishment of both autoimmunity and cardiac inflammation, providing the first clear link between parasite persistence and autoimmunity that could potentially reconcile the two camps.

However, subsequent reviews and reports resumed the ongoing debate, with some arguing that autoimmunity is an epiphenomenon of *T. cruzi* infection in some cases, and others maintaining that an autoimmune mechanism of pathogenesis has not been adequately tested.^{13,23,81,84–87} Although the data describing the existence of *T. cruzi*-induced autoimmunity and suggesting links to Chagas pathogenesis continue to grow, those who dismiss the significance seem confident that it is irrelevant to disease pathogenesis in the absence of direct reported evidence. Ongoing efforts to develop and administer *T. cruzi* antigen- and DNA-based vaccines will soon help to address whether protective immunity against *T. cruzi* can be achieved in large populations with no adverse health effects due to autoimmunity. In the case of at least one antigen-based vaccine, which targets a *T. cruzi* 24 kDa trypomastigote excretory-secretory protein (Tc24) and *T. cruzi* trypomastigote surface transialidase (TSA-1), preliminary data have been promising and will likely lead to the start of clinical trials within the next few years.⁸⁸ With other groups simultaneously working on several other antigen and DNA vaccine candidates, continued study of Chagas disease autoimmunity is important. Elucidating the mechanism of Chagas autoimmunity can help address potential complications of the treatments involving autoimmunity, and may serve as a model for studying infection-induced autoimmunity to gain insights, which may apply to other diseases.^{79,88–90}

Perspective

Despite extensive research, the ability to reliably predict and prevent disease outcome in *T. cruzi*-infected individuals remains elusive. Of the factors that contribute to the notable heterogeneity and complexity of Chagas disease, autoimmunity, and its unclear role in the pathogenesis of CHD, is one of the most interesting factors. Clearly, in some humans, autoantibodies and autoreactive T cells are produced during *T. cruzi* infection, with the autoantibodies having proven chronotropic effects on cardiac function. In experimental animals, many detailed studies have described humoral and cellular autoimmune responses, identified cardiac autoantigens and parasite mimic antigens, demonstrated the pathogenic potential of anti-receptor autoantibodies, and shown that autoimmune-mediated cardiac tissue rejection can occur.

The greatest challenge is to determine the significance of a potentially pathogenic mechanism, when multiple coincident mechanisms of tissue inflammation are likely. Of particular importance are the questions of how often autoimmunity develops in *T. cruzi*-infected individuals and whether the autoimmunity leads to cardiac inflammation. Cardiac myosin heavy chain is a particularly interesting target of humoral and cellular autoimmunity associated with *T. cruzi* infection, because it has been implicated in other cardiac infections, including chlamydial and coxsackieviral myocarditis. Because myosin autoimmunity may be pathogenic, we inhibited the myosin autoimmune response in A/J mice infected with the Brazil strain of *T. cruzi* using myosin-specific peripheral tolerance induction.⁹¹ Although both myosin- and *T. cruzi*-specific immunity were significantly decreased (the latter because of molecular mimicry), the mice still developed myocarditis, suggesting the involvement of other potential cardiac autoantigens and excluding autoimmunity. It is most challenging to address this in an antigen-specific manner because there are so many potential autoantigens involved. Further research needs to be conducted to substantiate the importance of autoimmunity in CHD pathogenesis.

We were initially surprised that immunization with *T. cruzi* lysate⁷³ or heat-killed *T. cruzi*⁹² did not induce histologically evident cardiac inflammation despite inducing cardiac myosin autoimmunity of a magnitude similar to that resulting from immunization with purified cardiac myosin. What are the factors that change an autoimmune response from nonpathogenic to pathogenic? This exact question has been addressed in virus-induced EAE.⁴⁰ Immunization of SJL/J mice with the major encephalitogenic myelin peptide PLP₁₃₉₋₁₅₁ in complete Freund adjuvant induces EAE, whereas immunization with a *Haemophilus influenzae* peptide sharing 6 of 13 amino acids with PLP₁₃₉₋₁₅₁ (HI mimic) in complete Freund adjuvant does not. However, when mice were infected with a nonpathogenic Theiler murine encephalomyelitis virus engineered to express PLP₁₃₉₋₁₅₁ or the HI mimic, all of the mice developed EAE. It is believed that the virus provides additional innate immune signals that convert nonpathogenic autoimmune responses to pathogenic ones. This possibility is testable in the context of experimental *T. cruzi* infection. Furthermore, the study of either disease may have implications for the investigation and development of treatments for other infection-induced, organ-specific diseases, such as multiple sclerosis and viral myocarditis, that may have an autoimmune component.

More important, it seems clear that the parasite is essential, for both initiating autoimmunity and maintaining it over time.³⁴ Although parasite persistence and parasite-specific immunity are clearly involved in pathogenesis in many cases, and parasite antigen and DNA may be detected in parasite-free areas of the heart, there are clearly situations in which there is inflammation in the absence of *T. cruzi*

parasites or molecules, as noted by generations of pathologists. The recent development of highly luminescent parasites and the finding of chronic myocarditis in the absence of *T. cruzi* further support this notion.⁹³

Finally, the mere presence of parasites in an inflammatory lesion does not preclude the presence of a coincident autoimmune response in the same lesion. Most authors conclude that Chagas is a complex disease with many mechanisms, which is an understatement at best. The individual mechanism in the setting of live infection can be dissected by inhibiting the individual mechanism; however, such an intervention often affects more than one potential mechanism. For example, interventions to reduce autoimmunity, such as peripheral tolerance induction, can inadvertently suppress protective parasite-specific immune responses. There are experimental models of individual mechanisms, such as autoimmunity, vaso-occlusion, and mitochondrial dysfunction. Although these models may recapitulate the aspects of pathogenesis in isolation, they may only be artificially robust and not adequately reflect the physiological aspects of infection. One such example is the induction of myocarditis via adoptive transfer of lymphocytes. However, despite a controlled setting, *ex vivo* stimulation with self-antigen or mitogen does not confirm that the same cells are inflammatory in the physiological setting.

Although the transmission of *T. cruzi* has significantly decreased in recent years due to improved vector control, *T. cruzi*-induced CHD remains a major health problem in vast areas of the western hemisphere. Much knowledge about the epidemiology, clinical disease progression, and pathogenesis of CHD has been gained during the past century, but there are many aspects of the disease that remain unclear and warrant further investigation. The study of a wide variety of animal models with different strain-strain combinations of parasite and host has allowed for the investigation of specific aspects of the disease that might manifest themselves specifically in infected individuals. From these studies, it is likely that both parasite-mediated damage and parasite-specific immunity and autoimmunity contribute to disease progression. Also, rather than being mutually exclusive, it is likely that these mechanisms act coincidentally or even synergistically (eg, by generating a proinflammatory environment that promotes both parasite killing and autoimmunity) to promote disease progression.

Further investigation into the potential interactions among the various mechanisms of Chagas pathogenesis described herein will allow us to develop safer, more efficient therapies and perhaps even cures for *T. cruzi* infection and Chagas disease. There have been some promising recent developments in the therapeutic area, including testing of cell-based therapies involving transfer of bone marrow cells or mesenchymal stem cells.⁹⁴⁻⁹⁶ This is an exciting avenue that has potential to slow or reverse cardiac dysfunction while maintaining robust parasite-specific immunity. A serious clinical trial investigating the incidence, quality, and

magnitude of humoral and cellular cardiac autoimmunity in infected individuals is needed to fully understand the potentially important clinical problem of *T. cruzi*-induced autoimmunity. Although not specifically focused on autoimmunity, recent genome-wide association studies and specific investigations of polymorphisms in cytokine genes^{97,98} may someday reveal the genetic basis for susceptibility and resistance in humans.

Although benznidazole and nifurtimox remain the mainstay of treatment of *T. cruzi* infection, there have been some recent developments in drug discovery,⁹⁹ and we are all hopeful that redoubled efforts in this area will lead to new individual or combination therapies to cure infection without the toxicity of the current agents. The determination of the *T. cruzi* genome sequence and comparative genomics of various *T. cruzi* strains should also reveal parasite-specific biosynthetic pathways revealing new targets for drug discovery. Vaccine development has enjoyed many recent advances, particularly because it is clear that reduction or elimination of the parasite load has great therapeutic benefit.^{79,88,90,100–102} Development of an effective *T. cruzi* vaccine may one day preclude the need for improving existing therapies. However, until an effective vaccine or improved therapies are widely available, global efforts to treat and prevent Chagas disease must also emphasize increased disease surveillance, including screening of potentially contaminated blood, organ, and food supplies in areas where infection is emerging. Vector control is also an area that deserves ongoing attention and support.

Conclusions

Chagas disease remains a major public health problem throughout the Americas, where transmission via transfusion/transplantation and through contact with infected insects continues to be a matter of concern. The tremendous variability in the outcome of infection and the multiple known mechanisms of tissue inflammation pose significant challenges to those trying to understand disease pathogenesis and work toward new therapies and vaccines.

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