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# IMMUNOLOGICAL TOLERANCE AND ITS BREAKDOWN IN CHAGAS' HEART DISEASE: ROLE OF PARASITOKINES

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## 1. ABSTRACT

Chagas' disease, a debilitating condition inflicting millions of people in Latin America, is caused by infection with the protozoan parasite *Trypanosoma cruzi.* One characteristic sequel to the subdued acute infection is electocardiographic alterations in about one third of the patients that reach the chronic phase of disease. Another feature of chronic Chagas' disease is the paucity of parasites in the diseased heart. There have been many debates whether chronic chagasic cardiomyopathy (CCC) is a consequence of parasite persistence or autoimmunity, a central question that will clearly influence the strategies for disease prevention and treatment. In this review, we summarize the pros and cons of each side and provide a novel view on the genesis, and hence treatment of, CCC. In particular, we emphasize the contribution of parasite-derived danger signal, such as parasitokines, to the breakdown of self-tolerance in *T. cruzi* infection. Accordingly, we argue that a more efficient way of countering immune subversion and autoimmune responses induced by the parasite would be targeting key parasitokines rather than blocking parasitic epitopes cross-reactive with host antigens. Finally, based on current knowledge on immune regulation, especially in transplantation models, we propose that future focus of CCC treatment should rely on efforts to restore the immunological tolerance to self-antigens concurrent with regimens to reduce the parasite load as much as possible through immunological and chemotherapy procedures.

## 2. INTRODUCTION

Chagas' disease, caused by infection with the protozoan parasite *Trypanosoma cruzi,* is well recognized a serious parasitic disease in the Americas. Its social and economic impact by far outweighs even the combined impact of other parasitic diseases throughout the American continents, such as malaria, schistosomiasis and leishmaniasis (1). It is estimated that about 16-18 million people are infected by *T. cruzi* with a further 100 million at risk (2).

The manifestations of the disease can be divided into two phases, namely, an acute infection that usually lasts for 4-8 weeks, and a chronic period that could maintain for decades [see (3-5) for extensive reviews]. Clinically apparent acute chagasic myocarditis may appear transiently in one third to half of chagasic individuals. A small percentage of individuals with acute disease will die of complications associated with acute myocarditis or meningoencephalitis, which usually develop in infants and very young children. The majority of the cases of acute myocarditis are mild and reversible (5). During this period, myocardial pathology is related to host cell damage induced by the parasite and/or immune responses towards the infected cells.

At the end of the acute infection, with host immune system mounting vigorous cellular and humoral responses that usually results in the virtual clearance the parasites, clinical symptoms become inapparent. However, approximately 20-30% of infected individuals will develop a dilated cardiomyopathy (electrocardiographic abnormalities, apical aneurysm and cardiac enlargement) that characterizes the cardiac form of the disease. Chronic chagasic cardiomyopathy (CCC) usually arises decades after the initial infection, thereby at a time when rare

parasites are detected by conventional histochemical examination (4,5). In Latin America, CCC-related heart failure is the leading cause of sudden death in adult chagasic patients (6).

## 3. CURRENT HYPOTHESES ON THE PATHOGENESIS OF CHRONIC CHAGASIC CARDIOMYOPATHY (CCC)

The cardiac form of Chagas' disease has long been suspected to be an autoimmune process (7,8). Histochemical examinations of cardiac muscles revealed that both in patients and in experimental animals, there is an intense infiltrate of mononuclear cells usually accompanied by tissue parasitism that is very scarce or virtually absent (3). It is hypothesized that immune cells are triggered to attack normal heart tissues because of the existence of parasite antigens cross-reactive with host antigens. Moreover, it is suggested that once elicited, autoimmune reactivities progress to cause disease pathology despite the paucity of tissue parasites. Up to date, researchers have identified a good number of parasite antigens that share similar epitopes with host cardiac proteins or other cellular components (9-14). Antibodies raised against these parasite proteins strongly react with counterpart self-antigens at correspondent epitopes, and vice versa (9,11,12). Most important, immunizing animals with the purified forms of either the parasite protein or the mimicked self-antigen in the absence of parasite reproduced cardiac inflammation and/or heart conduction abnormalities similar to that derived from chronic *T. cruzi* infection (15-19). One of such parasite proteins, for example, is cruzipain. This highly antigenic parasite antigen induces antibodies cross-reactive with host skeletal and cardiac myosin heavy chain (17,18). Anti-cruzipain antibodies not only cause inflammatory infiltrates, myopathic changes and electromyographic abnormalities in the immunized females, but also in their offspring when passed through placenta (17), suggesting a pathogenic role of a purified *T. cruzi* antigen in the development of CCC-like autoimmune responses. Observations on these parasite antigens, together with the nature of the disease, such as late onset and organ specificity, make some researchers believe that the pathogenesis of chronic Chagas' disease is due to autoimmunity induced by *T. cruzi* infection (hereafter referred as the "autoimmunity hypothesis") (20).

Recently, some investigators started to critically question the validity of the autoimmunity hypothesis to, instead, emphasize the persistence of parasites in the chronic lesions as the primary cause of CCC. Thus, for these investigators, the failure of the host to clear the infection should result in infection-induced, immune-mediated, tissue damage (hereafter referred as the "parasite persistence hypothesis") (21-23). Using sensitive techniques such as immunohistochemistry, in situ PCR and in situ hybridization, Tarleton et. al. provided solid evidence for a strong link between inflammatory loci at the chronic disease site and the presence of parasite DNA and/or antigens, which, otherwise, were elusive by standard histochemical methods (24). Studies by other groups also suggested a role for *T. cruzi* even in the chronic forms of Chagas' heart disease (25). The parasite persistence hypothesis is further supported by the observations that treatments to reduce parasite load correlate well with decrease in disease severity (26,27), whereas treatments resulting in increased parasite levels cause exacerbated disease (28,29). Such correlation is also evident in certain gene-deficient animal models where higher efficacy of anti-parasite immune responses leads to decreased tissue parasitism and reduced severity of chronic disease (30).

### 3.1. The focus of the debating hypotheses

Although both hypotheses agree on immune-mediated pathogenesis, the two sides have disparate views on how Chagas' disease should be treated and what preventive measures should be taken. Currently, chronically infected chagasic patients rarely receive chemotherapeutics in an effort to eliminate *T. cruzi*, as the supporters of the prevailing autoimmunity hypothesis argue that the disease is caused by autoimmune responses independent of the low level of parasitism (31). In terms of disease prevention, for quite a while, there have been no efforts to develop vaccines because of the concerns that anti-parasite immune responses boosted by vaccination would increase cross-reactivity and hence the severity of autoimmune disease at the chronic stage (32).

In sharp contrast, the parasite persistence hypothesis holds that CCC should be treated as an infectious disease, and chemotherapeutics should be used at any given time of the disease course to eliminate parasites (22,23). Moreover, it predicts that vaccines against the parasite will benefit disease prevention. Animal experiments and a long-term chemotherapy study so far support these views (33). However, advocates of this hypothesis also stipulate that CCC should not be treated as an autoimmune disease. The basis of such reasoning is that immunosuppressive drugs that are currently used for autoimmune disorders universally cause a comeback of parasitism and thus increase in disease severity of chronically infected individuals (28,34).

When studying the current literature on the pathogenesis of CCC, we noticed that some important issues have been left out and misconceptions were introduced into the debates. Thus, in the rest of this review, we hope to provide a more comprehensive model with balanced views (summarized in Table 1). First, we address how tolerance to self-antigens may be broken during *T. cruzi* infection and what role parasite-derived danger signals, such as parasitokines, may play in this process. Second, we discuss the difference between two misunderstood but important concepts, namely, immunosuppression and induction of immunological tolerance. Clarification of these issues will certainly impact the future direction of research and treatment for CCC.

## 4. POLYCLONAL LYMPHOCYTE ACTIVATION AND PARASITOKINES

An essential function of the immune system is to discriminate self and non-self. Lymphocytes whose recognition specificity is towards self-antigens (i.e.

**Table 1.** Summary of hypotheses proposed to explain pathogenesis of CCC

| Hypotheses | Autoimmunity [a] | Parasite persistence [b] | Immune deregulation by parasitokines [c] |
|---|---|---|---|
| Potential mechanisms | Molecular mimicry, cross-reactivity between host and parasite antigens | Persistent parasite infection evading immune surveillance | Breakdown of immune tolerance to self-antigen triggered and maintained by parasite infection |
| Predictions | Once initiated, autoimmunity self-perpetuates independent of parasitism | Inability to clear the infection causes chronic inflammation and immunopathology | Tolerance breakdown in the chronic phase results from immune deregulation in the acute phase |
| Major supporting observations | Intensive mononuclear cell infiltration with scarce parasitism; Abnormalities of heart conduction induced by immunizing cross-reactive antigens in the absence of infection | Correlation between inflammatory loci and parasite material by sensitive in situ PCR and immuno-histochemistry; Regimens that reduce the parasite load help check the severity of tissue inflammation and cardiomyopathy | Acute polyclonal lymphocyte activation that blunts an effective anti-parasite immunity; Parasitokines identified that directly activate naïve immune cells and facilitate infection |
| Implicated treatment strategies | Immunosuppression; not in favor of using chemotherapeutics to eradicate the parasite | Active regimens to eradicate the parasite throughout the course of disease | Active regimens to eradicate the parasite, plus re-establishment of immune tolerance |
| Implicated prevention strategies | Not in favor of vaccination for fear of aggravating autoimmune responses | Vaccination against major epitopes involved in establishing infection | Vaccination primarily against key immune deregulators (i.e., parasitokines) |

a. Ref. (20); b. Ref. (21-23); c. Proposed in this paper.

autoreactive) are potentially harmful to normal tissues and organs, and the majority of these precursor cells are deleted during development via apoptosis--negative selection--in thymus (for T cells) and in bone marrow (for B cells). Still, a small fraction of autoreactive lymphocytes escape negative selection and mature to the periphery (35). These cells are innocuous in normal situations but can cause various autoimmune diseases when improperly activated in processes described as tolerance breakdown to self-antigens. Therefore, the activation and the fate of autoreactive lymphocytes should be kept under tight control, which may encounter deregulation by factors from the host or the pathogen during an infection.

A characteristic feature of immune deregulation in acute *T. cruzi* infection is the profound polyclonal activation of B and T cells. Such activated B and T cells are not detectably reactive with the parasite. This type of response is thought to constitute an immune evasion mechanism because it masks/deviates the specific responses. Indeed, polyclonal lymphocyte activation correlates with disease susceptibility in *T. cruzi* infection (36,37). In addition, such response may as well be the "smoking gun" for the genesis of autoimmune phenomenon in the chronic form of the disease, as most of the lymphocytes activated during this process are not parasite specific but, instead, are reactive with host tissues (38-41). However, the molecular and cellular mechanisms for these events are not well understood.

Recently, others and we identified a group of parasite proteins that can mimic host cytokines or growth factors to directly activate normal lymphocytes unprimed for parasite antigens (42-45). We termed parasitokines for such parasite-derived mimetics of host cytokines/growth factors (46). A unique feature distinguishing parasitokines from nominal parasite antigens is that parasitokines can rapidly (prior to the development of parasitokine-specific adaptive immune responses) activate a wide range of host cells, in particular those that possess antigen presentation functions and belong to the innate immunity (45,47). This process does not need to involve processing and presentation of the antigenic peptides from parasitokines *per se*, as an appreciable primary response of a large population of responding lymphocytes, most likely polyclonal, is elicited (44,45).

*Trans*-Sialidase (TS) is the flagship parasitokine of *T. cruzi* (46). TS has been shown to directly stimulate the proliferation of primary B cells and the induction of nonspecific immunoglobulin secretion independent of T cells (45). TS also induces inflammatory cytokines (e.g. IL-6) from endothelial, epithelial cells and monocytes/macrophages (47). Moreover, TS can potentiate T cell activation by mobilizing co-stimulation from antigen-presenting cells (APCs) (44). In animal studies, subpopulation of *T. cruzi* parasites that expresses higher level of TS is more virulent in causing infection than the population that expresses no or lower level of TS (48). In addition, sensitization of mice with small doses of TS turns the mice into highly susceptible hosts to *T. cruzi* (49). In line with these findings, TS heterologously expressed in *Leishmania major* greatly enhances virulence of the parasites to mice (50). The parasitokine property of TS in aggravating the polyclonal lymphocyte activation may very likely underlie its virulence-enhancing activity in different infection models (46).

### 4.1. Parasitokines and immune tolerance breakdown

Besides their immune disturbance in acute infection, parasitokines like TS may play a pivotal role in

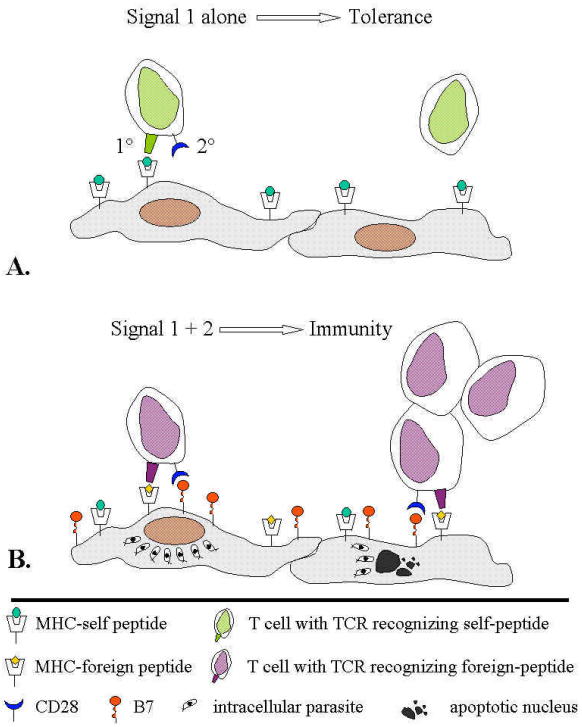


**Figure 1.** The requirement of both an antigen-specific signal and a co-stimulatory signal for naïve T cell activation plays a crucial role in preventing autoimmune responses to self-antigens. (A). Full activation of a naïve T cell requires at least two signals: an antigen-specific signal (Signal 1) provided through TCR engagement of the peptide-MHC complex and an antigen nonspecific signal (Signal 2) delivered by co-stimulatory receptors. A naïve, auto-reactive T cell that recognizes self-antigens on cells that do not provide co-stimulation become anergic. An anergic T cell does not differentiate into an armed effector cell, thus ensuring a state of tolerance to self-antigens. (B). During an infection, for example by intracellular parasites, infected cells express co-stimulatory molecules, such as B7. A naïve T cell that recognizes parasite peptides on an infected cell, and also receives co-stimulation through B7 interacting with its cognate receptor CD28, is fully activated. Such a T cell proliferates and differentiates into an effector lymphocyte capable of eliminating parasite-infected cells.

causing the autoimmune phenomenon in chronic stage. To better elaborate the relation between parasitokines and tolerance breakdown, it will be helpful to first briefly discuss the regulation of T cell activation. Full activation of a naïve T cell requires at least two signals: an antigen-specific signal (Signal 1) provided through TCR engagement of the peptide-MHC complex and an antigen nonspecific signal (Signal 2) delivered by co-stimulatory receptors. T cells stimulated by Signal 1 alone in the absence of co-stimulation become anergic, i.e., refractory to activation, or die by apoptosis (51). Thus, co-stimulation not only greatly reduces the activation threshold but also provides survival signals for naïve T cells. Most important, regulated expression of co-stimulatory activity is crucial to preventing destructive autoimmune responses to self-tissues. This is because the ligands for co-stimulatory receptors (B7.1/B7.2 for CD28, for instance) are usually not expressed by normal tissue cells, but only by professional APCs encountered with microbial constituents. Hence, such differential expression together with the requirement of simultaneous delivery of Signals 1 and 2 for clonal expansion of naïve T cells safeguards that autoreactive naïve T cells in contact with normal tissues are not accidentally activated (Figure 1).

During an infection, however, danger signals triggered by microbial constituents (e.g., parasitokines) can induce significant upregulation of co-stimulatory activities on professional APCs and on nonprofessional APCs as well, such as B cells and endothelial cells (52,53). The high co-stimulatory activities may reduce the activation threshold of autoreactive T cells, rendering their nonspecific bystander activation. Moreover, inflammatory cytokines (e.g., IFNs, IL-12, IL-15, and IL-18) released by innate and adaptive immune cells are able to drive bystander T cell activation that occurs in the absence of peptide-MHC recognition by TCR (54). Furthermore, some inflammatory cytokines, such as IL-6 and TNF-alpha, can substitute co-stimulation and provide B7/CD28-independent licensing signals in activating $CD8^+$ T cells (55). This may be crucial in the response to intracellular pathogens that are not cytopathic, especially in certain chronic infections, where the infected cells lack the expression of B7 or other cell surface co-stimulatory molecules that provide Signal 2 for $CD8^+$ T cell activation. On the other hand, similar mechanism may also operate to cause immunopathology when $CD8^+$ T cells, the main cell type responsible for immune activation in chronic human chagasic myocarditis (56), are licensed by inflammatory cytokines to kill normal tissue cells without the stringent requirement for co-stimulation. In this regard, it is interesting to note that significant amounts of IL-6 can be induced during *T. cruzi* infection in the parasitized tissues and in the circulation (57,58), and by the purified parasitokine TS in endothelial, epithelial cells (47) and monocytes/macrophages (45). Although IL-6 is required for host resistance against *T. cruzi* (59), the long-term secretion of such a cytokine capable of supporting polyclonal B and T cell activation (60) may be linked to the autoimmune status (56). Two different strains of IL-6 transgenic mice generated in different laboratories all show polyclonal hypergammaglobulinemia, massive plasmacytosis with autoantibody production and glomerulonephritis (61,62). Whether these mice are predisposed to $CD8^+$ T cell-mediated tissue damage when infected by intracellular pathogens, such as *T. cruzi*, will be of interest to observe.

Apart from the disturbances on co-stimulation and cytokine network by parasitokines that could contribute to autoimmunity, a more direct impact of parasitokines on tolerance breakdown potentially lies in the fact that some *T. cruzi* parasitokines, including TS and racemase (43), are mitogenic for B cells, causing polyclonal B cell activation. The importance of B cells as autoantigen-presenting cells has been extensively studied (63-65). There is accumulating evidence suggesting that autoreactive B cells

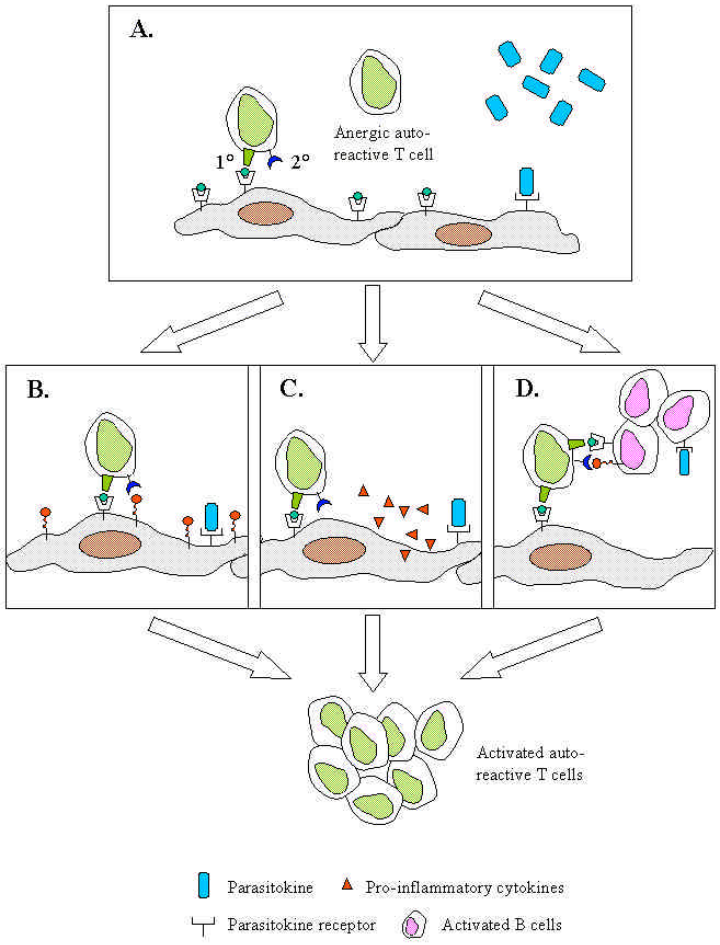


**Figure 2.** Parasitokines contribute to breakdown of tolerance to self-antigens, and bridges the observations on autoimmunity and parasite persistence. (A). An auto-reactive T cell recognizing self-peptide on normal tissue cells that can not provide co-stimulation becomes anergic. Parasitokines secreted by parasites can reach uninfected tissue cells far from the infection site, and bind to their cellular receptor on these cells. (B). The interaction between parasitokines and their receptors delivers a danger signal that directly up-regulates co-stimulatory molecules on target cells. After receiving a secondary co-stimulation signal, a naïve auto-reactive T cell evades anergy and becomes activated. (C). Upon parasitokine binding, the target cells can also secrete pro-inflammatory cytokines (such as IL-6). Pro-inflammatory cytokines can provide co-stimulation-independent signal to bystander activate T cells, and could also break the tolerance status of auto-reactive T cells. (D). Many parasitokines are mitogenic to naïve B cells, which become efficient antigen-presenting cells in vivo after activation. The activation of auto-reactive B cell clones is implicated in breaking tolerance of auto-reactive T cells by providing co-stimulation. The events of immune deregulation in (B-D) can occur in normal cells without direct parasite infection. Auto-reactive lymphocyte clones activated in acute phase can live on for many years, even though the level of parasitokines will be low in chronic phase, provided that persistent parasite infection tickles other danger signals to maintain their activation.

are capable of presenting autoantigen in activating autoreactive T cells (66), most likely through B7-dependent co-stimulation (64,67). For example, in B cell-deficient autoimmune-prone MRL *lpr/lpr* mice, populations of spontaneously activated T cells are virtually absent as compared with wild-type MRL *lpr/lpr* mice (65), suggesting that B cells play a central role in the activation of autoreactive T cells. Other studies using mice transgenic (Tg) for hen egg lysozyme (HEL) antigen (Ag) and/or antibody (Ab) have shown that HEL-specific T cells are tolerized in HEL Ag single Tg mice (66). However, T cells from Ag/Ab double Tg mice escape central deletion and experience a partial breakdown of peripheral tolerance presumably due to the presentation of HEL Ag by HEL-specific B cells in double Tg mice (66). Taken together, these studies illustrate that autoreactive B cells as APCs play an important role in tolerance breakdown of autoreactive T cells. Therefore, parasitokines from *T. cruzi* that activate APCs (particularly B cells) and their co-stimulation capabilities may very likely be the "missing link" between acute polyclonal lymphocyte activation and chronic autoimmunity in Chagas' disease (Figure 2).

### 4.2. Targeting parasitokines in Chagas' disease

It is safe to assess that there always are autoreactive lymphocytes activated during any infection

(especially in the acute phase). However, whether such bystander activation poses an autoimmunity threat is highly dependent on a responding lymphocyte repertoire (genetic makeup of the host), and how fast the pathogen is cleared in order to keep danger-triggered co-stimulation at pre-infection status. If the pathogen is not cleared promptly, long-term bystander activation and/or delivery of co-stimulation and cytokines to otherwise anergic autoreactive lymphocytes might inevitably break tolerance to self-antigens (68), as suggested by a strong correlation between various autoimmune diseases and chronic infections (69,70).

Two implications can be derived from the above concept in terms of preventing and treating CCC. First, as emphasized by supporters of the "parasite persistence hypothesis", chemotherapy and immunological means should be applied at any given time of the disease course to eliminate parasite infection or at least reduce the parasite load as much as possible. Second, for vaccine development, a more efficient strategy is to target *T. cruzi* parasitokines, in order to prevent both the pathogenic polyclonal lymphocyte activation in acute infection and the autoimmune responses in chronic phase. Currently, many studies on *T. cruzi*-host interactions focus on identifying parasite antigens that are cross-reactive with self-antigens, hoping to develop means to neutralize potential autoimmune responses. Yet, the efficacy of such a vaccination strategy will be dampened by immune subversion/deviation through the nonspecific polyclonal lymphocyte activation in acute infection (71). Hence, vaccination against parasitokines and other moieties mediating polyclonal lymphocyte activation should be on the top list for consideration (72).

## 5. RE-ESTABLISHING IMMUNOLOGICAL TOLERANCE IN CHRONIC CHAGASIC CARDIOMYOPATHY (CCC)

In terms of disease treatment, while we support the efforts to eliminate parasites and control the infection-induced immune disturbances that could lead to tolerance breakdown, we fail to agree with the advocates of the "parasite persistence hypothesis", who stipulate that CCC should not be treated as an autoimmune disease (23). The basis for the reasoning by these researchers is that immunosuppressive drugs currently used for autoimmune diseases universally cause a rebound of the parasitism and increase disease severity in chronically infected hosts (28,34). Nevertheless, systematic immunosuppression is not the correct way of treating autoimmune disease in the first place. Over the years, immunosuppression in organ transplantation and for autoimmune diseases has been associated with severe problems, such as drug toxicity and increased incidence of infections and malignancy. The relative ineffectiveness of the immunosuppressive drugs in the long run and the risk of graft rejection and autoimmune recurrence, once the drugs are withdrawn, require permanent drug treatment of patients. The *status quo* of practice strongly suggests that immunosuppression is not an ideal method to tackle the pathogenic immune response.

It is generally agreed that autoreactive lymphocytes are present in most normal individuals but are kept under control by multiple diverse peripheral tolerance mechanisms, including deletion, anergy and active regulation (73). Regulation of the immune response to self-antigens is a complex process that depends on maintaining self-tolerance while retaining the capacity to mount a robust immune response to foreign antigens. Intensive immunology studies in areas of transplantation and autoimmunity have shown that not only long-term stable tolerance can be induced to accept donor organs (74), but also restoration of self-tolerance is a feasible task to treat autoimmune disease (75). Therefore, the goal for treating CCC, as for any autoimmune disease with an infectious etiology, should always be to re-establish specific immunological tolerance towards the affected organ(s) without compromising immunity against tumor and infectious agents.

What can immunoparasitologists dealing with Chagas' disease learn from the recent advances in immune regulation? There is strong evidence that T cell-mediated dominant control of allo- and auto-reactive cells contributes to the maintenance of immunological tolerance, and its alteration can induce graft rejection and autoimmune diseases (76,77). In this regard, tremendous interest has been brought onto the specialized $CD4^+CD25^+$ T regulatory cells that are capable of silencing autoreactive T cells in the peripheral immune system (78). In normal individuals as well as in unimmunized naive mice, $CD4^+CD25^+$ T cells account for only 5-10% of the total peripheral $CD4^+$ T cells. Yet, depletion of this minor subpopulation by thymectomy of neonates on the third day of life or by treatment of adult $CD4^+$ T cells with anti-CD25 and complement results in the development of organ-specific autoimmunity (78). In cell mixing experiments, these cells can effectively suppress the activation of $CD4^+CD25^-$ T cells, as well as $CD8^+$ T cells (79), in a cell-cell contact dependent, soluble factors independent manner (78). In vivo experiments show that reconstitution of athymic nude (nu/nu) mice with $CD4^+CD25^-$ T cells causes various autoimmune diseases (such as thyroiditis, gastritis, insulitis, sialoadenitis, adrenalitis, oophoritis, glomerulonephritis, and polyarthritis), whereas co-transfer of $CD4^+CD25^+$ T cells prevents these autoimmune developments (80). Other studies suggest that the reason for some strains of gene-deficient mice to develop autoimmune diseases can be attributed to the defect in the $CD4^+CD25^+$ T cell population (81). Moreover, immune regulation by $CD4^+CD25^+$ T cells has been implicated in establishing long-term stable graft tolerance in transplantation models (82,83), whereas deactivation of such regulation is able to induce effective tumor immunity in otherwise non-responding hosts (84).

While many researchers are exploiting the full capacity of utilizing the $CD4^+CD25^+$ T regulatory cells for cell-based therapy in autoimmune disease and transplantation settings, others are investigating the possible use of recombinant proteins and monoclonal antibodies to specifically turn off unwanted immune responses. A potentially rewarding direction is to harness the so-called "negative regulators of co-stimulation" on T

cells. So far, there are two inhibitory receptors that can transmit negative signals in co-stimulation, in oppose to CD28-mediated positive co-stimulation. One is cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and the other is programmed death 1 (PD-1), both members of the immunoglobulin (Ig) superfamily and homologues of CD28. In contrast to $CD28^{-/-}$ mice that are deficient in certain immune responses, CTLA-4-deficient mice manifest autoimmune lymphoproliferative disorders and die at 3-4 weeks of age (85,86). Although the phenotype is milder, PD-1-/- mice in C57BL/6 background develop a lupus-like arthritis and glomerulonephritis and show hyper-responsiveness of B cells with increased serum IgG3 (87,88). Interestingly, PD-1-/- mice in BALB/c background develop a fatal dilated cardiomyopathy with severely impaired contraction and sudden death by congestive heart failure (89). All of the affected PD-1-/- mice exhibited high-titer circulating IgG autoantibodies reactive to a 33-kDa protein expressed specifically on the surface of cardiomyocytes (89). Whether chagasic cardiomyopathy arises from similar abrogation of the inhibitory receptor function, due to the infection by *T. cruzi* and/or polymorphism of the receptor gene in susceptible hosts, is a matter of conjecture. Taken together, the phenotypes of these knockout mice suggest the critical roles of the negative regulators of co-stimulation in preventing autoimmunity and maintaining peripheral tolerance. As a result, recombinant proteins and monoclonal antibodies that could transmit signals to block T cell activation through such inhibitory receptors are being pursued as potential therapeutic reagents.

Because sterile immunity against *T. cruzi* is difficult to achieve, induction of organ-specific immunological tolerance to the heart without compromising the normal immunity against *T. cruzi* should prove valuable in treating CCC. Future practice on chagasic patients may adopt both cell-based and protein-based therapies, plus priming the regulatory mechanisms with self-antigens, to acquire organ-specific immune tolerance. Till then, perhaps the debate on the pathogenesis of CCC, i.e., autoimmunity or parasite persistence, will resolve by itself.

## 6. SUMMARY

In summary, we believe that altered immune regulation towards self-antigens in Chagas' disease is maintained by the presence of chronic *T. cruzi* infection, but its trigger occurs as early as in acute infection where parasite-induced polyclonal lymphocyte activation initiated and/or perpetuated by parasitokines plays a pivotal role. Therefore, for preventing *T. cruzi* infection and its chronic complications, efforts should center on eliminating the parasites and blocking the detrimental effects of key parasite factors that globally mobilize the danger signal. In terms of future strategy for CCC treatment, it needs to be appreciated that maintaining and re-establishing organ-specific immune tolerance, but not applying generalized immunosuppression, should be the ultimate goal.

## 7. ACKNOWLEDGMENTS

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