MiniReview

Views on the autoimmunity hypothesis for Chagas disease pathogenesis

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Abstract

Initially, the notion that the pathogenesis of Chagas disease has an autoimmune component was based on the finding that sera from Trypanosoma cruzi-infected patients or laboratory animals contain antibodies that recognize both parasite and host tissue antigens. Subsequent work suggested that T lymphocytes from chagasic patients and animals also displayed such cross-reactivity. However, the autoimmunity hypothesis has remained controversial because of experimental pitfalls, incomplete or inadequate controls, difficulties in reproducing some key results, and a lack of persuasive evidence that the cross-reactive antibodies or lymphocytes can truly effect the multifaceted pathological features of Chagas disease. Whether the immunologic autoreactivities described to date cause chagasic pathology or result from it is another unresolved question. Discussed herein are the most recent contributions to this topic and the reservations they have raised.

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1. Introduction

The pathology of Chagas disease is as complex and multifaceted as the clinical manifestations [1–3] and the life cycle of its etiologic agent, the protozoan Trypanosoma cruzi [4]. Recent authoritative reviews on the clinical aspects of Chagas disease (ranging from severe cardiomyopathy or massive damage occurring in segments of the gastrointestinal tract to no apparent symptoms, as is the case in indeterminate Chagas disease [3,5]), its pathology [3], the unraveling biology of T. cruzi [4], and the frustrating attempts to develop effective chemotherapy [6] or immunoprophylaxis [7] against T. cruzi infection provide a comprehensive view of this major health problem. The histopathology of the various types of chagasic lesions has been well described, with minimal discrepancies [3,5] but this has not been the case for the mechanism(s) of pathogenesis, for which a number of hypotheses have been proposed [8,9].

In its mammalian hosts T. cruzi divides intracellularly and infected host cells eventually burst. The released organisms are then able to disseminate throughout the body, infecting additional cells and tissues. It is not difficult to envisage that the lysis of infected cells results in tissue damage, with direct and indirect pathological consequences depending on the affected tissues and organs. However, the presence of the parasite has not been demonstrated in many histologic studies of chronic chagasic lesions, suggesting that other processes might be at work besides cell invasion and lysis. As discussed below, this inference has been effectively challenged in recent years by results obtained using methods to identify parasite molecules that are much more sensitive than microscopic examination.

Although several hypotheses have been formulated to explain how chagasic lesions come about, the autoimmunity hypothesis is the one that has attracted most attention and elicited most controversy [8,10–17]. Because the author of this MiniReview has previously commented on this subject [8,18,19] the present article will focus preferentially on information published more recently.

Why is it important to resolve the controversy surrounding autoimmunity as a major mechanism of patho-
genesis in Chagas disease? If immune responses specific for \textit{T. cruzi} antigens cross-reacted with epitopes of key host tissues, the search for effective chemotherapy would be seriously complicated because drugs killing \textit{T. cruzi} would not necessarily suppress immune responses initially elicited by parasite antigens and subsequently boosted by host tissue antigens. Attempts to inhibit the autoimmune responses through the use of immunosuppressants would most likely be counterproductive, based on ample evidence that immunosuppression consistently exacerbates \textit{T. cruzi} infection in chagasic patients and infected laboratory animals, often reactivating latent chronic infection [20–25]. If immunologic cross-reactivity between \textit{T. cruzi} and host antigens resulted in pathology, efforts to develop a protective vaccine would also be seriously hampered by the need to prove that the selected \textit{T. cruzi} antigens will not elicit anti-self responses. Moreover, the fact that chagasic pathology in humans generally evolves slowly, often taking years or decades to become manifest, would represent another major obstacle in the testing of vaccines and possible deleterious effects. If, instead, immunologic cross-reactivities were not the root cause of pathogenesis in Chagas disease, or if autoimmune events were consequent to, rather than the primary cause of pathology, attempts to develop effective chemotherapy and vaccines would deserve much encouragement and support. To date, effective, non-toxic chemotherapy for \textit{T. cruzi} infection has remained elusive [6,26,27] and experimental anti-\textit{T. cruzi} vaccines, capable of increasing survival and decreasing parasitemia levels significantly relative to controls, have not produced sterile immunity [7].

2. Autoreactive humoral immunity

The detection of circulating anti-\textit{T. cruzi} antibodies that cross-react with host tissue antigens is not a novel finding in chagasic mammals and new examples are added to the literature sporadically (reviewed in [8]). In a recent report, Leon et al. [28] showed that A/J mice (a strain of mice with significant susceptibility to \textit{T. cruzi} infection) infected with the Brazil isolate of \textit{T. cruzi} produced detectable levels of anti-myosin IgG as soon as 7 days post-infection, i.e. during the acute phase of the infection. T cell autoreactivity in the acute phase was also seen in this model system, as denoted by ear swelling after injection of either myosin or a \textit{T. cruzi} antigen preparation. These authors were also able to show that immunization with purified myosin caused some heart lesions resembling those seen in \textit{T. cruzi}-infected mice. Interestingly, in C57BL/6 mice the levels of anti-myosin IgG found after \textit{T. cruzi} infection were small or undetectable [28]. This mouse strain is among the least susceptible to \textit{T. cruzi} infection and has been claimed not to develop cardiac autoimmunity or myocarditis after immunization with myosin [29].

The extent of ear swelling in response to myosin challenge was smaller in infected C57BL/6 than in infected A/J mice, suggesting that in mouse model systems of acute Chagas disease the ability to develop myosin-specific immunologic reactivity is linked to genetic make-up. The humoral and cellular levels of anti-myosin reactivity in \textit{T. cruzi}-infected mice were comparable with those of uninfected mice immunized with myosin. Both \textit{T. cruzi} infection and myosin immunization lead to heart tissue lesions, and it was implied that there was an involvement of myosin in chagasic pathology. The results of Leon et al. [28] extended to the acute phase of \textit{T. cruzi} infection findings that had been previously described for chronically infected mice [30–32] but did not establish whether such involvement would be initially causative (i.e. primary) or dependent upon (i.e. secondary or consequential) prior parasite-inflicted tissue damage.

It is noteworthy that that levels of anti-myosin antibodies are also significantly increased in patients with heart disease resulting from causes other than \textit{T. cruzi} infection such as, for example, myocardial infarction, coronary artery bypass, heart valve surgery, and viral myocarditis [33,34]. An increase in anti-myosin antibody titers, greater after cardiac surgery than after myocardial infarction or inflammatory heart disease unrelated to Chagas disease, has also been described [33]. Based on these reports, it would not seem unreasonable to infer that cardiac tissue damage resulting from tissue infection could cause the level of anti-myosin immunity to rise in cases of Chagas heart disease. Further complicating this issue is evidence that immunization with cardiac myosin induces myocarditis preferentially in genetically predisposed mice, including A/J mice [29], the susceptible strain used by Leon et al. [28].

As others before them [35], Leon et al. [28] were able to induce autoimmunity by immunizing normal A/J mice with myosin incorporated into complete Freund’s adjuvant (CFA). This aggressive vehicle, often used in attempts to induce autoreactive immunity [28,36,37], has deleterious effects of its own that are not commonly seen in the course of \textit{T. cruzi} infection. When overlapping with \textit{T. cruzi} infection, tissue destruction caused by CFA may have unintended consequences absent in uninfected controls receiving CFA. Also unclear is how the coincidental appearance of heart alterations in separate animals immunized with myosin or infected with \textit{T. cruzi} would be indicative of a primary role for anti-myosin immunity in the pathogenesis in Chagas disease. Relevant in this context are the findings of Neu et al. [35], who also used A/J mice and a myosin immunization protocol similar to that used by Leon et al. [28], and concluded that there were several reasons why anti-myosin antibodies could not have been involved in the ensuing myocarditis. Namely, the anti-myosin antibodies did not bind viable cardiac myocytes but, instead, reacted with detergent-permeabilized myocytes. Moreover, not only did immunosuppressed mice receiving myosin (whose anti-myosin humoral response had been
reduced to very low levels) develop myocarditis, but passive transfer of a high-titer anti-myosin antibody preparation failed to induce myocarditis. In their studies, Neu and colleagues identified deposits of anti-myosin IgG along damaged muscle myofibers but not on intact tissue.

As for a possible role for anti-myosin immunity in Chagas heart disease, several questions raised previously [8] remain unanswered: (i) is heart tissue damaged during T. cruzi infection different from heart tissue injury of a different etiology in eliciting anti-myosin immunity? (ii) in view of the results of Neu et al. [29,35], do anti-myosin antibodies truly contribute to chagasic pathology? (iii) if anti-myosin antibodies appeared after the occurrence of tissue damage, would they aggravate the pathology by mediating the destruction of intact heart cells? And (iv) if the answer to the previous question were yes, how would the anti-myosin antibodies lead to a predominantly mononuclear cell infiltrate (such as commonly seen in chagasic heart lesions) instead of the predominantly granulocyte cell infiltration that generally follows IgG deposition in host tissues?

Cunha-Neto and his collaborators have proposed a role for antibodies to the cardiac myosin heavy chain (CMHC) in the pathogenesis of Chagas heart disease [13,31]. In their work, antibodies to CMHC were found in chagasic sera regardless of whether the donors had overt symptoms of Chagas heart disease or were asymptomatic. However, a similar reactivity was demonstrable in healthy donors at levels comparable with those found in symptomatic and asymptomatic chagasic patients. Sera containing anti-CMHC antibodies recognized a 210,000-Da band on Western blots of a human heart tissue lysate; this band co-migrated with CMHC. Affinity-purified anti-CMHC antibodies from 23 symptomatic and 14 asymptomatic patients were tested for their ability to identify antigens on blots of a T. cruzi trypomastigote extract. Of these, 14 (i.e. 61%) and one (i.e. 7%) samples, respectively, identified two bands of 140,000 and 116,000 Da for which control sera showed no reactivity. The anti-CMHC from every patient with chronic Chagas heart disease and 14% of the asymptomatic chagasic patients recognized B13, a recombinant T. cruzi peptide [38]. In competitive enzyme-linked immunosorbent assay (ELISA) tests in which B13 was the solid-phase antigen, the reactivity of the serum of one chagasic heart patient was blocked by pre-incubation with p1439–1453, a synthetic CMHC peptide that includes the sequence AAALDK. This sequence presents partial homology with the B13 internal sequence AAAGDK. No competition was seen when the serum of an asymptomatic chagasic patient was tested. Pre-incubation with FGQAAAGDK, a segment of the B13 sequence, blocked B13 reactivity in both the symptomatic and asymptomatic sera. These results prompted Cunha-Neto et al. to postulate that the anti-CMHC activity found in both sera from asymptomatic patients and normal sera was attributable to ‘natural’ antibodies, distinct from the specific reactivity displayed by the sera from the symptomatic patients. So, in a hypothetical and somewhat arbitrary manner, the ‘specificity’ of anti-CMHC antibodies was linked to the development of chagasic myocarditis. However, there was a discrepancy between the observation that 100% of the sera from chronic patients with overt heart disease showed reactivity for CMHC but only 61% of the antibodies purified from these sera displayed anti-T. cruzi reactivity on Western blots. Again in this case, the authors felt that ‘natural’ antibodies present in the negative sera (39%) might account for the difference and did not carry out further work to clarify the puzzling questions that undermined their conjectural explanation. It is widely accepted that ‘natural’ antibodies belong to the IgM isotype, whereas the specific antibodies to T. cruzi or CMHC found in chronic patients are predominantly IgG. Since these two isotypes can be easily separated, the ‘natural’ antibody notion can be readily tested by using the IgM and IgG fractions from individual chagasic sera in the ELISA test. Also troublesome for the acceptance of both, the results and the assumptions made by Cunha-Neto et al. have been the observations of Levin and his associates (Levin, M.J., personal communication) that the B13 reactivity of sera from patients with overt Chagas heart disease and asymptomatic patients are, in fact, comparable, weak, and not unlike the reactivity displayed by sera from patients with idiopathic dilated cardiomyopathy or other non-chagasic cardiopathies. Additional conflict arises from Levin’s inability to reproduce Cunha-Neto’s observation that the AAALDK peptide inhibits the reactivity for B13 of sera from either patients with chronic Chagas heart disease or asymptomatic patients regardless of whether the peptide was used in free form or coupled to bovine serum albumin (Levin, M.J., personal communication). Quite illustrative of the nature of this aspect of the controversy are the published comments of Levin [39] pertaining an article by Kalil and Cunha-Neto [13] in which a case for the role for anti-CMHC antibodies in chagasic pathology had been made.

Tibbetts et al. [40] found that C57BL/6 mice infected with the Brazil isolate of T. cruzi develop severe cardiomyopathy and produce antibodies that recognize antigens in a C57BL/6 mouse heart extract. These antibodies were detectable 5 days after infection (5 days before the appearance of anti-T. cruzi antibodies). Anti-heart antibodies were also found in the sera of C57BL/6 mice infected with the Guayas T. cruzi isolate, a model system in which myocarditis tends to be mild. Antibodies from the C57BL/6 mice infected with Brazil T. cruzi reacted strongly with some heart tissue proteins and also with a skeletal muscle protein. In contrast, antibodies from animals infected with the Guayas isolate were weakly reactive with these antigens, implying that antibody autoreactivity was variable and linked to the severity of the ensuing cardiomyopathy. Unlike Cunha-Neto et al. [31], Tibbetts et al. [40] did not observe cross-reactivity of the mouse anti-heart antibodies...
with T. cruzi antigens. Furthermore, sera from mice hyper-immunized with a heart tissue homogenate or with myosin failed to recognize any antigen on blots of a lysate of T. cruzi epimastigotes [40], and sera from mice hyperimmunized with T. cruzi did not detect any muscle tissue antigen. These results suggested that the anti-heart antibodies might represent a response to cardiac cell antigens released by parasite-damaged cells rather than one initiated by T. cruzi antigens and subsequently perpetuated by mouse heart antigens. To be noted, T. cruzi epimastigotes, which are absent in mammalian hosts, share many but not all antigens with amastigotes and trypomastigotes, the mammalian forms of this parasite. Because the cross-reactivity experiments of Tibbetts et al. [40] had not been carried out using trypomastigote or amastigote lysates, molecular mimicry between heart and T. cruzi antigens could not be completely ruled out. Aside from this, there were obvious discrepancies between the results of Tibbetts et al. [40] and those of Cunha-Neto et al. [31]. The reason could conceivably be the use of different host species and procedural approaches in each study. Despite this confusion, brought to light in a previous review [8], Cunha-Neto and Kalil [41] have continued to refer to the reactivity of T lymphocytes from patients with chronic chagasic heart disease with B13 as if it were a well established fact.

Other autoantibodies have been identified in chagasic mammals. Levin and his co-workers [42] observed that the serum levels of ribosomal P protein-specific antibodies were higher in patients with chagasic heart disease than in patients with mild or no detectable myocarditis, suggesting a correlation between these for the appearance of these antibodies and the manifestation of heart disease [42,43]. Screening a T. cruzi kgt11 cDNA library with the serum of a patient with a high titer of anti-ribosomal P protein Levin’s group identified several clones. One of these, JL5, enabled them to map a relevant epitope to the C-terminal segment of a T. cruzi P ribosomal protein (termed TcP0). An internal peptide sequence (EDDD-MGFLGFDF) of the C-terminal region displayed partial homology with the SD(D/E)DMGFGLFD sequence present in the C-terminal region of human P ribosomal protein. Skeiky et al. [42] confirmed this immunologic cross-reactivity and observed that the deletion of six C-terminal amino acids modified the reactivity of the protein with the selected chagasic serum. The manner in which this deletion altered reactivity with TcP0 was reminiscent of that previously seen with autoantibodies from some patients with systemic lupus erythematosus (SLE; approximately 15% of SLE patients have autoantibodies to a shared epitope located in the C-terminal regions of the ribosomal proteins, P0, P1, and P2 [44]). Partial homology and the autoimmune nature of SLE hinted that epitope mimicry between T. cruzi and a mammalian ribosomal P protein might lead to an autoimmune condition in Chagas disease. Interestingly, the affinity constant of chagasic immunoglobulins for the JL5 peptide was found to be five times greater than that of SLE antibodies for H13 (the nearly homologous peptide specifically recognized by the antibodies from the SLE patients) [45]. The JL5 P ribosomal protein (currently named TcP2β) and its C-terminal R13 peptide (see below) are different from those of TcP0 (the 38-kDa T. cruzi P ribosomal protein). Thus, their sequences show little overlap and a JL5 DNA probe detects a 0.7-kb mRNA species, which is not long enough to be translated as a 38-kDa protein. However, anti-JL5 antibodies recognize the 38-kDa antigen on Western blots, indicating that there is immunologic cross-reactivity between TcP2β and TcP0 [42,46]. Sera from the SLE patients did not recognize the 38-kDa TcP0 protein [47,48]. Sera from patients with chronic Chagas heart disease have been shown to contain relatively high levels of anti-R13 but low levels of anti-H13 antibody [49] whereas the levels of anti-R13 and anti-H13 antibodies are generally comparable in SLE sera [50]. In sum, there is strong evidence that the anti-P ribosomal protein antibodies initiate myocardial cell damage would have to be supported, at the very least, by evidence that these antibodies can somehow engage cell-sheltered ribosomal P proteins or recruit effector components (e.g. complement activity, phagocytic cells). A paper that appeared in 1992 reported that affinity-purified anti-P autoantibodies can bind to the surface of human hepatoma and neuroblastoma cells and, to a lesser extent, to human fibroblasts [51].

Mice hyperimmunized with recombinant TcP2β have been shown to manifest electrocardiographic (EKG) alterations characterized by a net increase in the duration of the QRS EKG complex [49]. This is in keeping with the findings that (i) perfusion of isolated rabbit heart with IgG from chagasic patients causes EKG alterations unless the IgG preparation is pre-incubated with R13 peptide [52] and (ii) a murine monoclonal antibody reactive with the R13 peptide (a segment of the TcP2β C-terminal region) has a positive chronotrophic effect on cultured neonatal rat cardiomyocytes [53]. However, the mice hyperimmunized with JL5 did not show histological evidence of heart inflammation.

Aznar et al. [54] used an R13-specific ELISA to survey sera from three groups: chagasic patients (161 samples), blood bank donors from areas endemic for Chagas disease (285 samples), and donors serologically negative for T. cruzi (405 samples). Among the chagasic sera, seven obtained from acutely infected patients were negative whereas six out of seven sera from patients with congenital Chagas disease were positive. Among chronic patients and individuals with positive anti-T. cruzi serology, 60% and 49%, respectively, displayed reactivity with R13, although these percentages fluctuated widely in subsets of samples of different geographical provenience. Excluding a group of patients with digestive or asymptomatic forms of Chagas disease (who lacked R13 reactivity) this
study could not establish a precise link between R13 reactivity and other forms of Chagas disease, largely due to lack of definition of the clinical status of each donor. However, an attempt was made to look into a possible correlation between anti-R13 antibody and cardiomyopathy in a group of 14 patients from whom endomyocardial biopsies and blood samples had been taken at the same time. No statistically significant correlation could be observed in this subgroup. Nevertheless, this type of approach holds promise and should be undertaken again by using samples from a larger group of patients defined in serological and histopathological terms and selected from several endemic areas.

Given the uncertainties about a role of anti-P ribosomal antibodies in the production of Chagas heart disease, Sepulveda et al. [37] sought supportive evidence. To this end, they used synthetic peptides spanning the TcP2β molecule, trying to identify and define in molecular terms the specificity of antibodies obtained from mice chronically infected with T. cruzi and from mice hyperimmunized with the recombinant fusion proteins glutathione S-transferase-TcP2β and histidine (hist)-TcP2β. Antibodies to these fusion proteins recognized epitopes in various segments of the TcP2β molecule whereas the specificity of antibodies from infected mice was restricted to the C-terminus segment of this molecule. Anti-C-terminus antibodies were also present in sera from mice immunized with hist-TcP2β. Purified IgG, reactive with the C-terminus epitope of TcP2β, caused a significant increase in the beating frequency of primary neonatal rat heart myocyte cultures in an in vitro assay monitored microscopically. This functional activity appeared to involve selective stimulation of β1-adrenergic receptors since bisoprolol, a β1-blocker, abrogated the positive chronotropic effect of anti-TcP2β antibodies from the mice immunized with hist-TcP2β. These findings are highly reminiscent of results published by or in association with Levin’s group [55,56], showing that IgG from patients screened for autoreactivity against β1-adrenoceptors increased the beating frequency of rat myocytes in the same in vitro assay system. In this case, the relevant epitope included the AESDE amino acid sequence present in the second extracellular loop of the human β1-adrenergic receptor, which is partially homologous with the internal AESEE sequence of TcP0 [55].

An ability of antibodies from chagasic patients to recognize antigens of murine endothelial, vascular, interstitial, and nerve of tissues was described in the 1970s [57,58] but was recanted when the antibodies were determined to be heterophilic and unable to yield similar results when human tissues were used [59]. Since then, investigators have been cautious with results obtained by using heterologous assay systems or contrived in vitro conditions that preclude the normal regulatory mechanisms present in intact mammalian hosts. To circumvent this problem, the Levin group immunized BALB/c mice with P ribosomal proteins and monitored EKG patterns in these animals [60]. Groups of mice received six doses of recombinant TcP0 or TcP2β in incomplete Freund’s adjuvant over a 10-week period and EKG recordings were made at various times during, and once after completion of the immunization regimen. In the 66 days that followed initiation of the immunization regime the anti-TcP0 ELISA titers had reached as high as 1/6400 but no significant EKG abnormalities were seen. Surprisingly, 2.5 weeks later some of the mice with circulating anti-TcP0 antibodies started to present electrogenic or conduction disturbances in their EKG. At this time, some anti-TcP0 ELISA titers had reached 1/12800 and some of the animals were displaying supraventricular arrhythmia or abnormal repolarization. Two of the hyperimmunized mice, which had failed to produce antibodies, showed normal EKGS. Hyperimmunization with TcP2β turned out to be lethal for five of the six animals receiving this antigen. Although the cause for this outcome was unclear, the authors’ conjecture was that death in this group might have resulted from the supraventricular tachycardia that had been observed at an earlier time in their protocol. Alternative causes were not explored. Although the studies performed with the single surviving mouse were interpreted by the authors as confirming a link between anti-TcP2β antibodies and tachyarrhythmia, the obvious statistical constraint (a single animal) makes it advisable to await repetition of this part of the study. In any case, the study performed with the TcP0-immunized mice suggests that the specific antibodies could induce some alterations occurring in chagasic animals not precisely replicating the complexities of chronic T. cruzi infection.

Following a slightly different approach, Chiale and coworkers [61] used affinity-purified IgG from patients with ventricular arrhythmias (group I) or sinus node dysfunction (group II) to find out if they would affect the chronotropicism of cultured cardiomyocytes. Whereas groups I and II included chagasic and non-chagasic patients, group III consisted of healthy individuals used as controls. Whether or not from chagasic patients, IgG from group I patients increased both the beating rate of cultured cardiomyocytes and their cyclic AMP levels. IgG from patients in group II had the opposite effects and IgG from the control donors had no noticeable consequence. Because group I sera tended to contain relatively low levels of anti-β1-adrenergic receptor antibodies and low levels of anti-M2 cholinergic receptor antibodies, and the opposite relationship was demonstrable in group II sera, Chiale et al. suggested a link between the presence of anti-autonomic membrane receptor antibodies and cardiac arrhythmia. Since the etiology of the heart conditions was not confined to T. cruzi infection, no link could be established between the appearance of the anti-receptor antibodies and T. cruzi antigens. In this context, it should be mentioned that, for many years, it has been known that IgG from chagasic patients can increase the beating frequency of isolated rat atrial preparations [62]. Chiale’s results [61] make it difficult to
convincingly postulate at this time that these or other anticholinergic receptor antibodies found in chagasic sera [63–65] originate in immune responses initiated by *T. cruzi* antigens. Recently, Perez Leiros et al. [66] reported that IgG from chagasic patients whose sera contained antibodies capable of activating rat atrial muscarinic acetylcholine receptors in an atropine-sensitive manner, immunoprecipitated purified as well as reconstituted human M₂ muscarinic cholinergic receptor molecules without blocking the binding of a specific ligand. This IgG also had an agonist effect on CHO cells stably transfected with M₂ muscarinic acetylcholine receptors, reducing binding affinity for the agonist and causing partial desensitization. The original stimulus for the formation of these antibodies was not ascertained and whether they could cause heart dysfunctions of the types seen in chagasic patients remains an open question.

The literature on autoimmunity and Chagas disease is strewn with reports of heart and other anomalies occurring in laboratory animals immunized with *T. cruzi* antigens under conditions that are far from resembling a natural immune response (reviewed in [8,36]). A diverse collection of putative autoantigens has been uncovered in experiments using complete and incomplete Freund’s adjuvants or contrived in vitro approaches [8,17,67]. Doubts also arise when legitimate questions and constructive criticisms go unaddressed or when pathology elicited by certain experimental protocols develops within days or a few weeks, far outpacing the time frame of development of typical pathology in chronic chagasic patients (measured in years and decades) or chronically infected animals (measured in months and years). Molecular mimicry as a basis for pathologic autoreactivity has been invoked in a number of infectious diseases [68], including Chagas disease [12,55,69–71]. Five stringent, objective criteria have been proposed for credibly arriving at the conclusion that molecular mimicry underlies pathology in infectious diseases [68]. Investigators who have studied examples of molecular mimicry in *T. cruzi* infection have concluded that these criteria have not been fully met in the case of Chagas disease [17].

3. Autoreactive cell-mediated immunity

Cunha-Neto et al. have been among the most prolific contributors to the notion that there is an autoimmune component to the pathogenesis of Chagas disease (reviewed in [8,13]). These authors have contended that the production of chronic chagasic heart tissue lesions involves deleterious effects mediated by cross-reactive T lymphocytes infiltrating heart tissue lesions [31,32]. These T cells were claimed to become activated upon suitable recognition of B13, the *T. cruzi* epitope putatively cross-reactive with murine CMHC [32,70]. However, as noted above for the corresponding antibody cross-reactivity, acceptance of the view that chagasic pathology is a result of molecular mimicry between *T. cruzi* and a heart tissue component has now been made contingent upon an explanation for the presence of a similar autoreactivity in patients with non-chagasic cardiomyopathies or in asymptomatic chagasic patients lacking heart anomalies (Levin, personal communication). Recently, Cunha-Neto and Kalil [41] reported that peripheral blood mononuclear cells from chagasic patients with overt heart disorder or asymptomatic chagasic patients responded to in vitro stimulation with B13 or other *T. cruzi* antigens with increased interferon-γ and reduced interleukin (IL)-4 production [41,72], as would be consistent with a Th1 type cytokine profile. From these observations the authors inferred that heart damage in chagasic cardiomyopathy could be secondary to inflammatory cytokines and a delayed type hypersensitivity process initiated by B13. These conclusions are somewhat premature because uncontested evidence of the cross-reactivity of B13, a parasite antigen, with host tissue antigens has not been forthcoming. Additionally persuasive would be some proof that CMHC-specific T cells are present in heart tissue lesions. It is noteworthy that both the levels of the response to B13 and the cytokine production profile of lymphocytes from asymptomatic chagasic patients were similar to those of T cells from patients with overt heart disease [41], weakening the notion that anti-B13 reactivity correlates with pathogenesis. Hopefully, these comments will stimulate much research to resolve the noted ambiguities and conflicts.

Ribeiro dos Santos and his co-workers [73] used a heart graft model system previously developed by Fulmer et al. [74] to study the involvement of autoreactive cellular immunity in the production of heart lesions in experimental *T. cruzi* infection. In their protocol, hearts taken from normal newborn BALB/c mice were transplanted into the dorsal base of the pinna of one ear of syngeneic recipients. Under these conditions, the subcutaneously implanted hearts start contracting a week to 10 days later. Grafts placed in the ears of mice chronically infected with *Y* strain or Colombia strain *T. cruzi* rejected their transplants within 20 days of implantation [73], but grafts placed into normal mice or animals that had been hyper-immunized with *T. cruzi* were not affected. In the chronic mouse model, administration of anti-CD4 antibodies, but not anti-CD8, two weeks prior to heart implantation prevented rejection, indicating that CD4 lymphocytes were responsible for heart rejection. In these animals, termination of the anti-CD4 treatment and reconstitution of CD4 cells did not restore heart rejection capability. Ribeiro dos Santos et al. also injected unfractionated T cells, purified CD4, or CD8 cells from chronically infected mice in the immediate vicinity of grafted newborn hearts placed in the ears of normal syngeneic mice. Graft destruction was noted within 4 days in mice that had been injected with unfractionated T cells or CD4 cells but not in mice that had received CD8 cells. Injection of cells from normal
mice or from mice immunized with an avirulent strain of *T. cruzi* failed to affect the transplanted hearts. In addition, unfractionated T cells or CD4 lymphocytes from chronically infected mice, but not CD8 cells from these animals, engaged in in vitro proliferation when incubated with a myocardial antigen. This proliferation was inhibited by anti-CD4 antibody, suggesting that an autoimmune CD4 cell-mediated phenomenon was critical to heart graft rejection in mice chronically infected with *T. cruzi*. The inability of mice hyperimmunized with *T. cruzi* to reject the transplanted hearts was taken as evidence of a lack of involvement of anti-parasite immune responses in heart graft rejection, placing the emphasis on a heart-specific autoreactivity associated with chronic Chagas disease. Since heart damage was seen only in the chronically infected mice, it did not take much time for other investigators to wonder whether *T. cruzi* might have infected the heart transplants. Using a similar model system, Tarleton’s group [75] was able to show that that *T. cruzi* was systematically present in heart grafts undergoing rejection. This result raised questions about an exclusive role for heart-specific CD4 cells in the rejection event. In their work, Tarleton’s group used various combinations of mouse strain (C57BL/6J and C3H/HeSnJ) and *T. cruzi* isolates (Brazil and Y, and the Sylvio X10/4 clone of *T. cruzi*), making it possible to study the fate of heart transplants in animals undergoing infections of varying degrees of severity and characteristics. The results indicated that the grafts survived in chronic chagasic mice for extended periods of time without displaying signs of rejection or inflammation. Furthermore, in situ polymerase chain reaction (PCR) tests performed on the grafts failed to detect the presence of parasite DNA [75]. Survival of the heart transplant was seen in the vast majority of C3H/HeSnJ mice chronically infected with the Sylvio X10/4 clone and C57BL/6J mice chronically infected with the Brazil or Y strain. The few rejections observed in normal and chronic chagasic mice were traced back to contamination or mechanical damage caused during graft excision. It is noteworthy that infection of C3H/HeSnJ mice with the Sylvio X10/4 *T. cruzi* clone leads to severe heart tissue inflammation but undetectable parasitemia, as generally seen in humans with chronic Chagas heart disease [73,76]. Therefore, the C3H/HeSnJ/Sylvio X10/4 combination enabled Tarleton to monitor the fate of heart grafts in a model system in which the likelihood of graft infection was very low. Instead, Ribeiro dos Santos and his colleagues had infected their mice with the Y or Colombia *T. cruzi* isolates, which are considerably more invasive and persistent than the Sylvio X10/4 clone [76,77]. Ribeiro dos Santos and his co-workers have attributed the differences between their own results [78] and those of Tarleton [75] to the use of different strains of mice and *T. cruzi*. This explanation awaits substantiation but, even if autoreactive CD4 cells were involved in chagasic pathogenesis in some, but not all, murine model systems, this very constraint would present as a laboratory oddity and not as a generic mechanism.

Ribeiro dos Santos et al. [78] attempted to rule out a possible role for *T. cruzi* in the CD4 cell-mediated carditis he had previously described [73]. To this end, they used CD4-enriched spleen cells from DBA/2 mice chronically infected with *T. cruzi* maintained in feeder cell cultures supplemented with recombinant IL-2 and containing a crude syngeneic mouse heart tissue antigen. The cell preparations, consisting of approximately 95% CD4 cells, proliferated in response to stimulation with either a crude *T. cruzi* antigen or heart tissue antigens from different animal species. In vitro, the CD4-enriched cells caused the beating of fetal heart cell clusters to stop. Direct injection of the CD4 cells into syngeneic neonatal heart grafts placed into the pinna of the recipients’ ears also caused beating to cease. At first glance, these results appeared to indicate that parasite infection was not a requisite for heart cell damage by the CD4 cells. However, both the experimental approaches and some of the results raised questions. Thus, in the assay designed to ascertain the reactivity of the CD4-enriched cells for a lysed *T. cruzi* preparation the antigen was a parasite-containing supernatant of infected monkey kidney epithelial cell cultures, i.e. one that may have contained allogeneic cell components. Parallel tests using a control preparation made from non-infected cell cultures were apparently not carried out. Therefore, the possibility that the observed proliferation might have been elicited by host cell material could not be ruled out. One of the most critical experiments included in this study involved the transfer of CD4-enriched cells derived from chronically infected DBA/2 mice to *T. cruzi*-infected BALB/c nu/nu mice. Although intense inflammation developed in this model system, the meaning of it was difficult to interpret because of the rather intense *T. cruzi* infection that typically occurs in thymus-deficient mice [79–81]. Not less problematic is the interpretation of the heart inflammation seen in mice that had received both the CD4 cell-enriched preparation and exogenous crude heart antigen incorporated into CFA. Control mice that had not been infected or injected heart antigen in CFA did not display heart inflammation after receiving the CD4-enriched cells. So, why did infected mice receiving the heart-sensitized CD4 cells require stimulation with exogenous heart material to develop heart inflammation? In other words, why did their native heart antigens not constitute a sufficient stimulus for activation of the transferred CD4 cells? And, were the transferred CD4 cells (or their progeny, if any) actually present in the inflammatory cell infiltrates of heart tissue lesions? And, assuming that they were, were they primary protagonists in causing tissue damage, secondary contributors, or not at all involved?

Pontes de Carvalho et al. [82] assessed inflammation and fibrosis in the hearts of mice subjected to treatment with both, a cardiac myosin-rich antigen emulsified in CFA and an anti-CD4 cell monoclonal antibody. This
treatment, expected to induce immunologic tolerance to cardiac myosin, caused mice to become resistant to subsequent induction of experimental autoimmune myocarditis but was unable to affect the production of anti-myosin IgG. Microscopic examination of heart tissues from T. cruzi-infected mice given the tolerizing treatment revealed less intense inflammation or fibrosis than seen in control mice receiving anti-CD4 antibody and CFA but not cardiac myosin. The authors were hopeful that their observations would bring to an end the ongoing dispute over the involvement of autoreactive CD4 cells in the pathogenesis of Chagas disease. But this was not the case since the tolerized mice produced normal levels of anti-cardiac myosin IgG. Since isotype switching to IgG is a Th (i.e. CD4)-dependent event, it would appear that the regime to make the mice tolerant was not as effective as had been anticipated. Because of this, it remains conjectural that the occurrence of mild chagasic heart lesions is a reflection of inability to mount a significant immune response to cardiac myosin.

Gironès et al. [83] have described an antigen, Cha, recognized by sera from many chagasic patients or T. cruzi-infected mice. A segment of the Cha molecule was found to be partially homologous with one found in an expressed sequence tag of the parasite, and also with SAPA, the shed acute-phase antigen of T. cruzi [84,85]. T lymphocytes from T. cruzi-infected mice mounted proliferative responses when stimulated in vitro with recombinant Cha. Transfer of splenic T cells from chronically infected mice to naive syngeneic mice was found to lead to production of anti-Cha antibodies, detectable 60 days later. Since transferred T cells were described as consisting of up to 99% CD3 cells, depleted of B cells by treatment with anti-IgM and anti-IgG plus complement, and lacking macrophages, the provenance of the B lymphocytes responsible for antibody production in this experiment was unclear. The presence of a few contaminating Cha-specific B cells and/or parasite material in the T cell preparations could be a plausible explanation. This possibility would not be inconsistent with the additional finding by Gironès et al. that the myocardium of mice receiving the T cells showed heart lesions similar to those occurring in infected mice. The use of cloned Cha-specific T cells in similar transfer type experiments would preclude contaminations with B lymphocytes or parasites.

4. Other considerations

Much of the information pertinent to the autoimmunity hypothesis of Chagas disease has been derived from experiments using murine model systems. Some features of human chagasic pathology can be reproduced in mice provided that the appropriate strains of mouse and parasite are selected [40] or the model system is somehow manipulated [86]. But some investigators disagree with the often-repeated assertion that murine hosts of T. cruzi develop a disease that closely replicates the features and pathological characteristics of human Chagas disease [30].

Alternative hypotheses for the pathogenesis of Chagas disease have been discussed [8,9,87]; none of them excluding the others.

In 1995, it was reported that none of two heart transplant recipients exhibited signs of myocarditis over a 6-year post-operative period [88]. These patients received immunosuppressive therapy to control adverse T cell responses that would imperil the grafted hearts, a precaution unlikely to have obliterated pre-existing humoral autoimmune responses targeting heart antigens. Another article described the follow up of 10 chagasic patients for a 10-year period after heart transplantation [89]. At this time, seven patients remained alive and were classified within class I according to New York Heart Association guidelines. The rate of heart rejection was lower among these patients than among individuals of an age- and sex-matched control group. Myocardial biopsies showed no signs of disease recurrence in the hearts given to the chagasic patients. In another study, the subjects were 22 patients with orthotopic heart transplantation [90]. Of these, nine had received hearts between 1985 and 1991 whereas the rest of the patients did so between 1991 and 1995. Although both groups received post-operative immunosuppressive treatments, they differed in the dose of cyclosporin A received. The second group was given a smaller dose to minimize the increase in parasite burden seen during immunosuppression [91–93]. Chagas disease was reactivated in five of the initial nine patients but only in one of the 13 patients in the second group. These results have made it difficult to reconcile the fact that transplanted hearts given to patients with the most severe cases of Chagas heart disease remained essentially undamaged for so many years with the tenets of a theory that postulates that deterioration of the native heart is due in large measure to autoimmunity.

Proponents of the autoimmunity hypothesis for Chagas disease have often sought support in the observation that T. cruzi is hard to find microscopically at lesion sites, suggesting that an indirect mechanism may be at work. This view has been challenged by a substantial and still growing body of literature showing parasite presence when more sensitive methods are used [94–100].

The controversy surrounding the autoimmunity hypothesis for Chagas disease has slowed down and even discouraged attempts to develop effective vaccines and anti-T. cruzi chemotherapy, as neither one is thought to stand a realistic chance against persistent autoimmunity. In the meantime, substantial progress has been made in endemic areas through vector control efforts [101–103]. Hopefully, nature will not teach us again the painful lesson learned from the fight against malaria, that insecticides alone are unlikely to contain indefinitely the transmission of pathogenic parasites by biologically resourceful insect vectors.
So, a query raised previously [8] is worth repeating here: should scientists and health authorities continue to put all their hopes on vector control strategies and continue to argue about the putative role of autoimmunity and other hypotheses while postponing support for approaches aimed at establishing the benefits of modern vaccines and novel chemotherapy for Chagas disease?

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References


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