Etiology of Chagas disease myocarditis: autoimmunity, parasite persistence, or both?

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In Chagas disease, caused by *Trypanosoma cruzi*, the scarcity of parasites in the chronic phase of the disease contrasts with the severe cardiac pathology observed in ~30% of chronic patients, and suggests a role for autoimmunity as the origin of the pathology. Recent studies on parasite detection, however, suggest that parasite persistence is the main cause of Chagas disease. To date, there is no conclusive evidence for the pathogenic role of either autoimmunity or parasite-specific immunity. Here, the most recent evidence in favour of each hypothesis is discussed.

The autoimmune hypothesis for Chagas disease arises from the fact that parasites are scarce in the chronic phase of disease. Therefore, it has been suggested that the disease could be the result of an autoimmune reaction caused either by autoantibodies or autoreactive T cells, derived by molecular mimicry between parasite and host antigens [1–5], or by bystander activation [6]. Molecular mimicry implies the sharing of specific antigenic epitopes between *Trypanosoma cruzi* and host antigens. By contrast, in bystander activation, no particular *T. cruzi* antigen is involved. Rather, *T. cruzi* infection causes tissue destruction that results in the release of autoantigens to which the autoimmune response is directed.

The parasite persistence hypothesis arises from the fact that *T. cruzi* does persist in the chronic phase of Chagas disease, and that antiparasite treatments to reduce parasite burden also result in a decrease in the severity of the disease (reviewed in Ref. [7]). Consequently, in this model, disease is thought to be a result of mostly to an antiparasite response. However, if parasite replication, which takes place in several organs, is exclusively responsible for pathology, then why do inflammatory lesions develop (although not exclusively) in the heart [8,9]? Moreover, why does parasite burden not always correlate with the degree of myocarditis [10,11]?

Molecular mimicry

It has been proposed that to prove the involvement of epitope mimicry in an infectious disease of suspected autoimmune etiology, five criteria need to be confirmed experimentally [12] (Box 1). In *T. cruzi* infection, the first three criteria have been clearly established, and this has allowed the identification of several (perhaps too many) candidate autoantigens. Among them, it is noteworthy that crossreactive antibodies between *T. cruzi* ribosomal proteins and the β-adrenergic receptor and the muscarinic receptor can cause electrocardiographic alterations in noninfected hearts that suggest a possible involvement of crossreactive antibodies in pathology [5,13]. If there were a unique crossreactive antigen, infection with parasite lacking the inducing antigen, or infection of knockout mice lacking the crossreactive autoantigen, would prevent the disease. However, because multiple autoantigens seem to be involved in the pathology of Chagas disease, such experiments are difficult to perform; therefore, criteria number 4 has yet to be demonstrated.

Criteria number 5 is considered to be the decisive test of the autoimmunity concept [12]. In this respect, the presence of autoreactive T cells against cardiac myosin and/or B13 proteins in heart lesions has been described, but any contribution that these T cells makes to the pathology has not been demonstrated [14]. As an alternative way of investigating the roles of autoimmunity and parasite persistence in chronically infected mice, the popular syngeneic heart transplant model has been used. Initial studies showed that in chronically infected mice there was rejection of transplanted syngeneic hearts, although these studies failed to rule out that the transplants were infected [15]. On the contrary, subsequent reports showed that injection of live parasites was required to cause rejection of syngeneic hearts transplanted in chronically infected mice [16]. These contrasting results might be the result of different mice and parasite strains used, but both anti-self and antiparasite

Box 1. Criteria for demonstrating the involvement of epitope mimicry in an autoimmune disease

(1) Association of the disease with a particular microorganism.
(2) Identification of the culprit microorganism epitope(s) that elicits the crossreactive response.
(3) T-cell or B-cell populations against that epitope(s) should be expanded in the infection.
(4) Elimination of the crossreactive epitope(s) from the microorganism should result in nonpathogenic infection.
(5) Autoreactive T cells should be able to transfer the disease.

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immune responses could represent inducing agents for rejecting implanted hearts. Another way to determine the pathological effect of autoreactive T or B cells would be to immunize mice with crossreactive antigens to see if this induces pathology. However, immunization with an autoantigen (injected together with adjuvants and via different routes) might not reflect the way the autoantigen is presented during natural infection.

The best approach is thought to be the transfer of putative autoreactive T cells from chronically infected mice, or of T cells specific for a given autoantigen. However, this approach does have its limitations in that it might also be unrelated to the way in which naive T cells respond to antigens in a natural infection. We have recently shown that reactivity against the Cha autoantigen in human and mouse *T. cruzi* infections is the result of molecular mimicry between distinct Cha T-cell and B-cell epitopes, and highly immunogenic parasite antigens, which triggers strong T-cell and B-cell responses [17]. More interestingly, adoptive transfer of T cells purified from the spleens of chronically infected mice induced heart infiltrates in naive recipients, and triggered anti-Cha antibody production in the absence of the parasite [17] (Fig. 1; Table 1). This indicates that autoreactive T cells activated during infection are sufficient to induce myocarditis in mice, and is, to our knowledge, the best evidence to date for an autoimmune-based mechanism.

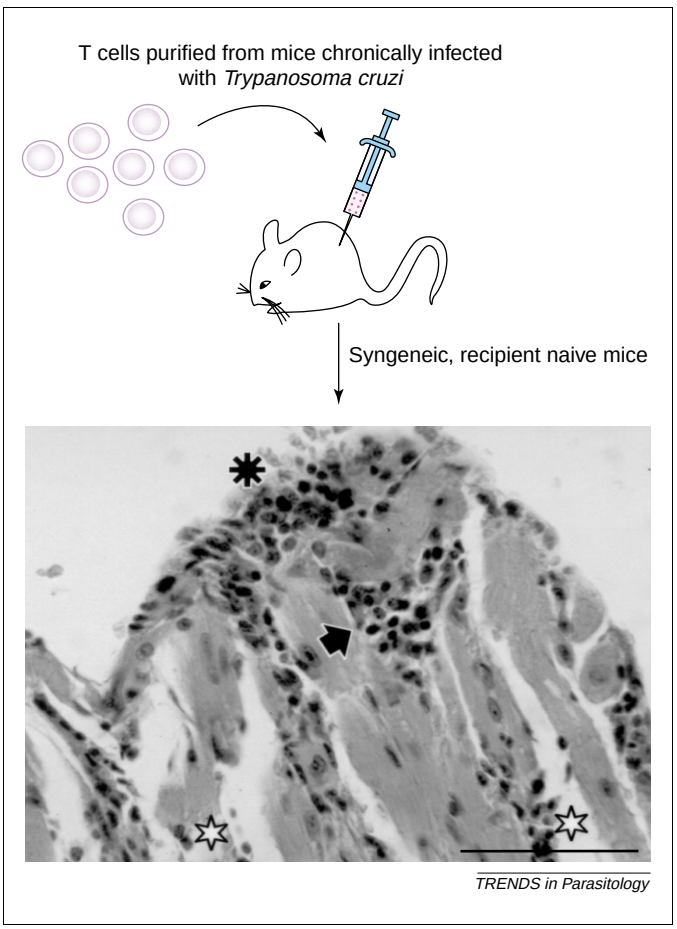

Fig. 1. Adoptive transfer of purified T cells from chronically infected mice triggers myocarditis in syngeneic recipient naive mice. Key: black arrow, subendocardial lymphocytic cumulus; black star, limited myocarditis zone; white star, general edema. Scale bar = 25 μm

Parasite detection

Parasite persistence and its correlation with myocarditis have been well documented in the chronic phase by PCR and *in situ* PCR [18,19]. In addition, intact parasites or parasite antigens have been reported in the injured heart [20]. However, controversy still exists about whether parasites or parasite-derived products are associated to all inflammatory loci. A recent study of hearts from chronic chagasic patients showed that there was no significant correlation between the intensity of the lesions and the quantity of parasite antigen [11]. Similar results were obtained in mice 120 days after infection with *T. cruzi* [10], although the specificity (self or parasite) of the T cells found in the vicinity of inflamed tissue is unknown. The lack of correlation between intensity of lesions and quantity of parasite antigens in some cases [10,11] suggest that, in addition to these parasite antigens, self-antigens could also contribute to support the extent of T-cell stimulation required for the tissue destruction seen in injured hearts.

Decrease of clinical symptoms

A strong argument in favour of the parasite persistence hypothesis comes from the fact that treatments to decrease the parasite burden in the acute phase are associated with a decrease in clinical symptoms [7]. In a review on long-term benznidazole treatment of chronic patients, a cure rate of 8% was reported [21]. Accordingly, we have found that in humans, the titer of anti-Cha autoantibodies and antiparasite antibodies decrease in parallel with

Table 1. Relationship between anti-*Trypanosoma cruzi* and anti-heart responses in *T. cruzi*-infected mice

Mouse strain	Susceptibility to myocarditis	Anti-*Trypanosoma cruzi* response		Anti-heart response		Anti-Cha response	
		T cell	B cell	T cell	B cell	T cell	B cell
Infected C57BL/6	No[a,b]	Yes[a]	Yes[b,c]	Yes[a]	Yes[a,b]	ND	No[b]
Infected A/J	Yes[a]	Yes[a]	Yes[a]	Yes[a]	Yes[a]	ND	ND
Infected CBA/J	Yes[c]	ND	Yes[c]	ND	ND	Yes[c]	Yes[c]
Transferred CBA/J[d]	Yes[c]	ND	ND	ND	ND	ND	Yes[c]

The absence or presence of myocarditis, anti-*Trypanosoma cruzi* (against non-crossreactive *T. cruzi* antigens), anti-heart (heart antigens) and anti-Cha responses in susceptible (CBA/J, A/J) and resistant (C57BL/6) mouse strains are indicated. Abbreviation: ND, not determined.

[a]T-cell response was measured by delayed-type hypersensitivity [6].

[b]N. Girones and M. Fresno, unpublished.

[c]T-cell response was measured by specific proliferation [17].

[d]Transferred CBA/J are mice adoptively transferred with activated, autoreactive T cells (purified from chronically *T. cruzi*-infected mice).

Table 2. Myocarditis and antibody responses in chagasic patients

Chagasic patients	Myocarditis[a]	Anti-Cha antibodies[b]	Anti-*Trypanosoma* antibodies[b]
Symptomatic	Yes	+++	+++
Asymptomatic (untreated)	No	++	++
Asymptomatic (treated)	No	+	+

[a]The absence or presence of myocarditis symptoms was determined from the clinical history of patients.
[b]Data from Ref. [22]. Antibody response was calculated at OD 450 nm, as follows: +, low level of response (<0.3); ++, intermediate level of response (0.3–1.0); +++, high level of response (>1.0).

benznidazole treatment, and increase with symptomatology [22] (Table 2). Therefore, benznidazole treatment in the chronic phase might be controlling parasitaemia, which also lowers autoreactivity as a result of molecular mimicry. Thus, regardless of the underlying etiological mechanism, antiparasite drugs are the best available treatment for chronic patients.

Comparing Chagas disease with autoimmune myocarditis

Another autoimmune disease characterized by myocarditis is that caused by the Coxsackie B3 virus. In fact, the current debate on autoimmunity and parasite persistence in Chagas disease resembles that in the Coxsackie B3 virus infection [23]. Early after viral infection in mice, no infectious virus can be isolated, only viral RNA can be detected in myocardial cells, but afterwards, cardiac myosin becomes the major autoantigen in autoimmune myocarditis [23]. This might result from stimulation of autoreactive T cells because myocarditis-inducing epitope of myosin is associated with the major histocompatibility complex of antigen-presenting cells (APC) in the heart of susceptible A/J mice, in the absence of infection [24]. However, no viral antigens that are crossreactive with cardiac myosin have been described. Chagas disease myocarditis is different because several myocardial crossreactive antigens have been described (among them, cardiac myosin), and parasite persistence has been documented. Despite these differences, similar strains of mice present the same susceptibility to both infective agents and development of myocarditis (BALB/c, CBA/J, A/J are susceptible, whereas C57BL/6 are resistant) [6,25–27]. Moreover, in *T. cruzi* infection, this phenomenon might be related to higher parasitaemia levels and/or inflammation, but is not related to anti-*T. cruzi* antibodies, which are similar in susceptible and resistant strains (Table 1). These results suggest that the genetic background might have a role in the development of myocarditis.

Antibodies against β-adrenergic and muscarinic receptors that are crossreactive with *T. cruzi* ribosomal proteins present in chagasic sera have been shown to cause electrocardiographic alterations in rabbit hearts [5]. More recently, *T. cruzi* ribosomal proteins, which crossreacted with β1-adrenoreceptor, caused changes in electrocardiograms in immunized mice [13]. Also, T cells reactive to cardiac tissue had been isolated from chagasic hearts, although the specificity of these T cells has not been determined [14]. Interestingly, immunization with cruzipain, a major cysteine protease from *T. cruzi*, also triggered anti-myosin antibodies and produced cardiac pathology, although the mechanism involved is unknown [28]. The Cha autoantigen, present in myocardial cells, is another candidate involved in the induction of myocarditis by autoreactive T cells [17]. Finally, the induction of immunological tolerance to myocardial antigens (highly enriched in cardiac myosin) prevents experimental chronic Chagas disease myocarditis [29], supporting an autoimmune-mediated pathogenic mechanism.

Coexistence of parasite persistence and autoimmunity

We believe that the two hypotheses of parasite persistence and autoimmunity can be reconciled. Thus, we suggest that the parasite is the trigger that activates certain T cells (specific for autoantigen and/or crossreactive parasite antigen). Once these are activated, they secrete inflammatory cytokines that mediate some cardiac damage. This liberates autoantigens (such as myosin) that are recognized by another set of autoreactive T cells and autoantibodies, which damage the cardiac tissue further. Simultaneously, cardiac damage could favour the induction of co-stimulatory molecules needed for the correct activation of autoreactive T cells.

However, myocarditis usually takes decades to be clinically apparent, and then only in a subset of *T. cruzi*-infected people. Viruses involved in autoimmune myocarditis initiate inflammation via local induction of pro-inflammatory cytokines: before exacerbation of the disease, relapses are preceded by an increase in tumour necrosis factor (TNF)-α and interferon (IFN)-γ levels, suggesting a new infection by the same or different pathogens [30]. Hypothetically, it is possible that another triggering event has to occur for the emergence of myocarditis in the chronic phase of *T. cruzi* infection. Thus, resurgence of infection (perhaps, caused by immunosuppressive drugs) and/or cardiac damage (i.e. infarct, re-infection, another infectious agent in genetically predisposed individuals) could represent the other triggering events that have to occur for emergence of myocarditis in the chronic phase. They would act to increase inflammatory conditions and co-stimulatory molecules, and to recruit APC and activate them to present the antigen correctly. In some instances, such as HIV infection [31] or treatment with immunosuppressive drugs [32], re-emergence of myocarditis has been reported.

Another aspect not taken into account in this debate is the role of regulatory T (Tr) cells, which suppress potentially pathogenic autoimmune responses [33]. Tr cells have stronger avidity than autoreactive naive $CD4^+$ cells, and therefore, at low concentrations of autoantigen, are the only cells activated and they can suppress activation of T cells of the same specificity. However, if the concentration of mimetic parasite antigen and/or autoantigen increases, there would be sufficient antigen to stimulate pathogenic autoreactive T cells [33]. This situation is likely to take

place in the acute phase of the *T. cruzi* infection, thus allowing autoreactive T cells to be expanded.

At the same time, it has been shown that deposition of complement in the heart triggers the activation, via complement receptor (CR)1 and CR2, of a subset of $CD44^{hi}$ $CD62^{lo}$ T cells and causes autoimmune myocarditis [34]. It is noteworthy that these cells are also found in the hearts of mice chronically infected with *T. cruzi* [10]. This would provide a putative link between autoantibodies, which could fix complement, and the activation of T cells. Along these lines, the finding that Cha has B-cell and T-cell crossreactive epitopes [17] makes it a leading candidate for the induction of disease.

Conclusions

We suggest that *T. cruzi* persistance in chronic Chagas myocarditis, together with the immune response to multiple myocardial antigens, point to the participation of anti-self and/or antiparasite immune responses in the induction of myocarditis by *T. cruzi*. Thus, parasites are the trigger, but anti-self and/or antiparasite T and B cells are the actual effector cells. Accordingly, when there is molecular mimicry between host and parasite antigens, treatment with antiparasite drugs inhibits parasite replication, and hence anti-self and/or antiparasite immune responses that cause myocarditis. In addition, other factors, such as Tr cells, fixation of complement by autoantibodies and secondary infections, which have not been explored in detail, might have some role in the pathology of Chagas disease.

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