

Where do we stand on the autoimmunity hypothesis of Chagas disease?

Felipe Kierszenbaum

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA

The question posed in the title elicits as much controversy today as it did when I wrote about this subject in the first issue of *Parasitology Today* 20 years ago. A consensus is now emerging that *Trypanosoma cruzi*, the etiological agent of Chagas disease, bears primary responsibility for producing chagasic pathology. Whether one or more of the autoimmune events described in human and experimental Chagas disease can contribute to, or aggravate, this pathology is the current question.

Is there autoimmunity in Chagas disease?

I asked this question in 1985 [1]. Now, we know that the answer is 'yes' but the key question has shifted to 'is this autoimmunity relevant to the pathology of Chagas disease?' Recent reviews have condensed the various positions on this issue [2,3]. Therefore, this article focuses mainly on the evidence that keeps the controversy alive.

The notion that autoimmunity underlies pathogenesis in Chagas disease (for reviews, see Refs [3–6]) has had a negative impact on efforts to develop effective chemotherapy and vaccines against *Trypanosoma cruzi* infection. Chemotherapy targeting *T. cruzi* would not be able to stop the course of a disease perpetuated by a continuous immune response to host tissue antigens. Vaccines would have to overcome the impossible – that is, proving, through neverending, futile attempts, that they would not induce the disease that they are designed to prevent. Establishing whether autoimmunity is a primary cause of chagasic pathology or a secondary aggravating factor is important because autoimmunity would be circumvented if infection were to be prevented or rapidly controlled. However, should autoimmunity be a major concern in Chagas disease? To examine the main issues surrounding this question, it is useful to separate the proposed mechanisms according to the possible mediators: autoantibodies and autoreactive cellular immunity. However, it must be acknowledged that these mechanisms are not entirely independent of each other.

Are autoantibodies involved in the pathogenesis of Chagas disease?

In human or experimental *T. cruzi* infection, most autoantibodies described to date fit the concept of

molecular mimicry, although responses to cryptic antigens have also come into play. In molecular mimicry, epitopes of parasite antigens elicit antibodies that can crossreact with epitopes of host tissue molecules. Cryptic antigens are generally intracellular molecules that become exposed to the immune system when host cells, bursting from infection or damaged by other means, release their contents. Typical examples of both are cardiac myosin and ribosomal proteins [7–9]. Neu *et al.* [10] have listed several reasons why antimyosin antibodies are unlikely to be involved in myocarditis; a salient reason being that these antibodies do not bind intact myocytes, although they readily find their specific target in detergent-permeabilized cells. Because antibodies that mediate damage of intact cells must first bind to cell surface antigens, Neu's points undermine acceptance of the notion that autoantibodies to intracellular antigens are responsible for pathology in Chagas disease. Moreover, a recent study by Leon *et al.* [11] has concluded that myosin autoimmunity is not essential for cardiac inflammation, at least in acute experimental *T. cruzi* infection. Reproducing the main features of chagasic pathology with passively transferred antibodies specific for epitopes shared by *T. cruzi* and mammalian hosts or elicited by cryptic antigens would go a long way towards supporting an autoimmune pathological mechanism but so far there is no persuasive evidence that such is the case. An alternative protocol, based on actively immunizing mice with cross-reactive antigens, has been tried and the results have been mixed [3,5,11,12]. Thus, Lopez Bergami *et al.* [12] recorded altered electrocardiographic patterns in some of the mice immunized with *T. cruzi* ribosomal P proteins. Because antibodies to these proteins also bind G protein-coupled receptors linked to the induction of supra-ventricular arrhythmia, these authors suggested that they might be involved in pathogenesis in chronic chagasic heart disease, despite the fact that inflammatory lesions or fibrosis, typical of Chagas heart disease, could not be detected in the immunized mice. Proving a role in chagasic pathology for antibodies to cardiac myosin, which occur in *T. cruzi*-infected mice and patients [3], has been similarly difficult [5,11]. To complicate matters, the specific antigens for most autoreactive antibodies found in chagasic patients and laboratory animals have a wide tissue distribution, making it difficult to explain why lesions occur at just one or a few of the many localizations [3–5]. It

Corresponding author: Kierszenbaum, F. (kierszen@msu.edu).

Available online 25 August 2005

is noteworthy that antimyosin antibodies are not unique to *T. cruzi* infection. Thus, in A/J mice, the strain used to study the effects of anticardiac myosin antibodies appearing after *T. cruzi* infection [5,7], the same antibodies were detected after coxsackievirus infection [13]. Moreover, antimyosin antibodies have been described in patients with heart problems as varied as myocardial infarction, viral myocarditis, nonchagasic inflammatory heart disease and in individuals who had undergone several types of cardiac surgery, including coronary artery bypass [14,15] (M.J. Levin, personal communication). In addition, Baig *et al.* [16] could not establish a correlation between the levels of cardiac-specific antibodies and the presence or extent of heart damage in chagasic patients. In the light of all this information, any conceivable role of a cardiac myosin-specific immune response in chagasic pathology would have to be considered as secondary, and probably a result of cardiac tissue damage. If so, immunoprophylaxis and promptly applied chemotherapy would stand a real chance against Chagas disease.

Circulating antibodies from chronic chagasic patients have been shown to affect certain heart cell functions *in vitro*. For example, these antibodies can display muscarinic cholinergic activity [17], alter the beat rate of cultured cardiac cells [18] or induce atrioventricular conduction block in isolated whole rabbit hearts [19]. Although these effects bear resemblance to anomalies seen in some cases of Chagas disease, an actual role for these antibodies in tissue pathology has not been demonstrated. In addition, experiments testing the effects of human antibodies on animal tissues have met with a healthy dose of skepticism ever since early studies linking autoimmunity and chagasic pathology, based on the binding of putative autoantibodies from chronic patients to murine tissues [20,21], had to be recanted because they could not be reproduced with human tissue [22].

Several unresolved issues have hindered acceptance of the autoimmunity hypothesis for Chagas disease. Thus, there is the contrast between the relatively short amounts of time it takes for autoantibodies to appear after *T. cruzi* infection (generally a few weeks) and the extended periods of time elapsing between infection and the appearance of chagasic pathology (generally years). Moreover, the autoantibodies claimed to contribute to chagasic pathology have been also found in patients with indeterminate Chagas disease (i.e. asymptomatic) and not infrequently in healthy individuals [3,23,24]. Also enigmatic is the ability of autoantibodies from chagasic patients to produce deleterious effects *in vitro* that are not demonstrable in many of the patients from whom the autoantibodies are obtained.

In previous reviews [3,4], I raised some questions about anticardiac myosin autoimmunity which are applicable to other antigens implicated in Chagas disease pathogenesis. Namely, can any of the autoantibodies that have been described reproduce chagasic pathology? Do autoantibodies appearing *after* tissue damage has occurred aggravate the pathology of Chagas disease? Should damage to the heart or other tissues ensuing during *T. cruzi* infection be viewed differently from similar injuries seen in other infectious diseases or noninfectious

diseases? Even if we knew now that autoantibodies contribute to chagasic pathology, how would we explain that the autoantibodies, generally IgG, bring about the typical mononuclear cell infiltrate that is commonly seen in chagasic heart lesions instead of the predominantly granulocyte cell infiltration that follows tissue deposition of IgG? These questions are not new but the answers have not been forthcoming.

Autoreactive cell-mediated immunity in Chagas disease?

Not long after the initial reports that circulating autoantibodies had been detected in chagasic patients, cellular autoreactivities began to appear in the literature (for review, see Refs [3,4]). A couple of salient examples will help to illustrate the current status of this side of the autoimmunity controversy. Cunha-Neto *et al.* have been among the staunchest proponents of the involvement of autoreactive T cells in the pathogenesis of Chagas heart disease [25]. This group reported that cardiac myosin induced *in vitro* proliferation by CD4⁺T cell clones derived from cells infiltrating the heart lesions of a single chronic Chagas cardiomyopathy patient [26], and obtained similar results using B13, a recombinant peptide containing the immunodominant FGQAAAGDK epitope, claimed to be crossreactive with the internal AAALDK sequence of cardiac myosin. It should be noted that others have not been able to corroborate this particular immunological crossreactivity (M.J. Levin, personal communication). Aside from this disagreement, acceptance of a major role for antimyosin as a primary cause of chagasic heart injury, or its attribution to molecular mimicry with a *T. cruzi* epitope, will require explanations for the absence of cardiac tissue damage in asymptomatic patients producing antimyosin and the presence of antimyosin antibodies in patients with nonchagasic cardiomyopathies.

Cunha-Neto and Kalil [27] described experiments in which peripheral blood mononuclear cells from chagasic patients with overt heart disease produced interferon- γ but exhibited decreased interleukin-4 production after *in vitro* stimulation with B13. Because these results fit a typical T helper cell type 1 (Th1) cytokine production pattern, it was surmised that heart tissue damage in chagasic patients might result from inflammatory cytokines modulating a delayed-type hypersensitivity event triggered by B13. However, a close look at the results reveals that the levels of both anti-B13 antibody and cytokine produced by cells from patients with overt heart disease were comparable with those of cells from asymptomatic chagasic patients.

Some attempts have been made at inducing immunological tolerance to heart antigens in mice, to establish whether this might reduce the extent of heart tissue damage after *T. cruzi* infection [28]. Tolerance seemed to reduce the extent of myocardial damage in these animals. These results merit attention and independent corroboration efforts, preferably using an alternative protocol that does not involve injection of complete Freund's adjuvant, a rather aggressive reagent with its own effects on the immune system, whose effects would be difficult to

separate in control noninfected animals receiving only the adjuvant.

A major splash in this field was caused by a study showing that hearts from healthy newborn BALB/c mice grafted into the dorsal base of the pinna of one ear of syngeneic mice were rejected by mice surviving infection with highly invasive *T. cruzi* isolates [29]. By contrast, the transplanted hearts adapted well and survived in non-infected mice. Rejection in the chronically infected mice was known to involve CD4⁺T cells because treatment with anti-CD4, but not anti-CD8, antibodies prevented it. In a variation of this experimental approach, unfractionated T cells, or purified CD4⁺ or CD8⁺ cells from chronically infected mice were injected near the sites at which the hearts had been grafted, and rejection followed in short order but only in the animals that had received either unfractionated T cells or CD4⁺T cells. In addition, unfractionated T cells or CD4⁺T cells from the chronically infected mice proliferated *in vitro* upon incubation with a myocardial antigen, denoting their particular specificity. This effect was also inhibited by anti-CD4 antibodies. These findings seemed to separate the parasite from the immunological reaction that was affecting the transplanted hearts, emphasizing a key role for heart-specific cellular autoimmunity in *T. cruzi*-infected mice. However, the presence of *T. cruzi* in the rejected grafts had not been ruled out. Using a nearly identical protocol, Tarleton *et al.* [30] found that *T. cruzi* was indeed present in the heart grafts undergoing rejection. By contrast, rejection did not occur when the Sylvio X10/4 clone of *T. cruzi* (which causes severe heart disease but extremely low, if any, parasitemia [31]) was used to reduce the chances of infecting the grafted hearts [30]. Parasite material was not detectable in these grafts by using a highly sensitive PCR test. The striking differences between the results of dos Santos *et al.* [29] and those of Tarleton have been attributed to the use of different strains of mice and parasite isolates in their experiments. Even if this explanation were to be accepted on face value, an autoimmune cellular contribution to heart pathology would have to be perceived as occurring under unique experimental conditions.

A recently described *T. cruzi* antigen, Cha, is recognized by sera from either chagasic patients or *T. cruzi*-infected mice [32]. A segment of the Cha molecule is partially homologous with one found in an expressed sequence tag of *T. cruzi* and one present in the shed acute-phase *T. cruzi* antigen [33]. T cells from infected mice were found to proliferate upon stimulation with Cha, and splenic T cells from chronically infected mice transferred to naive syngeneic mice were shown to enable production of anti-Cha antibodies, detectable two months after the cell transfer. The transferred cells were described as 'up to 99%' CD3⁺T cells, being depleted of B cells, and essentially free of macrophages. When B cells are so thoroughly excluded, the source of the B cells or plasma cells secreting the anti-Cha antibodies is not apparent, raising the possibility that some Cha-specific B cells or some parasite materials might have contaminated the injected T cell preparation.

Several other cellular crossreactivities between *T. cruzi* and host tissue antigens have been examined (for review,

see Ref. [3]). At present, their possible role in the pathogenesis of Chagas disease is not much clearer than in the examples summarized above.

Concluding remarks

The reservations about the autoimmunity hypothesis for pathogenesis in Chagas disease, noted above and sometimes based on experimental pitfalls or insufficient controls, have fueled a long-lasting controversy. Admittedly, this is a rather difficult field of research, but it is has been unnecessarily complicated in some cases by data overinterpretation, attribution of significance to miniscule differences, and premature conclusions. After so many years of controversy, key experiments are still pending. If the autoantibodies and/or autoreactive cells that have been postulated to contribute to producing chagasic pathology actually do so, their passive transfer to healthy hosts should reproduce at least some of it. There are animal model systems in which this approach can be reasonably tested, and the results would be more conducive to resolving the controversy than those obtained with the contrived *in vitro* assays used to date (for review, see Ref. [3]).

In the meantime, an ever-increasing body of literature documenting the presence of *T. cruzi* in affected tissues, and long-term studies of chagasic patients receiving heart transplants (for review, see Ref. [3]), have gone a long way towards associating the pathology of Chagas disease with the presence of the parasite. In this context, it should be noted that Higuchi *et al.* [34] have formulated an interesting hypothesis linking parasite levels and the immune status of the host as possible factors impinging on the production of overt manifestations of Chagas disease.

The controversy surrounding the autoimmunity hypothesis has had consequences, discouraging support for efforts aimed at developing effective chemotherapy and vaccination. In the meantime, health authorities concerned with autoimmunity in Chagas disease seem to have found some comfort in the progress achieved with vector control measures. However, if the history of other vector-borne diseases serves as a precedent, neglect of alternative approaches will probably be regretted sooner or later, when the insect vectors of *T. cruzi* evolve a way to emulate their malaria-carrying counterparts and show that they remain a force to be reckoned with.

Acknowledgements

I thank Mariano J. Levin for generously giving me access to unpublished results from his laboratory. I regret that a fair amount of work that would have added enriching details to the present discussion has been left out because of space limitations.

References

- 1 Kierszenbaum, F. (1985) Is there autoimmunity in Chagas' disease? *Parasitol. Today* 1, 4–6
- 2 Engman, D.M. and Leon, J.S. (2002) Pathogenesis of Chagas heart disease: role of autoimmunity. *Acta Trop.* 81, 123–132
- 3 Kierszenbaum, F. (2003) Views on the autoimmunity hypothesis for Chagas disease pathogenesis. *FEMS Immunol. Med. Microbiol.* 37, 1–11
- 4 Kierszenbaum, F. (1999) Chagas' disease and the autoimmunity hypothesis. *Clin. Microbiol. Rev.* 12, 210–223

- 5 Leon, J.S. and Engman, D.M. (2003) The significance of autoimmunity in the pathogenesis of Chagas heart disease. *Front. Biosci.* 8, e315–e322
- 6 Tarleton, R.L. (2003) Chagas disease: a role for autoimmunity? *Trends Parasitol.* 19, 447–451
- 7 Leon, J.S. *et al.* (2004) A cardiac myosin-specific autoimmune response is induced by immunization with *Trypanosoma cruzi* proteins. *Infect. Immun.* 72, 3410–3417
- 8 Kaplan, D. *et al.* (1997) Antibodies to ribosomal P proteins of *Trypanosoma cruzi* in Chagas disease possess functional auto-reactivity with heart tissue and differ from anti-P autoantibodies in lupus. *Proc. Natl. Acad. Sci. U. S. A.* 94, 10301–10306
- 9 Mahler, E. *et al.* (2004) Structural and functional complexity of the humoral response against the *Trypanosoma cruzi* ribosomal P2 beta protein in patients with chronic Chagas' heart disease. *Clin. Exp. Immunol.* 136, 527–534
- 10 Neu, N. *et al.* (1990) Cardiac myosin-induced myocarditis. Heart autoantibodies are not involved in the induction of the disease. *J. Immunol.* 145, 4094–4100
- 11 Leon, J.S. *et al.* (2003) Myosin autoimmunity is not essential for cardiac inflammation in acute Chagas' disease. *J. Immunol.* 171, 4271–4277
- 12 López Bergami, P. *et al.* (2001) Antibodies against the carboxyl-terminal end of the *Trypanosoma cruzi* ribosomal P proteins are pathogenic. *FASEB J* 15, 2602–2612
- 13 Neu, N. *et al.* (1987) Autoantibodies specific for the cardiac myosin isoform are found in mice susceptible to Coxsackievirus B3-induced myocarditis. *J. Immunol.* 138, 2488–2492
- 14 de Scheerder, I.K. *et al.* (1989) Anti-myosin humoral immune response following cardiac injury. *Autoimmunity* 4, 51–58
- 15 Nomura, Y. *et al.* (1994) Relationship between the degree of injury at operation and the change in antimyosin antibody titer in the postpericardiotomy syndrome. *Pediatr. Cardiol.* 15, 116–120
- 16 Baig, M.K. *et al.* (1997) Human chagasic disease is not associated with an antiheart humoral response. *Am. J. Cardiol.* 79, 1135–1137
- 17 Goin, J.C. *et al.* (1997) Interaction of human chagasic IgG with the second extracellular loop of the human heart muscarinic acetylcholine receptor: functional and pathological implications. *FASEB J.* 11, 77–83
- 18 Masuda, M.O. *et al.* (1998) Functionally active cardiac antibodies in chronic Chagas' disease are specifically blocked by *Trypanosoma cruzi* antigens. *FASEB J.* 12, 1551–1558
- 19 de Oliveira, S.F. *et al.* (1997) Sera from chronic chagasic patients with complex cardiac arrhythmias depress electrogenesis and conduction in isolated rabbit hearts. *Circulation* 96, 2031–2037
- 20 Cossio, P.M. *et al.* (1974) Chagasic cardiopathy. Demonstration of a serum gamma globulin which reacts with endocardium and vascular structures. *Circulation* 49, 13–21
- 21 Khoury, E.L. *et al.* (1979) Circulating antibodies to peripheral nerve in American trypanosomiasis (Chagas' disease). *Clin. Exp. Immunol.* 36, 8–15
- 22 Khoury, E.L. *et al.* (1983) Heterophile nature of EVI antibody in *Trypanosoma cruzi* infection. *Clin. Immunol. Immunopathol.* 27, 283–288
- 23 Cunha-Neto, E. *et al.* (1995) Autoimmunity in Chagas disease cardiopathy: Biological relevance of a cardiac myosin-specific epitope crossreactive to an immunodominant *Trypanosoma cruzi* antigen. *Proc. Natl. Acad. Sci. U. S. A.* 92, 3541–3545
- 24 Girones, N. *et al.* (2001) Antibodies to an epitope of the Cha human autoantigen are markers of Chagas' disease. *Clin. Diagn. Lab. Immunol.* 8, 1039–1043
- 25 Kalil, J. and Cunha-Neto, E. (1996) Autoimmunity in Chagas disease cardiomyopathy: fulfilling the criteria at last? *Parasitol. Today* 12, 396–399
- 26 Cunha-Neto, E. *et al.* (1996) Autoimmunity in Chagas' disease. Identification of cardiac myosin-B13 *Trypanosoma cruzi* protein crossreactive T cell clones in heart lesions of a chronic Chagas' cardiomyopathy patient. *J. Clin. Invest.* 98, 1709–1712
- 27 Cunha-Neto, E. and Kalil, J. (2001) Heart-infiltrating and peripheral T cells in the pathogenesis of human Chagas' disease cardiomyopathy. *Autoimmunity* 34, 187–192
- 28 Pontes-de-Carvalho, L. *et al.* (2002) Experimental chronic Chagas' disease myocarditis is an autoimmune disease preventable by induction of immunological tolerance to myocardial antigens. *J. Autoimmun.* 18, 131–138
- 29 dos Santos, R.R. *et al.* (1992) Anti-CD4 abrogates rejection and reestablishes long-term tolerance to syngeneic newborn hearts grafted in mice chronically infected with *Trypanosoma cruzi*. *J. Exp. Med.* 175, 29–39
- 30 Tarleton, R.L. *et al.* (1997) "Autoimmune rejection" of neonatal heart transplants in experimental Chagas' disease is a parasite-specific response to host infected tissue. *Proc. Natl. Acad. Sci. U. S. A.* 94, 3932–3937
- 31 Postan, M. *et al.* (1986) A histopathological analysis of the course of myocarditis in C3H/He mice infected with *Trypanosoma cruzi* clone Sylvio X10/4. *Trans. R. Soc. Trop. Med. Hyg.* 80, 50–55
- 32 Girones, N. *et al.* (2001) Dominant T- and B-cell epitopes in an autoantigen linked to Chagas' disease. *J. Clin. Invest.* 107, 985–993
- 33 Pollevick, G.D. *et al.* (1993) Members of the SAPA/trans-sialidase protein family have identical N-terminal sequences and a putative signal peptide. *Mol. Biochem. Parasitol.* 59, 171–174
- 34 Higuchi, M.L. *et al.* (2003) Pathophysiology of the heart in Chagas' disease: current status and new developments. *Cardiovasc. Res.* 60, 96–107

AGORA initiative provides free agriculture journals to developing countries

The *Health Internetwork Access to Research Initiative* (HINARI) of the WHO has launched a new community scheme with the UN Food and Agriculture Organization.

As part of this enterprise, Elsevier has given 185 journals to *Access to Global Online Research in Agriculture* (AGORA). More than 100 institutions are now registered for the scheme, which aims to provide developing countries with free access to vital research that will ultimately help increase crop yields and encourage agricultural self-sufficiency.

According to the Africa University in Zimbabwe, AGORA has been welcomed by both students and staff. 'It has brought a wealth of information to our fingertips' says Vimbai Hungwe. 'The information made available goes a long way in helping the learning, teaching and research activities within the University. Given the economic hardships we are going through, it couldn't have come at a better time.'

For more information visit:
<http://www.healthinternetwork.net>