

Invited Review

Parasite persistence in the aetiology of Chagas disease

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Abstract

Two primary hypotheses are proposed to account for pathogenesis in chronic *Trypanosoma cruzi* infections: that the persistence of *T. cruzi* at specific sites in the infected host results in chronic inflammatory reactivity and that *T. cruzi* infection induces immune responses which are targeted at self tissues. The data supporting parasite persistence as the primary cause of disease in *T. cruzi* infection have been recently reviewed and the reader is referred to this review for extensive documentation of most of the arguments outlined herein. This manuscript will briefly reiterate the main points of this previous review, adding additional data that have been presented since its publication. Then, philosophical and practical arguments on why Chagas disease should be investigated and treated as a parasitic infection and not as an autoimmune disease are presented. This is admittedly an 'opinion piece' and not a balanced review of the literature on Chagas disease. There are substantial data other than those reviewed here, which have been presented in support of the autoimmunity hypothesis. It is left to others to review that body of literature. © 2001 Published by Elsevier Science Ltd. on behalf of Australian Society for Parasitology Inc.

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1. Introduction

The mechanisms of pathogenesis in Chagas disease really distil down to two primary hypotheses: (1) that the persistence of *Trypanosoma cruzi* at specific sites in the infected host results in chronic inflammatory reactivity (hereafter call the parasite persistence hypothesis) and (2) that *T. cruzi* infection induces immune responses which are targeted at self tissues and are independent of the persistence of *T. cruzi* (hereafter referred to as the autoimmunity hypothesis). In both cases, the immune-based pathology results in the cumulative, focal destruction of tissues, and the signs and symptoms of clinical disease. There are other proposed mechanisms of pathogenesis, including the direct destruction of tissues by the parasite or by parasite-derived molecules, the 'starving' of tissue due to vascular blockage, denervation of muscle tissues or the polyclonal activation of the immune system. However these mechanisms can be either discarded as unlikely or be treated as the after-effects or the end effectors of either the parasite persistence or autoimmunity models.

2. The autoimmunity hypothesis

The prevailing dogma has long been that Chagas disease has an autoimmune aetiology. The communal strength of belief in the autoimmunity hypothesis is reflected in how human Chagas disease is treated and in how research in Chagas disease is funded. Thus chronically infected individuals with frank Chagas disease rarely receives chemotherapeutics in an attempt to control or eliminate *T. cruzi*. The basis of this treatment decision is the argument that disease is not a direct result of the presence or the level of parasites (Bestetti, 1997). In terms of prevention, there have been no international programs for vaccine development although similar programs have been implemented for related pathogens such as *Leishmania*. Again, the justification seems to be based upon the argument that enhancing the anti-parasite immune response in *T. cruzi* infection may increase the severity of autoimmune disease in the chronic phase of the infection (Kierszenbaum and Hudson, 1985; Mosca, 1986).

The main support for the autoimmune hypothesis is the conclusion that signs of disease are evident in tissues in the apparent absence of parasites. This observation has led to the suggestion that inflammatory responses present in Chagas-ic lesions are not directly induced by or targeted for *T. cruzi* but rather are specific for other, perhaps 'self', cross-reactive or mimicked, antigens. The observation that

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anti-self antibodies and lymphocytes are demonstrable in *T. cruzi*-infected hosts supports this hypothesis as does other characteristics of the disease, including its delayed onset, organ specificity and spectral nature. However, definitive proof of anti-self responses as the primary cause of disease in *T. cruzi*-infected hosts remains elusive.

3. Parasite persistence hypothesis

The contrasting view that parasite persistence is the primary cause of disease in *T. cruzi* infection has been recently reviewed (Tarleton and Zhang, 1999). The parasite persistence hypothesis rests upon two sets of experimental and clinical results: (1) evidence that Chagas disease is inextricably linked to the persistence of *T. cruzi* at or near the disease site, and (2) evidence that treatments which decrease parasite load result in a concomitant decrease in disease severity while treatments which result in increased parasite levels lead to exacerbated disease.

The link between the presence of *T. cruzi* and sites of disease is incredibly strong. Although standard histochemical techniques often fail to reveal *T. cruzi* at sites of disease, immunohistochemistry, whole tissue and in situ PCR and in situ hybridisation techniques have provided indisputable evidence of parasite persistence at sites of disease (reviewed in Tarleton and Zhang, 1999). The organ specificity of parasite association with disease is nearly perfect. Jones et al. (1992) reported that *T. cruzi* DNA was not detectable in heart tissue from seropositive cadavers that lacked evidence of Chagas-ic cardiopathy but was consistently detected in heart specimens from patients diagnosed with chronic Chaga's-ic cardiomyopathy. Vago et al. (1996) detected *T. cruzi* DNA in oesophageal tissue from seropositive patients with megaesophagus or with inflammatory lesions in the oesophagus but not from three patients who died of chronic Chagas-ic cardiomyopathy without evidence of megaesophagus. It has been argued that parasite DNA and antigen may persist for extended periods of time in lesions and thus may not be indicative of an active site of infection. However Anez et al. (1999) reported the persistence of intact amastigotes in 22 of 26 endomyocardial biopsies from chronic Chagas-ic patients. In various mouse models of chronic disease, we have found an absolute correlation between the presence of disease and the presence of parasite DNA in muscle tissue (Zhang and Tarleton, 1999). Considering the combination of data from murine and human infections and from examination of heart, skeletal muscle and gut tissues, it is difficult to arrive at any conclusion other than that disease severity is directly related to the presence and level of parasites within the affected tissue.

4. Predictions of the parasite persistence and autoimmunity hypotheses

The parasite persistence and autoimmunity hypotheses

have different predicted outcomes for situations where the immune system is boosted non-specifically or by vaccination or, alternatively, compromised by immunosuppressants. The parasite persistence model predicts that enhancement of the immune response to *T. cruzi* would result in decreased disease severity by reducing parasite load. In contrast, the autoimmune hypothesis would predict that enhancement of the immune response, either specifically or non-specifically, would exacerbate disease. Likewise, diseases with an autoimmune aetiology generally respond positively to immunosuppressive therapy while immunosuppression would likely increase parasite load and disease severity according to the parasite persistence model.

Results of experiments in which immune enhancement or immunosuppression therapies have been applied during *T. cruzi* infection firmly support the predictions of the parasite persistence hypothesis. A variety of immunosuppressive situations, including treatments to prevent transplant rejection (Almeida et al., 1996; Jardim and Takayanagui, 1994) and in patients of AIDS (Ferreira et al., 1991; Rocha et al., 1994; Sartori et al., 1995) correlate with exacerbation of the infection and disease. In experimental models, defects in immune function almost uniformly result in increased susceptibility to *T. cruzi* infection and death of animals during the acute phase of the infection and increased parasite load and disease symptoms during the chronic phase of infection (Andrade et al., 1997; Andrade et al., 1987; Boulton et al., 1988; Melo, 1999; Melo and Machado, 1998; Okumura et al., 1994; Rocha et al., 1994; Sartori et al., 1995; Silva and Rossi, 1990; Tarleton et al., 1996; Tarleton et al., 1994). Alternatively, enhancing the efficiency of the anti-parasite response by immunotherapy, gene-deletion, or vaccination, results in decreased severity of chronic disease, not an exacerbation of disease as predicted by the autoimmune hypothesis (Kumar, 2000; Tarleton et al., 2000) Garg and Tarleton, unpublished observation). The ability of both prophylactic and therapeutic vaccination to reduce the severity of chronic disease in experimental animals is particularly important and provides convincing evidence for the ability of *T. cruzi* vaccines to modulate chronic phase disease (Garg and Tarleton, unpublished observations).

One aspect of the relationship between the immune response to *T. cruzi* and disease severity in the chronic stage of the infection that has not received sufficient attention is that of the quality of the immune response evoked by the infection. The 'efficiency' of the anti-*T. cruzi* response is determined, among other factors, by the type 1 and type 2 cytokine bias of the response. In murine infections, a type 1 biased response results in significantly better control of the infection and a reduced level of disease (El Cheikh et al., 1992; Minoprio et al., 1993; Tarleton et al., 2000). In contrast an equally vigorous type 2-biased or a mixed type 1/type 2 response is less efficient at controlling *T. cruzi*, allowing for greater parasite persistence and an increased level of disease.

Inflammation is often equated with disease. However if the inflammatory response is productive and results in control or even elimination of parasites from a site, then inflammation is also a disease-preventing response. Alternatively, an ineffective immune response characterised by substantial but non-productive inflammation and resulting tissue destruction without efficient clearance of parasites, is clearly disease-promoting. The spectral nature of Chagas disease in humans is likely the result of variation in the efficiency of the immune response in different individuals: efficient immune responses control the level of parasites and thus limit tissue damage while inefficient responses fail to adequately control the parasite burden, thus promoting more persistent inflammatory reactions and more severe disease. One key to the treatment or prevention of Chagas disease may be to more fully understand the components of an efficient anti-*T. cruzi* immune response and to promote that subset of responses by immunotherapy. The observation in experimental models that making immunity to *T. cruzi* more efficient through vaccination (Garg and Tarleton, unpublished observation) or through genetic manipulation of the host (Tarleton et al., 2000) provide strong support for the therapeutic potential of such approaches.

Lastly, with respect to the association between parasite persistence and disease severity, the results of chemotherapeutic treatment of hosts in the chronic stage of *T. cruzi* infection deserve mention. Particularly impressive are the results of one long-term follow-up study of 131 patients examined nearly a decade after treatment with benznidazole (Viotti et al., 1994). This study found a significant decrease in clinical deterioration in treated Chagasic patients when compared with untreated patients. The decreased severity of disease was also associated with decreased titres of anti-*T. cruzi* antibodies, suggestive of parasitological cure. Drug therapy should have no effect on the presumptive anti-self response but could substantially alter the level of parasites in tissues. Thus, this study, as well as others in humans and in experimental animals (Andrade et al., 1996; Andrade et al., 1992; Andrade et al., 1991) provide additional support for parasite persistence and not autoimmunity as the cause of Chagas disease. More importantly, these studies suggest that consideration should be given to the use of chemotherapy delivered at any time during the infection as a means to modulate the severity of Chagas disease (Andrade et al., 1996).

5. But could autoimmunity still be responsible for Chaga's disease?

The vast majority of data in the literature firmly support parasite persistence over autoimmunity as the cause of Chagas disease. But none of the data disproves the autoimmunity hypothesis. And I suspect that it is in fact impossible to disprove the autoimmunity hypothesis. Immune responses, and the antigenic repertoire of host and parasite

are all sufficiently complex that it will likely be impossible to exclude the participation of cross-reactive or anti-self responses to disease in *T. cruzi* infection. However, the lack of disproof should not be taken as the presence of support for a hypothesis. Nevertheless, if one believes that the data are still equivocal on this issue, then how should one proceed?

First, one can ask what sorts of experiments are most likely to provide the definitive proof for one of these hypotheses or the other. Among these experiments, I would place at the top of the list those that would determine whether disease persists or decreases in severity in the absence of parasites in the host (i.e. cure). As reviewed previously and reiterated above, substantial evidence supports a link between the severity of disease and the relative level of parasites in tissues. However all of these experiments are correlative but none are definitive. The definitive experiment may await an experimental system in which one can completely rid a chronically infected host of *T. cruzi* and then observe the progression or the lack of progression of disease in the absence of parasites. The autoimmunity hypothesis would predict that in this circumstance, disease would continue to progress while the parasite persistence model would predict an arresting of the disease. Even more definitively with respect to human disease would be more and clearer documentation of the course of disease in chronically infected patients 'cured' of the infection. However the latter experiment can not yet be done as far as I know (that is, there is not a sufficiently effective treatment or way to monitor 'cure') and would take decades to perform. Evidence of the transfer of disease in the absence of parasites will never be definitive. Likewise, the identification of presumptive mimicked or cross-reactive antigens that are the target of immune responses in *T. cruzi*-infected hosts is not supportive of the autoimmune hypothesis; such antigens and responses are evident in most, if not all, infections.

Second if one considers that these two hypotheses are equally supported by the existing data, one can apply a more general scientific principle to assist in determining the relative validity of one over the other. Ockham's Razor, or the doctrine of simplicity is often useful in such cases. This principle states in effect 'Why assume that things are complex if a more simple theory can explain the observations?' Further distilled, Ockham's Razor says that if there are two or more competing theories with similar explanatory power, the simplest of the theories is more likely to be correct. Ockham's Razor does not guarantee that the simplest theory will be correct, rather, it prioritises the competing hypotheses.

Applying Ockham's Razor to the question of the basis of Chaga's disease, the simplest explanation for disease is that the persistence of parasites induces a chronic immune response. Invoking autoimmunity or other alternative hypotheses requires an additional level of complexity on the system. Thus, we should accept that Chagas disease is

a problem of parasite persistence until it is proven that this is not the case. There is no need to invoke, invent or conceive of alternative hypotheses to explain the basis of disease. We have a perfectly good, reasonable and simple hypothesis that is supported by the vast majority of experimental data. This is the most compelling reason to accept persistence over autoimmunity in the etiology of Chagas disease.

6. Practical issues: research, treatment and prevention

From a practical standpoint the autoimmunity and parasite persistence hypotheses suggest very different directions for research in *T. cruzi* infection and, more importantly, different avenues for the treatment of both acutely and chronically infected individuals. Advocates of the autoimmune hypothesis would likely support continued research to define putative 'autoantigens' with the hope that such knowledge would lead to the identification of ways to block these specific anti-self responses. In sharp contrast, the parasite persistence hypothesis would support research into immune enhancing therapies, including a focus on vaccine development that has been noticeable lacking for *T. cruzi*. The parasite persistence hypothesis would also support the use of chemotherapeutics to reduce parasite load at any point during the infection, including during advanced stages of the chronic disease. A final practical point is that even if Chagas disease does have an autoimmune component, treating it as an autoimmune disease is not an option. Generalised immunosuppression, the method of treatment of most autoimmune diseases, is clearly impractical as a treatment for Chagas disease. *Trypanosoma cruzi* infection and Chagas disease must be treated as a parasitic disease with the primary goal of enhancing effective immune responses and reducing the parasite load.

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References

- Almeida, D.R., Carvalho, A.C., Branco, J.N., Pereira, A.P., Correa, L., Vianna, P.V., Buffolo, E., Martinez, E.E., 1996. Chagas disease reactivation after heart transplantation: efficacy of allopurinol treatment. *J. Heart Lung Transplant.* 15, 988–92.
- Andrade, Z.A., Andrade, S.G., Sadigursky, M., 1987. Enhancement of chronic *Trypanosoma cruzi* myocarditis in dogs treated with low doses of cyclophosphamide. *Am. J. Path.* 127, 467–73.
- Andrade, S.G., Stocker, G.S., Pimentel, A.S., Grimaud, J.A., 1991. Reversibility of cardiac fibrosis in mice chronically infected with *Trypanosoma cruzi*, under specific chemotherapy. *Mem. Inst. Oswaldo Cruz* 86, 187–200.
- Andrade, S.G., Rassi, A., Magalhaes, J.B., Ferrioli, F.F., Luquetti, A.O., 1992. Specific chemotherapy of Chagas disease: a comparison between the response in patients and experimental animals inoculated with the same strains. *Trans. R. Soc. Trop. Med. Hyg.* 86, 624–6.
- Andrade, A.L., Zicker, F., de Oliveira, R.M., Almeida Silva, S., Luquetti, A., Travassos, L.R., Almeida, I.C., de Andrade, S.S., de Andrade, J.G., Martelli, C.M., 1996. Randomised trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection. *Lancet* 348, 1407–13.
- Andrade, S.G., Carneiro Filho, A., de Souza, A.J., de Lima, E.S., Andrade, Z.A., 1997. Influence of treatment with immunosuppressive drugs in mice chronically infected with *Trypanosoma Cruzi*. *Int. J. Exp. Pathol.* 78, 391–9.
- Anez, N., Carrasco, H., Parada, H., Crisante, G., Rojas, A., Fuenmayor, C., Gonzalez, N., Percoco, G., Borges, R., Guevara, P., Ramirez, J.L., 1999. Myocardial parasite persistence in chronic Chagasic patients. *Am. J. Trop. Med. Hyg.* 60, 726–32.
- Bestetti, R.B., 1997. Should benznidazole be used in chronic Chagas disease? [letter; comment]. *Lancet* 349, 653.
- Bouillon, F., Sinagra, A., Riarte, A., Lauricella, M., Barra, J., Besanson, M., Lejour, C., Lopez, B.O., Favalaro, R., Segura, E.L., 1988. Experimental cardiac transplantation and chronic Chaga's disease in dogs. *Transplant. Proc.* 20, 432–7.
- El Cheikh, M.C., Hontebeyrie, J.M., Coutinho, A., Minoprio, P., 1992. CD5 B cells. Potential role in the (auto)immune responses to *Trypanosoma cruzi* infection. *Ann. NY Acad. Sci.* 651, 557–63.
- Ferreira, M.S., Nishioka, S.A., Rocha, A., Silva, A.M., Ferreira, R.G., Olivier, W., Tostes Jr., S., 1991. Acute fatal *Trypanosoma cruzi* meningoencephalitis in a human immunodeficiency virus-positive hemophilic patient. *Am. J. Trop. Med. Hyg.* 45, 723–7.
- Jardim, E., Takayanagui, O.M., 1994. Chagasic meningoencephalitis with detection of *Trypanosoma cruzi* in the cerebrospinal fluid of an immunodepressed patient. *J. Trop. Med. Hyg.* 97, 367–70.
- Jones, E.M., Colley, D.G., Tostes, S., Lopes, E.R., Vnencak, J.C., McCurley, T.L., 1992. A *Trypanosoma Cruzi* DNA sequence amplified from inflammatory lesions in human Chagas-ic cardiomyopathy. *Trans. Assoc. Am. Physicians* 105, 182–9.
- Kierszenbaum, F., Hudson, L., 1985. Autoimmunity in Chagas Disease: Cause or Symptom? *Parasitol. Today* 1, 4–9.
- Kumar, S.A.R.L.T., 2000. Antigen-specific Th1 but not Th2 cells provide protection to lethal *Trypanosoma cruzi* infection in mice. *J. Immunol.*, in press.
- Melo, R.C., 1999. Depletion of immune effector cells induces myocardial damage in the acute experimental *Trypanosoma cruzi* infection: ultrastructural study in rats. *Tissue Cell* 31, 281–90.
- Melo, R.C., Machado, C.R., 1998. Depletion of radiosensitive leukocytes exacerbates the heart sympathetic denervation and parasitism in experimental Chagas disease in rats. *J. Neuroimmunol.* 84, 151–7.
- Minoprio, P., el Cheikh, M.C., Murphy, E., Hontebeyrie Joskowicz, M., Coffman, R., Coutinho, A., O'Garra, A., 1993. Xid-associated resistance to experimental Chagas disease is IFN-gamma dependent. *J. Immunol.* 151, 4200–8.
- Mosca, W., 1986. Autoimmunity should not limit the search for a Chagas disease vaccine. *Parasitol. Today* 2, 122.
- Okumura, M., Mester, M., Iriya, K., Amato, N.V., Gama, R.J., 1994. Effects of immunosuppression and benznidazole on *Trypanosoma cruzi* parasitism during experimental acute Chagas disease. *Transplant. Proc.* 26, 1587–9.
- Rocha, A., de Meneses, A.C., da Silva, A.M., Ferreira, M.S., Nishioka, S.A., Burgarelli, M.K., Almeida, E., Turcato, J.G., Metz, K., Lopes, E.R., 1994. Pathology of patients with Chagas disease and acquired immunodeficiency syndrome. *Am. J. Trop. Med. Hyg.* 50, 261–8.
- Sartori, A., Lopes, M.H., Caramelli, B., Duarte, M., Pinto, P., Neto, V.A., Shikanai, Y.M., 1995. Simultaneous occurrence of acute myocarditis and reactivated Chagas disease in a patient with AIDS. *Clin. Infect. Dis.* 21, 1297–9.
- Silva, J.S., Rossi, M.A., 1990. Intensification of acute *Trypanosoma cruzi* myocarditis in BALB/c mice pretreated with low doses of cyclophosphamide or gamma irradiation. *J. Exp. Pathol.* 71, 33–39.

- Tarleton, R.L., Zhang, L., 1999. Chagas disease etiology: Autoimmunity or parasite persistence? *Parasitol. Today* 15, 94–99.
- Tarleton, R.L., Sun, J., Zhang, L., Postan, M., 1994. Depletion of T-cell subpopulations results in exacerbation of myocarditis and parasitism in experimental Chagas disease. *Infect. Immun.* 62, 1820–9.
- Tarleton, R.L., Grusby, M.J., Postan, M., Glimcher, L.H., 1996. *Trypanosoma cruzi* infection in MHC-deficient mice: further evidence for the role of both class I- and class II- restricted T cells in immune resistance and disease. *Int. Immunol.* 8, 13–22.
- Tarleton, R.L., Grusby, M.J., Zhang, L., 2000. Increased susceptibility of Stat4-deficient and enhanced resistance in Stat6-deficient mice to infection with *Trypanosoma Cruzi*. *J. Immunol.* 165, 1520–5.
- Vago, A.R., Macedo, A.M., Adad, S.J., Reis, D.D., Correa-Oliveira, R., 1996. PCR detection of *Trypanosoma cruzi* DNA in oesophageal tissues of patients with chronic digestive Chagas disease. *Lancet* 348, 891–2.
- Viotti, R., Vigliano, C., Armenti, H., Segura, E., 1994. Treatment of chronic Chagas disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up. *Am. Heart J.* 127, 151–62.
- Zhang, L., Tarleton, R.L., 1999. Parasite persistence correlates with disease severity and localization in chronic Chagas disease. *J. Infect. Dis.* 180, 480–6.