

Invited Review

Autoimmunity in Chagas heart disease

J.S. Leon, D.M. Engman*

Northwestern University Medical School, Departments of Pathology and Microbiology-Immunology, Feinberg Cardiovascular Research Centre,
Chicago, IL 60611, USA

Received 9 October 2000; received in revised form 9 January 2001; accepted 9 January 2001

Abstract

The possibility that cardiac autoimmunity contributes to the pathogenesis of Chagas heart disease is controversial. In this paper, we address the following questions regarding the genesis of autoimmunity in Chagas heart disease: (i) What mechanism(s) are potentially responsible for the generation of self-directed antibodies and lymphocytes? (ii) What is the evidence that any of these mechanisms actually can occur? (iii) What are the implications of the presence of autoimmunity for other mechanisms of cardiac inflammation? © 2001 Published by Elsevier Science Ltd. on behalf of Australian Society for Parasitology Inc.

Keywords: Myocarditis; Chagas heart disease Myosin; Autoimmunity

1. Introduction

After several decades of productive investigation on the subject, the etiology of Chagas heart disease, both in humans and in experimental animal models, is not completely understood. Many pathogenic mechanisms have been described for what is in essence a heterogeneous set of infections with highly varied outcomes. These include lysis of parasitised cells, immunity to parasite antigens persisting in the tissue, autoimmunity and microvascular spasm, among others. It is presumed, by us as well as others, that parasite-specific immunity is operative in virtually all cases and may (logically) account for most tissue inflammation as well. However, the possibility that pathogenic autoimmunity, induced by any number of mechanisms (see below) contributes to tissue damage has been suggested by a number of investigators (reviewed most recently in Kierszenbaum 1999). This view is supported by a large amount of relatively weak circumstantial evidence, including some of our own (Tibbetts et al., 1994). Of particular relevance to the new evidence for pathogenic autoimmunity presented below, are previous reports of autoimmunity to cardiac myosin developing in *T. cruzi*-infected mice (Rizzo et al., 1988; Tibbetts et al., 1994) and humans (Cunha-Neto et al., 1996, 1995). It should be emphasised that not a single report published to date indicates that autoimmunity is pathogenic. However, this is formally true of parasite-speci-

fic immunity as well. The challenges inherent in dissecting the mechanisms of pathogenesis in infection-induced disease are outlined in Section 4.

We followed the autoimmunity debate played out in numerous reviews and back-and-forth letters to the editor in *Parasitology Today* and became aware of a central problem in the field. Each research group contributing to the parasite persistence/immunity versus autoimmunity struggle (i) employed a different combination of mouse and parasite strains and (ii) focused on a particular mechanism of pathogenesis without testing any others. We thought that we could contribute to the issue by developing a murine model of infection that exhibited (i) severe myocarditis, (ii) strong parasite-specific immunity and (iii) strong autoimmunity. If we were thoughtful, and addressed both mechanisms simultaneously, we might be able to determine the relative roles of both mechanisms in tissue inflammation. We initiated the search by choosing the A/J strain of mouse because it develops myosin autoimmunity in response to coxsackievirus infection and immunisation with cardiac antigens—that is, it is ‘predisposed’ to develop myosin autoimmunity. In the end, infection of A/J mice with the Brazil strain of *T. cruzi* provided the best model, developing all three essential disease properties within a mere 21-days after infection. Of particular importance is the fact that the quality, magnitude and kinetics of the autoimmune response are similar to those induced by immunisation with cardiac myosin in complete Freund’s adjuvant—a model in which autoimmunity is clearly pathogenic (Figs. 1 and 2: see legends for description). This strongly suggests, but by no

* Corresponding author. Tel.: +1-312-503-1268; fax: +1-312-503-1265.
E-mail address: d-engman@northwestern.edu (D.M. Engman).

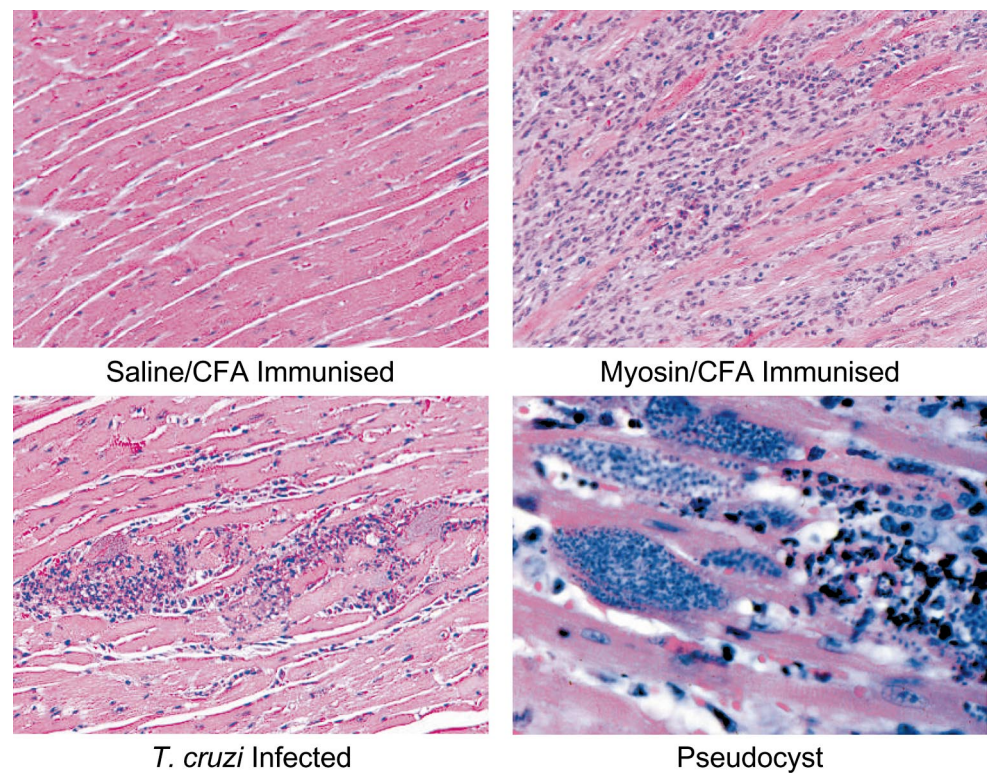
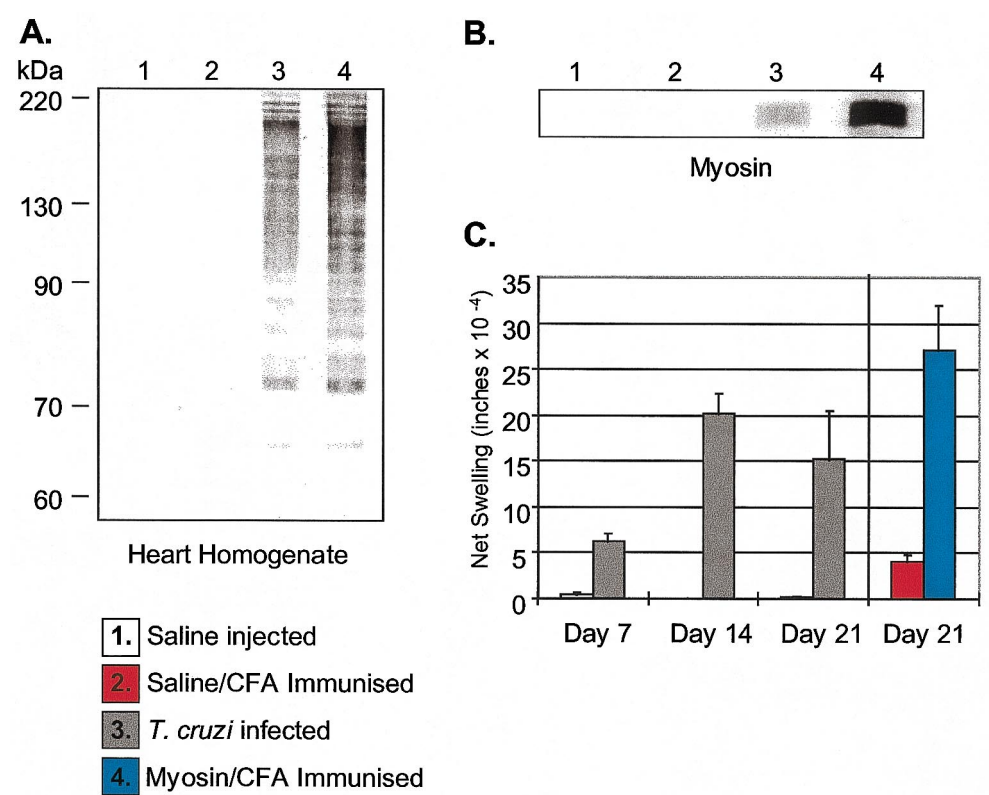


Fig. 1. Cardiac histopathology in A/J mice 21 days post immunisation/infection. Myocarditis was induced by immunisation with myosin/CFA or by intraperitoneal infection with the Brazil strain of *T. cruzi*. Hearts of myosin-immunised mice show diffuse, severe mononuclear cell infiltration, myocyte swelling and fibrosis. Hearts of *T. cruzi*-infected mice show massive mononuclear infiltration, parasite pseudocysts and myocyte swelling and necrosis.



means proves, that autoimmunity is pathogenic in this model of Chagas heart disease. How autoimmunity develops as a result of *T. cruzi* infection and how it contributes to tissue inflammation are questions we hope to answer, at least in part, in the coming months (Kierszenbaum, 1999; Tarleton and Zhang, 1999). Our goal for the remainder of this presentation is to share our own perspective on the pathogenesis question and to outline our view of what experimental findings may be necessary to conclude that any of the proposed mechanisms is inflammatory, i.e. contributes directly to tissue inflammation in this illness.

2. Characteristics of *T. cruzi*-induced autoimmunity

2.1. Onset of autoimmunity

When is autoimmunity first induced by *T. cruzi*? One possibility is that autoimmunity is induced immediately after the initial contact of the parasite with the host, during the acute phase of disease. In support of this hypothesis, autoantibodies against myosin, actin and laminin can be detected in acute disease (Grauert et al., 1993; Ternynck et al., 1990). In addition, cardiac autoantibodies and cardiac myosin-specific delayed type hypersensitivity (DTH) develop as early as 7 days p.i. (Fig. 2). These results suggest that tissue damage caused by the parasite and/or cross-reactive immunity with *T. cruzi* antigens (molecular mimicry) are the initial trigger for autoimmunity. The polyclonal, polyspecific nature of the autoantibody response supports the former hypothesis.

Autoimmunity may also develop later in the disease course. There are reports that serum and splenocytes from chronically infected mice promote in vitro cell lysis while serum and splenocytes from acutely infected animals do not (Acosta and Santos-Buch, 1985; Laguens et al., 1988). The caveat to these reports is that only the lytic responses were assessed -not antigen-specific humoral and cellular autoimmunity. It is possible that non-pathogenic autoimmune responses were present in both acute and chronic disease yet became pathogenic (directly responsible for inflammation) only in chronic disease. Moreover, several mechanisms can be envisioned for the elaboration of cytolytic responses that do not involve antigen-specific autoimmunity. Persistent, chronic inflammation may be necessary to overcome the threshold of cardiac damage or produce the correct inflammatory environment for the stimulation and expansion of autoreactive cells.

2.2. Autoimmunity depends on host immunogenetics

Is the autoimmunity induced by *T. cruzi* influenced by the genetics of the host? Several autoimmune models including multiple sclerosis, diabetes, and rheumatoid arthritis have implicated several genes in autoimmune diseases (Griffiths et al., 1999). In the myosin-induced myocarditis model shown above, only certain strains of mice develop myocarditis upon immunisation (Neu et al., 1987c). Interestingly, the same strains susceptible to myosin myocarditis are also susceptible to autoimmunity induced by coxsackievirus B3 infection (Neu et al., 1987a). We have shown that the A/J strain of mouse, which develops autoimmunity in response to myosin immunisation or coxsackievirus infection, also develops autoimmunity upon *T. cruzi* infection. The C57BL/6 strain which is resistant to myosin immunisation or coxsackievirus infection, is also resistant to autoimmunity induced by infection with the Brazil strain of *T. cruzi*, further supporting but not confirming a common mechanism of autoimmune myocarditis in the three models. In myosin myocarditis in the A/J mouse, no single gene appears to confer susceptibility to autoimmunity, but major histocompatibility complex associated genes, cytokine genes, and cardiac myosin genes among others have been implicated (Malkiel et al., 1996). Thus it is likely that several host genes may be involved in susceptibility and resistance to autoimmunity induced by *T. cruzi*.

2.3. Autoimmunity depends on parasite genetics

In addition to host genetics, the genetics of the parasite may influence the development of infection-associated autoimmunity. It is well documented that genetically distinct *T. cruzi* strains and clones cause highly varied disease in the presence or absence of autoimmunity. For example, two reports using the Guayas and Brazil strain of *T. cruzi* have demonstrated differences in the degree of myocarditis and autoantibodies induced (Rowland et al., 1995; Tibbetts et al., 1994). Specifically the Guayas strain did not induce myocarditis or autoantibodies to either cardiac myosin or a 43 kDa cardiac glycoprotein. These genetically distinct parasites may or may not induce autoimmunity because: (i) the threshold of damage or inflammation necessary to induce autoimmunity may or may not be overcome by different parasites, (ii) crossreactive proteins participating in molecular mimicry are absent or vary in different parasites. We consider the latter highly unlikely, because differ-

Fig. 2. Induction of strong myosin-specific humoral and cellular autoimmunity during acute infection of A/J mice with the Brazil strain of *T. cruzi*. Groups of mice were examined: (1) injected with saline, (2) immunised with saline in CFA, (3) infected with *T. cruzi*, (4) immunised with myosin in CFA. (A) Western blot of A/J heart homogenate with sera from groups 1–4 obtained 21 days p.i. Antibodies to a number of cardiac proteins are produced in both *T. cruzi*-infected and myosin-immunised mice. (B) Western blot of purified A/J cardiac myosin sera from groups 1–4 obtained 21 days p.i. Myosin autoantibodies are produced in both *T. cruzi*-infected and myosin-immunised mice. (C) Myosin-specific delayed-type hypersensitivity (DTH) in groups 1–4 measured 21 days p.i. DTH was elicited by injection of 10 µg myosin or PBS into ear and net swelling (myosin - PBS) measured after 24 h. Myosin DTH is detectable as early as 7 days p.i. and is of magnitude similar to that in myosin-immunised mice by 14–21 days p.i. *T. cruzi*-specific antibodies and DTH responses also develop during this time period (not shown).

ent clones are very similar antigenically despite having different genetic and biological properties.

3. Mechanisms of initiation of autoimmunity induced by *T. cruzi*

What are the mechanisms that may initiate autoimmunity in a host infected with *T. cruzi*? There are several mechanisms that might explain autoimmunity induced by *T. cruzi* (Malkiel et al., 1996) (Fig. 3). All are based on the observation that an immunocompetent host possesses circulating, autoreactive T cells and B cells that are normally tolerant to self antigens (Dighiero and Rose, 1999).

3.1. Bystander activation

There are two components to the mechanism of bystander

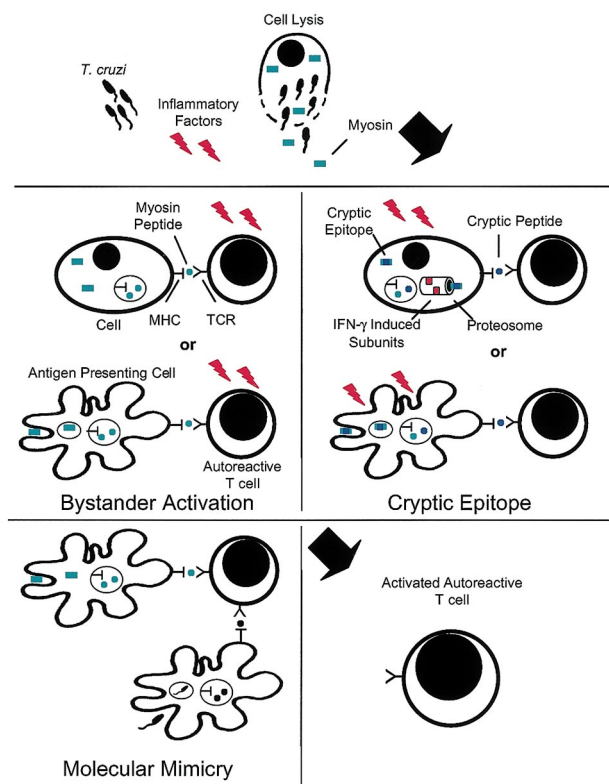


Fig. 3. Possible mechanisms of *T. cruzi* induced autoimmunity. Initially, *T. cruzi* infection causes lysis of cardiac myocytes, releasing cardiac antigens, such as myosin. Infection also induces the elaboration of inflammatory factors such as chemokines and cytokines, which contribute to the creation of a proinflammatory environment. Bystander activation: inflammatory factors can promote the activation of autoreactive T cells encountering cognate myosin peptide in the context of MHC, which may be enhanced by increased availability of free myosin released during myocytolysis. Cryptic epitope: inflammatory factors cause altered processing and presentation of self antigen. For example, IFN- γ induced replacement of proteosome subunits may result in the presentation of novel peptides, cryptic epitopes/peptides, and stimulation of autoreactive T cells. Molecular mimicry: *T. cruzi* derived peptides resemble host peptides and initiate a cross-reactive T cell response leading to the activation of autoreactive T cells.

activation. Both depend on the inflammatory environment induced by *T. cruzi* infection of host tissue (Talvani et al., 2000). One idea is that the proinflammatory environment in the host tissue during *T. cruzi* infection, rich in cytokines, nitric oxide and chemokines, is sufficient to activate autoreactive T cells by lowering the threshold of activation (Fedoseyeva et al., 1999). These cells then proliferate in response to self antigen presented on host antigen presenting cells (APC). The second component is that myocardial cytolysis resulting from *T. cruzi* infection leads to the release of self proteins. The elevated levels of myocardial antigens, in a proinflammatory environment, lead to increased presentation of self peptide and stimulate the expansion of autoreactive cells. In support of this mechanism, there are several examples of autoimmunity occurring after cardiac damage including cardiac surgery (de Scheerder et al., 1989), cardiac transplant rejection (Fedoseyeva et al., 1999), and infection with viruses (Neu et al., 1987b) and protozoa (given by and referenced in this presentation).

3.2. Cryptic epitope

The hypothesis of cryptic epitope assumes that host T cells are centrally or peripherally tolerised to peptide epitopes normally processed and presented on host APC. However, autoreactive T cells specific for peptides not normally presented, cryptic epitopes, escape tolerisation. In *T. cruzi* infection, the tissue inflammation may cause new cryptic epitopes to be presented by APC. Because the circulating T cells are not tolerant to these 'novel' epitopes, they become activated and initiate autoimmunity. Cryptic epitopes may be produced when processing and presentation of peptides is altered. A well studied example is the effect of IFN- γ on proteases involved in antigen processing and the proteasome. After in vitro IFN- γ treatment, novel subunits are added to the proteasome leading to modification of the protease composition involved in the production of peptides for presentation. Both effects result in the altered presentation of peptide sequences on APC (York et al., 1999).

3.3. Molecular mimicry

The hypothesis of molecular mimicry is popular in the *T. cruzi* field. This hypothesis suggests that a 'crossreactive' *T. cruzi* and self protein have similar amino acid sequences or three-dimensional epitopes. T cells specific for the cross-reactive *T. cruzi* proteins also react to self proteins with the same epitope and initiate autoimmunity. Many papers have attempted to identify putative crossreactive *T. cruzi* proteins by identifying crossreactive antibodies or similarities in amino acid sequences between *T. cruzi* and self proteins (Kierszenbaum, 1999). One popular candidate is the B13 epitope of *T. cruzi* that shares peptide sequence similarity with cardiac myosin (Cunha-Neto et al., 1995). One criticism of these 'crossreactive' proteins is that while indirect evidence suggest the presence of host responses against both the parasite protein and the putative self

protein, there is no direct evidence demonstrating that the crossreactive *T. cruzi* protein can induce autoimmunity. Two controversial reports have addressed this criticism by demonstrating that immunisation with *T. cruzi* lysate (Laguens et al., 1989) or the ribosomal P protein induces functional changes in the heart (Motran et al., 1999). If such a 'crossreactive' protein is shown to participate in a molecular mimicry mechanism to induce autoimmunity, then this protein may be used in specific interventions to prevent autoimmunity.

In conclusion to this section, these mechanisms are not mutually exclusive and may also operate in series. For example, autoimmunity generated via molecular mimicry may lead to the release of self proteins, thereby promoting bystander activation and elaboration of autoimmunity against additional targets. The temporal 'redirection' of autoimmunity to different epitopes is called epitope spreading and is observed in several other models of organ-specific inflammation (Vanderlugt et al., 1998).

4. Discussion

4.1. Pathogenesis of Chagas disease

Infection of humans with *T. cruzi* can lead to a large number of clinical outcomes, ranging from lifelong, asymptomatic infection in the majority of cases to acute myocarditis and sudden death in the minority. Development of chronic disease-cardiomyopathy and/or megadisease occurs in approximately one-third of infected individuals and develops at a wide range of times after infection. This huge variation in the potential consequence of human *T. cruzi* infection indicates that Chagas disease is, in fact, a heterogeneous assortment of diseases. There are likely many factors that contribute to this heterogeneity which, although grounded in the genetic variation of both parasite and host, are poorly understood even today. Moreover, since individuals may be infected with a mixture of parasite clones of differing pathogenic potential, it is possible that the disease course in these cases is actually a composite of pathogenically distinct infections. These mixed infections may result either from primary infection with a mixed population or from reinfection.

The study of animal models of Chagas disease has permitted the definition of a number of distinct mechanisms of pathogenesis, including parasite antigen-specific inflammation, parasite-induced myocardial cell necrosis and repair, microvascular spasm and autoimmunity. As is observed in human infections, there is a wide variety of outcomes of the animal infections; a single strain of mouse may develop many different types of disease depending on the parasite isolate used and a single parasite clone can cause very different disease in different strains of mice. Therefore, no single strain-strain combination or mechanism defined within is reflective of all human Chagas disease.

While some may view this as a shortcoming of the murine models, we believe that it is a strength, since the variation in outcome is precisely what is observed in humans. However, it is equally important that conclusions drawn from the study of one strain-strain combination not be interpreted as representing all of Chagas disease. Furthermore, the different pathogenetic mechanisms are not mutually exclusive, a point overlooked by some. Finally, and perhaps most important, when both parasite and host antigens are present in the inflamed myocardium, it is difficult to determine whether anti-parasite immunity, autoimmunity, both or neither are responsible for the tissue damage. It may be logical to presume that immunity to foreign antigen in the tissue is inflammatory and also that one need not invoke an autoimmune hypothesis to explain the damage. However, reasonableness and conclusive determination are not the same, even without considering the presence of additional, coexistent inflammatory mechanisms.

4.2. The *T. cruzi* Brazil - A/J mouse model of Chagas disease

This strain-strain combination possesses several key features: (i) severe myocarditis (in the presence of parasite pseudocysts), (ii) strong parasite-specific immunity and (iii) strong autoimmunity. Several findings suggest that myocardial inflammation in this model has a pathogenic autoimmune component: the quality, kinetics and magnitude of the antimyosin autoimmunity are similar to that observed in a purely autoimmune model induced by myosin immunisation. Also, preliminary survey of additional mouse strains revealed the same pattern of susceptibility/resistance in *T. cruzi* Brazil strain infection as that observed in coxsackievirus infection and myosin immunisation, both of which involve myosin autoimmunity. The challenge now is to use this model to address the numerous questions that remain about cardiac autoimmunity, including those of the mechanism of its genesis and pathogenic potential. Assuming we can eventually prove that autoimmunity is pathogenic in this model (see below), what is the relevance of the model to human Chagas disease? At present, no one can say whether or not a certain fraction of human Chagas heart disease results from autoimmunity. We presume, but also do not know for certain, that some inflammation results from parasite-directed mechanisms.

4.3. Defining the mechanism(s) of pathogenesis: special challenges in infection-induced inflammatory disease

The major challenge with infection-induced models of tissue inflammation is that it is impossible to attribute any aspect of the inflammatory process to a single mechanism of pathogenesis. In the case of *T. cruzi* infection, as long as living organisms are present it is impossible to rule out parasite-induced microvascular spasm or parasite-specific inflammation when concluding that antigen-specific autoimmunity may be present.

We feel it most likely that myosin autoimmunity in the A/

J mouse develops via a bystander mechanism initiated by parasite-induced myocardial cytolysis. It is not known whether molecular mimicry involving the B13 antigen occurs in these mice and, if so, what its genesis and pathogenic potential would be. For example, in molecular mimicry, it is not necessary that immunity to the 'foreign' peptide develop first. Bystander activation-mediated autoimmunity to self peptide may simply happen to cross-react with the parasite-derived peptide. It should be relatively simple to test whether cross-reactive immunity to, say, the B13 peptide participates in tissue inflammation. Provided the B13 protein is not essential for the organism's replication and invasive capacity, a B13 null mutant can be generated to determine the role of the protein in pathogenesis.

Another set of challenges involves the experiments required to affirm the various hypothetical mechanisms of pathogenesis. Because the antigen specificities of the infiltrating lymphocytes in the inflamed heart are difficult to determine (and likely a mixture of parasite, self and non-specific targets), how does one prove that one mechanism is operative. In vitro measures of immunity do not necessarily prove in vivo functionality. Our argument that the myosin autoantibodies and DTH are significant is based exclusively on the similarity to myosin-myocarditis and not because we have shown that autoimmunity is pathogenic in the infected mice. Likewise, how do we actually prove that parasite immunity causes tissue inflammation? How do the parasite-directed antibody, DTH and T cell proliferative responses, which also are present in the Brazil-infected A/J mice, reflect processes in the inflamed heart? Adoptive transfer of sensitised lymphocytes is one approach, but there are caveats for both the design and the interpretation of these experiments. Since adoptive transfer is typically successful only if an in vitro activation step is included, successful induction of myocarditis via transfer of myosin-activated splenocytes from infected A/J donors into naïve recipients might be interpreted as confirming the autoimmunity hypothesis. However, the A/J strain is so predisposed to develop myosin myocarditis, that result may, in fact, be misleading (even if we show that no parasites are present, which we believe possible). Can adoptive transfer be used to confirm the parasite persistence hypothesis? Myocarditis resulting from adoptive transfer of lymphocytes from infected donors into transgenic mice expressing parasite antigens in the heart, but not from transfer into non-transgenic littermates, might confirm this hypothesis provided the right antigens are selected.

4.4. Final remarks

Other groups have contributed substantially to our understanding of Chagas disease pathogenesis and it is logical that parasite persistence is a major factor in tissue inflammation. We would like to contribute to the issue by rigorously testing the autoimmunity hypothesis suggested by vast amounts of weak circumstantial evidence published by

others and us in the past. The *T. cruzi* Brazil - A/J mouse combination offers an attractive model for study in this regard, since it possesses very strong autoimmune features. New approaches for the selective inhibition of antigen-specific cellular immunity may also help to determine the relative contributions of different types of immunity to Chagas disease pathogenesis.

Acknowledgements

This work was done during the tenure of a fellowship from the American Heart Association, to JSL. DME is an Established Investigator of the American Heart Association.

References

- Acosta, A.M., Santos-Buch, C.A., 1985. Autoimmune myocarditis induced by *Trypanosoma cruzi*. *Circulation* 71, 1255–61.
- Cunha-Neto, E., Duranti, M., Gruber, A., Zingales, B., de Messias, I., Stolf, N., Bellotti, G., Patarroyo, M.E., Pilleggi, F., Kalil, J., 1995. Autoimmunity in Chagas disease cardiomyopathy: biological relevance of a cardiac myosin-specific epitope crossreactive to an immunodominant *Trypanosoma cruzi* antigen. *Proc. Natl. Acad. Sci. USA* 92, 3541–5.
- Cunha-Neto, E., Coelho, V., Guilherme, L., Fiorelli, A., Stolf, N., Kalil, J., 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, 1709–12.
- de Scheerder, I.K., de Buyzere, M.L., Delanghe, J.R., Clement, D.L., Wieme, R.J., 1989. Anti-myosin humoral immune response following cardiac injury. *Autoimmunity* 4, 51–58.
- Dighiero, G., Rose, N.R., 1999. Critical self-epitopes are key to the understanding of self-tolerance and autoimmunity. *Immunol. Today* 20, 423–8.
- Fedoseyeva, E.V., Zhang, F., Orr, P.L., Levin, D., Buncke, H.J., Benichou, G., 1999. De novo autoimmunity to cardiac myosin after heart transplantation and its contribution to the rejection process. *J. Immunol.* 162, 6836–42.
- Grauert, M.R., Houdayer, M., Hontebeyrie-Joskowicz, M., 1993. *Trypanosoma cruzi* infection enhances polyreactive antibody response in an acute case of human Chagas disease. *Clin. Exp. Immunol.* 93, 85–92.
- Griffiths, M.M., Encinas, J.A., Remmers, E.F., Kuchroo, V.K., Wilder, R.L., 1999. Mapping autoimmunity genes. *Curr. Opin. Immunol.* 11, 689–700.
- Kierszenbaum, F., 1999. Chagas disease and the autoimmunity hypothesis. *Clin. Microbiol. Rev.* 12, 210–23.
- Laguens, R.P., Meckert, P.C., Chambo, J.G., 1988. Antiheart antibody-dependent cytotoxicity in the sera from mice chronically infected with *Trypanosoma cruzi*. *Infect. Immun.* 56, 993–7.
- Laguens, R.P., Cabeza Meckert, P.M., Chambo, J.G., 1989. Immunologic studies on a murine model of Chagas disease. [Spanish]. *Medicina (Buenos Aires)* 49, 197–202.
- Malkiel, S., Kuan, A.P., Diamond, B., 1996. Autoimmunity in heart disease: mechanisms and genetic susceptibility. *Mol. Med. Today* 2, 336–42.
- Motran, C.C., Cerban, F.M., Rivarola, H.W., Vottero de Cima, E., 1999. Characterisation of autoantibodies generated in mice by immunisation with the C-terminal region of *Trypanosoma cruzi* ribosomal P1 and P2 proteins. *Clin. Immunol.* 91, 17–24.
- Neu, N., Beisel, K.W., Traystman, M.D., Rose, N.R., Craig, S.W., 1987a. Autoantibodies specific for the cardiac myosin isoform are found in

- mice susceptible to coxsackievirus B3-induced myocarditis. *J. Immunol.* 138, 2488–92.
- Neu, N., Craig, S.W., Rose, N.R., Alvarez, F., Beisel, K.W., 1987b. Coxsackievirus induced myocarditis in mice: cardiac myosin autoantibodies do not cross-react with the virus. *Clin. Exp. Immunol.* 69, 566–74.
- Neu, N., Rose, N.R., Beisel, K.W., Herskowitz, A., Gurri-Glass, G., Craig, S.W., 1987c. Cardiac myosin induces myocarditis in genetically predisposed mice. *J. Immunol.* 139, 3630–6.
- Rizzo, L.V., Cunha-Neto, E., Teixeira, A.R., 1988. Autoimmunity in Chagas disease: immunomodulation of autoimmune and T. cruzi-specific immune responses. *Mem. Inst. Oswaldo Cruz* 83, 360–2.
- Rowland, E., Luo, H., McCormick, T., 1995. Infection characteristics of an Ecuadorian *Trypanosoma cruzi* strain with reduced virulence. *J. Parasitol.* 81, 123–6.
- Talvani, A., Ribeiro, C.S., Aliberti, J.C., Michailowsky, V., Santos, P.V., Murta, S.M., Romanha, A.J., Almeida, I.C., Farber, J., Lannes-Vieira, J., Silva, J.S., Gazzinelli, R.T., 2000. Kinetics of cytokine gene expression in experimental chagasic cardiomyopathy: tissue parasitism and endogenous IFN-gamma as important determinants of chemokine mRNA expression during infection with *Trypanosoma cruzi*. *Microbes Infect.* 2, 851–66.
- Tarleton, R.L., Zhang, L., 1999. Chagas disease etiology: autoimmunity or parasite persistence? *Parasitol. Today* 15, 94–99.
- Ternynck, T., Bleux, C., Gregoire, J., Avrameas, S., Kanellopoulos-Langevin, C., 1990. Comparison between autoantibodies arising during *Trypanosoma cruzi* infection in mice and natural autoantibodies. *J. Immunol.* 144, 1504–11.
- Tibbetts, R.S., McCormick, T.S., Rowland, E.C., Miller, S.D., Engman, D.M., 1994. Cardiac antigen-specific autoantibody production is associated with cardiomyopathy in *Trypanosoma cruzi*-infected mice. *J. Immunol.* 152, 1493–9.
- Vanderlugt, C.L., Begolka, W.S., Neville, K.L., Katz-Levy, Y., Howard, L.M., Eagar, T.N., Bluestone, J.A., Miller, S.D., 1998. The functional significance of epitope spreading and its regulation by co-stimulatory molecules. *Immunol. Rev.* 164, 63–72.
- York, I.A., Goldberg, A.L., Mo, X.Y., Rock, K.L., 1999. Proteolysis and class I major histocompatibility complex antigen presentation. *Immunol. Rev.* 172, 49–66.