

AUTOIMMUNITY IN CHAGAS' DISEASE

Gabriel A. Schmuflis

Communicable Disease Program  
Pan American Sanitary Bureau/  
World Health Organization  
525 Twenty-third Street, N.W.  
Washington, D.C. 20037, U.S.A.

Recent human infection by Trypanosoma cruzi is mostly asymptomatic or symptoms are so mild or uncharacteristic that, even in the highly endemic areas, they are not recognized as such (1-3). If infected individuals are not treated, it is accepted that a cure will not occur. They may or may not develop clinical symptoms, but will remain nonetheless infected throughout their lives. In fact, parasitemia has been detected by xenodiagnosis years after the infection in up to 50% of untreated individuals (4). Some of those infected, having or not having symptoms during the acute infection, will remain asymptomatic. Others, up to 50% depending on the geographical area, may develop the clinical symptoms that characterize Chagas' disease (5-7).

In the few cases of humans with recent infections who died, it is possible to find nests of amastigotes in the heart. Therefore, it is easy to establish a relationship between parasites and the local inflammatory lesions (5,8-10). On the other hand, parasites are not frequently demonstrated in the hearts of patients who died with chronic chagasic myocarditis (5,8,9). Similarly, in chronic cases with the digestive form of the disease, anatomical lesions are characterized by the neurolysis of the parasymphathetic nerve plexus without parasites (11-13). Therefore, several attempts have been made to explain the fact that cardiac and hollow viscera pathology characterizing chronic Chagas' disease occur in the absence or scarcity of parasites.

The lack of a relationship between tissue lesions and parasite foci, as well as the mononuclear inflammatory foci in the myocardium which characterize the chronic chagasic myocarditis, have been considered as an indirect indication that an "allergic reaction" might be involved in the pathogenesis of tissue injuries

(14-16). The possibility that an autoimmune reaction may be responsible for the chronic myocarditis that appears in Chagas' disease was also expressed by Mazza and Jorg and by Jafee (17,18). Some early experimental data also led to the same conclusion. Monkeys sensitized with several doses of dead epimastigotes, or lysates from epimastigotes, later on developed focal inflammatory reactions resembling those found in human Chagas' disease (19). Additional studies in mice have shown not only a lack of correlation between parasites and the inflammatory response, but also indicated that inflammation in immune animals is an allergic reaction associated with the production of antibodies against T. cruzi or a tuberculin-type of cellular hypersensitivity (20,21).

Another hypothesis to explain the pathogenesis of tissue lesions in chronic patients, asserts that T. cruzi may produce toxins capable of destroying certain target cells (22), or that the destruction of the parasympathetic nervous system is responsible for the pathological alterations in the myocardium or the hollow viscera (13,23-25).

In recent years it has been reported that T. cruzi may share antigens with host tissues, thus inducing a cross-reacting immune response similar to an autoimmune reaction (reviewed in 2,26-29). Some of these findings have been controversial (30-35), and there always exists the possibility that the immune response to self-antigens is secondary to the destruction of tissues by the intracellular parasites. Another possibility, is that parasite antigens bind to host cells and that these cells become targets of the host immune response (36,37).

Although present knowledge is based on human and experimental studies on the cell mediated and on humoral response to parasite and host tissues, it is difficult to correlate experimental data, with what happens in the human infection. In fact,

a completely satisfactory animal model that could develop all features of chronic Chagas' disease still needs to be evaluated (38).

#### Cell Mediated Immunity To Host Tissues

Experimental work indicates that lymphocytes from T. cruzi chronically infected rabbits or rabbits immunized with a small particle or membrane fraction obtained from T. cruzi, were able to destroy allogeneic parasitized or non-parasitized cells in vitro. On the other hand, lymphocytes from infected rabbits did not react with allogeneic kidney cells, thus suggesting that the target cells were specific. Using the macrophage migration inhibition test, it became apparent that a cross-reacting antigen was present in both the T. cruzi particulate material and in the allogeneic heart cells (26,39,40). However, since lymphocytes were obtained from only a small number of animals (39), doubts have been raised about whether these experiments should be considered as a valid indication that sensitized lymphocytes in the absence of parasites may be responsible for the persistence of mononuclear infiltrates in the heart. It has also been reported that T. cruzi trypomastigotes from tissue culture, as well as subcellular fractions from the parasite, induce in rabbits chronic myocarditis similar to that occurring in chronically infected humans (41). Although in further experiments it was shown again that T. cruzi inoculated rabbits did develop chronic myocarditis (42), other authors have not confirmed the suitability of rabbits as models for chronic T. cruzi infection (19,43).

An indication of the possible deleterious role of lymphocytes on heart cells is that spleen lymphocytes from chronically infected mice, but not from normal ones, adhere to primary embryo heart muscle cells in vitro (44). Not only heart cells could be affected, but also skeletal muscle and the peripheral nervous system. A significant occurrence of acquired T. cruzi antigen dependent spleen

T-cell cytotoxicity to syngeneic skeletal muscle myoblasts, was established in T. cruzi-infected mice. These animals developed an apparently sterile lymphoid polymyositis (45). Evidence has been also presented indicating that the neuropathy that appears in chronically infected mice, which is characterized by granulomatous cellular infiltrates and demyelination, could be transferred to naive recipients by inoculation of T-helper cells from chronically infected animals. On the other hand, antibodies cross-reacting with peripheral myelin did not seem to play any role (46). The opinion that an autoimmune phenomenon is involved in the pathogenesis of tissue damage is also supported by the finding that myocarditis, which resembles chronic experimental Chagas' heart disease, could be induced in mice by the inoculation of homologous heart in complete Freund adjuvant (47), and that normal mice receiving mononuclear spleen cells from infected mice developed myocardial lesions (48). Furthermore, it was shown that adoptive transfer of lymphocytes from mice with long-term T. cruzi infections produced autoimmune anti-heart lymphocyte cytotoxicity in syngeneic recipients, which is abolished by glucocorticoid therapy (49). These findings suggest that at least in mice, sensitized lymphocytes could be cytotoxic to muscle cells in the absence of parasites (45,46,48).

Common antigenic determinants present in several T. cruzi strains and in purified preparations of mammalian striated muscle sarcoplasmic reticulum (SR) of several animal species have been described (50,51). The shared parasite-muscle antigen (SRA) is present in purified preparations of SR rich in the magnesium-activated adenosine triphosphate. Taking advantage of these findings, mice were used to test whether T. cruzi gives rise to anti-heart autoimmune reactions; whether this autoimmunity is elicited by SRA; and whether SRA induces an autoimmune reaction that may reproduce cardiac lesions found in Chagas' disease (49). Data provided by this study indicate that hearts of mice infected for 150 days showed a

mononuclear cell myocarditis with myocytolysis in the absence of T. cruzi, and that inoculation of mice with SRA induced similar types of lesions. In addition, mice infected for 150 days exhibit splenic lymphocyte cytotoxicity to normal syngeneic cardiac myofibers. Similarly, lymphocyte cytotoxicity to normal syngeneic heart cells was induced in mice given SRA (49).

In vitro studies using leukocytes from T. cruzi infected or uninfected humans show that only the former are inhibited to migrate when incubated with allogeneic heart antigen (52). In addition, only mononuclear blood cells from individuals with a positive leukocyte migration inhibition test are capable of attaching to and destroying non-parasitized murine heart cells (53). Along the same line was the work demonstrating the comparatively higher lytic effect of lymphocytes from T. cruzi infected donors, as shown by the chromium release of non-infected allogeneic fetal heart cells, when compared with cells from normal individuals (54).

Obviously, it is difficult to extrapolate on these findings when comparing an allogeneic system with what may happen in vivo. However, the target cells seem to be quite specific. Mononuclear cells from infected individuals did not attach to mice liver cells (53), and lymphocytes that lysed heart cells did not have any effect on Vero or kidney cells (54). In addition, if histocompatibility antigens were involved in the lysis of the allogeneic fetal heart cells, kidney cells may have been also destroyed (54).

The leukocyte migration inhibition response to mouse and guinea pig heart, skeletal muscle, kidney, liver and brain was tested with mononuclear cells from asymptomatic individuals infected with T. cruzi (55). Unexpectedly, the inhibition of cell migration was detected only when kidney, liver, and brain antigens were included in the system. This lack of migration inhibition induced by heart antigens may be explained by the asymptomatic condition of all of the patients

(55). On the other hand, heart antigens inhibited the migration of leukocytes from patients with chagasic cardiopathy (56).

Delayed hypersensitivity to rat heart and T. cruzi epimastigote antigens was tested comparatively by blast transformation of lymphocytes from infected individuals with or without chagasic cardiomyopathy. In this case, no relationship was established between chagasic heart lesions and delayed hypersensitivity to heart tissues (57,58). In addition, no differences have been found in the capabilities of lymphocytes from infected or uninfected individuals to mount a blastogenic response against human heart antigen (59).

In a different approach, the lymphoproliferative response of non-infected and infected individuals was investigated using homologous tissues and subcellular fractions of T. cruzi (60). While non-infected individuals did not show a lymphoproliferative reaction against heart tissue extracts, some of the infected individuals did. However, no differences in blast transformation between the infected individuals with or without symptoms were seen when heart, liver, and kidney were used as antigens. This suggests that the lack of organ specificity of the reaction is similar to that reported using the leukocyte migration inhibition test (55). On the other hand, the lack of correlation between a positive blast transformation and clinical status of the patient contrasted with the differences detected when several T. cruzi subcellular fractions were used as antigen in the in vitro system. A positive reaction against a flagellar and cell sap fraction was present in those individuals with overt cardiopathy. The cell sap fraction induced a higher proportion of positive reactions in those individuals with minimal heart damage, while the percentage of a positive reaction against a microsomal fraction was similar in all groups (60).

The data reported from humans (57,59,60), including a follow-up study (58), have opened doubts on the assertion that a relationship exists between delayed hypersensitivity against heart antigens and chagasic cardiopathy. At the same time, a positive relationship between disease and the presence of CMI in vitro against constituents of the parasite has been suggested (60).

#### Tissue-reacting Immunoglobulins

In the late fifties it was shown that sera from two individuals infected with T. cruzi formed precipitin lines when incubated with human heart extracts (61). This finding has been further confirmed with a higher number of serum samples from individuals with chronic chagasic heart lesions (62,63). Heterophil antibodies and anti- $\gamma$ -globulin antibodies also appear as a consequence of the infection. However, their presence has been mostly related with recent infections, and even in this case their significance is not clear (64-67). Although the passive transfer of sera from infected mice to normal recipients induced myocardial lesions, it was difficult to rule out the transfer of the infection to the recipient mice by the inoculated sera (67).

Later studies in humans indicate that patients with Chagas' heart disease have circulating immunoglobulins which react by indirect immunofluorescence with endocardium, interstitium, and vascular structures. Evidence indicates that this endocardial-vascular-interstitial factor (EVI) fixed complement, and that the reaction could be obtained by using the serum and tissues from the same patient. Specificity was not related to AB blood group systems, or to Forssman or Wassermann antigens. The reacting factor was effectively absorbed from sera with organ homogenates and with guinea pig red blood cells, although EVI was considered independent of heterophil antibodies (68). By ultrastructural immunochemical methods it was observed that the EVI antibody reacted with the plasma membrane of



the heart and skeletal muscle fiber as well as with endothelial cells of blood vessels. (65). Absorption of the serum with T. cruzi epimastigotes resulted in the negativization of the EVI reaction, suggesting that the genesis of the reacting gamma globulin is related to T. cruzi antigens (68,69). Morphologic alterations, which coincided with the presence of the EVI antibodies, were found also in skeletal muscle biopsies from infected individuals. The lesions also presented autologous immunoglobulins bound to the plasma membrane of muscle fibers and endothelial cells (70).

When similar studies were carried out in myocardial biopsies of four chagasic individuals with EVI antibodies, in vivo deposits of immunoglobulins were found in the plasma membrane of working myocardial cells and endothelial cells. The location of the in vivo bound  $\alpha$  globulin was coincident with the specificity of the EVI antibody, while intracellular alterations were compatible with hypoxia of the fibers (71).

EVI antibodies were more prevalent in individuals with chagasic cardiomyopathy, but were also detected in some asymptomatic individuals with positive serology for T. cruzi and in a few individuals with negative serology from the endemic area. On the other hand, EVI was not detected in those with negative serology, with or without other cardiovascular diseases from the non-endemic area (68). Further studies on congenitally T. cruzi infected cases, or on children with acute infection, indicate that EVI antibodies first appear in the IgM fraction of the Igs and later in the IgG fraction (72-74).

Evaluation of the specificity of the EVI antibody was made with sera from patients with other parasitic diseases collected in areas non-endemic for T. cruzi. Only two out of 60 sera were EVI positive (75). Another study confirmed

the presence of EVI antibody in sera of individuals from Chile and Brazil. It also indicated that 23% of 63 cutaneous leishmaniasis had EVI titers of  $\geq$  16 (76).

The above studies (68-76) plus the fact that the detection of EVI antibodies during routine screening for antinuclear antibodies allowed for the detection of unsuspected T. cruzi infection (77), suggested that the presence of the EVI factor may be of help for a more accurate diagnosis of T. cruzi infection and of chagasic myocardopathy (68). The following, also indicated that EVI antibodies may have a pathological role: (1) Their higher prevalence in adults with chagasic cardiopathy than in those with asymptomatic T. cruzi infections (68,78); (2) The presence of immunoglobulins bound to the plasma membrane of muscle cells in EVI positive patients (68-71). Such binding may be responsible for functional alterations of the cells (71). For example, EVI antibodies induced morphologic and functional alterations in rat myocardial cells in vitro, apparently through modification of the  $\beta$ -adrenergic receptors at the cell plasma membrane level (79-81); and (3) The presence of EVI antibodies in some cases coincided with the existence of significant skeletal muscle lesions (70) or intracellular alterations of the myocardial cells (71).

It has been thought that EVI may develop soon after infection as a response to parasite antigens (73,74,82), and it could later develop from the release of antigens due to tissue damage (71-74). Support for this possibility comes from findings on recently infected humans who became negative for T. cruzi antibodies after specific treatment (83,84). Although negativization of serology in these patients had indicated a parasitological cure, some of them had remained positive for EVI, thus indicating that EVI antibodies may become self-perpetuated in the absence of parasites (83,84).

The majority of acute or chronic chagasic individuals with EVI also had an antibody reacting with Schwann sheaths of myelinated somatic and unmyelinated autonomic peripheral nerve from human and murine origin (69,74). However, no reaction was seen against central nervous tissue such as neurons, glial cells or periaxonal sheaths. The peripheral nerve (PN) antibody(ies) fixed complement and were absorbed out by lyophilized epimastigotes of T. cruzi (85). On the other hand, antibodies against neurons have been found in chronic Chagas' disease by other authors (86). In this case, however, cross-absorption experiments indicate that there were no common antigens between T. cruzi and the reacting cells (79).

The hypothesis that EVI and PN antibodies were raised as a response to T. cruzi antigens that cross-react with host tissues has been supported by various absorption experiments (68,69,78,87). In addition, the results obtained using monoclonal antibodies from mice immunized either with rat dorsal root ganglia preparations (88) or with T. cruzi (89) also indicated a possible cross-reactivity between parasites and tissues. Monoclonal CE5 produced by inoculation of rat nervous tissue reacted with T. cruzi epimastigotes and amastigotes, as well as with Purkinje neurons, dorsal root ganglia neurons, motoneuronal cell bodies, neuronal cells of laminae IV and V of the dorsal horn and with cardiac muscle (89). On the other hand, monoclonal antibodies produced by inoculation with T. cruzi reacted with central and peripheral nervous tissue and with glia (89).

One of the criticisms in considering that a true cross-reaction exists between parasites and host tissues is that, in some cases, parasites used to infect (40,41) and to test the reactivity of cross-reacting antisera (49,51,88), or in the absorptions (68,69,87), were cultivated in such a way that they could incorporate into the parasite components responsible for stimulating the cross-reacting immune response: for example, culture media with brain, heart, or liver extracts. In the

case of EVI, this possibility seems unlikely. Lyophilized epimastigotes did absorb EVI actively from the sera while lyophilized culture media unseeded with T. cruzi did not (68,69). The reactivity of the CE5 monoclonal antibody with epimastigotes (88) has been explained because the epimastigotes were grown in culture media containing brain extracts (34). However, CE5 antibody was shown to immunoprecipitate a polypeptide from the in vitro translation products of T. cruzi-epimastigotes' mRNA. This indicates that the CE5-defined epitope is specified by the parasite genome (90).

More fundamental questions have been raised in regard to the EVI reaction. First, heterophil antibodies in sera of individuals from areas free of T. cruzi infection have given a fluorescent pattern on murine heart and skeletal muscle similar to that described for the EVI (91). Second, the reassessment of reactivity of chagasic sera from individuals with or without chagasic cardiopathy in human heart or skeletal muscle biopsies did not show an EVI pattern. Third, contrary to previous reports, the presence of spontaneously bound Igs on the surface of muscular fibers and endothelial cells from biopsies of chagasic patients with circulating EVI antibodies was not established. Fourth, the capacity of positive chagasic sera to react with striated muscle or PN of murine origin, was lost after absorption of the sera by guinea pig RBC (92,93). The conclusion of this work is that EVI antibodies are of heterophil nature. In addition, since EVI did not react with human tissues, their pathogenic effect is doubtful (92).

Some of the above findings should be re-examined in the light of later reports. The lack of Igs bound to human tissues that was attributed to the use of labelled polyvalent and whole antisera in the previous work, contrasted with the finding of Igs in the heart tissue of three chagasic patients with cardiopathy, using labelled F(ab')<sub>2</sub> antihuman IgG (68,92,94). Moreover, antibodies against

laminin, a component of basement membrane, have been found in humans and monkeys infected with T. cruzi. These anti-laminin antibodies were absorbed by laminin and found to react with T. cruzi trypomastigotes and amastigotes grown in cells that do not have laminin. In contrast, these antibodies did not react with epimastigotes (95). Purified anti-laminin antibodies from humans and monkeys reacted on mouse heart and skeletal muscle showing an EVI pattern (95) similar to that produced in the same tissues by sera from rabbits immunized with mouse heart or skeletal muscle, rat kidney or T. cruzi epimastigotes (96). Recently, it has also been demonstrated that circulating antibodies to mouse laminin in Chagas' disease, American cutaneous leishmaniasis and normal human sera recognize galactosyl(α1:3)-galactose epitopes present in normal and pathological human tissues (97). The aforementioned findings could be considered as additional evidence of the presence of cross-reacting antigens between T. cruzi and host tissues.

EVI and anti-laminin antibodies were not the only tissue-reacting immunoglobulins described in humans infected with T. cruzi. Patients who developed moderate to severe cardiomyopathy have abnormally high levels of IgG anti-muscle SRA (cited in reference 51). In addition, antibodies to nidogen, another basement membrane protein, were also detected in chronic chagasic patients (98). Evidence indicates that human antibodies reacting with nidogen also cross-react with laminin (98).

#### Conclusions

Available information demonstrates that inoculation of mice and rabbits with antigens from parasite or tissues may induce a chronic myocarditis with some resemblance to that produced by the inoculation of live parasites. Therefore, it is possible that parasite products inoculated into the host may elicit an immune response that could produce tissue damage. Whether or not this possibility truly

exists in other hosts should be tested when, for example, a candidate antigen for a vaccine is found. This view is reinforced by the finding that a microsomal fraction of T. cruzi epimastigotes induced myocardial lesions in mice, and that this effect is not related to any toxic activity of the preparation (99).

Experimental evidence indicates that cell mediated immunity to host tissues exists in rabbits and mice infected with T. cruzi, and that this phenomenon could be linked with the presence of tissue lesions. Whether it does exist in humans is questionable. Conflicting reports have been published in relation to the detection of delayed hypersensitivity to homologous or heterologous heart, using the leukocyte migration inhibition or the blast transformation test (52,55-60). On the other hand, there seems to be no doubt that tissue-reacting immunoglobulins appear in response to T. cruzi infection, and that some antigenic determinants are common between parasite and host tissue. However, until now, there is no proof that tissue reacting Igs were involved in the pathogenesis of lesions in humans. In fact, one of the problems in interpreting the original studies with EVI is that only one serum dilution was tested (68,84). Other studies were made attempting to establish a relationship between EVI titers and the clinical status of patients using serial serum dilutions. It was confirmed that EVI titers were lower in seronegative individuals for T. cruzi, and that the presence of EVI was linked with positive serology, but not with the presence or absence of chagasic cardiopathy (100-102). Therefore, the possibility of using EVI detection for prognosis has been discarded. In addition, the fact that antilaminin and antinidogen antibodies were found in patients with American cutaneous leishmaniasis in whom cardiac damage was not reported, makes it also improbable that these antibodies have a role in the pathogenesis of chagasic cardiopathy (98,102). However, the sera from patients with chronic chagasic cardiomyopathy having significantly higher titers of anti-sarcolemmal Igs than uninfected individuals, also recognize a 25-Kda T. cruzi

polypeptide (P-25) (104). The fact that individuals with other skeletal muscle chronic diseases (polymyositis, Duchenne muscular dystrophy) and that mice immunized with the sarcolemmal antigen had anti-P-25 activity in their sera are further indications of cross-reactivity between parasites and muscular antigens (104). The possibility that this P-25 antibody could indicate existing cardiomyopathy and could even be of prognostic value in asymptomatic individuals deserves further attention.

In the near future we can expect that more data will become available using T-cell clones to test whether T. cells recognize T. cruzi, muscle and nerve antigens, and what would be the effect of these cell lines when transferred to syngenic recipients. However, the main challenge concerns the translation of this knowledge to what happens in the human infected with T. cruzi.

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