Brazilian Journal of Medical and Biological Research (2000) 33: 253-258
ISSN 0100-879X Overview

How are antibodies involved in the protective mechanism of susceptible mice infected with *T. cruzi*?

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Abstract

Host resistance to *Trypanosoma cruzi* is dependent on both natural and acquired immune responses. During the acute phase of the infection the presence of IFN-γ, TNF-α, IL-12 and GM-CSF has been closely associated with resistance, whereas TGF-ß and IL-10 have been associated with susceptibility. Several investigators have demonstrated that antibodies are responsible for the survival of susceptible animals in the initial phase of infection and for the maintenance of low levels of parasitemia in the chronic phase. However, how this occurs is not yet understood. Our results and other data in the literature support the hypothesis that the protective role of antibodies in the acute phase of infection is dependent mostly on their ability to induce removal of bloodstream trypomastigotes from the circulation in addition to other concomitant cell-mediated events.

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Research supported by FAPESP, FUNDAP and Fundação Butantan.

Received April 9, 1999
Accepted December 8, 1999

Key words
- *Trypanosoma cruzi*
- Chronic mouse antibodies
- Clearance
- Cytokines
- Acute phase
- Susceptible mice

Trypanosoma cruzi, the causative agent of Chagas' disease, is a protozoan parasite that affects millions of people in Latin America. The parasite assumes different morphological, physiological and biochemical characteristics during its life cycle. Ingested trypomastigotes differentiate in the insect gut into epimastigotes, the replicative form, which will then differentiate into metacyclic trypomastigotes, the infecting form, inside the insect. Once in the vertebrate host, infective trypomastigote forms readily enter cells where they replicate as amastigotes and differentiate into bloodstream trypomastigotes which are released and can either infect other cells, other mammalian hosts or new insects when they suck infected blood.

After experimental infection of normal mice the parasites are able to survive and replicate in a variety of nucleated cells and are easily detected in peripheral blood during the acute phase. This is followed by a considerable reduction of parasitemia with the establishment of an apparent equilibrium between the parasite and the host in the chronic phase of the infection. Animals in the chronic phase of the infection do not present a new acute phase even when reinfected with a very large infective dose of parasites. Susceptible mice do not survive the acute phase. To control infection in the acute and chronic phase several components of the innate and acquired immune responses are required simultaneously through still unclear mechanisms.

Many strains of mice have been studied and different experimental approaches have been used to obtain a better understanding of

the immune response to *T. cruzi.* Evidence has accumulated over the years that cytokines play key roles in the control of the acute infection. IFN-γ, TNF-α, IL-12 (1) and GM-CSF (2) seem to be the cytokines most closely associated with the control of the parasitemia and mortality of resistant mice in the acute phase of the disease. This occurs due to an increase of oxidative metabolism (3,4), stimulation and proliferation of cells involved in the production of IFN-γ and TNF-α (5), and an increase of the cytotoxic activity of lymphocytes (6), although a direct nitric oxide-mediated killing of *Trypanosoma cruzi* has also been suggested (7). Other investigators have suggested that GM-CSF and TNF-α have a direct trypanocidal effect independent of nitric oxide liberation (8). Furthermore, transgenic mice with high levels of soluble TNF-α receptors that neutralize the *in vivo* effect of TNF-α are highly susceptible to *T. cruzi* infection (9). Class I MHC-restricted CD8+ T cells have been added to the list of critical effector mechanisms in immunity to *T. cruzi* (10-12). Furthermore, results of experiments in mice with induced defects in the cytolytic function of CD8+ T cells suggest that the control of *T. cruzi* infection by this population of T cells is not dependent on the granzyme perforin cytolytic pathway (11). However, there is also experimental evidence suggesting that TGF-ß (13) and IL-10 (14) increase the susceptibility to the infection by inhibiting the cytokines induced by macrophage activation and suppressing the production of reactive oxygen and nitrogen intermediates (15). Nitric oxide has also been shown to be involved in suppression of host immunity by participating in the modulation of the immune response by mediating apoptosis (16).

On the other hand, it has been known for many years that antibodies are very important in the control of the chronic phase of infection. Several investigators have shown that protection induced by adoptive transfer of spleen cells from chronically infected mice is prevented by depletion of B cells and that passive transfer of serum or IgG antibody isotypes collected from chronically infected mice protects naive recipients (17-19).

Two biological activities of *T. cruzi* antibodies are their ability to 1) induce *in vitro* lysis of the parasites (19) and 2) induce clearance of the parasites when injected together with trypomastigote forms into normal mice (20).

Attempts to characterize the parasite antigens, target of lytic antibodies, have identified a wide range of membrane polypeptides from 72 to 160 kDa (21-23). However, the mechanism by which these antigens participate in the lytic process is not well known. It has been suggested that some antigens may be molecules that prevent the interaction of C3b with factor B suppressing C3 convertase formation (24,25). In this case specific antibodies may inhibit this activity rendering the trypomastigotes susceptible to lysis.

Studies from our laboratory have demonstrated that trypomastigotes are easily removed from the mouse circulation in the presence of antibodies collected during the chronic phase of the infection and complement. In this situation the Fc fragment of the antibody molecule plus the C3 factor of the complement system are essential for parasite clearance (20). However, C5 is not involved, suggesting that lysis by complement activation is not implicated in parasite clearance (26).

An interesting biological aspect observed during the immune clearance of trypomastigotes is a significant decrease in platelet number (27). In addition, animals previously depleted of platelets show significantly decreased immune parasite clearance. In this respect, it is important to point out that platelets are involved in the antibody activity against *Schistosoma mansoni* larvae (28), *Brugia malayi* (29), sheep erythrocytes (30), and *Trypanosoma musculi* (31) and in chagasic cardiomyopathy (32).

Experiments performed in our laboratory

by incubating opsonized trypomastigotes and platelets showed an immediate adherence of platelets to the parasites, but 4 h were required to produce lysis (33). This observation seems to rule out the possibility that platelet-induced lysis is involved in the clearance of bloodstream trypomastigotes since this is a very rapid phenomenon *in vivo*. Platelet adhesion to bloodstream trypomastigotes was only observed in the presence of antibody and complement.

Considering data reported by Norris et al. (22) suggesting that epimastigote forms of *T. cruzi* are not infective due to the absence of a 160-kDa membrane complement regulatory protein (CRP), it was interesting to determine the role of mouse platelets in epimastigote clearance. Curiously, the incubation of epimastigotes with fresh mouse whole blood, serum or plasma in the presence of platelets results in the immediate adherence of platelets to the parasites through a mechanism dependent on the C3 component of complement, although no lysis was induced. However, epimastigote forms are cleared from the circulation of mice at an extremely rapid rate (34).

The ultrastructural characterization of the fate of parasites 15 min after intravenous injection of an epimastigote suspension (1-2 x 10^8) into normal mice showed clumps of parasites and platelets in direct contact with phagocytes in the lumen of capillaries. Parasites which were intact or in different stages of disintegration inside phagocytic cells were observed mostly in the pulmonary microvasculature (35).

Recent advances in the genetic manipulation of trypanosomes have shown that epimastigotes may be transfected with a plasmid encoding the trypomastigote-specific CRP, inducing conversion from a complement-sensitive to a complement-resistant state (36). These studies showed the critical function of this membrane protein in protecting the parasite from the effect of complement. Anti-CRP antibodies with the capacity to inhibit the C3b-CRP interaction were capable of supporting high levels of complement-mediated lysis of trypomastigotes and were also protective when adoptively transferred to mice prior to a lethal *Trypanosoma cruzi* challenge (36). On the other hand, Franchin et al. (37) showed that a sialylated epitope on the surface of bloodstream trypomastigotes is the target of a protective monoclonal antibody. This mAb did not lyse trypomastigotes *in vitro* in the presence of human complement or mouse spleen cells. The precise mechanism of action of this antibody is unknown.

When monitoring the appearance of lytic and clearance antibodies during the course of the infection, it can be seen that, whereas lytic antibodies appear early in the acute phase, clearance antibodies are detected later in the acute phase and in the chronic phase. Krettli et al. (38) showed that lytic antibodies present in trypomastigotes collected in the acute phase can be completely eluted when the parasites are incubated at 37°C for 2 h (further incubation of the parasites in the presence of complement does not induce lysis). Similar results were obtained by De Gaspari et al. (39) who were also able to elute antibodies from trypomastigotes collected in the acute phase. Garcia et al. (40) showed that in the acute phase most antibodies on the membrane of trypomastigotes are of the IgM class, which is very efficient in inducing lysis (38,39) but very inefficient in inducing clearance (40,41).

In contrast to the total elution of antibodies bound to parasites collected in the acute phase we found that antibodies can only be partially eluted from trypomastigotes sensitized with serum obtained during the chronic phase of the disease (42).

Two remarkable characteristics of anti-*T. cruzi* antibodies with lytic and clearance abilities are that they are induced mostly by live parasites and are specific for each parasite strain (17,18).

One pertinent question at this point in-

volves the role of lysis and clearance in protection against *T. cruzi* infection. However, it is difficult to answer since clearance antibodies usually also have lytic ability, although these two abilities could be distinguished by the use of heterologous antisera (43). In this regard, it should be pointed out that antisera from the acute phase, in spite of their high lytic ability (38,39), have no clearance effect (40,41). Furthermore, the behavior of anti-*T. cruzi* antibodies after recurrence of acute infection also suggests a protective role for clearance antibodies (44).

Earlier studies by MacAskill et al. (45) have shown that the liver plays a major role in immune clearance of *T. brucei* from the circulation of mice. Similarly, Dempsey and Mansfield (46) and Scott and Moyes (47) reported that the liver was the primary organ of immune clearance of *T. rhodesiense* and *T. cruzi,* respectively. Gregory and Wing (48) suggested that the principal mechanism for clearing pathogens from the bloodstream and eliminating them from the liver depends upon the complex interaction of Kupffer cells with neutrophils that migrate into the liver following infection. However, in experiments on mice, in which Kupffer cells were excluded surgically from the circulation, the clearance of *T. musculi* was not affected (49).

In our recent study about the fate of bloodstream forms of *T. cruzi* in mouse tissues after immune clearance we observed that parasites were present more frequently in the lung (50), most of them interiorized by neutrophils, eosinophils and monocytes in various stages of degeneration. However, platelets, that are important in the clearance of both epimastigote and trypomastigote forms, were not found inside phagocytic cells although they were abundant in the capillaries, sometimes occluding them completely. Indeed the vascular endothelial cells, which are important targets for parasites, are among the cells that are first encountered by invading organisms, are closely associated with platelet function, and may also mediate parasite elimination (51). The fact that endothelial cells have both Fc and C3 receptors (52,53) and secrete nitric oxide following cytokine stimulation has important implications for the host defense mechanism dependent on antibody, complement and platelets (54). In this context, experiments of intravital microscopy performed during the clearance of epimastigote forms of *T. cruzi* (55) have confirmed the critical role of endothelial cells in the clearance of these parasite forms. Experiments with opsonized bloodstream trypomastigote forms are under way in our laboratory.

In summary, there is evidence that different cell populations may be responsible for the elimination of *T. cruzi* from the circulation, suggesting that the protective role of antibodies with clearance ability *in vivo* is dependent on the presence of concomitant cell-mediated events. As outlined previously, trypomastigote forms infect most nucleated cells, and thus susceptible mammalian hosts represent a favorable environment for the development and replication of the parasite. It is reasonable to suggest that clearance-inducing antibodies can change the course of infection of susceptible hosts by directing the parasites to cells that recognize the opsonized organisms and help to destroy them through intra- and extracellular events involving cytokine-activated cells or their secreted products.

Acknowledgments

We are grateful to Dr. Harumi A. Takehara for a critical reading of the manuscript.

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