BRAZILIAN JOURNAL OF MEDICAL AND BIOLOGICAL RESEARCH
www.bjournal.com.br

ISSN 0100-879X
Volume 44 (2) 84-181 February 2011
BIOMEDICAL SCIENCES

doi: 10.1590/S0100-879X2011007500005

Braz J Med Biol Res, February 2011, Volume 44(2) 84-90

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The Brazilian Journal of Medical and Biological Research is partially financed by

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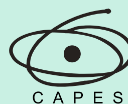

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Brazilian Journal of Medical and Biological Research (2011) 44: 84-90
ISSN 0100-879 Review

Evasion of immune responses by *Trypanosoma cruzi*, the etiological agent of Chagas disease

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Abstract

Infection with the protozoan parasite *Trypanosoma cruzi* leads to Chagas disease, which affects millions of people in Latin America. Infection with *T. cruzi* cannot be eliminated by the immune system. A better understanding of immune evasion mechanisms is required in order to develop more effective vaccines. During the acute phase, parasites replicate extensively and release immunomodulatory molecules that delay parasite-specific responses mediated by T cells. This immune evasion allows the parasite to spread in the host. In the chronic phase, parasite evasion relies on its replication strategy of hijacking the TGF-β signaling pathway involved in inflammation and tissue regeneration. In this article, the mechanisms of immune evasion described for *T. cruzi* are reviewed.

Key words: *Trypanosoma cruzi*; T cells; GPI-anchors; TGF-β; Mucin; Apoptosis

Introduction

Human infection with *Trypanosoma cruzi* results in multiple immunological dysfunctions, chronic persistence of the parasite, and inflammatory destruction of either the heart muscle, or autonomic neurons innervating the digestive tract. The condition is known as Chagas disease, and affects millions of people in Latin America (1). The immune responses to *T. cruzi*, the immunological dysfunctions induced by the infection, and the mechanisms of cardiac injury have been reviewed previously (2,3). This review focuses on mechanisms of parasite evasion from immune responses.

T. cruzi has evolved sophisticated strategies to evade host immune responses. It is estimated that *T. cruzi* emerged over 150 million years ago, and infected primitive mammals in the region that originated the Americas (4). Contact with humans occurred much more recently. Infection of isogenic mouse strains with *T. cruzi* isolates adapted to laboratory conditions reproduces certain characteristics of Chagas disease. However, drawing general conclusions from any specific combination of parasite and mouse strain can be misleading, due to the genetic variability found in nature. Findings range from virulent isolates that induce systemic proinflammatory responses to isolates of low virulence that induce a mild inflammatory response and do not kill the host. These distinct parasite populations coexist in nature. Virulence and pathogenicity differ among clones isolated from Chagas disease patients (5). In addition, the stock populations are less virulent than isolated clones (5). Co-infection of mice with virulent CL Brener and avirulent JG isolates produces intermediate virulence, which correlates with increased expression of interleukin-10 (IL-10) by macrophages and $CD4^+$ T cells, compared with infection by CL Brener alone (6). These data suggest that infection with heterogeneous populations of parasites, which better reflect natural infections, induces a mixture of pro- and anti-inflammatory immune responses in the host.

Parasite survival is favored by immune regulation that follows the initial immune responses

Following infection, the initial rise in parasitemia is contained by proinflammatory cytokines and microbicidal mediators released by macrophages and natural killer cells.

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Received November 17, 2010. Accepted January 3, 2011. Available online January 14, 2011. Published February 7, 2011.

These innate immune responses are followed by polyclonal activation of major lymphocyte subsets, and the onset of acquired immunity against the parasite mediated by $CD4^+$ T cells, $CD8^+$ T cells, and B cells (2). Together, these robust immune responses reduce, but do not eliminate the parasite burden. Studies with genetically deficient mice indicate that both $CD4^+$ and $CD8^+$ T cells are equally essential for control of parasitemia and survival of the host (7). Regarding $CD4^+$ T cells, Th1 responses mediate protection against *T. cruzi* infection, while Th2 responses correlate with parasite persistence (8).

Toll-like receptors (TLRs) comprise a family of pattern recognition receptors able to sense microbial pathogens (9). TLRs are expressed by phagocytes and other cell types that patrol the body, and induce secretion of proinflammatory cytokines (9). TLRs play an important role in early innate immune responses against *T. cruzi* (10). In addition, *T. cruzi* activates innate immune responses through TLR-independent pathways (11,12). TLRs link innate and adaptive immunity by up-regulating the ability of accessory cells to generate immune responses mediated by T and B lymphocytes (9). In the absence of TLR signaling, $CD4^+$ T-cell responses against *T. cruzi* are reduced, but $CD8^+$ T-cell responses are preserved (13).

In order to avoid life-threatening injury to organs, proinflammatory responses triggered by microorganisms are counteracted by anti-inflammatory responses mediated by IL-10 and transforming growth factor-β (TGF-β) (14). In agreement, both IL-10 and TGF-β play critical roles in regulation of host immune responses to *T. cruzi* (15,16). The anti-inflammatory responses induced by *T. cruzi* are strong enough to inhibit development of experimental autoimmune encephalomyelitis in mice (17), and play a major role in the promotion of parasite persistence (2). Parasite persistence could be the price paid by down-regulating proinflammatory cytokine secretion. At peak parasitemia, the inflammatory cell infiltrates in the heart contain T cells expressing both Th1 and Th2 cytokines (18). Furthermore, infiltrating macrophages express arginase I, a marker of alternative macrophage activation, in a manner related to susceptibility to *T. cruzi* infection (18).

The acute phase: immunomodulatory molecules are released by the parasite

Parasite persistence depends on a combination of factors, including release of molecules that interfere with the immune responses. Early work demonstrates that *T. cruzi* suppresses lymphocyte activation (19), an effect that depends on the density of parasites. Therefore, suppression induced by parasite molecules is more relevant at the acute phase, when the concentration of such molecules can be fairly high. In addition, T-cell depletion can be observed at peak parasitemia (20). Together, these factors contribute to immunosuppression, and promote the spread of infection. Perhaps as a consequence of early immunosuppression, the onset of antigen-specific cytotoxic T-cell responses against *T. cruzi* is delayed, compared to other infections (21). This delay in $CD8^+$ T-cell responses could be responsible for unchecked spread of the parasite in the host. The best characterized parasite molecules are surface glycosylphosphatidylinositol (GPI)-anchors, to which both pro- and anti-inflammatory effects were ascribed.

GPI-anchored mucins

GPI-anchored mucins (GPI-mucins) (22) and glycoinositolphospholipids (23) comprise some of the most abundant *T. cruzi* surface molecules. GPI-mucins are involved in parasite adherence to host tissues (24) and are encoded by a large family containing 6% of all predicted *T. cruzi* genes (22). GPI-mucins are polymorphic proteins consisting of conserved and variable regions. It has been suggested that the large array of GPI-mucin genes is responsible for parasite surface variability, leading to differential tissue adherence, and evasion of host immune responses (22). The role of GPI-mucins in the induction of host protective responses is controversial. GPI-mucins require expression of TLR2 to activate cytokine and mediator release (25). Furthermore, signaling through both TLR2 and TLR9 accounts for the control of parasitemia (10). However, studies with TLR2-deficient animals have indicated that GPI-mucins and TLR2 play a predominantly regulatory rather than proinflammatory role *in vivo* (26). Perhaps one explanation for this paradox is that TLR2 requires additional signaling pathways to exert proinflammatory effects *in vivo*. In agreement with this possibility, TLR2 cooperates with the bradykinin B_2 receptor for the induction of a protective Th1 response against *T. cruzi* (27).

On the other hand, analysis of adaptive responses induced by GPI-mucins reveals immunosuppressive and parasite evasive effects. Human T cells exposed to GPI-mucin TcMuc-e2 become anergic and fail to produce IL-2 (28). In addition, AgC10, a GPI-mucin derived from epimastigotes, inhibits mouse T-cell activation and IL-2 secretion (29). This *T. cruzi* mucin binds L-selectin on T cells, and L-selectin expression is required for inhibition of T-cell responses (29). The above-mentioned effects are probably related to intact GPI-mucins. But what happens with antigenic processing of GPI-mucins? It is expected that polymorphic GPI-mucins are processed and generate a large number of partially related T-cell epitopes. Simultaneous exposure to several GPI-mucin epitopes reduces the expression of each epitope below a threshold level required to stimulate interferon-γ (IFN-γ) secretion by $CD4^+$ T cells (30). However, IL-4 responses are preserved (30), suggesting that bulk responses to GPI-mucins induce a shift toward Th2 responses. One important question regarding polymorphism of GPI-mucins is whether individual parasites express all possible mucin epitopes, or whether individuals

express unique variant GPI-mucins (22).

GPI-mucins and induction of dysfunctional host dendritic cells

One important mechanism of evasion is through the modulation of the immunogenic properties of dendritic cells (DCs). Infection with *T. cruzi* increases the number of splenic DCs (20). However, most splenic DCs remain immature, as suggested by decreased expression of CD86 and failure to migrate toward the T-cell zone in response to injection of lipopolysaccharide (LPS) (20). In agreement with these findings, exposure to *T. cruzi in vitro* fails to induce maturation, and blocks DC maturation induced by LPS, which correlates with increased secretion of IL-10 and TGF-β (31). These results suggest that exposure to *T. cruzi* molecules renders DCs dysfunctional for protective responses. Although the nature of *T. cruzi* molecules that modulate DC differentiation is unclear, GPI-mucins are likely candidates. *T. cruzi* is unable to synthesize sialic acid. However, *T. cruzi* expresses *trans*-sialidase (TS), an enzyme that transfers sialic acid from host glycoproteins to parasite GPI-mucins (22). A virulent *T. cruzi* strain expresses high TS activity and is able to suppress both IL-12 production by DCs and induction of T-cell activation (32). The extent of parasite sialylation correlates with binding to Siglec-E on DCs, and cross-linkage of Siglec-E blocks IL-12 production (32). These results suggest that parasite virulence correlates with TS activity and with the extent of GPI-mucin sialylation.

Trans-sialidase

The TS protein family itself corresponds to a subset of the GPI-mucin superfamily (22). In addition to its effects on GPI-mucins, TS is released and interacts directly with host lymphocytes through desialylation and resialylation of acceptor glycoproteins (33). TS co-stimulates $CD4^+$ T-cell proliferation and increases cytokine production through engagement of the lymphocyte mucin CD43 (34). However, TS also induces lymphocyte apoptosis through target cell resialylation (33). The mechanisms of virulence are unclear, but a recent study indicates that FLY, a conserved cell-binding peptide from TS family proteins, increases parasitemia, mortality and the number of $CD4^+CD25^+FoxP3^+$ regulatory T cells in the hearts of infected animals (35).

Following T-cell activation, surface expression of sialic acid is down-regulated. Decreased sialylation plays an important role by increasing cellular contacts and enhancing reactivity of $CD8^+$ T cells to their cognate peptide-MHC class I ligands (36). One important target of *T. cruzi* TS appears to be $CD8^+$ T cells. Treatment with TS resialylates $CD8^+$ T cells *in vitro* and *in vivo*, including resialylation of CD43 (37). Increased resialylation compromises the ability of activated $CD8^+$ T cells to kill targets bearing *T. cruzi* epitopes (37). These results suggest that, during the acute phase, *T. cruzi* manipulates host T-cell sialylation to evade immune responses.

Glycoinositolphospholipids

Glycoinositolphospholipids (GIPLs) are free GPI anchors derived from distinct *T. cruzi* life stages, with modulatory effects on host immune responses (23). GIPLs suppress $CD4^+$ T-lymphocyte activation *in vitro* and *in vivo* through the ceramide domain (38). In addition, GIPLs suppress IL-2, but not IL-4 secretion, suggesting that GIPLs shift the immune response toward a Th2 profile (38). GIPLs polyclonally activate B-cell Ig secretion, and the activity is located in the glycan moiety (39). Therefore, different biological actions can be assigned to distinct moieties of the GIPL molecule. In agreement with a general suppressive function in cell-mediated immunity, GIPLs down-regulate LPS-induced expression of co-stimulatory molecules and proinflammatory cytokine secretion in human macrophages and DCs (40).

Other modulatory molecules from *T. cruzi*

The major *T. cruzi* cysteine proteinase cruzipain induces IL-10 and TGF-β secretion, as well as arginase expression by macrophages, leading to increased intracellular replication of *T. cruzi* (41). Tc52, a *T. cruzi* glutathione thioltransferase, activates the immune system through TLR2, and elicits protective immune responses when combined with conventional adjuvants (42). In the absence of adjuvants, Tc52 increases IL-10 mRNA in macrophages (43). Moreover, Tc52 expression appears to be required for optimal *T. cruzi* replication in the host (43).

The chronic phase: an equilibrium between parasite killing and replication

Chronic infection induces long-lasting activation of parasite-specific $CD8^+$ T cells in mice, while chronic infection in man induces a reduction of the ability to respond against *T. cruzi* antigens (21). The difference is ascribed to the different time frames of infection, i.e., two years in mice versus several decades in man (21). Despite inducing robust immune responses in mice and humans, chronic infection with *T. cruzi* cannot be eliminated by the immune system. In chronic infection, the number of parasites is dramatically reduced. Therefore, it is unlikely that molecules released by the parasite play an important role in mechanisms of evasion. One possible mechanism of evasion is the selection of escaping parasites due to the differential display of antigenic epitopes by infected cells (22). A novel multigene family of *T. cruzi* surface proteins is the mucin-associated surface protein (MASP) family (44). Immunofluorescence studies with a MASP-derived peptide have suggested that

the expression of a particular MASP is limited to a fraction of individuals within the parasite population (44). This finding supports the possibility of selection of parasites based on escape from CD8+ T-cell responses directed against variable epitopes. However, in spite of thousands of proteins expressed, the CD8+ T-cell response against *T. cruzi* is highly focused on a few peptides encoded by the TS gene family (21). The reason for this immunodominance is unclear. In fact, it has been suggested that immunodominance is an evasion strategy aimed at reducing the frequencies of the most effective anti-parasite lymphocyte clones (45). If GPI-mucin or TS gene-encoded peptides are involved

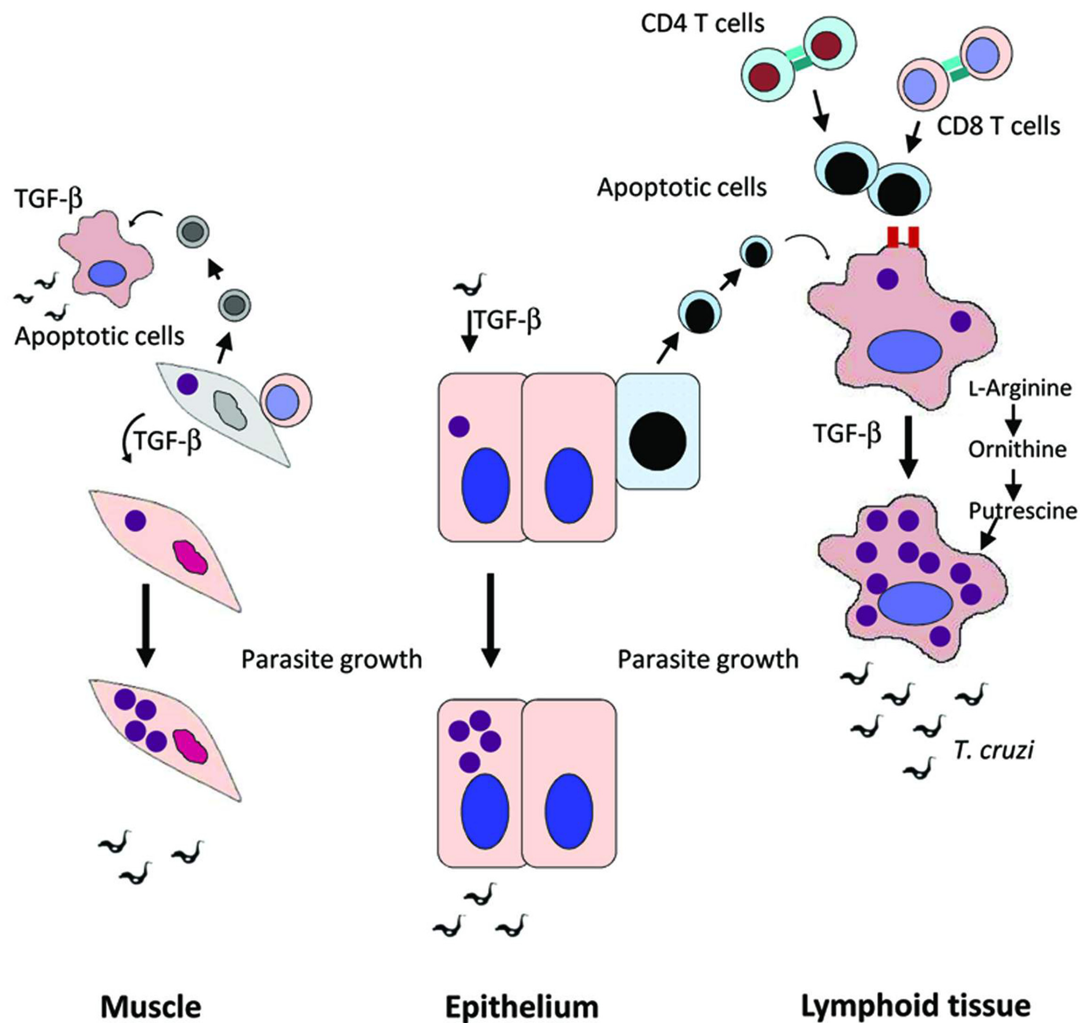

Figure 1. Role of the transforming growth factor-β (TGF-β) signaling pathway in the replication of *Trypanosoma cruzi*. Parasite replication is driven by TGF-β originating from tissue turnover and from resolving inflammation. *Center*, Penetration and replication of *T. cruzi* in epithelial cells require signaling of TGF-β through TGF-β receptors TβRI or TβRII (47). Amplifying mechanism: apoptotic cell death releases either active or latent TGF-β into the extracellular medium (48,49). In addition, TGF-β induces apoptosis in epithelial cells and hepatocytes (50). *Left*, In heart muscle cells and fibroblasts, TGF-β is important for parasite replication (51), and parasite replication leads to apoptosis in parasites, macrophages and cardiomyocytes (52). *Right*, TGF-β drives replication of *T. cruzi* in host macrophages (56). In both lymphoid tissue and inflammatory infiltrates, apoptotic lymphocytes and apoptotic bodies derived from dying epithelial and muscle cells are engulfed by macrophages. Engulfment leads to release of TGF-β, induction of ornithine decarboxylase and a shift in arginine metabolism towards production of putrescine (56). Putrescine production drives parasite replication in macrophages (56). Note that replicating intracellular *T. cruzi* amastigotes are indicated by small intracellular purple circles. At the chronic phase of infection, parasite replication is counteracted by parasite killing mediated by cytotoxic CD8+ T-cell responses (indicated in Figure 1, top left), and by additional effector mechanisms.

in parasite evasion, then cyclical shifts in the frequency of recently activated CD8$^+$ T-cell clones should be observed, similar to some chronic autoimmune diseases. There is no evidence, so far, for these recurrent shifts in the clonal frequencies.

In addition, effector CD4$^+$ T cells should not be forgotten in host defense against *T. cruzi*. Effector CD4$^+$ T cells can be more efficient at tumor rejection than CD8$^+$ T cells (46). The mechanisms are not completely understood, but indirect mechanisms are involved and require cells expressing MHC class II molecules (46).

In the chronic phase, the number of parasites killed by the immune response should be roughly similar to the number of new parasites arising by replication. As summarized in Figure 1, a likely mechanism of escape relies on the positive correlation between replication of *T. cruzi* and TGF-β production by the host. Production of TGF-β is a common feature of cell death, phagocytosis of apoptotic cells following immune responses or tissue turnover, and anti-inflammatory immune responses. Signaling by TGF-β is required for *T. cruzi* replication in epithelial cells (47). Apart from the properties of the parasite (47), apoptosis could drive this process, since apoptotic cells release TGF-β (48,49), and TGF-β induces apoptosis in epithelial cells and hepatocytes (50). In addition, TGF-β appears to be important for parasite replication in cardiac fibroblasts and myocytes, as well as fibrosis and cardiac remodeling in the course of infection (51). *T. cruzi* itself undergoes apoptosis and induces apoptosis in cardiomyocytes and macrophages (52). Apoptosis of leukocytes, cardiomyocytes and parasites is a common feature of cardiac inflammatory infiltrates in dogs acutely infected with *T. cruzi* (53). Similar to apoptotic cells, trypomastigote forms of *T. cruzi* express phosphatidylserine on the surface, and engage TGF-β signaling pathways in macrophages (54). However, the macrophage receptor involved remains unidentified. This study suggests that apoptotic mimicry through exposure of phosphatidylserine deactivates macrophages and helps parasite replication (54). Furthermore, infection with *T. cruzi* triggers apoptosis of T and B lymphocytes, and lymphocyte apoptosis has immunoregulatory implications for host immune responses (55). Following apoptosis, dead cells are engulfed by macrophages. Phagocytosis of apoptotic lymphocytes drives replication of *T. cruzi* in macrophages through a biochemical cascade that includes PGE_2, TGF-β, ornithine decarboxylase, and polyamine synthesis (56). Increased replication of *T. cruzi* depends on production of TGF-β by macrophages engulfing dead cells (56). Injection of apoptotic cells increases parasitemia *in vivo*, and treatment with the cyclooxygenase inhibitors aspirin or indomethacin reduces parasitemia (56). Correlation between induction of apoptosis and growth of *T. cruzi* is demonstrated by the increased parasitemia elicited by injection of a modified *T. cruzi* recombinant protein that induces apoptosis (57). In agreement with a pathogenic role, *in vivo* treatment with a caspase inhibitor reduces lymphocyte apoptosis and improves protective immune responses in mice infected with *T. cruzi* (58). Interestingly, mice chronically infected with *T. cruzi* develop IgG antibodies against apoptotic cells that reduce, but do not prevent the deleterious effect of apoptotic cells on intramacrophage parasite replication (59). In addition, under inflammatory conditions, phagocytosis of apoptotic cells induces a regulatory phenotype in macrophages, which is permissive for intracellular pathogen replication (60).

Concluding remarks

During the acute phase of infection, *T. cruzi* replicates extensively and releases immunomodulatory molecules that delay parasite-specific responses mediated by effector T cells. This mechanism of evasion allows the parasite to spread in the host. In the chronic phase, parasite evasion most likely relies on hijacking of the host TGF-β signaling pathway of tissue regeneration. As a consequence, the rates of parasite killing and replication remain roughly the same in the chronic phase of infection. *T. cruzi* is adapted to coexist with a vigorous immune response mounted by CD8$^+$ T cells, calling into question the efficacy of conventional vaccines. A better understanding of the mechanisms of evasion employed by the parasite is necessary. In the near future, a combination of strategies aimed at both early killing of parasites and neutralizing suppressive mechanisms could be necessary for effective therapies and vaccines.

Acknowledgments

I thank Dr. Michelle M. Diniz for help with the artwork. Research supported by CNPq, FAPERJ, and Programa Institutos Nacionais de Ciência e Tecnologia (INCT).

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