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Chapter IX

Molecular and Cellular Mechanisms Leading to Pathogenesis of *Trypanosoma Cruzi*, The Agent of Chagas'Disease

Ali Ouaissi[1], *Margarida Borges* [1,2]
and Anabela Cordeiro-Da-Silva[2,3]

[1]Institut de la Recherche pour le Développement, Unité de Recherche n° 008
« Pathogénie des Trypanosomatidae », Montpellier, France
[2]Biochemical Laboratory, Faculty of Pharmacy, University of Porto, Portugal
[3]Institut of Molecular and Cellular Biology, University of Porto, Portugal

Abstract

Trypanosoma cruzi, the causative agent of Chagas'disease is a parasitic protozoa that infects more than eighteen million people in South and Central America. Chagas'disease is characterized by a heart defect and megaviscera in a proportion of patients, and these clinical signs are associated with extensive destruction of parasympathetic, enteric and other neurons and degeneration of cardiac muscle. Chagas'disease is often associated with the presence of autoantibodies (autoAb) against host tissues, including specialized components of striated muscle, neurons and connective tissue, which make autoimmune reactions likely. Interestingly, a remarkable polyspecificity of the autoAb was observed. Indeed, the autoAb have been found to cross-react with animal erythrocytes and distinct structural basement membrane proteins such as laminin and collagen. The purpose of the present review is to summarize some of the current data on the pathophysiology of *T. cruzi* infection. Special attention is given to recent data mainly from our own laboratory illustrating the important role of an immunomodulatory factor released by the parasite, the Tc52 protein, in the induction and perpetuation of chronic disease.

Introduction

Chagas'disease, or American trypanosomiasis due to the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), is endemic in Latin America. Eighteen million individuals are infected and 100 million of people are at risk (WHO, 2002). The life cycle of *T. cruzi* involves obligatory passage through a definitive vertebrate host (mammals, including man) and an intermediate invertebrate host (hematophagous triatomine bugs). The trypomastigote, ingested by the insect, differentiates into the proliferative epimastigote in the midgut of the insect vector and transforms into an infective metacyclic trypomastigote in the posterior intestine (hindgut) and released in the faeces of the bug during or just after the blood meal (Bonaldo, 1988). This latter form, following invasion of vertebrate host cells, undergoes differentiation into amastigotes, which after several reproductive cycles, transform into the infective trypomastigote forms released in the blood circulation after the rupture of the host cell.

The two available drugs (nifurtimox and benznidazole) present some toxic effects and cure a very low percentage of chronic cases, likely due to the resistance of some strains (Rodrigues Coura & Castro, 2002). Thus, the search of an effective vaccine against the disease is still needed.

Different strategies were developed in order to identify parasite components which could be used as a vaccine candidate. The use of inactivated parasites (Andrews *et al.*, 1985), fractionated parasite material (Gonzales Cappa *et al.*, 1981) and irradiated non infectious *T. cruzi* trypomastigotes (Zweerink *et al.*, 1984) have been shown to induce partial protection of mice against a lethal *T. cruzi* challenge. Due to the cross reactivity between some parasite and host components (Khoury *et al.*, 1979) and toxicity of some parasite molecules, identification and isolation of antigens that induce protective immune mechanisms is needed for the development of an effective convenient vaccine. In this way, the use of defined parasite surface antigens has already been shown to induce partial protection against lethal infection with reduced parasite levels (Scott & Snary, 1979; Snary, 1983).

Moreover, several experiments performed using the mouse model, such as passive transfer of specific anti-*T. cruzi* polyclonal antibodies (Kretteli & Brener, 1976), different anti-*T. cruzi* antibody classes (Takehara *et al.*, 1981), or monoclonal antibodies against the *T. cruzi* flagellar fraction (Segura *et al.*, 1986), have already demonstrated the importance of the humoral response in host's defense against the *T. cruzi* challenge. Fischer rats have also been shown to be susceptible to *T. cruzi* infection (Rivera-Vanderpoas *et al.*, 1983), and could be considered as an experimental model for studies of the immune response involved in resistance against the *T. cruzi* infection. Using this model, Rodriguez *et al.* (1981) showed that the neonatally initiated injections of anti-μ antibodies in rats resulted in a loss of antibody production and an increase in rat susceptibility to the acute infection.

Important immune dysfunction is observed during the acute phase of Chagas'disease, namely polyclonal activation of B and T cell populations, whose autoreactive sub-populations may lead to severe chronic tissue damage (Minoprio *et al.*, 1986; Engman & Leon, 2002). Paradoxically, a state of immunosuppression could be observed during the acute phase of the disease (Tarleton, 1988; Santos Lima & Minoprio, 1996).

Molecular cloning of relevant *T. cruzi* genes involved in the host-parasite relationships has produced a whole series of significant details on the mechanisms of the action of certain *T. cruzi* virulence factors and their interaction with the cells of the immune system (Ouaissi *et al.* 2004). The purpose of this review is to summarize some of the current data related to the *T.cruzi*-host cell interplay.

Immunology of *T. Cruzi* Infection

Trypomastigotes are able to invade and multiply within different host cells, including macrophages, endothelial cells, smooth and striated muscles, fibroblasts and even neurons (Ouaissi, 1993). After the recognition phase and adhesion, the trypomastigote is internalized, evades the phagolysosome, undergoes transformation into amastigotes and multiplies within the cytoplasm of the infected host cell (Ouaissi, 1993; see Figure 1).

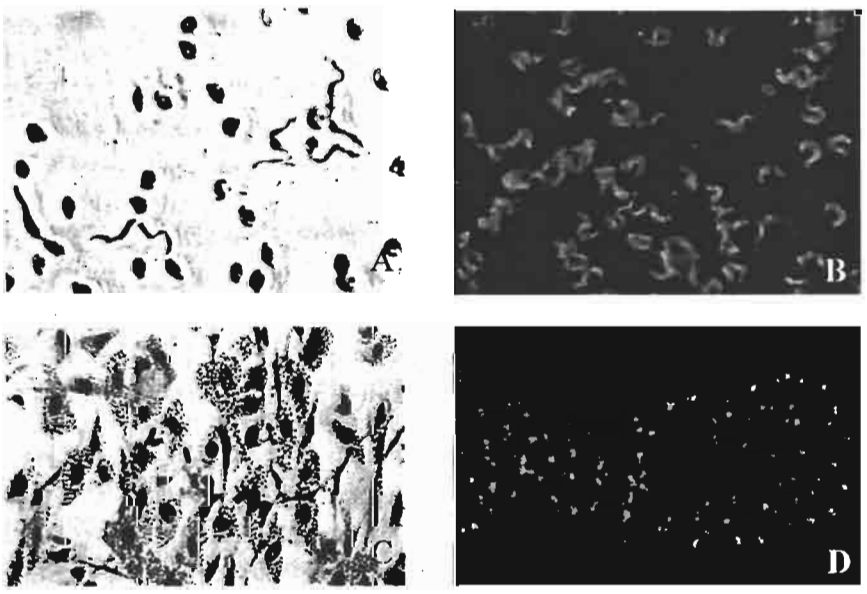

Figure 1. Different *T. cruzi* developmental stages. A) phase contrast microscopy showing an heterogenous population comprising trypomastigotes (slender forms with flagellum) and extracellular amastigotes (round forms without flagellum); B) trypomastigotes reacting with a monoclonal antibody (mAb VG3/G11, Ouaissi *et al.*, 1991) directed against a carbohydrate epitope carried by an 85 kDa surface polypeptide; C) heavily *in vitro*-infected L929 mouse fibroblast cell line showing high density of intracellular amastigotes; D) VG3/G11 immunofluorescence staining of L929-infected fibroblasts showing intracellular trypomastigotes originating from amastigotes after a step of differentiation reacting with the mAb.

In man, the immune system is able to induce a reduction in parasitaemia during acute phase and to actively control the infection for years, but the infection persists for life. In the last several years, a number of studies have provided a better understanding of some characteristics of the anti-*T. cruzi* immune response in man and experimental models. A number of humoral and cellular mediators have been shown to be important in resistance (Krettli & Brener, 1982; Tarleton, 1991). However, the mechanisms by which this machinery interacts with the parasite and vice-versa still needs to be better clarified.

The immune control of *T. cruzi* is mediated by different cell populations: T cells, B cells, macrophages and NK cells (Kierszenbaum & Pienlowsky, 1979; Schmunis *et al.*, 1971; Kierszenbaum & Howard, 1976; Kierszenbaum *et al.*, 1974, Rottenberg *at al.*, 1988; Trishmann, 1983).

There are several mechanisms by which T cells contribute to the eradication of *T. cruzi*: (i) direct destruction of infected cells (Nickell *et al.*, 1993); (ii) stimulation of antigen specific antibody production (Rottenberg *et al.*, 1992); (iii) secretion of lymphokines which in turn modulate different parasiticidal mechanisms (Nogueira & Cohn, 1978; Frosch *et al.*, 1997). It is now clear, that both $CD4^+$ and $CD8^+$ T cells mediate effector functions that control parasite growth and dissemination into the host leading therefore to protection against Chagas'disease (Tarleton, 1991).

It has been shown that mice treated with anti-$CD4^+$ (Araujo, 1989; Rottenberg *et al.*, 1992) or anti-$CD8^+$ antibodies (Tarleton, 1990) exhibited a higher susceptibility to infection. Similar observations were obtained by infecting gene knockout mice lacking CD4+ or CD8+ molecules (Rottenberg *et al.*, 1993) or α_2-microglobulin (Tarleton *et al.*, 1992). Moreover, it has also been reported that mice depleted of $CD4^+$ and $CD8^+$ T cells have increased susceptibility to infection and exacerbation of chronic pathology, and also enhanced myocardial parasitism (Tarleton *et al.*, 1994). Further, mice lacking the major histocompatibility complex (MHC) class I and class II molecules exhibited a high rate of mortality compared to the control mice (Tarleton, 1996), demonstrating the critical importance of both CD8+ and CD4+ T cell-mediated immune responses in the control of *T. cruzi* infection. Given that *T. cruzi* lives free within the cytoplasm of the infected host cell and that the parasite antigens are processed and presented on MHC class I, inducing $CD8^+$ T cytolytic cells. This induction mechanism would be an important arm of the immune system to control *T. cruzi* infection (Buckner *et al.*, 1997).

There are two distinct subsets of $CD4^+$ T cells, T helper type 1 (Th1) and T helper type 2 cells (Th2), which produce distinct and restricted patterns of cytokines that cross regulate each other and thus mediate different types of immune responses (Townsend & McKenzie, 2000). Th1 subset produces abundant interleukin 2 (IL-2) and interferon γ (IFN-γ). These cytokines are known to be involved in classic cell-mediated functions such as clonal expansion of cytotoxic T lymphocytes (CTLs), macrophage activation and class-switching to IgG isotypes that mediate complement lysis of sensitized cells (reviewed by Murray, 1998). Th2 subset produces the functionally opposite cytokines, as IL-4 and IL-5, which are known to activate B cells to switch to neutralizing antibodies (IgG1 in the mouse) and IgE, the initiator of immediate hypersensitivity (Murray, 1998).

Unlike the situation observed in murine infections of *Leishmania major* in which the resistant and susceptible mouse strains exhibit a dominance of either Th1 (IL-2, IFN-γ, IL-12)

or Th2 (IL-4, IL-10) type cytokine responses (Sher & Coffman, 1992) respectively. During the acute and chronic phases of infection by *T. cruzi* both type 1 and type 2 patterns of cytokine are developed in both resistant and susceptible mouse strains (Eksi *et al.*, 1996). Nevertheless, recent studies have shown that antigen-specific Th1 (but not Th2) cells provide protection against lethal *T. cruzi* infection in mice (Kumar & Tarleton, 2001).

Macrophage activation by lymphokines, secreted by T lymphocytes, leads to increased trypanocidal activity (Roitt, 1985a). The production of nitric oxide (NO) by IFN-γ-activated macrophages and also in synergism with TNF-α, is a major effector mechanism during an experimental *T. cruzi* infection (Muñoz-Fernández *et al.*, 1992). Indeed, NO-mediated trypanocidal activity can be blocked by L-arginine analogs that inhibit the induced nitric oxide synthase (iNOS) pathway (Hölscher *et al.*, 1998). Moreover, chemoattractant molecules, such as leukotriene B (4) or platelet-activating factor, may also activate macrophages to induce NO-mediated killing of *T. cruzi* (Talvani *et al.*, 2002). Moreover, studies have shown that other factors such as myoglobin may facilitate a *T. cruzi* infection by acting as a scavenger of NO (Ascenzi *et al.*, 2001). Chemokines such as a macrophage inflammatory protein 1 alpha (MIP-1α), has been shown to play a role in macrophage/monocyte influx in infected organs during infection by *T. cruzi* (Petray *et al.*, 2002). Others have demonstrated that MIP-1α, RANTES and MIP-1β, released by macrophages following *T. cruzi* infection, are able to increase the *T. cruzi* trypomastigote uptake and destruction *in vitro* and in mouse inflammatory macrophages both *in vivo* and *in vitro*, through the cytotoxic NO-dependent mechanism (Brenier-Pinchart *et al.*, 2001).

Dendritic cells (DC) are crucial in the initiation of the immune response and are distinguishable from the other antigen presenting cells by their high efficient antigen presentation. DCs are specialized to acquire and process antigen in peripheral non-lymphoid sites, and to transport the antigen to the secondary lymphoid organs where the stimulation of naïve lymphocytes occurs. During their migration, DCs enter a process of maturation that determines whether adaptative immune response occurs and the nature of that immune response. Moreover, DCs are early producers of IL-12 for the initiation of Th1 responses (Banchereau *et al.*, 2000). It is only in recent years that investigations were developed to explore the relationship between *T. cruzi* and DC. In fact, it has been shown that *T. cruzi* released soluble factors could prevent DC maturation and secretion of IL-12 and TNF-α (Van Overtvelt *et al.*, 1999). Further, it was demonstrated that *T. cruzi* is able to inhibit the LPS-induced up-regulation of MHC class I molecules at the surface of human DC (Overtvelt *et al.*, 2002). Studies with the glycoinositolphospholipid (GIPL) from *T. cruzi* have demonstrated that this molecule leads to a down-regulation of human DC surface antigens, such as CD80, CD86, HLA-DR, CD40 and CD57 that are important for T cell activation (Brodskyn *et al.*, 2002). Taken together, these observations led investigators to propose a novel efficient mechanism leading to the alteration of DC function and maturation, that may be used by *T. cruzi* to escape the host immune response. However, although these investigations are interesting and provocative, it is important to remind that the GPI anchors express biological activities similar to those of LPS. Given the fact that LPS induces the maturation of dendritic cells, one would expect that *T. cruzi*-derived LPS-like substances could activate rather than inhibit DC maturation. Therefore, the observations showing DC-inhibition await further explanation. In this regard, it is noteworthy that GPI anchors and

GIPLs from *T. cruzi* are potent activators of the human and mouse macrophage Toll-like receptor 2 (TLR2) (Campos *et al.*, 2001). Interestingly, the TLR2 activation by GPI led to the synthesis of IL-12 and TNF-α when using LPS as a triggering agent. Moreover, it has been shown that *trans*-sialidase (TS) from *T. cruzi,* a parasite-secreted protein, was able to activate mouse DC (Todeschini *et al.*, 2000). Furthermore, in recent studies we found that a *T. cruzi* released protein related to the thiol-disulfide oxidoreductase family, called Tc52, which is crucial for parasite survival and virulence, induces human DC maturation. Tc52-treated immature DC acquire CD83 and CD86 expression, produce inflammatory chemokines (IL-8, MCP-1, and MIP-1α, and present potent costimulatory properties. Tc52 binds to DC by a mechanism with the characteristics of a saturable receptor system and signals via TLR2. While Tc52-mediated signaling involves its GSH-binding site, another portion of the molecule is involved in Tc52 binding to DC (Figure 2). In contrast to GPI and GIPLs-induced DC activation via TLR2, TLR2 activation by Tc52 resulted in the secretion of IL-8, MCP-1 and MIP-1α no increased production of TNF-α occurred. This provides the first evidence, to our knowledge, that a protozoan parasite-derived molecule which belongs to the thiol-disulfide oxidoreductase family, by interacting with TLR2 leads to selective release of inflammatory chemokines, a pattern distinct from the classical profile observed in the case of LPS and related molecules such as GPI and GIPLs. Together these data evidence complex molecular interactions between the *T. cruzi*-derived molecule, Tc52, and DC, and suggest that Tc52 and a related class of proteins might constitute a new type of pathogen-associated molecular patterns (PAMPs).

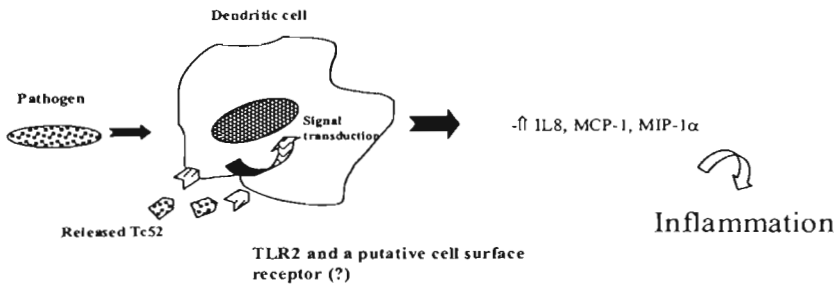

Figure 2. A model for *T. cruzi* Tc52-DC interaction and signaling pathway. The binding of as yet uncharacterized DC surface structure (Tc52R) followed by the interaction of the glutathion binding domain of Tc52 to the Toll-like receptor two (TLR2) led to the activation of the signaling cascade and NF-κB nuclear translocation and regulation of nuclear gene expression.

Mechanisms Leading to the State of Immunosuppression

In mice and humans, the acute phase of Chagas'disease is marked by a state of immunosuppression (Ouaissi *et al.*, 2001). This phenomena is characterized by a decrease of interleukin-2 (IL-2) production, increase of suppressive activity by splenic T cells and macrophages, down regulation of CD3, CD4 and CD8 T cell markers and inhibition of IL-2 receptor (IL-2R) expression in the case of human peripheral blood mononuclear cells (Sztein & Kierszenbaum, 1993). *In vitro* activated T cells from infected mice showed low levels of IL-2 production and proliferative response to mitogens, characteristic of the immunosuppressive response (Tarleton, 1988). In mice, the immunosuppressive activity has been directly involved in the immunodeficiency. In fact, several investigators have documented the notion of suppressive cell populations (T cells, NK cells or macrophages) being involved in the immunosuppression phenomena (Tarleton, 1988; Cerrone *et al.*, 1992; Takle & Snary, 1993). Investigators have demonstrated that prostaglandins were also able to mediate suppression of lymphocyte proliferation and cytokine synthesis during acute *T. cruzi* infection (Pinge-Filho *et al.*, 1999).

Furthermore, several parasite suppressive factors contained in, or released by *T. cruzi* could be responsible for immunological abnormalities (Liew *et al.*, 1988; Serrano & O'Daly, 1990). Indeed, a trypanosomal immunosuppressive factor (TIF), released by the parasite, has been found to suppress the lymphoproliferation and the expression of several molecules involved in lymphocyte activation such as CD3, CD4, CD8, CD25 and the transferrin receptor. Furthermore, this factor (s) with a molecular mass between 30 and 100 kDa was able to curtail a mechanism controlling cell progression through G1 phase (Kierszenbaum *et al.*, 1990, 1998).

Among these parasite-released molecules, a *T.cruzi* protein termed Tc52 (a polypeptide of molecular mass 52 kDa, sharing structural and functional properties with the thioredoxin and glutaredoxin family involved in thiol-disulphide redox reactions), expressed by all *T. cruzi* developemental stages (Figure 3), was shown to suppress T cell proliferation induced by anti-CD3 stimulation (Ouaissi *et al.*, 1995), and exerted several cytokine and chemokine-like activities able to synergize with IFN-γ to stimulate NO production by macrophages (Figure 4) and to modulate IL-1α, IL-12 and IL-10 encoding genes (Fernandez-Gomez *et al.*, 1998) as well as DC chemokine secretion pattern (see above and also Figure 2). NO has a number of other physiological effects that may play a role in the complex host-parasite relationships (James, 1995). Indeed, it has been shown that NO production during Toxoplasmosis in C57BL/6 mice has two opposite effects: being protective against *Toxoplasma gondii* and down-regulating the immune response, suggesting its possible contribution in the establishment of chronic infections (Hayashi *et al.*, 1996).

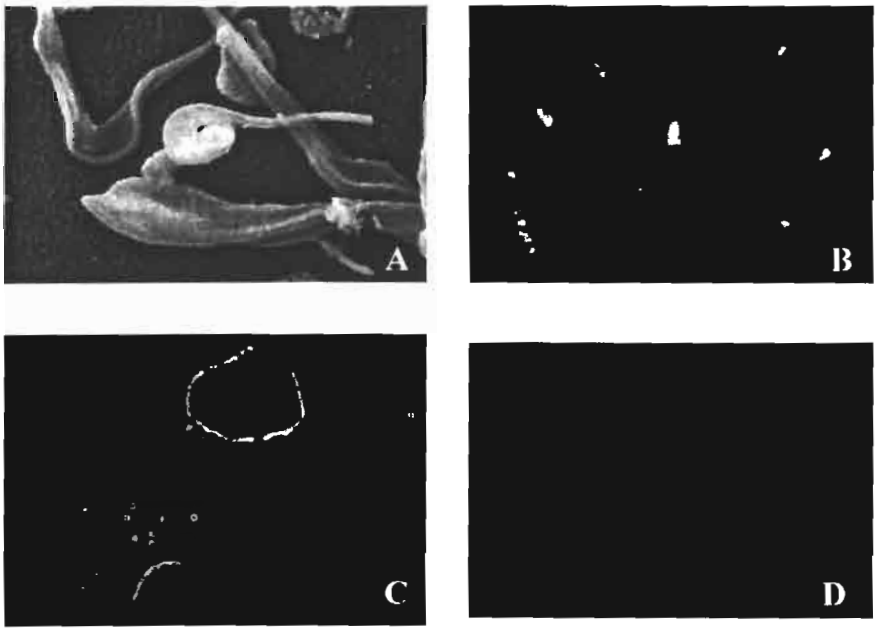

Figure 3. A) scanning electron microscopy analysis of a *T. cruzi* trypomastigotes preparation; B) immunofluorescence staining of *T. cruzi* epimastigotes with anti-Tc52 mouse immune serum showing high density intracellular vesicles containing the reactive Tc52 molecule; C) amastigotes released from ruptured-infected J774 mouse macrophages and the macrophage surface exibited positive immune reactivity with anti-Tc52 immune serum; D) immunofluorescence staining of Tc52-treated J774 macrophages with anti-Tc52 immune serum showing positive labelling on the surface as well as inside the cell.

In the case of *T. cruzi*, previous studies have shown that experimental infection induces NO production, and suggest that IFN-γ and TNF-α are involved in this phenomenon (Munoz-Fernandez *et al.*, 1992a; Petray *et al.*, 1994). Furthermore, independent experiments have shown that IFN-γ and TNF-α mediated activation of macrophages leads to increased production of NO which in turn suppress T cell activation (Abrahamson & Coffman, 1995). Moreover, the participation of NO in the suppression of T cell activation has been reported in a number of other biological systems (James, 1995). Furthermore, NO markedly inhibited the induction of IL-2 promoter, which could account for most of the reduction in IL-2 production, and weakly increased the activation of IL-4 promoter (Chang *et al.*, 1997). This mechanism could be involved in the down-regulation of IL-2 gene expression observed during *T.cruzi* infection (Soong & Tarleton, 1994). Therefore, it is likely that NO production during the initial phase of acute *T.cruzi* infection might participate in the clearance of parasites by macrophages whereas its overproduction during the late phase of acute infection would account for the immunosuppression observed.

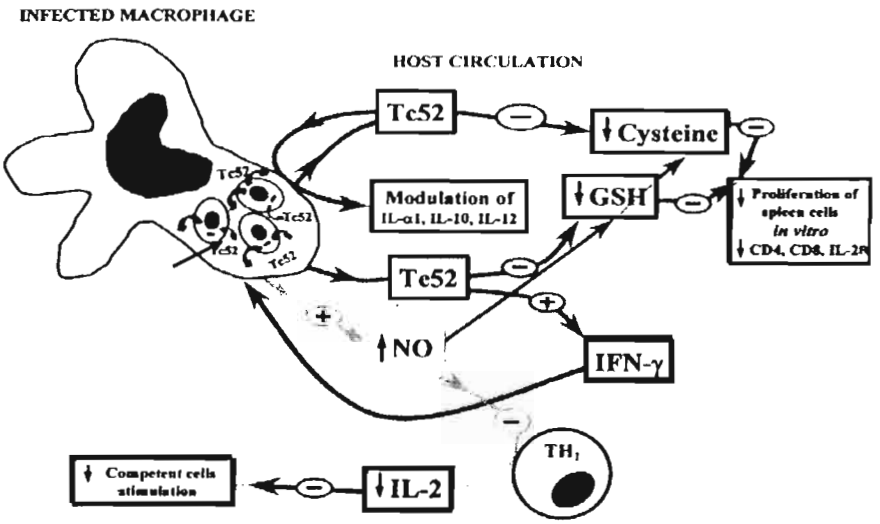

Figure 4. Schematic representation of Tc52 released protein -macrophage interplay.

Another interesting observation is that Tc52 acts directly on macrophages to modulate IL1-α expression. The proinflammatory cytokine IL-1α has potential deleterious effects. Indeed, inappropriate expression of IL-1α in the blood during sepsis correlates with hypotension, shock and death (Dinarello & Wolff, 1993; Ferreira, 1993). Moreover, evidences showing that NO stimulates the release of IL-1α from activated macrophages has beeen recently reported (Hill *et al.*, 1996). Taken together, these observations and our data may suggest that Tc52 could trigger a feed-back loop amplification for the production of the major proinflammatory cytokine IL-1α. However, due to the potential deleterious effects of IL-1α, its activity is regulated at the levels of synthesis, processing and release, and by a naturally occurring receptor antagonist (IL-1Rα) and serum proteins such as α2-macroglobulin (Dinarello, 1993; Arend *et al.*, 1994) whose production has been shown to increase during *T.cruzi* infection (Araujo-Jorge *et al.*, 1992).

These observations may have some implications *in vivo*. Indeed, we showed that elevated levels of circulating Tc52 in the blood of *T. cruzi* experimentally infected mice occurred during the acute phase of the disease and were associated with decreased responsiveness of T cells to mitogen or anti-CD3 stimulation (Ouaissi *et al.*, 1995). Thus, it is reasonable to assume that exogenous Tc52 might participate directly or indirectly at least via NO production in the immunosuppression observed during *T. cruzi* infection. However, it is noteworthy that Chagas'disease almost exclusively involves intracellular amastigotes, which also expressed Tc52 protein. Experiments carried out with murine macrophages harboring a eukaryotic plasmid carrying the *Tc52* gene, showed increased IL-10 mRNA levels (Borges *et*

al., 2001). It is tempting to speculate that Tc52-induced increased IL-10 secretion might participate in the downregulation of IL-2 production. This is in agreement with previous studies showing that murine IL-10 can downregulate the host immune response by decreasing the production of IL-2 (Fiorentino *et al.*, 1991) and inhibiting mitogen driven T-cell proliferation (Ding & Shevach, 1992).

Although the basic molecular and immunological approaches have revealed interesting features regarding the cytokine and chemokine-like activities of Tc52, we thought that further *in vivo* functional studies were needed to ascertain its biological function (s). Thus, genetic manipulation of *T. cruzi* allowed us to produce parasite clones lacking a Tc52 protein-encoding allele ($Tc52^{+/-}$) (Allaoui *et al.*, 1999). Subsequently, the disease phenotype in $Tc52^{+/-}$- infected BALB/c mice, during the acute and chronic phases of the disease was examined. The results obtained are in agreement with the observations made when using *in vitro* experimental models. Indeed, a lack of suppression of IL-2 production and of T-cell proliferation inhibition was observed in the case of spleen cells from $Tc52^{+/-}$-infected mice when compared to the wild-type (WT) parasite infected ones. Also, increased production of IL-10 was observed in the case of spleen cells from WT-infected mice, whereas the levels measured in the case of $Tc52^{+/-}$-infected mice were comparable to those of normal mice spleen cells, suggesting therefore, that Tc52 play a role in IL-10 cytokine regulation during *in vivo T. cruzi* infection (Garzon *et al.*, 2003). Therefore, it is reasonable to suggest that the reduction of Tc52 production by gene targeting which in turn downregulates the IL-10 synthesis could be among the mechanisms participating in the immunoregulatory mechanisms leading to the control of IL-2 production.

We have already shown that Tc52, under conditions of experimental infections, appeared relatively immunologically silent during the early acute phase, failing to elicit significant levels of antibodies and lymphocyte proliferation (Ouaissi *et al.*, 1995). This observation allowed us to make the hypothesis that the analysis of structure-function relationship in the Tc52 molecule could reveal discrete domains, which either contribute to minimize its antigenicity and/or act as an immunoregulatory factor. Studies conducted showed that, indeed, a major peptide fragment of 28 kDa molecular mass (Tc28k) localized in the carboxy-terminal portion of Tc52 carry the inhibitory capacity in T cell activation (Borges *et al.*, 2003). Synthetic peptides spanning the amino terminal or carboxy-terminal domain of Tc52 protein indicated that the activity mapped to the 432-445 aa residues of Tc52 sequence. Moreover, the peptide when coupled to a carrier protein, exhibited increased inhibitory activity on T lymphocyte activation. Interestingly, the coupled peptide significantly down regulated IFN-γ and IL-2 secretion. Likewise, in immunized mice, the coupled peptide 432-445 was a very poor B and T cell antigen compared to the other Tc52 derived peptides. Therefore, the immunomodulatory portion of *T.cruzi* Tc52 virulent factor seems to reside, at least in part, in a conserved sequence within its carboxyl-terminal domain which could minimize its antigenicity (Borges *et al.*, 2003). Such molecules may permit parasites to escape immune surveillance and to grow unimpeded by normal immune responses. Moreover, the impairment of multiple immune effector functions by blocking the signal transduction pathways utilized by cytokines such as IL-2 and IFN-γ, the host may become more susceptible to opportunistic infections as well.

Autoimmune Reactions

A number of investigators have proposed autoimmunity as the major contributor to Chagas'diease pathology (Kierszenbaum, 1999). *T. cruzi* infection causes lysis of cardiac myocytes, releasing cardiac antigens, such as myosin, considered to be the major autoantigen. Moreover, the pro-inflammatory environment induced by the presence of inflammatory factors as chemokines and cytokines are also fundamental conditions to this process (Cunha-Neto *et al.*, 1995). Indeed, the pro-inflammatory reactions may induce proliferation of autoreactive T cells, in response to self antigens presented on the host APC. Further, the elevated levels of myocardial antigens, in the presence of pro-inflammatory cytokines, lead to increased presentation of self antigens and stimulation and expansion of autoreactive cells (Fedoseyeva *et al.*, 1999). Furthermore, 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 became activated and initiate autoimmunity (Leon & Engman, 2001).

The "molecular mimicry" is another possibility leading to the autoimmune reactions. Indeed, *T. cruzi* antigens may share amino-acid (aa) sequences or three-dimensional epitopes with host components (Table 1). These shared peptides may initiate a cross-reactive T cell response leading to activation of autoreactive T cells. However, others argue that this mechanism is less than convincing (Benoist & Mathis, 2001).

Table 1. Molecular mimicry in Chagas'disease

T. cruzi molecule	Host component
α-Gal residues	α-Gal, EVI [(1)]
Sulphated glycolipids	Neurons [(1)]
FI-160	47 kDa neuronal protein [(1)]
Ribosomal P protein	Ribosomal P protein (Hela cell line) [(1)]
23 kDa ribosomal protein	23 kDa ribosomal protein (Hela cells reticulocytes) [(1)]
Microtubule-associated proteins	Microtubule-associated proteins (brain) [(1)]
Ribosomal P0 protein	β1-adrenoreceptor [(1, 2)]
Carboxyl-terminal end of ribosomal P proteins	β1-adrenoreceptor [(3, 4)]
B13 protein	Human cardiac myosin heavy chain [(1, 5)]

[(1)] Kalil & Cunha-Neto, 1996. [(2)] Skeiky *et al.*, 1992. [(3)] Motrán *et al.*, 1998. [(4)] Lopez Bergami *et al.*, 2001. [(5)] Cunha-Neto *et al.*, 1995.

The precise mechanism responsible for myocardial and conducting damages in Chagas'disease is not completely understood. The existence of autoantibodies directed against host tissues during *T. cruzi* infection is well documented. On the other hand, several observations suggested the possible involvement of autoreactive cell-mediated immunity in the production of Chagasic cardiomyopathy (Ouaissi *et al.*, 2001). The presence of antoanti-Idiotype (Id) T cells in Chagas'patients has been demonstrated (Gazzinelli *et al.*, 1988). The authors suggested that Id-anti-Id interactions may play a role in determinning the pathogenesis of Chagas, cardiomayopathy. In a previous report we have shown that

acetylcholinesterase (AChE) exhibited immunological cross-reactivity with *T. cruzi* (Ouaissi *et al.*, 1988). Moreover, antibodies to AChE and anti-idiotypic antibodies were detected in sera of patients presenting the chronic cardiac form of the disease. The data provide a biochemical basis supporting, in part, the denervation hypersensitivity in Chagas'disease. In addition, it provided the notion of an Id-anti-Id regulation of conducting tissue damage during the course of *T. cruzi* infection.

Induction of effector functions by T cells requires two signals provided by antigen-presenting cells (APC) (Mueller *et al.*, 1989): signal 1 is delivered by T-cell receptor (TCR), which recognizes specific peptides presented on MHC molecules; signal 2 is provided by some integral membrane proteins: APC-like lymphocyte functional antigen 3 (LFA-3), intercellular adhesion molecule 1 (ICAM-1), ICAM-2 and ICAM-3, or B7-1/2 and co-receptor molecules such as CD2, LFA-1, and CD28 (Damle *et al.*, 1992; Freeman *et al.*, 1993). It was shown that infection with *T. cruzi* was able to up-regulate B7-2 molecules on macrophages and, in this way, enhance their costimulatory activity (Frosch *et al.*, 1997) and increase the expression and secretion of ICAM-1 in inflammatory cells, as well as cardiac myocytes in infected mice (Laucella *et al.*, 1996). Moreover, an increase in the expression of ICAM-1, LFA-1, integrin VLA-4 and vascular cellular adhesion molecules (VCAM-1) in chagasic hearts from infected experimental animals has been demonstrated and associated with a persistent production of inflammatory and anti-inflammatory cytokines from the early acute stage through the late chronic stage of infection (Zhang & Tarleton, 1996).

Polyclonal Activation

T. cruzi infection is associated with a poly-isotypic production of non-specific immunoglobulins as well as parasite specific response. This response is characterized essentially by a predominance of IgM, IgG2a and IgG2b that persists during all the chronic phase (El Cheikh *et al.*, 1992). During the acute phase of infection, the parasitaemia level decreases quickly coinciding with increased antibody levels, suggesting the protective role of antibodies against blood forms of the parasite (Krettli & Brener, 1976; Brener, 1980). Moreover, animals producing weak levels of antibodies exhibited a high susceptibility to *T. cruzi* infection (Kierszenbaum & Howard, 1976). Indeed, the transfer of immune sera to athymic mice delayed the appearance of parasites and increased mouse survival (Kierszenbaum, 1980). Indirect evidence supported the implication of specific antibodies in the control of infection. For example, *in vivo* transfer of spleen cells from mice which had recovered from the acute phase of infection to naïve mice induce protection against challenge infection, removal of B lymphocytes abolished the immune protection (Scott, 1981). Nevertheless, studies during *T. cruzi* mouse infection have demonstrated clearly an important non-specific B and T lymphocytes polyclonal activation (Ortiz-Ortiz *et al.*, 1980; D'Imperio Lima *et al.*, 1985, 1986).

The non-specific polyclonal lymphocyte activation that occurs during infection plays a role in the pathogenesis of Chagas' disease. Extensive polyclonal B and T cell activation occurred during the acute and chronic phases of *T. cruzi* infection (Minoprio *et al.*, 1986a, 1986b). Simultaneously, massive polyclonal activation also occurred in minor lymphocyte subsets, such as CD5B and γδ T lymphocytes, which are associated with autoimmune

reactivity and pathological processes (El Cheikh *et al.*, 1992; Santos Lima & Minoprio, 1996). In the early stages of infection, the number of immunoglobulin-secreting cells in the spleen and peripheral lymph nodes is very high and the majority of activated B cells secrete antibodies nonspecific for the parasite antigens (Reina-San-Martin *et al.*, 2000; el Bouhdidi *et al.*, 1994). Isolation of lipopolysaccharide (LPS)-like substances from *T. cruzi* has been reported (Goldenberg *et al.*, 1983). An octadecapeptide derived from the sequence of an 85-kDa surface protein from *T. cruzi* trypomastigotes has been shown to be able to act as a comitogenic molecule (Pestel, *et al.*, 1992). Recent studies have also shown that *T. cruzi* Tc24 released protein induced an increase in the number of B cell secreted immunoglobulins mainly of IgM isotype in Tc24 treated mice (Da Silva *et al.*, 1998). It is well known that the antigenic complex composition of *T. cruzi* parasites can contribute to the polyclonal activation resulting in a "hyperstimulation" of lymphocyte clones directed against a multitude of challenging antigens. As a consequence, a panclonal activation of the immune system develops resulting in a nonspecific B cell response during experimental infections (Cordeiro-da-Silva *et al.*, 2002; Requena *et al.*, 2000). These responses lead to the expansion of antiself clones that may be responsible for the killing of parasitised and nonparasitised cells. High cellularity (60-70% of lymphocytes) is concomitant with very large lymphocyte activation (Minoprio, 1986a). In this phase of infection, the B cell polyclonal proliferation is characterised by a typical isotypic profile, IgG2a and IgG2b, in spleen and lymph nodes. The hybridomas produced by spleen B cells or lymph node T cells of infected mice fail to bind parasite Ag but are autoreactive clones, in such a way that the antibodies recognize natural structures like actin, tubulin, myosin, keratin, myoglobulin, thyroglobulin or myelin (Reina-San-Martin *et al.*, 2000 and Ouaissi *et al.*, 2001). Moreover, a recent study has demonstrated that a protein from *T. cruzi* named TcPA45 induced polyclonal activation. Moreover, the TcPA45 was shown to be a eucaryotic proline racemase hortologue, allowing the parasite to synthesize and express itself on its surface proteins containing D-proline, an aminoacid resistant to host-induced proteolytic mechanisms, and that this enzymatic activity was necessary for mitogenic activity (Reina-San-Martin *et al.*, 2000).

Apoptosis as a Possible Process Involved in the Immune System Dysegulation

Programmed cell death (PCD) seems of great importance in the field of immunology and cell biology of protozoan parasites. Indeed, in the last few years, a large amount of evidence supported the notion that apoptosis of immune cells could be among factors which help the parasite to avoid the elimination favoring, therefore, its persistence in the immune host. Apoptosis can occur in a variety of cell types during the acute phase of *T. cruzi* infection (Zhang *et al.*, 1999; Ameisen *et al.*, 1995). However, apoptosis observed in immune cells, such as macrophages, lymphocytes and interstitial dendritic cells, may have functional significance different from that found in cardiac myocytes and endothelial cells, such as regulation and modulation of inflammatory responses (Zhang *et al.*, 1999). Lopez and co-workers have observed that splenic CD4+ T cells from mice infected with *T. cruzi* undergo apoptosis *ex-vivo* upon stimulation (Lopes *et al.*, 1995), a fact that allowed the authors to

suggest that this phenomenon may participate in the immune deficiency *in vivo*, helping, the parasite in this way to persist during the chronic phase of the disease. Moreover, spleen CD4+ T cells apoptosis in *T. cruzi*-infected BALB/c mice, was due to increased expression of Fas and Fas ligands (FasL), two markers known to be essential to the PCD. It was also demonstrated that, Fas: FasL pathway controls parasite replication *in vitro* and prevents an exacerbated Th2 type immune response to the parasite (Lopes *et al.*, 1999). Furthermore, it was dcmonstrated that IFN- γ is able to modulate Fas and Fas-L expression and nitric oxide-induced apoptosis during *T. cruzi* infection (Martins *et al.*, 1998). Furthermore, parasite or host-derived factors could be involved in apoptosis of immune cells.

During the last few years, a number of investigations have focused on the study of an important family of proteins from *T. cruzi*: Trans-sialidse (TS) and mucin families (Frasch, 2000). *T. cruzi* is unable to synthesize *de novo* the monosaccharide sialic acid, but can incorporate sialic acid derived from the host (Frash, 1994). The parasite expresses TS able to catalyse the transfer of sialic acid from host glycoconjugates to mucin-like molecules located on the parasite surface membrane. TS is released by the parasite into the external milieu as a soluble factor (Frasch, 2000). TS is expressed in the invasive trypomastigote stage and is defined by two regions: a globular amino-terminus of about 640 amino acids containing the catalytic activity and a variable number of repeated highly antigenic motifs of 12 amino acid named SAPA located at the C terminus (Frasch, 2000). Members of the TS-like family in the intracellular amastigote stage stimulate a CD8+ cytotoxic T-cell response (Wizel *et al.*, 1997) demonstrating that these antigens enter the class I pathway in infected cells. Moreover, it was found that members of TS and TS-like families induced an antibody response against the parasite (Cazzulo & Frasch, 1992). However, it was demonstrated that TS, through its C-terminal of long tandem repeats induced an abnormal polyclonal B cell activation and Ig secretion (Gao *et al.*, 2002). SA85-1.1 surface protein belonging to TS family of *T. cruzi*, has been shown to stimulate a polarized Th1 response to become anergic (Millar *et al.*, 1999). Further study has identified an epitope of SA85-1.1, named epitope 1, able to protect mice and stimulating 4% to 6% of the splenic CD4+ cells during *T. cruzi* infection (Millar *et al.*, 2000).

Furthermore, independent investigators have shown that TS was able to induce apoptosis *in vivo* in spleen, thymus and peripheral ganglia (Leguizamon *et al.*, 1999). In contrast, another study showed that TS blocked activation-induced cell death in CD4+ T cells from *T. cruzi*-infected mice requiring CD43 signaling (Todeschini *et al.*, 2002). Further, depending of the host cell type infected by *T. cruzi*, TS could exert an anti or pro-apoptotic function. For example, TS is able to enter into synergism with neurocytokines to prevent neuron and Schwann cell apoptosis (Chuenkova & Pereira, 2000; Chuenkova *et al.*, 2001). Alternatively, TS was responsible for the early thymic alterations via apoptosis induction in the "nurse cell complex" (Mucci *et al.*, 2002).

Further, GIPL-derived ceramide from *T. cruzi* has been reported to be able to synergize with IFN- γ to induce intense macrophage apoptosis (Freire-de-Lima *et al.*, 1998). Moreover, the demonstration of increased parasite replication inside macropahges as a result of apoptotic cells uptake, confirms the important contribution of apoptosis in the maintenance of *T. cruzi* in the immune host (Freire-de-Lima *et al.*, 2000). However, it has also been postulated that infected cells were still able to counteract the parasite by either initiating their

own death by apoptosis and/or being more easily recognized and phagocytosed by macrophages. This led to the parasite elimination from the infected cell. It has also been reported that the protozoan parasite, by activating the expression of heat shock proteins or NF-kappa B, could interfere with molecules of cellular death machinery, thus regulating the transcription of anti-apoptotic molecules (Heussler *et al.*, 2001).

Conclusion

T. cruzi can elicit a complex series of cellular interactions which result in specific and non-specific immune responses. The immunosuppression appears to predominate at certain phases of the infection. Relieving these immunological alterations could provide the means to restore the efficacy of the immune protection mechanisms. It is hoped that the identification of target genes that induce immune dysfunction and their mode of action will help to develop new therapeutic interventions.

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