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Review

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Molecular basis of *Trypanosoma cruzi* and *Leishmania* interaction with their host(s): exploitation of immune and defense mechanisms by the parasite leading to persistence and chronicity, features reminiscent of immune system evasion strategies in cancer diseases

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Summary

A number of features occurring during host-parasite interactions in Chagas disease caused by the protozoan parasite, *Trypanosoma cruzi*, and Leishmaniasis, caused by a group of kinetoplastid protozoan parasites are reminiscent of those observed in cancer diseases. In fact, although the cancer is not a single disease, and that *T. cruzi* and *Leishmania* are sophisticated eukaryotic parasites presenting a high level of genotypic variability, the growth of the parasites in their host and that of cancer cells share at least one common feature, that is their mutual capacity for rapid cell division. Surprisingly, the parasitic diseases and cancers share some immune evasion strategies. Consideration of these immunological alterations must be added to the evaluation of the pathogenic processes. The molecular and functional characterization of virulence factors and the study of their effect on the arms of the immune system have greatly improved understanding of the regulation of immune effectors functions. The purpose of this review is to analyze some of the current data related to the regulatory components or processes originating from the parasite that control or interfere with host cell physiology. Attempts are also made to delineate some similarities between the immune evasion strategies that parasites and tumors employ. The elucidation of the mode of action of parasite virulence factors toward the host cell allow not only provide us with a more comprehensive view of the host-parasite relationships but may also represent a step forward in efforts aimed to identify new target molecules for therapeutic intervention.

Key words: **trypanosomatids • virulence factors • cancer cells • immunoregulation**

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INTRODUCTION

Trypanosoma cruzi, the causative agent of Chagas' disease, is a parasitic protozoan that infects more than 18 million people in South and Central America[53]. The parasite replicates as an epimastigote in the midgut of its insect vector and transforms to a metacyclic infective trypomastigote in the hindgut. In the mammalian host cells, dividing amastigotes are found which transform into infective trypomastigotes prior to release into the bloodstream. The 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 as well as degeneration of cardiac muscle[12, 86].

Leishmaniasis comprises a group of several different diseases which are widely distributed in tropical and subtropical areas and also are commonly found in the Mediterranean basin[37]. The extent and severity of this group of diseases designate Leishmaniasis as an important health problem. *Leishmania* sp., which causes the diseases, are kinetoplastid protozoan parasites transmitted by a blood-feeding dipteran vector of the sub-family *Phlebotominae*. This parasite has a complex life cycle that includes different morphological forms. Within the insect vector, the parasite replicates as a non-infective promastigote which transforms into an infective metacyclic promastigote. In the mammalian host, the infective promastigotes invade the macrophages and differentiate into amastigotes, which are the proliferative forms within the vertebrate host.

The parasites *T. cruzi* and *Leishmania* sp. produce a number of interesting pathogenic features in man and different animal models and offer the opportunity of studying parameters involved in pathogenesis and disease. Although the exact role of the host immune system in the pathogenesis is still not fully understood, it is generally accepted that the immune system plays an important role in protection from parasitic infection. This is supported by evidence, at least in experimental models, that a deficiency or defect in cellular and/or humoral immune responses to *T. cruzi* or *Leishmania* contributes to the progress of Chagas' disease and Leishmaniasis[37, 86]. It is the purpose of the present report to review some of the data concerning the parasite factors that interfere with the host immune protective mechanisms and their mode of action, and put them in perspective with points of interest for further studies that will more fully explore possible ways to interfere with their biological activities. Attempts will also be made to delineate some similarities shared by parasites and tumor cells in terms of immune evasion strategies.

COMPLEMENT EVASION BY PARASITES

It has been known for many years that *T. cruzi* and *Leishmania* evade and subvert the complement system of their vertebrate hosts[35]. This process is among the first means whereby the parasites avoid direct killing by serum factors, such as the complement system. The complement resistance is developmentally regulated. Indeed, whereas the vector-stage epimastigotes of *T. cruzi* and the *Leishmania* promastigotes (which will not be naturally exposed to complement) are susceptible to complement-mediated killing through an apoptosis-like death[4], infective metacyclic trypomastigotes and metacyclic promastigotes, the vertebrate infective forms, are resistant[38].

In the case of *T. cruzi*, different laboratories have reported that trypomastigotes produce complement--regulatory molecules which interfere with the complement activation cascade[38]. In fact, the pioneering observations of Joiner et al.[45] have shown that tissue culture-derived trypomastigotes produce factors that interfere with the function of classical and alternative pathway C3 convertases. These studies also indicated that the parasites shed into the culture medium a limited number of components, ranging 87–155 kDa, the most prominent molecules being 87–93 kDa, which accelerate the intrinsic decay of the alternative and classical pathway C3 convertases. By exhibiting these functions, the parasite proteins behave in a manner analogous to the human membrane regulatory protein decay-accelerating factor. In subsequent studies, Norris and Schrimpt[63] characterized a parasite membrane (185 kDa) and a soluble form (160 kDa) of a complement regulatory protein (CRP) that mimics the host CRP protein family which serves to prevent complement-induced lysis of autologous cells. The parasite CRP interferes with the activation of the alternative and classical complement pathways by binding complement components C3b and C4b, thus preventing the assembly of the C3 convertases. In a series of elegant studies it was shown that the antibodies that block the CRP-C3b interaction were able to induce high levels of complement-mediated lysis of trypomastigotes[61, 62]. Furthermore, stable transfection of the complement-sensitive *T. cruzi* epimastigote forms with a plasmid carrying the trypomastigote--specific CRP gene confers complement resistance[60], thus demonstrating the CRP to be among the *T. cruzi* virulence factors.

In previous studies we have shown that *T. cruzi* trypomastigotes express on their surface and release a glycoprotein (gp58) able to inhibit the formation of the cell-bound and fluid-phase alternative pathway

C3 convertases[34]. The protein has no inhibitory effect on classical pathway C3 convertase activity. gp58 differs from CRP and the 87–93 kDa components by its molecular size and mechanism of action: 1) gp58 did not accelerate decay-dissociation of preformed C3b, Bb and C3b, Bb and P sites; 2) it did not inhibit the formation of classical pathway C4b, 2a convertases sites; and 3) it did not incorporate into membranes of sheep red blood cells. No specific binding of gp58 to cell-bound C3b could be evidenced; functional studies favored the possibility of gp58 exerting its inhibitory activity through an interaction with factor B rather than C3b.

In addition to the inefficient activation of the complement cascade, the C5b-9 complex could be generated in some cases, but the parasites still remain resistant to complement-induced lysis. This is the case of the infective *L. major* metacyclic promastigotes which, although able to assemble C5b-9 on their surface, resist complement lysis, whereas the non--infective log-phase promastigotes are killed[35]. C3b is deposited on the prominent surface glycolipid, lipophosphoglycan (LPG), a developmentally regulated surface component of the parasite which changes in terms of chemical composition, being densely packed and extending further from the membrane of the infective promastigote form compared with the log-phase parasites, thus rendering the membrane distant from the C5b-9 complex. Moreover, LPG and C5b-9 are spontaneously released from the promastigote surface, an additional factor leading to complement resistance[35]. Another intriguing finding was the fact that a protein kinase-1 (LPK-1) isolated from *L. major* promastigotes was able to phosphorylate purified human C3, C5, and C9[41]. Phosphorylation of C3 by LPK-1 reduces the rate at which trypsin cleaves C3 to C3a and C3b. Given that trypsin mimics the action of C3 convertase, it might be postulated that phosphorylation can regulate the susceptibility of complement proteins to proteolytic cleavage, leading to the activation cascade and, ultimately, to the parasite killing.

In a recent study it was shown that a *Leishmania* surface antigen 2 (PSA-2), sharing leucine repeat motifs with another major component of the promastigote surface, proteophosphoglycan (PPG), binds to the murine and human complement receptor CR3, thus participating in the invasion of the host cell by the parasite[47]. Since LPG, PPG, and PSA-2 are expressed extracellularly, in secreted and membrane-bound forms, it might be interesting to explore whether PSA-2 could interfere with the complement activation pathways and, if so, whether this could participate in the evasion mechanisms leading to parasite survival. Taken together, these observations support the notion that the infective stages of *T. cruzi* and *Leishmania* have developed mechanisms that confer to the parasite the capacity to avoid the deleterious effect of the first barrier, i.e. complement-mediated killing.

ANTIGENIC "DISGUISE"

Although the acquisition of host molecules by certain parasites, such as schistosomes, during their life in their vertebrate host, a fact which was assumed to limit access of immune effectors to target antigens[16], nonspecific or specific binding of the host serum of tissue components have also being reported in the case of *T. cruzi* and *Leishmania* parasites[68, 69, 90]. We have also observed (Ouaissi, unpublished data) that *T. cruzi* trypomastigotes, when cultured *in vitro* with the human T lymphocyte clone LDG4 isolated from a chronic systemic lupus erythematous patient[73], could acquire the CD2 cell surface marker (Fig. 1). Whether this molecule could help the parasite to interact with other cell types is not yet known. Furthermore, *L. major* amastigotes derived from BALB/c mice lesions were found to be covered with different immunoglobulin isotypes, such as IgG, IgM, and IgA[52]. Reversible acquisition of a sarcoma-associated antigen by *T. cruzi* amastigotes when cultured in a mesodermal WOS cell line was also reported[19]. Although it is not known whether the host molecules acquired by *T. cruzi* and *Leishmania* could contribute

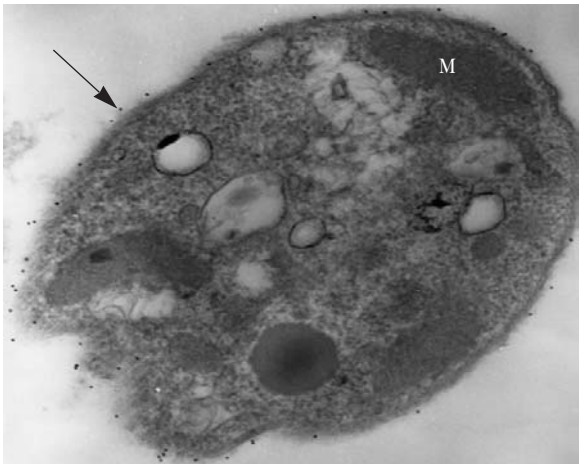

Figure 1. Electron micrograph of *T. cruzi* trypomastigote sections showing surface localization of acquired CD2 epitopes. A human T cell clone (LDG4) was incubated *in vitro* with *T. cruzi* trypomastigotes for 36 h at 37°C. The parasite-cell suspensions were washed and fixed in 2% paraformaldehyde-0.1% glutaraldehyde, dehydrated in ethanol, and embedded in Lowicryl K4M at –30°C. Ultrathin sections were collected on carbon-parlodion-coated nickel grids and floated on PBSO (1% ovalbumin in PBS). They were then treated with anti-CD2 mAb and protein-A-gold as reported elsewhere[71]. The gold particles bound to the parasite membrane indicates the presence of CD2 epitopes (arrow). M – mitochondria.

as an "antigenic disguise" to escape host immune attack, it is noteworthy that most of these molecules help the parasite to enter the host cell and reach the sanctuary of subcellular compartments or tissues (Fig. 2).

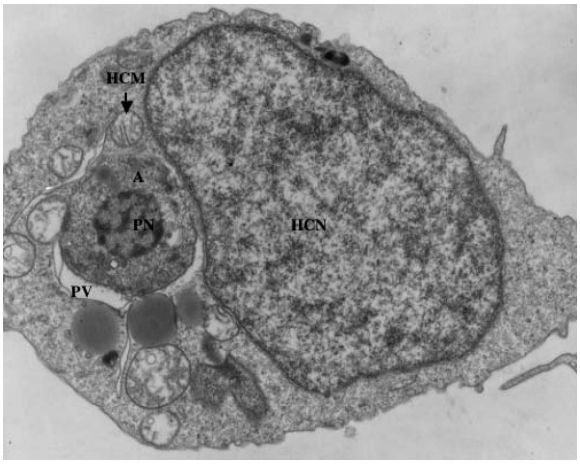

Figure 2. Transmission electron microscopy (TEM) of a murine macrophage J774 cell line infected *in vitro* with *T. cruzi*. Cells were processed for TEM as described elsewhere[71]. The electron micrograph shows an amastigote (A) inside a prasitophorus vacuole (PV). In some places the parasite is in contact with the host cell cytoplasm. Abbreviations: HCM – host cell mitochondria, HCN – host cell nucleus, PN – parasite nucleus. Magnification: ×11000.

In contrast to the antigenic variation that occurs in the case of African trypanosomes, *T. cruzi* and *Leishmania* sp. express antigenic polymorphism. For example, the *T. cruzi* SA85-1 proteins (a subfamily of the *T. cruzi* sialidase surface protein superfamily) encode variant epitopes that may also inhibit T cell responses[46].

Cancer cells also have the capacity to undergo antigenic modulation so that antibodies may induce the disappearance of membrane-associated determinants of neoplasic cells, a mechanism which allows, for example, murine leukemia cells to "escape" antibody-mediated destruction[22]. Tumor-associated antigens (TAAs) can modulate their expressions on the cell surface depending on different phases of the cell-cycle. This phenotypic variation and modulation of TAA expression among, and within, carcinomas may have important implications in the therapy of cancer[39].

IMPAIRMENT OF PROTECTIVE IMMUNITY

Infection of inbred mice with *T. cruzi* leads to an acute infection characterized by a severe immune depression[70]. The suppressive effects of *T. cruzi* infection decreases in magnitude once the host has entered the chronic phase. This is of particular relevance to humans because the chronic phase of Chagas' disease in man can last for 20 years or more. Furthermore, a state of immunosuppression also occurred during Leishmaniasis. Indeed, in human visceral Leishmaniasis, antigen-specific immunosuppression during the acute phase of the disease appears to be induced by a cell-mediated immune response[17].

In recent years there has been much interest in determining the mechanisms by which *T. cruzi* and *Leishmania* parasites suppress or evade the immune system of the host. Both parasites have been reported to shed significant amounts of soluble molecules into the external milieu both *in vitro* and *in vivo*. Some of these shed molecules act as immunosuppressive agents, thus indirectly promoting parasite survival and growth.

ALTERATION OF NITRIC OXIDE PRODUCTION AND CYTOKINE SECRETION PROFILES

Some of the released parasite molecules cause derangement of growth-control processes (i.e. cell--cycle inhibitors) and/or exert chemotactic activity towards mobile inflammatory cells. The mononuclear phagocyte system comprises a wide range of cell types, and cells of this lineage are involved in host defense against microbial invaders and in the recognition phase of specific immunity, among other functions[57, 87]. In recent years we have examined the mechanism(s) of action of a number of *T. cruzi*- and *Leishmania* sp.-released molecules found to act as regulatory factors (activation/inhibition) of host immune cells (i.e. T and/or B cells, macrophages, dendritic cells)[23, 66, 70].

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) was shown to suppress T cell proliferation induced by anti-CD3 stimulation[65] and exerted several cytokine- and chemokine-like activities, being able to synergize with interferon (IFN)-γ to stimulate nitric oxide (NO) production by macrophages and to modulate genes encoding interleukin (IL)-1α, IL-12, and IL-10[31]. A large number of reports have documented the role of NO in host defense against pathogens, including viruses, bacteria, parasites, fungi, and helminthes. However, NO has a number of other physiological effects that may play a role in the complex host-parasite relationships[44]. Indeed it has been shown that NO production during toxoplasmosis in C57BL/6 mice has two opposing effects, being pro-

tective against *Toxoplasma gondii* and down-regulating the immune response, suggesting its possible contribution in the establishment of chronic infections[40].

In the case of *T. cruzi*, previous studies have shown that experimental infection induces NO production and suggest that IFN-γ and tumor necrosis factor (TNF)-α are involved in this phenomenon[56, 72]. 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[1]. Moreover, the participation of NO in the suppression of T cell activation has been reported in a number of other biological systems[44]. 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[18]. This mechanism could be involved in the down-regulation of IL-2 gene expression observed during *T. cruzi* infection[82]. 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 observed immunosuppression.

Another interesting observation is that Tc52 acts directly on macrophages to modulate IL-1α 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 mortality[28, 32]. Moreover, evidence showing that NO stimulates the release of IL-1α from activated macrophages has been recently reported[42]. Taken together, these observations and our data may suggest that Tc52 could trigger feedback-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 naturally occurring receptor antagonist (IL-1Rα) and serum proteins such as α2-macroglobulin[6, 27], whose production has been shown to increase during *T. cruzi* infection[5].

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[65]. 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 *Tc52* gene showed increased IL-10 mRNA levels[11]. It is tempting to speculate that Tc52-induced increased IL-10 secretion might participate in the down-regulation of IL-2 production. This is in agreement with previous studies showing that murine IL--10 can down-regulate the host immune response by decreasing the production of IL-2[33] and inhibiting mitogen-driven T cell proliferation[29].

Although the basic molecular and immunological approaches have revealed interesting features regarding the cytokine- and chemokine-like activities of Tc52, we though that further *in vivo* functional studies were needed to ascertain its biological function(s). Given that we have already established by genetic manipulation *T. cruzi* clones lacking a Tc52 protein-encoding allele ($Tc52^{+/-}$)[3], we decided to examine the disease phenotype in *Tc*$52^{+/-}$-infected BALB/c mice during the acute and chronic phases of the disease. 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 *Tc*$52^{+/-}$--infected mice when compared with wild-type (WT) parasite-infected ones. Moreover, 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 *Tc*$52^{+/-}$-infected mice were comparable to those of normal mice spleen cells, thus suggesting that Tc52 plays a role in IL-10 cytokine regulation during *in vivo T. cruzi* infection[36]. Therefore it is reasonable to suggest that the reduction in Tc52 production by gene targeting, which in turn down-regulates IL-10 synthesis, could be among the mechanisms participating in the immunregulatory mechanisms leading to the control of IL-2 production (Fig. 3).

Given that Tc52, under conditions of experimental infections, appears immunologically relatively silent during the early acute phase, failing to elicit significant levels of antibodies and lymphocyte proliferation, we hypothesized that analysis of the structure--function relationship in the Tc52 molecule could reveal discrete domains which either contribute to minimize its antigenicity and/or act as immunoregulatory factors. The studies conducted showed that indeed a major peptide fragment of 28 kDa molecular mass (Tc28k), localized in the carboxy-terminal portion of Tc52, carries the inhibitory capacity on T cell activation. Synthetic peptides spanning the

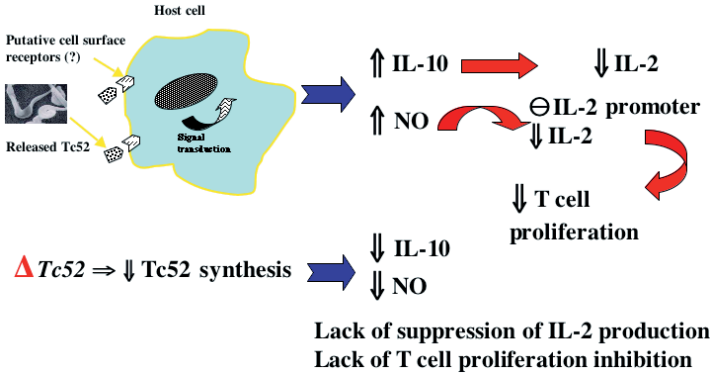

Figure 3. A model of *T. cruzi* Tc52-host cell interaction and signaling pathways.

amino terminal or carboxy-terminal domain of Tc52 protein indicated that the activity mapped to Tc52 residues 432-445. Moreover, the peptide, when coupled to a carrier protein, exhibited increased inhibitory activity on T lymphocyte activation. Interestingly, the coupled peptide significantly downregulated IFN-γ and IL-2 secretion. Likewise, in immunized mice the coupled 432-445 peptide was a very poor B and T cell antigen compared with the other Tc52--derived peptides. Therefore the immunomodulatory portion of the *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[10]. Such molecules may permit parasites to escape immune surveillance and to grow, unimpeded by normal immune responses. Moreover, with the impairment of multiple immune effectors functions by blocked signal transduction pathways utilized by cytokines such IL-2 and IFN-γ; the host may become more susceptible to opportunistic infections as well.

Since dendritic cells (DCs) are the most potent antigen-presenting cells (APCs), we reasoned that it might be interesting, when considering the development of vaccination strategies, to explore the possible interaction of Tc52 with DCs and, if there is such, whether this could modulate DC activity. The experiments conducted revealed interesting features: 1) Tc52 induces human DC maturation as assessed by up-regulation of costimulatory surface antigens such as CD54 and CD86 and HLA-DR molecule; 2) inflammatory chemokine synthesis (IL-8, monocyte chemoattractant protein (MCP)-1, and macrophage-inflammatory protein (MIP)-1α); 3) Tc52-treated DCs present potent costimulatory properties; and 4) binding experiments showed complex molecular Tc52-DC interactions that involved Toll-like receptor 2 (TLR2) and Tc52 glutathione-binding site which mediated intracellular signaling, whereas another, unidentified portion of the Tc52 molecule is involved in its binding to DCs[66]. In fact, Tc52 is made up of two homologous domains comprising a glutathione binding site (G-site) and a hydrophobic C-terminal region (H-site). The molecule may act as a dimeric-like complex where the two "pseudo-subunits" area arranged in an antiparallel fashion separated by a strong β-turn motif (Ala225-Pro-Gly-Tyr228). The Tc52 G-site binds to TLR2, and the other portion of the molecule, likely the H-site; interacts with a putative DC surface structure. Binding to TLR2 activates the signaling cascade leading to nuclear factor (NF)--κB nuclear translocation and regulation of nuclear gene expression. Figure 4 shows a model for Tc52 binding to the DC that could explain several aspects of its action and accommodates the above-mentioned finding. It might be that the H-site interacts first with the still unknown DC surface structure, the membrane-bound receptor-Tc52 complex then moves to reach the TLR2 and binds to it through the Tc52

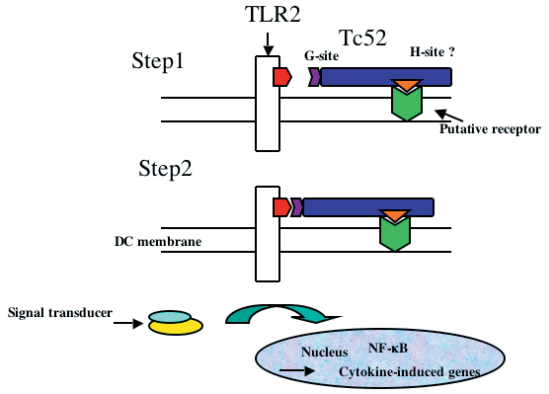

Figure 4. A model representing the two putative sequential steps of the interaction of Tc52 with DC membranes: The *T. cruzi* Tc52 is composed of two homologous domains comprising a glutathione binding site (G-site) and a hydrophobic C-terminal region (H-site). The molecule may act as a dimeric-like complex where the two "pseudo-subunits" area arranged in an antiparallel fashion separated by a strong β-turn motif (Ala225-Pro-Gly-Tyr228). For clarity, half of the Tc52 protein containing G and H sites is shown interacting with a DC. Step 1, binding of as yet uncharacterized DC surface structure (Tc52R) to the Tc52 H-site; step 2, interaction of the TLR2 with the Tc52 G-site after lateral movement of the Tc52R--Tc52 H-site complex in the plane of the membrane. Binding to the TLR2 activates the signaling cascade leading to NF-κB nuclear translocation and regulation of nuclear gene expression.

G-site, resulting in the activation of intracellular signaling cascades leading to NF-κB nuclear translocation and the regulation of DC gene expression.

Taken together, the data obtained allowed us to propose that Tc52 and the related class of proteins might constitute a new type in the pathogen-associated molecular patterns. Moreover, it is reasonable to suggest that targeting of DC with Tc52 may favor local recruitment and activation of leukocyte and then DC migration to the lymph node, where they could initiate specific B and T cell immune responses. Therefore, Tc52 could be added to the list of *T. cruzi* antigens that trigger the innate immune system. Indeed other *T. cruzi* molecules have been the subject of active investigations. This is the case of the glycosylphosphatidylinositol (GPI) anchors and the glycoinositolphospholipids (GIPLs), which are potent activators of human and mouse macrophage TLR2[15]. However, although GPI and Tc52 both trigger activation via TLR2, the profiles of inducible cytokine synthesis appear to be different: IL-12 and TNF-α in the case of GPI, whereas TLR2 activation by Tc52 resulted in IL-8, MCP-1, and MIP-1α production, with no significant increase in TNF-α synthesis. Altogether, these observations suggest that the parasites secrete molecules which, upon interaction with the host cell, may trigger signaling pathways leading to different patterns of cell activation/inhibition.

In the case of *Leishmania* parasites, it has been shown that lipophosphoglycan glycoconjugates (LPP), a parasite surface and released molecule, was capable of modulating the *iNOS* gene and NO production[78]. However, although the host immune system has to produce a certain amount of NO to eliminate the parasites, and even certain tumors, its overproduction may have adverse effects, leading to serious pathological alterations[51], including the down-regulation of T cell response as mentioned above. This feedback regulatory mechanism has also been demonstrated in other parasitic diseases. Indeed it has been reported that NO-mediated suppression of T cells during *T. brucei* infection could result from a synergistic effect of soluble trypanosome products and IFN-γ on *iNOS* expression[83]. *Toxoplasma gondii* uses this kind of mechanism to generate a transient immunosuppression that probably helps in the establishment of the chronic infection in animals and humans[40].

LeIF, another parasite factor that modulates human monocytes as well as monocyte-derived macrophages and DCs, is a gene product homologous to the eukaryotic initiation factor 4A and has been shown to be a strong inducer of IL-12 and, to a lesser extent, of IL-10 and TNF-α in macrophages and DCs. Moreover, up-regulation of the co-stimulatory and intercellular adhesion molecules B7-1 and CD54 (ICAM-1) by monocytic APCs in response to LeIF was also demonstrated, suggesting that LeIF could be among the immunomodulatory factors of *Leishmania* parasites[77].

Of the evolutionarily conserved antigens of *Leishmania*, the ribosomal proteins LiP2a, LIP2b, LiP0/LcP0, and LeIF have been shown to be recognized by the immune system of the host with high frequency[80]. Other parasite components belonging to the large ribosomal protein family, such as S3a (*Leishmania* S3a: LmS3a), could also be among the candidate antigens for recognition by the immune system[24]. However, when studying the effect of LmS3a on T and B cells in a murine model, a striking bivalent effect was demonstrated, the protein being stimulatory and inhibitory toward B and T cells, respectively[23]. Moreover, analysis of cytokine production revealed a significant down-regulation of IFN-γ, IL-2, and IL-12 secretion by LmS3a, thereby suggesting that the molecule, in contrast to LeIF, is acting as a Th1 down-regulating factor. Since the induction of a Th1 immune response and the simultaneous lack of activation of a Th2 response are required for the generation of immunity to Leishmaniasis, it is tempting to speculate that LmS3a could be among the candidate molecules to be tested for the optimal design of vaccines against *Leishmania* parasites.

Many types of cancer take advantage of the immune-modulating activities of cytokines because of their capacity to act on gene expression, to down-regulate certain immune responses that might destroy cancer cells[81]. Thus many tumor types secrete IL-10, which shares many of the same immunosuppressive effects on T cells as transforming growth factor (TGF)-β. Indeed, in recent study it was shown that tumor-derived mucin profoundly affects the cytokine repertoire of monocyte-derived DCs and switches them into IL-10 (high) IL-12 (low) regulatory APCs with limited capacity to trigger protective Th1 responses[54]. Moreover, recent data showed that supernatants from ovarian carcinoma cell lines exerted a profound inhibition of proliferation, a lower level of IFN-γ, and a higher level of IL-10 gene expression in $CD8^+$ T cells, most of the $CD8^+$ T cells being arrested in the G0/G1 phase of the cell cycle[89].

Numerous endocrine tumors, including medullary thyroid carcinomas[30, 49], parathyroid adenomas, insulinomas, carcinoids, pheochromocytomas[84], sarcoma cells[43], and numerous lung cells, express calcitonin gene-related peptide (CGRP) that, once bound

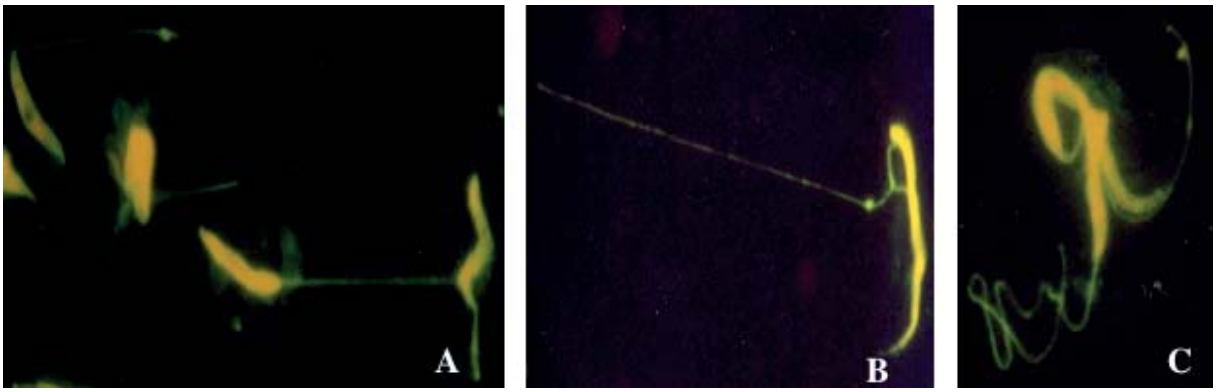

Figure 5. Immunofluorescence using a mouse immune serum to *L. major* LmS3a recombinant protein reacting with *L. major* promastigotes. Positive reactions allowed distinguishing thin trails of variable length that reacted with the antibodies (**A**); trails can be smooth or beaded in appearance (**B**); trails suggestive of rotational movement are also clearly distinguishable (**C**).

to its cognate receptor on T lymphocytes, inhibits the production of IL-2[88]. The circulating level of CGRP increased significantly in cancer patients such that it may correlate with the degree of malignancy[85]. Thus the tumor cells may evade destruction by the immune system through down-regulation of IL-2 production. Furthermore, shed tumor cell vesicles have been shown to be immunosuppressive *in vitro*[8, 79]. The mechanism leading to such immunosuppression seems to be related to the inhibition of MHC class II antigen expression by macrophages upon their interaction with vesicles shed from the tumor cell surface[76].

In the case of *Leishmania* parasites it has been demonstrated that infection with *L. donovani* inhibited the induction of IFN-γ of H-2 Aβ gene transcription, class II mRNA accumulation, and H-2 A^d protein expression in cells of the murine macrophage cell line P388D1[48]. Furthermore, decreased expression of B7 molecules on *L. infantum*-infected canine macrophages seems to be among the mechanisms leading to the down-regulation of T cell proliferation[74]. These observations suggest that down-regulation of costimulatory molecules is a shared mechanism that may help both the pathogen and the tumor cells to escape the host immune effector mechanisms.

In the case of *T. cruzi*, using different monoclonal antibodies (the 155D3 mAb to an 85 kDa polypeptide epitope and a mAb to the Tc24 flagellar calcium-binding protein), we conducted immunoelectron microscopy studies on sections of *T. cruzi* trypomastigote forms. Both mAbs revealed reactive material associated with the parasite surface and on vesicles, which were released from the parasites[64, 71]. Our electron micrographs suggest that the vesicles originated from both the parasite surface and the flagellar pocket. The release of *T. cruzi* components onto

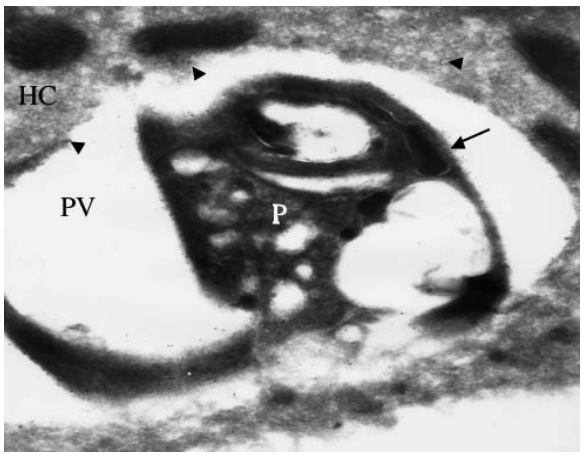

Figure 6. Electron micrograph of a cross section of a *L. major* promastigote inside a J774 mouse macrophage cell line reacted with a polyclonal antibody against *Leishmania* antigens associated with cytoplasmic vesicles[91]. The gold particles indicative of the presence of parasite antigens were found at the parasite surface (arrow), and in association with the parasitophorus vacuole, and even inside the host cell cytoplasm (arrowhead). Abbreviations: PV – parasitophorus vacuole, P – promastigote after early invasion, HC – host cell cytoplasm.

modified surfaces and mammalian cells has been reported by other investigators[7]. Furthermore, in independent studies[92] using immunofluorescence assays with antibodies to *Leishmania* LmS3a recombinant protein which down-regulate the Th1 immune response[23] we found that the protein is released in association with thin trails of variable length, being smooth or beaded in appearance (Fig. 5). The release of parasite material inside the host cell by intracellular amastigotes could also be visualized (Fig. 6). Therefore it is tempting to suggest that, like tumor cells, the released parasite antigens in association with vesicles might in some way interact with the host cell surface receptors, leading to the host cell phenotypic variability. This might result in the activation of

intracellular signaling pathways leading to deactivation/activation of the host cell immune system.

INDUCTION OF APOPTOSIS IN THE HOST IMMUNE CELLS

Another mechanism leading to homeostasis disorder in the host is the fact that the invading parasites can release factors which kill the cells of the immune system by activating the cellular death machinery, thus inducing apoptosis. Indeed, many efforts in the last years have focused on the study of an important family of proteins from *T. cruzi*: trans-sialidase (TS) and GPI-anchored mucin-like proteins. TS is released by the parasite into the external milieu as a soluble factor, being a modified sialidase able to transfer sialic acid from sialoglycoconjugates from the host to β--galactoses in the glycoconjugates of the parasite. It has been shown that under certain circumstances TS could act as an apoptosis inducer in cells of the immune system *in vivo*[50]. Furthermore, evidence reported supports the notion that TS is a virulence factor responsible for thymic alterations via apoptosis of the "nurse cell complex"[21].

Paradoxically, there is a large amount of experimental evidence showing that parasites may inhibit apoptosis of host cells. For instance we showed that the intracellular production of native Tc52 stimulated the growth of macrophages and fibroblasts[67]. In fact, a number of other studies have shown that parasite-derived molecules could interfere with the growth of host cells. In the case of *T. cruzi*, strong evidence indicates that the parasite's TS synergizes with the cytokine ciliary neurotropic factor and leukemia inhibitory factor to prevent apoptosis of neuronal cells[21]. TS also triggered *bcl-2* gene expression, leading to the protection of rat pheochromocytoma PC12 cells, a cell line that exhibits several characteristics of neurons, from apoptosis induced by growth factor deprivation. Furthermore, TS has been reported to act as a survival factor in Shawn cells by protecting them from apoptosis[20]. In fact, TS activates Shawn--cell phosphatidylinositol 3-kinase (P13K)/Akt protein kinase signaling, a cell survival pathway. Moreover, it has been shown that *L. donovani* infection of macrophages led to their resistance to apoptosis induced by the removal of growth factors[55]. In addition, treatment of bone marrow-derived macrophages with exogenous *L. donovani* promastigote surface LPG inhibits apoptosis, thus suggesting that LPG might be involved in the pathway leading to the apoptosis inhibition. This phenomenon seems to be correlated with an increased transcription of macrophage cytokine genes encoding GM-CSF, TNF-α, TGF-β, and IL-6. More recently, it has been reported that co-incubation of polymorphonuclear neutrophil granulocytes (PMNs) with *L. major* promastigotes resulted in significant inhibition of PMN apoptosis. This phenomenon is associated with a significant reduction in PMN caspase-3 activity. Since parasites were found inside mice skin PMNs after a subcutaneous challenge infection, it has being suggested that infection with *L. major* prolongs the survival time of PMNs *in vivo*[2].

Other intriguing observations relate to the general suppression of macrophage gene expression during *Leishmania* infection[13]. In fact, these authors, using cDNA expression array analysis, have reported that although ~40% of the genes whose expressions were detected in macrophages were down-regulated during infection with *L. donovani*, some genes were induced during the infection. The proposed hypothesis was that the overexpressed gene products might play a role in the recruitment of additional macrophages to the site of infection, allowing the parasite to survive within the host cell. In a more recent study, an interesting and unexpected finding relates to the *L. donovani* elongation factor-1α[58]. Indeed, this factor could be exported from the phagosome and bound to the macrophage Scr homology 2 domain containing tyrosine phosphatase-1 (SHP-1), leading to its activation. As a consequence, a down-regulation of macrophage-inducible NO synthase expression in response to IFN-γ occurred, a process which may contribute to the deactivated phenotype of infected macrophages.

Members of the tumor necrosis factor family of receptors such as TNFRI and Fas, also called CD95 or APO-1, are involved in apoptotic pathways used by the immune system to kill cancerous cells. Many types of cancer cells have developed strategies to evade and exploit the Fas and TNFRI apoptotic pathways through the expression of non-functional Fas receptors that bind Fas-ligand (FasL) without triggering the biochemical cascades leading to apoptosis[25]. Moreover, cancerous cells such as lung and colon tumors could secrete a soluble decoy receptor that binds to FasL and inhibits FasL-induced apoptosis[75]. Thus, like *T. cruzi* and *Leishmania* parasites, interference with the apoptotic pathways is also a strategy exploited by tumors to prevent their destruction by the host immune effector cells.

"PANCLONAL" ACTIVATION OF CELLS OF THE IMMUNE SYSTEM

T. cruzi and *Leishmania* sp. release a large number of molecules which could act as mitogenic substances inducing polyclonal lymphocyte responses and, con-

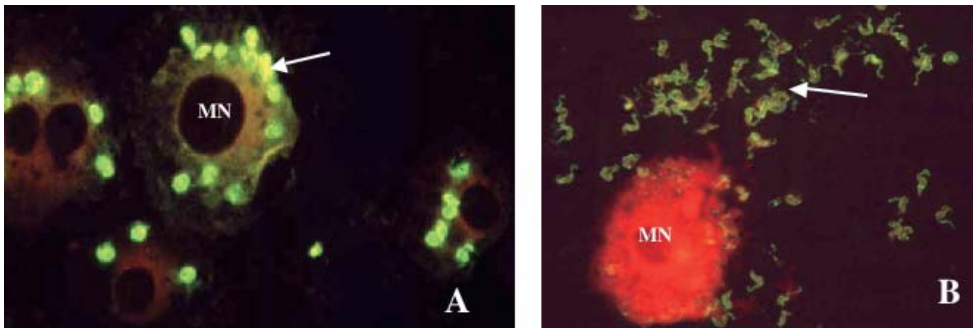

Figure 7. Immunofluorescence using a mAb to *T. cruzi* Tc24 protein showing a high reactivity against intracellular amastigotes inside a J774 mouse macrophage cell line (**A**). Positive signal diffusing in the cytoplasm of the infected cell could also be seen. Positive reactions were also observed on trypomastigotes released from heavily infected and ruptured cell (**B**). Abbreviation: MN – macrophage nucleus.

sequently, a general lack of specificity of antibodies and T cell responses during the infection. The mechanisms by which *T. cruzi* and *Leishmania* sp. activate "panclonal" B lymphocyte response are fundamental, and may contribute to the immune deregulation. A number of *T. cruzi*-released proteins behave as B cell activators[70]. Among these factors, a Tc24 flagellar calcium-binding protein is highly expressed in all the parasite's developmental stages (Fig. 7). Investigations on the immunogenicity of released Tc24 protein have revealed that *in vivo* treatment with the recombinant Tc24 protein induces a quick increase in the number of B cell-secreted immunoglobulins, mainly of the IgM isotype. The IgM response is mostly unrelated to the antigens present in total parasite extracts or to the protein itself. The use of euthymic and athymic mice is suggestive of a mitogenic activity of Tc24 on B cells that is T cell independent[26]. Tc24 induces a strong B lymphocyte response in *in vitro* and *in vivo* assays, indicating that the nature of the B cell responses following Tc24 injection is indeed the polyclonal expansion of non-specific, non-parasite-directed B cell clones, similarly to other mitogen stimulations. A single injection of Tc24 without any adjuvant induces the production of immunoglobulins that are not specific to parasite antigens, as described for other mitogenic products released by several microorganisms such as bacteria, viruses, and parasites.

Soluble parasite-derived antigens from *L. major* and *L. donovani* are mitogenic and trigger the production of immunoglobulins with autoantibody activity[9]. Thus, crude extracts of *L. donovani* and *L. mexicana amazonensis* contain components which cause strong *in vitro* polyclonal activation of hamster spleen cells[14]. Moreover, an excreted factor derived from the culture medium of *L. major* was found to suppress ConA-induced polyclonal activation of mouse T cells[37]. These observations suggest that the state of immunosuppression could be in part a consequence of the intense B cell polyclonal activation observed during the early stages of infection.

CONCLUDING REMARKS

Parasitic infections lead to the development of a complex network of cross-regulatory mechanisms. The recent discovery of *T. cruzi* mitochondrial DNA, which is termed kinetoplast DNA, and its integration into the human as well as other mammalian genomes[59] adds new levels of complexity to the pathogenesis of intracellular protozoan infections. In fact, although significant progress has been made in our understanding of the immune response to parasites, no definitive step has yet been successfully made in terms of operational vaccines against parasitic diseases. Therefore, it seems likely that further elucidation of the molecular events which tightly regulate the processes of immune cell activation and disruption of the regulatory mechanisms that limit effective immune responses will shed light on the possible means to coordinate an effective attack against parasitic diseases and tumor cells.

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