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Review Article

Regulatory Cells and Immunosuppressive Cytokines: Parasite-Derived Factors Induce Immune Polarization

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Parasitic infections are prevalent in both tropical and subtropical areas. Most of the affected and/or exposed populations are living in developing countries where control measures are lacking or inadequately applied. Although significant progress has been made in our understanding of the immune response to parasites, no definitive step has yet been successfully done in terms of operational vaccines against parasitic diseases. Evidence accumulated during the past few years suggests that the pathology observed during parasitic infections is in part due to deregulation of normal components of the immune system, mainly cytokines, antibodies, and immune effector cell populations. A large number of studies that illustrate how parasites can modify the host immune system for their own benefit have been reported in both metazoan and protozoan parasites. The first line of defense against foreign organisms is barrier tissue such as skin, humoral factors, for instance the complement system and pentraxin, which upon activation of the complement cascade facilitate pathogen recognition by cells of innate immunity such as macrophages and DC. However, all the major groups of parasites studied have been shown to contain and/or to release factors, which interfere with both arms of the host immune system. Even some astonishing observations relate to the production by some parasites of orthologues of mammalian cytokines. Furthermore, chronic parasitic infections have led to the immunosuppressive environment that correlates with increased levels of myeloid and T suppressor cells that may limit the success of immunotherapeutic strategies based on vaccination. This minireview briefly analyzes some of the current data related to the regulatory cells and molecules derived from parasites that affect cellular function and contribute to the polarization of the immune response of the host. Special attention is given to some of the data from our laboratory illustrating the role of immunomodulatory factors released by protozoan parasites, in the induction and perpetuation of chronic disease.

1. INTRODUCTION

There is increasing evidence that immune mechanisms are involved in the pathogenesis of many parasitic infections. The initial stages of the disease are generally characterized by the induction of a nonspecific lymphoproliferation, which is believed to disrupt antigen recognition and interfere with protective immune responses. Paradoxically, in most cases a state of immunosuppression can be evidenced. This hyporesponsiveness to antigen-specific and polyclonal stimuli in chronic parasitic infections could be related to immunosuppressive cytokines (i.e., IL-10 and TGF-β) secreted by antigen presenting cells and regulatory T cells (Treg cells). A growing list of parasite-derived molecules able to exert immunomodulatory activities on the cells of the innate immunity leading to such polarized cytokine secretion has been reported [1, 2]. Interestingly, these immunosuppressive regulatory responses resulting from repeated exposure to pathogens and/or their released products have been postulated to be responsible for protection against inflammatory diseases such as allergy or autoimmunity leading to the germless theory of allergic diseases and the hygiene hypothesis [3, 4]. This has led investigators to search for parasite molecules which could be used as a new therapy for immunological disorders [5].

In fact, complex feedback loops could explain the properties of a suppressor activity seen in parasitic infections. For instance, the parasites cannot only induce the production of host immunomodulatory lipids, the best characterized being the endogenous eicosanoids, but are also able to synthesize/secrete their own glycoproteins and lipids, which in turn activate cells of innate immunity towards the anti-inflammatory cytokine response. This physiological microenvironment may favor the development of Treg cells. A number of excellent reviews were devoted to the Treg cells

and their physiological role at the level of peripheral self-tolerance, avoidance of autoimmune diseases, tumor, and infectious disease immunology [6–13].

The purpose of this minireview is to analyze some of the current data related to the regulatory components or processes originating from the parasite (protozoa and some helminth pathogens) that affect host cellular functions leading to an immunosuppressive state that counteract proinflammatory cytokine production which may lead to excessive host tissue damage. The outcome of a parasitic infection will depend on the final balance of the protective and pathological properties of the cytokine network.

2. INDUCTION OF SUPPRESSOR CELLS

Immunosuppression involves an act that reduces the activation or efficacy of the immune system. Some components of the immune system itself have immunosuppressive effects on other parts of the immune system, and immunosuppression may occur as an adverse reaction to treatment or other conditions like infection processes. The ability of parasites to survive in the immune hosts depends on a variety of escape mechanisms. One of these is the inhibition or the suppression of the immune responses of their hosts. Several possible explanations have been put forward, such as antigenic competition, acquired tolerance, as well as the possible blocking role of soluble antigens or circulating immune complexes. The release by the parasites themselves of excretory-secretory products, which have potent immunosuppressive activity, represents another possible explanation. Moreover, a large number of reports have described the presence of suppressor cells (T lymphocytes and macrophages) in humans and animals infected with various parasites.

For instance, during onchocerciasis due to a pathogenic human filarial worm, *Onchocerca volvulus*, a state of cellular immune unresponsiveness develops in patients with the generalized form of the disease [14]. Moreover, it has been reported that parasite antigens are present in breast milk of *O. volvulus*-infected women which activated cells that suppressed the proliferative response of autologous lymphocytes to mitogens and antigens, suggesting therefore that this may induce tolerance and/or suppression in infants born of infected mothers [15].

A number of years ago, we have shown that total antigens from *O. volvulus* (OVA) markedly inhibited the proliferation of normal human lymphocytes stimulated with polyclonal activators such as phytohaemagglutinin (PHA). The inhibition was not due to a cytotoxic effect of OVA and was not abrogated by removal of the adherent cell population. Interestingly, we showed that the in vitro response of normal human lymphocytes was suppressed by coculture with allogeneic or syngeneic lymphocytes, which had previously been exposed to OVA. A significant reduction of the suppression was however observed when OVA pretreated cells were depleted of T cells by centrifugation of E rosettes. Moreover, the passage of OVA through an immunoadsorbant column containing a monoclonal antibody to OVA epitope abrogated its immunosuppressive effect. These observations allowed us to postulate that a parasite antigen(s) was responsible for the induction of T suppressor cells [16]. Since the molecular mechanisms of suppressor cells were difficult to characterize, interest in such cells was lost.

Recent progress in the identification of $CD4^+$ T cell populations, together with the use of genetically modified animal models have led to significant advances in the understanding of the immunosuppression phenomenon at the cellular and molecular levels. The concept of T regulatory cells (Treg) suppressing immune responses via cell-cell interactions and/or the production of suppressor cytokines are currently well documented [6–9]. At least two main Treg cell populations were defined: "naturally" occurring regulatory T cells ($Foxp3^+$ $CD4^+$ $CD25^+$) and the "adaptive" regulatory T cells (e.g., T_R1 or T_H3) [10–12]. Although some controversy has been reported in the literature, evidence which accumulated over the years has undoubtedly shed light on the importance of Treg cells in health and disease. Thus, in the case of onchocerciasis, a series of reports has shown that the hyporesponsiveness in individuals with the generalized form of the disease is not due to a shift towards a T_H2 response. Rather, it results from *O. volvulus* antigen-specific T cells having a cytokine profile with no IL-2 and high IL-10 and TGF-β production similar to the adaptive Treg cells also known as T_R1 and T_H3 which suppress ongoing inflammation [17, 18]. Cloning procedures allowed obtaining T cell clones bearing T_R1 suppressor cytokine profile producing significant amounts of IL-10 but no IL-2 or IL-4 and expressing high levels of cytotoxic T lymphocyte antigen (CTLA-4) after stimulation.

Although the examination of T cell lines or clones derived from the peripheral blood mononuclear cells of infected individuals has the great advantage that the cells producing the cytokines can be accurately defined, in this case the cytokines derived from "neighboring" cells in vivo are no longer represented in the system. Nevertheless, the generation of T cell clones has provided valuable information on human responses in general and to infections in particular.

However, when considering a wide range of autoimmune and inflammatory manifestations, the mechanisms by which regulatory Treg cells exert their activity remain unclear. For instance, studies in vivo have demonstrated that regulation is dependent on cytokines such as IL-10 and TGF-β as well as the expression of CTLA-4 molecule [19, 20]. However, in vitro studies have shown that neither soluble cytokines nor CTLA-4 is required for the suppressive effects of Treg [21–23]. Moreover, it has been shown that regulation of the ileal inflammatory process resulting from *Toxoplasma gondii* in murine model is dependent on TGF-β producing intraepithelial lymphocytes suggesting therefore that these cells represent an essential component in gut homeostasis after oral infection with this parasite [24]. Furthermore, in the susceptible BALB/c mouse experimental model of filarial infection with *Litomosoides sigmodontis*, a parasite closely related to *Brugia* and *Wuchereria* species causing human lymphatic filariasis, the Treg cells have been shown to be responsible for susceptibility to parasite. However, although treatment of infected mice with antibodies to CD25 and

glucocorticoid-induced TNF receptor family-related gene reduced Treg activity and led to increased antigen-specific immune responses, this results in the significant reduction of parasite numbers, in vivo neutralization of IL-10 receptor, but did not restore the ability of the immune system to kill parasites, supporting the notion that Treg cells act in an IL-10-independent manner [25].

Nevertheless, a recent study has shown that in a murine model of coinfection with a gastrointestinal nematode parasite *Heligmosomoides polygyrus* and the blood-stage malaria parasite *Plasmodium chabaudi* increased levels of IL-10. This occurred in concurrent infections, whereas high levels of TGF-β were seen during *P. chabaudi* single infections [26]. Interestingly, anthelminthic drug treatment of mice before *P. chabaudi* infection reduced TGF-β levels and restored antimalarial immunity. Thus, induction/expansion of the suppressor function is a complex process depending on multiple factors, among which concurrent infections are highly prevalent in many endemic tropical and subtropical regions of the world.

Hyporesponsiveness also occurs during human schistosomiasis. In fact, the infection downregulates both T_H1 and T_H2 cytokines [27]. In more recent studies, it has been shown that in a mouse schistosomiasis, Treg cells and IL-10 inhibited T_H1 development [28] and egg-induced pathology [29]. However, a number of studies have pointed to the role of IL-10 and TGF-β (two cytokines being released by adaptive Treg cells) as immunomodulatory cytokines in helminth infections. Parasite-specific activation of natural Treg cells has been reported in mice *Leishmania major* infection [30]. The cells were positive for Foxp3, produce IL-10 in response to *Leishmania*-infected dendritic cells, and exerted strong suppressive activity in vitro. In fact, previous observations have shown that in the case of *L. major* infection of genetically resistant C57BL/6 mice which spontaneously heal their dermal lesions with persistence of latent parasites, $CD4^+$ T cells are the main producers of IFN-γ and IL-10 in the dermis, although $CD8^+$ T cells were also able to produce either cytokine with appropriate stimuli [31]. Similar to T_R1 cells, the majority of $CD4^+$ T cells in the dermis and a proportion of $CD4^+$ T cells in the draining lymph nodes were able to produce both IL-10 and IFN-γ. Thus, in the chronic sites of infection, the release of IL-10 and IFN-γ by T cells led to the establishment of a latency with persistence of low number of viable parasites within lymphoid tissue and skin lesion after self-cure.

Although the T cell network seems to play a key role in the immunosuppression process, the existence of other T-cell-independent mechanisms has been clearly demonstrated. Indeed, recent investigations have shown that helminth and protozan infections can elicit a myeloid population characterized as $Gr\text{-}1^+/CD11b^+$ cells that substantially impaired antigen-specific T cell responses [32–34].

The myeloid suppressor cell-induced immunosuppression is mediated by nitric oxide production (NO), a messenger known to be involved in diverse signaling pathways including smooth muscle relaxation, platelet inhibition, neurotransmission, immune regulation, and destruction of microbes and tumor cells [35]. The participation of NO in the suppression of T cell activation has been reported in a number of biological systems (reviewed in [36]). In fact, NO production during toxoplasmosis in C57BL/6 mice has two opposite effects being protective against *Toxoplasma gondii* and downregulating the immune response, suggesting its possible contribution in the establishment of chronic infections [37]. In the case of *Trypanosoma cruzi*, previous studies have shown that IFN-γ and nonoxidative molecules (TNF-α and NO) could play a role in the control of *T. cruzi* infection in mice [36]. Furthermore, a series of experiments supports the notion that IFN-γ and TNF-α mediated activation of macrophages which leads to increased production of NO, and in turn suppresses T cell activation [38]. The involvement of NO in apoptosis of thymocytes and macrophages has also been documented [39, 40] and NO markedly inhibits the induction of IL-2 promoter, which can account for most of the reduction in IL-2 production, and weakly increases the activation of IL-4 promoter [41]. This mechanism could be involved in the downregulation of IL-2 gene expression observed during *T. cruzi* infection [42]. Therefore, it is likely that NO production during the initial phase of acute infections 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.

Although significant advances have being made regarding the Treg subsets, a recent exiting review pointed to the importance of B cells possessing regulatory functions and suggested these be called Breg cells (reviewed in [43]). Indeed, in addition to the pathogenic role of B cells, which produce autoantibodies that contribute to the development of autoimmune diseases, the existence of regulatory B cells capable of inhibiting inflammatory responses through the production of regulatory cytokines IL-10 and TGF-β has been demonstrated in a number of experimental models of chronic inflammation [43].

In the case of parasitic infections, a number of studies have reported several alterations in B cell functions. For instance, in the mouse model of *Schistosoma mansoni* infection, splenic B cells have been shown to proliferate in response to an oligosaccharidic antigen (see also the next section) and were triggered to secrete high levels of IL-10 [44]. Furthermore, spleen B cells from mice infected with the L3 larval stage of filarial nematode *Brugia pahangi* contributed substantially to IL-10 production that in turn downregulated the expression of B7 molecules on the B cell surface [45]. This led to the decrease of their efficiency as antigen presenting cells to $CD4^+$ T cells and restricting their expansion, suggesting therefore that B-cell-derived IL-10 could participate in the regulation of proinflammatory CD4 responses as in other various models of autoimmune diseases [46].

2.1. Induction of apoptosis in the host immune cells

Another mechanism leading to the 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. Therefore, apoptosis seems to represent a fundamental feature with particular relevance in the maintenance of protozoan infections [47]. Indeed, studies in experimental mouse *T. cruzi* infections have shown that apoptosis of T cells play an important role in the immunossupression that occurs during the acute phase of Chagas' disease [48]. In a canine model of acute Chagas myocarditis, Zhang *et al.* [49] have reported that programmed cell death (PCD) occurs in cardiac myocytes, endothelial cells, macrophages, interstitial dendritic cells, and lymphocytes, suggesting that parasite-derived factors could be responsible of the apoptosis observed in the immune cells. In the same way, a correlation between the extent of PCD and the level of suppression of $CD4^+$ T-cell proliferative responses was observed [50]. Moreover, it has been suggested that the upregulation of Fas and Fas ligand play an important role in the induction of $CD4^+$ T cell death. This pathway is able to control and modulate the immune response against *T. cruzi* [51].

Parasite-derived substances are believed to be key factors in the immunossupression phenomena observed by exerting a proapoptotic activity against some immune cells. Thus, it has been reported that the *T. cruzi*-secreted trans-sialidase (TS) was able to induce apoptosis features in cells of the immune system in vivo [52]. Furthermore, evidences reported support that TS is a virulence factor responsible for thymic alterations via apoptosis of "nurse cell complex" [53]. Moreover, the glycoinositolphospholipid (GIPL) from *T. cruzi*, in the presence of IFN-γ, induced murine macrophage apoptosis leading to increased parasite release from these cells [54]. Surprisingly, internalization of apoptotic T lymphocytes by macrophages increased the replication of *T. cruzi* amastigotes inside macrophages [55]. Another additional mechanism that may amplify the immune suppression process is the fact that apoptotic cells release their TGF-β during their suicidal act. They flip intracellular phosphatidylserine onto the outer leaflet of their membranes rendering them target of phagocytic cells signaling the macrophages to release significant amounts of TGF-β which in conjunction with IL-10 and PGE-2 may act as a feedback amplification loop mediating immune suppression.

2.2. PD-1 as active suppressor of T regulatory cells

Programmed death-1 (PD-1) [56], a member of the CD28 family, is an immunoreceptor tyrosine-based inhibitory-motif- (ITIM-) containing receptor induced on T, B, and myeloid cells upon activation in vitro [57]. Interaction of PD-1 ligands (PD-L1 and PD-L2, members of the B7 family) with PD-1 may lead to the inhibition of proliferation and cell division of activated T cells expressing PD-1. Thus, the absence of PD-1 induced proliferation of effector T cells in the adenovirus-infected liver and resulted in rapid clearance of the virus. The blockage of the PD-1 pathway can augment antiviral immunity [58]. Recent investigations have reported that PD-1/PD-L systems may play a role during parasitic infections. Indeed, it has been shown that PD-L1 and PD-L2 have distinct roles in regulating host immunity to cutaneous leishmaniasis [59]. In fact when compared to wilt-type mice (WT), the PD-$L1^{-/-}$ and PD-$L2^{-/-}$ exhibited distinct disease outcomes following infection with *L. mexicana*.

PD-$L^{-/-}$mice developed resistance, whereas PD-$L2^{-/-}$ showed exacerbated disease. Although both PD-$L1^{-/-}$ and PD-$L2^{-/-}$ produced similar levels of IFN-γ as the WT mice, the development of IL-4 producing cells was reduced in PD-$L1^{-/-}$ mice suggesting that impairment of T_H2 response due to PD-L1 deficiency could be related to increased resistance to *L. mexicana* infection. Furthermore, in the case of CBA/J mouse *Schisitosoma mansoni* chronic infection, the parasites can induce increased expression of PD-L2 on splenic $CD11c^+/B220^-$ dendritic cells leading to moderate morbidity [60]. Taken together, these observations suggest that PD-1/PD-L systems may play a role in negative regulation of immune responses during parasitic infections.

2.3. Parasite released molecules as immunoregulatory factors

2.3.1. Lipids

A number of reviews pointed to the importance of endogenous as well as parasite-derived lipids as immunoregulatory factors [2, 62, 63]. It is well known that eicosanoids such as prostaglandins PGE2, PGD2, and LipoxinA4 can act not only on antigen-presenting cells through defined or putative surface receptors and strongly modify their pattern of cytokine synthesis (IL-1, TNF-α, IL-12, and IL-10), but also on T cells at the level of IL-2 synthesis, IL-2 receptor expression, and cellular proliferation [2]. Glycoinositolphospholipids (GIPLs) are some of the major glycoconjugates present on the cellular surface of *Leishmania* [63] and different strains of *T. cruzi* [64]. *T. cruzi* GIPL blocks T cell responses induced by different polyclonal activators, the suppressive domain being assigned to the ceramide portion of the molecule. Indeed, purified GIPLs from *T. cruzi* inhibit in vitro $CD4^+$ and $CD8^+$ T cell proliferation induced by bacterial superantigen and anti-TCR;CD3 antibodies. The inhibition leads to loss of IL-2 responsiveness, with inhibition of CD25 expression on both $CD4^+$ and $CD8^+$ subsets [65].

Dendritic cells (DCs) are crucial in the initiation of the immune response and are distinguishable from the other antigen presenting cells by their highly efficient antigen presentation. DCs are specialized to acquire and process antigen in peripheral nonlymphoid 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 adaptive immune response occurs and the nature of that immune response. Studies with the glycoinositolphospholipid (GIPL) from *T. cruzi* have demonstrated that this molecule led to a downregulation of human DC surface antigens, such as CD80, CD86, HLA-DR, CD40, and CD57 that are important for T cell activation [66]. These observations allowed 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, it is important

to remember that the GPI anchors express biological activities similar to those of lipopolysaccharides (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 [67]. 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) [68].

2.3.2. Polysaccharides

A number of saccharides (from oligosaccharides to complex polysaccharides) derived from parasites have been identified and were shown to be implicated in host cell signaling systems. Complex polysaccharides for example which are not digested by the macrophage lysosomal enzymes can be retained intracellularly for a long period of time, interfering therefore with the presentation of peptide antigens to T cells [69]. Moreover, these molecules can act directly on the cell of the immune system. For instance, lacto-*N*-fucopentaose III (LNFP-III) and lacto-*N*-neotetraose (LNnT), sugars of egg antigens of *S. mansoni*, also found in human milk were shown to participate in the T_H2 polarization and immune suppression. Indeed, intraperitoneal injection of LNnT-Dex into mice expanded a cell population, phenotypically defined as $Gr1^+/CD11b^+/F4/80^+$ producing high levels of IL-10 and TGF-β ex vivo [32].

$Gr1^+$ cells suppressed naïve $CD4^+$ T cell proliferation in vitro in response to anti-CD3/CD28 antibody stimulation. Suppression involved cell contact and was dependent on IFN-γ and NO, with a discrete role played by IL-10 [32]. Furthermore, LNFP-III stimulated splenic B cells from parasite-infected mice to proliferate and produce IL-10 and PGE2, two molecules known to downregulate T_H1 cells [44]. The major source of IL-10 was the B-1 subset ($CD5^+$ $B220^+$) [70].

Oligosaccharide structures from other parasites have been shown to modulate B cell activity. Indeed, the *T. cruzi* GIPL was found to be a stimulatory factor for B cells, inducing the production of IgG3 in the absence of any costimuli, the active portion being present in the oligosaccharide fraction [71].

In the case of *Leishmania* parasites, the major cell surface molecule, phosphoglycan (PG), has been shown to selectively inhibit the synthesis of IL-12 (p. 40, p. 70) by activated murine macrophages. The inhibition was dependent on the galactose (beta1-4) mannose (alpha1)-PO4 repeating units and not the GPI lipid anchor of lipophosphoglycan [72].

2.3.3. Polypeptides

In addition to lipids and polysaccharides, parasite-derived proteins and even small RNA molecules could interfere with the cell of the immune system. Thus, the glutathione-S-transferases (GSTs), which are ubiquitous housekeeping enzymes found in nearly all animals and some parasites, appeared to have immunomodulatory functions [73]. Indeed, the dimeric form of GST present in the excretory-secretory products of *Fasciola hepatica* exerted a significant inhibition of rat T cell proliferation in vitro and a downregulation of NO production by normal peritoneal macrophages. Furthermore, in *O. volvulus*, a novel type of GST possessing the characteristic of secreted protein has been identified. In fact, the parasite has two GSTs (ovGST1 and ovGST2), the ovGST2 functions as an intracellular cytosolic housekeeping enzyme, whereas the ovGST1 is found in the media surrounding adult worms maintained in culture, suggesting therefore that the enzyme is released from the parasite. Recent investigations have shown that *O. volvulus* extracellular GST produces PGD2, a known anti-inflammatory molecule [74].

In accordance with these findings, we have also demonstrated the ability of a *T. cruzi* released protein, Tc52, containing a tandemly repeated structure characteristic of glutathione S-transferases (GSTs) to induce nonspecific suppression of T lymphocyte activation [75]. Furthermore, our studies have provided evidence demonstrating that purified Tc52 acted directly on macrophages to increase IL-10 gene expression [76]. In addition, experiments carried out with murine macrophages harboring a eukaryotic plasmid carrying Tc52 gene showed increased IL-10 mRNA levels [77]. Moreover, using synthetic peptides spanning the amino terminal or carboxy-terminal domain of Tc52 protein, we found that the sequence encapsing the carboxy-terminal residues 432–445 when coupled to a carrier protein, ovalbumin, exhibited increased inhibitory activity on T lymphocyte activation and significantly downregulated IFN-γ and IL-2 secretions [78].

The in vivo immunomodulatory effect of Tc52 has been investigated in mice. Given that we have already established by genetic manipulation *T. cruzi* clones lacking a Tc52 protein-encoding allele ($Tc52^{+/-}$) [79], we decided to examine the disease phenotype in $Tc52^{+/-}$-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, these studies showed a reversion of the suppressive phenotype in vivo during the infection with mutant parasites lacking one Tc52 gene allele. Moreover, a lack of increased secretion of IL-10 correlates with decreased in vivo Tc52 production [80]. Therefore, it is reasonable to suggest that this reduction by gene targeting which in turn downregulates the IL-10 synthesis could be among the immunregulatory mechanisms operating during *T. cruzi* infection. It is tempting to speculate that Tc52-inducing 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 and inhibiting mitogen-driven T cell proliferation [81, 82]. Furthermore, the effect of Tc52 on the allergic airway inflammation induced by OVA in the BALB/c strain of mouse was evaluated. While the OVA challenge induced increased cellular infiltrates in the bronchoalveolar lavage fluid, simultaneous injection of Tc52 with OVA significantly reduced inflammation (Lamkhioued, personal communication).

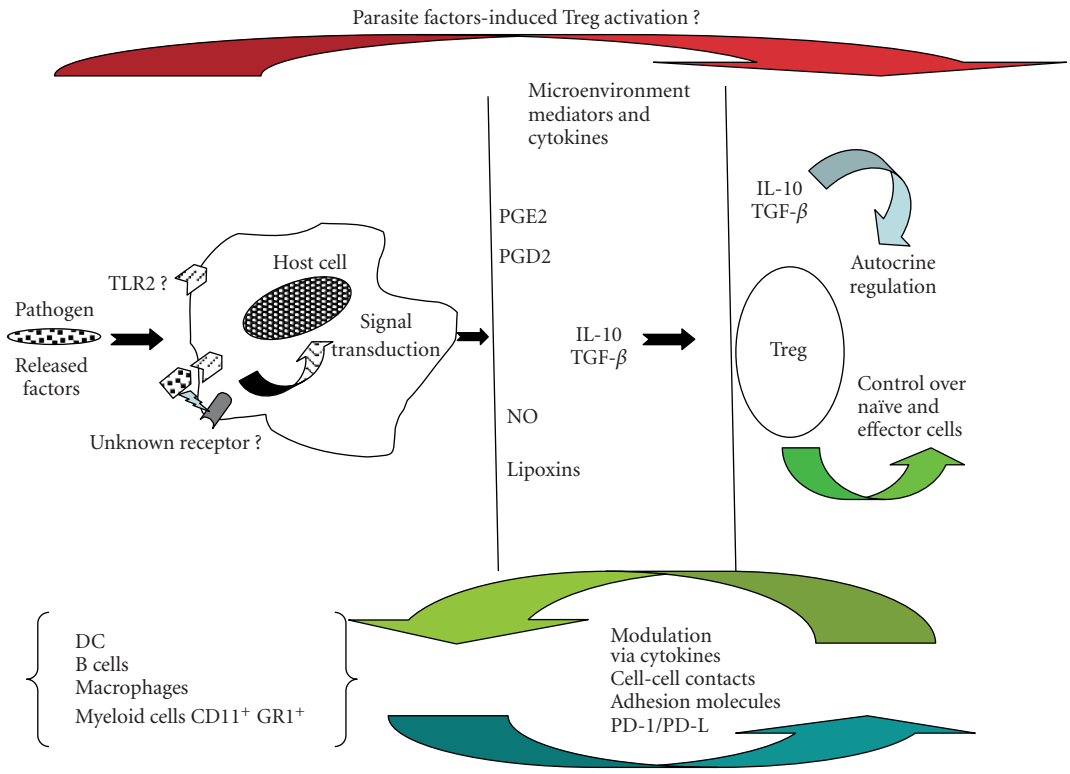

Figure 1: A model for parasite-derived factors-host cell interaction and signaling pathways.

In view of the fact that increased NO production by splenic macrophages has been involved in the suppression of lymphocytes proliferation in mice infected with *T.cruzi* [38], we have investigated whether Tc52 modified macrophage NO production. We first showed that Tc52 could be bound to the macrophage surface as evidenced by FACS analysis. Moreover, while Tc52 alone had no effect, addition of IFN-γ induced the production of high amounts of NO by macrophages correlating with increased levels of *iNOS* transcripts [76]. It is known that IFN-γ by itself is a relatively poor stimulator of NO production by macrophages and the addition of a second signal can significantly enhance NO production [83]. Since high levels of IFN-γ have been recorded during acute phase mouse *T.cruzi* infection, and that Tc52 could be detected in the blood of infected mice, it is reasonable to suggest that Tc52 may act as a "secondary signal" for NO secretion by macrophages in vivo, which in turn could modulate T cell function. This kind of mechanism also occurred in T. *brucei* infection. 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 [84].

Modulation of *iNOS* gene and NO production by soluble proteins has also been reported in the case of *Entamoeba histolytica* [85].

Although Tc52 could induce the secretion of the suppressive cytokine IL-10 by macrophages, recent investigations have shown that the protein could trigger human and mouse DC maturation as evidenced by an upregulation of costimulatory surface antigens such as CD54, CD86, and HLA-DR molecules. Moreover, incubation of DC with Tc52 led to increased inflammatory chemokine synthesis (IL-8, monocyte chemoattractant protein-1, and macrophage-inflammatory protein-1α). Interestingly, binding experiments showed complex molecular Tc52-DC interactions that involved toll-like receptor 2 and Tc52 glutathione-binding site which mediated intracellular signaling, whereas another unidentified portion of the Tc52 molecule is involved in its binding to DC [86]. In fact, the Tc52 is made 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" areas are arranged in an antiparallel fashion separated by a strong β-turn motif (Ala225-Pro-Gly-Tyr228). The Tc52 G-site binds to TLR2, the other portion of the molecule, likely the H-site, interacts with a putative DC surface structure. Binding to the TLR2 activates the signaling cascade leading to NF-κB nuclear translocation and regulation of nuclear gene expression. 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 G-site resulting in the activation of intracellular signaling cascades leading to NF- κB nuclear translocation and regulation of DC gene expression.

On first examination, the fact that Tc52 activity resulted in the induction of gene encoding both anti-inflammatory

(IL-10) and proinflammatory (IL-6, IL-8) mediators may appear paradoxical. Yet, in common with other physiological systems, it is apparent that a counter-regulatory mechanism is essential to provide the balance and regulation that are necessary to control the inflammation cascade. Within the site of an inflammation reaction, IL-10 produced locally would act to counter the stimulatory effects of IL-8 and IL-6, and thereby enable the balance to be established.

Other parasite-derived molecules which subvert immune regulation have been described: the *T. cruzi* antigen molecule SAPA (shed acute phase antigen) which exhibited a neuraminidase-transsialidase activity downregulated T lymphocyte proliferation as a consequence of T suppressor/cytotoxic cell activation and secretion of PGE2 [87]. A *T. cruzi* membrane glycoprotein inhibited the expression of IL-2 receptor chains and secretion of cytokines by subpopulations of activated human T lymphocytes [88] among others.

Moreover, parasite-derived polypeptides could act directly on B cells either as specific or nonspecific activators. Indeed, we have shown that in vivo treatment of mice with a flagellar Ca^{+}-binding protein, Tc24 from *T. cruzi,* induced a quick increase in the number of B cell secreted immunoglobulins of IgM isotype, suggestive of a mitogenic activity of Tc24 on B cells that is T cell independent [89]. Moreover, we have identified an *L. major* gene encoding a protein sharing significant homology to mammalian ribosomal protein S3a named LmS3a exhibiting dual activity being stimulatory and inhibitory towards T and B cells, respectively [90]. Analysis of cytokine production revealed a significant downregulation of IFN-γ, IL-2, and IL-12 secretion by LmS3a. These results are compatible with mitogenic induction of the immune system accompanied by a state of immunosuppression.

Another intriguing aspect in the parasite relationship is the fact that parasites could release factors that mimic host cytokines. For instance, (1) hydatid fluid fractions mimicked IL-1, IL-2, and IL-6 [91]; (2) an IFN-γ homolog that binds to the IFN-γ receptor and induced change in lymphoid cells has been identified in an intestinal nematode [92]; (3) two homologs of the human macrophage migration inhibitory factor (MIF) have been characterized in the human parasitic nematode *Brugia malayi* and termed Bm-MIF-1 and Bm-MIF-2, both having functional properties similar to the MIF human counterpart [93]; (4) *Toxoplasma gondii* releases cyclophilin-18 (C-18) that signals through the chemokine receptor CCR5 leading to the IL-12 synthesis by dendritic cells and a strong protective response [94]; (5) the tapeworm *Hymenolepis diminuta* has been shown to express an IL-12-like peptide, one of the suggested hypothesis being that the peptide could act as a competitive antagonist for the IL-12 receptor, thus contributing to the general immunosuppression [95].

Taken together, these examples raise the distinct possibility that the production of parasite factors that interact with cell surface receptors may be one mechanism whereby the parasite is able to interfere with the regulation of the induction/initiation phase of the host immune response that may protect the host from excessive inflammation and may potentiate the parasite's own survival.

2.4. Concluding remarks

The soluble parasite factors can elicit a complex series of cellular interactions leading to an immunosuppression state (Figure 1). The fact that immunoregulatory parasite-derived substances may have additional roles in driving early immunological events towards T_H2-type or anti-inflammatory responses has opened new areas of investigation looking for molecules that may represent novel potential therapeutic agents for the treatment of T_H1-mediated inflammatory and autoimmune diseases.

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