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MINIREVIEW

Host-Parasite Interactions in Trypanosomiasis: on the Way to an Antidisease Strategy▽

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TOWARD AN ANTIDISEASE STRATEGY

African trypanosomiasis (AT) is a family of parasitic conditions affecting both humans and livestock, impairing development in sub-Saharan Africa, throughout the 10 million-km^2 habitat zone of the common vector, *Glossina* spp. According to the WHO's estimations (2), 300,000 to 500,000 people are affected by AT and annual economic losses due to animal AT are about $4.5 billion (U.S. dollars). AT also represents an economic constraint in other parts of the world, despite the absence of tsetse flies in these other locations. Some AT-related *Trypanosoma* species are indeed adapted to mechanical transmission, which does not require any biological cycle to be completed in the vector and thus allows transmission by non-tsetse blood-feeding flies. In the quest to control AT, various control methods have been developed, although chemotherapy still represents the principal one. Because of the ongoing dissemination of trypanocide-resistant trypanosome strains, the research for alternative control methods has been intensified. Among these, the development of a vaccine has long been impeded by the existence of the variable surface glycoprotein (VSG) coat switch, which will probably keep frustrating this project for many years (126). Trypanotolerant breeds represent a real hope for the development of more efficient animal production in the tsetse belt. Besides a better control of parasite multiplication in the vascular compartment, these breeds are able to limit the amount of lesion development for a given parasite burden. As the complete elimination of the agent from the infected organism is rendered impossible by VSG coat switch, animals showing some ability to limit consequences of infection (trypanotolerance) are thus favored through natural selection. Over the past decade, the new concept of an antidisease vaccine has gained in popularity since its introduction by Playfair et al. (127) in the context of malaria. The goal of such a vaccine is no longer to allow a rapid elimination of the parasite but to neutralize the pathological effects of trypanosomal factors. Furthermore, where classic chemotherapy fails or is poorly sustainable, support treatments can be envisaged, which would aim at countering part of the pathogenesis. For these so-called "antidisease strategies," precise knowledge of host-parasite interactions and parasitic factors involved in pathogenesis is needed. Significant work has been achieved in this field since the last paper reviewing biologically active parasite products was published in 1978 (151). The present paper thus aims at presenting recent advances regarding parasite factors involved in AT, with special reference to prospects for curative or preventive interventions at the level of host-parasite interaction.

AT AND COMPARATIVE PATHOLOGY

The definition of AT actually encompasses very different types of pathogenesis (4), relating to the broad range of trypanosome species belonging to three different subgenera (*Trypanozoon*, *Dutonella*, and *Nanomonas*). In the present context, it is crucial that this fact be kept in mind, as one therapeutic agent or a vaccine could be potent for only one of these various trypanosome species. On the other hand, the species within the *Trypanosoma* genus naturally share an important core genome and proteome. Although interactions involving parasite factors might ultimately differ between them, it seems reasonable to envisage the potential role of a factor in one trypanosome species if this factor is described to play some role in another. As great achievements in AT have been made by using comparisons with malaria and leishmaniasis, such a comparative approach among trypanosomiases could be fruitful (62, 79, 146). In this context, the Tritryp project, which involves jointly analyzing genomes and proteomes of *T. brucei brucei*, *Trypanosoma cruzi*, and *Leishmania major*, highlights the remarkably conserved genetic core between these species (23, 38, 62, 125). Several genome projects are also under way for *T. brucei gambiense*, *T. congolense*, and *T. vivax* (62). Besides the significant success of the *T. cruzi* proteome project (6), the recent description of the *T. brucei* plasma membrane subproteome was a unique breakthrough (24, 60). These achievements should bring insights into host-parasite interactions and the identification of new vaccine candidates or chemotherapeutic targets. New insights are already being gained from research into the trypanosome flagellar pocket, the main host-parasite interface (51). As divergences should not mask convergences and vice versa, in an attempt to leave a place for such comparative thinking, the authors will briefly men-

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tion here factors described for *T. cruzi* and the eventual existence of orthologs among African trypanosome species and will highlight differences between trypanosome species where necessary.

THE MAJOR CANDIDATES

VSGs and Glycosyl-Inositol-Phosphate Anchors. The immune response and immunopathology in AT have been thoroughly investigated during the last few decades (84, 146). In addition to the fascinating VSG switch immune evasion mechanism (126), immune suppression is a classical feature of AT, a phenomenon upon which much research effort has been concentrated (9, 84, 114, 146). Macrophage activation has been highlighted as a critical point of immune function or dysfunction in both *T. brucei brucei* and *T. congolense* infection mouse models (9, 114).

Pathogenesis is partly related to macrophage overactivation and uncontrolled production of harmful substances, such as tumor necrosis factor (TNF), which induces immune suppression, anemia, organ lesions, and cachexia (32, 78, 81). While soluble VSGs (sVSGs), actively shed by live parasites, have been identified as major TNF-inducing factors through the glycosyl-inositol-phosphate (GIP) moiety, membrane-fixed VSGs, which are abundantly released into the bloodstream at each parasite lysis phase, have been shown to contribute to macrophage overactivation through their dimyristoylglycerol compound (146). TNF does not, however, play the same pathological role based on the disease-causing species (105). This difference could relate directly to differences in VSG structure leading to different abilities to trigger TNF production, with GIP-associated galactose side chains being potentially involved (80). Moreover, phospholipase C (PLC), which is responsible for sVSG release by live *T. brucei brucei*, might not have the same role in *T. congolense* or *T. vivax* infection, minimizing the role played by sVSG in pathogenesis in *T. vivax*. A PLC-deleted *T. brucei brucei* strain ($PLC^{-}/^{-}$) has shown this enzyme to be necessary to affect the accurately timed and controlled macrophage activation needed for defense (9).

Cysteine Peptidases. Cysteine peptidases (CPs), which are known to play a role in the pathogenesis of *T. cruzi* and *Leishmania* spp. infections (91), could also be involved in AT-induced immune depression, anemia, thrombocytopenia, or central nervous system (CNS) invasion (7, 8, 42, 63, 111, 152). In addition, CPs are considered potential targets for future drug design (trypanocide and antidisease) as well as antidisease vaccine candidates (29, 64, 92). Although most of the literature has dealt with *T. cruzi* CP (cruzain or cruzipain), CPs from *T. brucei brucei*, *T. congolense*, and *T. brucei rhodesiense* have also been extensively studied (8, 28, 42, 56, 67, 90, 119, 134, 152). Progress in proteomics and genomics will further allow rapid evolution in the field, as several new proteases are already available in gene databanks. Simultaneously, important efforts are being undertaken for the development of CP-inhibiting molecules for *T. congolense*, *T. brucei brucei*, and *T. brucei rhodesiense* (34, 35, 48, 49, 59, 82, 157).

Most work on CPs from an antidisease point of view has been conducted in *T. congolense*, based on the initial observation of a better immune response directed against these molecules in trypanotolerant cattle in comparison with susceptible breeds (8). Structurally, *T. congolense* CPs have a molecular mass of about 33 kDa and contain two major domains: a catalytic domain and a C-terminal extension, which is not present in mammalian homologs and which is not involved in enzymatic activity (22). Two families of related CPs have been described, CP1 and CP2, for which distinct roles in pathogenesis have been suspected from an immunization study conducted in cattle (7). This study suggests a greater role for CP2, which could contribute to anemia as well as to immunosuppression, while the role of CP1 is more restricted to the housekeeping duties in the cell. This greater role for CP2 was linked by those authors to its capacity to degrade protein substrates at physiological pH. Procongopain (*T. congolense* CP precursor) has also been shown to be processed at basic pH (141). A similar observation has been made regarding the *T. brucei rhodesiense* CP, rhodesain (and cruzain of *T. cruzi*), which have been found to retain significant activity and stability up to pH 8, and this is thus said to be consistent with a possible extracellular function (28). Interestingly, *T. brucei brucei* CP has been shown to be released extracellularly by viable, actively motile, intact, and highly infective trypanosomes (117, 119). The pH activity profile for this protease has also been shown to range from 7.0 to 9.0. However, doubts regarding the possible activity of CPs released into a host's bloodstream arise from their sensitivity to plasmatic inhibitors, such as cystatin, α-macroglobulin, and kininogen (152). Quite surprisingly, activation of a *T. brucei* CP by a kininogen-like molecule from rat serum (called rat trypanopain modulator) has also been reported (69). Recently, the first report of the in vivo activity of bloodstream-released CP has been published with a description of a pyroglutamyl peptidase (PGP) type I from *T. brucei brucei* (96). In that study, this enzyme showed optimal activity at bloodstream pH and proved to be insensitive to plasma CP inhibitors. Substrates for PGP are N-terminally blocked with the pyroglutamyl moiety, such as tyrotrophin-releasing hormone or gonadotrophin-releasing hormone. Both hormones show dramatically reduced activity in infected hosts, and PGP proved to be responsible for total reduction of tyrotrophin-releasing hormone activity and partial reduction for gonadotropin-releasing hormone.

Oligopeptidases. CPs are not the only parasite protease family to be possibly involved in AT (135). Several other peptidases have now been described in *T. brucei brucei*, *T. congolense*, or *T. evansi* which are suspected of playing a role in pathogenesis (95, 98, 99, 101). Trypanosomal serine oligopeptidases (OPs) were first described in *T. brucei brucei* (152). This family (composed of 80-kDa proteins) is known to catalyze the degradation of several peptide hormones, such as neurotensin or atrial natriuretic peptide. They have further been shown to retain full catalytic activity in the bloodstream of an infected host, being insensitive to plasma peptidase inhibitors (97, 101, 152). The release of OP into the bloodstream is thought to occur during parasite lysis phases, as no active secretion has been observed in vitro (the protein lacks a signal peptide) (97). Thus, OPs are strongly thought to be responsible for the observed endocrine dysfunctions following parasite lysis (144) and more precisely, for the reduction in plasma levels of atrial natriuretic factor (107). This reduction would explain the reported hypervolemia (3, 155), and thereby the cardiomyopathy, known to occur in early AT (19). In vivo treatments with

OP inhibitors have been shown to improve the survival rate during *T. brucei brucei* infection. However, no correlation between inhibitory potency and in vitro trypanocide effect has been observed (100). Inhibitors are thus now thought to act by neutralizing free released enzymes, rather than through an intraparasitic effect (97). Recently, tropolysin, a newly described OP of *T. brucei brucei* and *T. congolense*, has been shown to abrogate bradykinin prohypotensive properties in vivo and to contribute to the dysregulated kinin metabolism in an infected host (101).

NEW CANDIDATES

Anemia and Sialidases. The pathogenesis of anemia in AT is not completely understood. Different nonexclusive mechanisms are thought to be involved: impaired erythropoiesis, increased erythrophagocytosis, and direct alteration of red blood cells (RBC) (146). The first mechanism might involve nitric oxide secretion (75), while the second has been shown to be induced by macrophage overactivation in the spleen and liver (104). These mechanisms have more or less importance according to the trypanosome species involved. In this respect, it is currently well-known that anemia in *T. brucei brucei* infection is TNF dependent (macrophage overactivation) (81), while anemia in *T. congolense* is not (105). Interestingly, in a *T. evansi* model, only late anemia was shown to partially depend on TNF (15).

In infections by *T. congolense*, *T. vivax*, and potentially *T. evansi*, direct alteration of RBC is thus expected to be an important mechanism, in which parasite sialidases could play a central role. RBC surface alterations have indeed been shown to be caused by bloodstream *T. vivax*, *T. congolense*, and *T. evansi* sialidases, thus leading to their subsequent phagocytosis (26, 40, 115, 116). Although the experimental demonstration of sialidase activity in *T. congolense* bloodstream forms was quite recent (116), a direct interaction of this trypanosome with RBC resulting in alteration of these cells had already been shown in earlier studies (13, 14). *T. vivax* sialidase was shown to present some host specificity, desialating camel, goat, and zebu RBC, while those from mice, dogs, and N′Dama bulls were shown to be resistant (26). In *T. evansi*, an isolated sialidase was found to desialate ghost RBC in the following order of efficiency: mouse, rat, camel, goat, and dog (115). This host specificity could be linked to different sialic acid compositions between hosts, as was shown to be the case between N′Dama and zebu cattle (143). Indeed, sialic acid was shown to contain less *O*-acetyl than glycolyl groups in zebu cattle, while the inverse situation occurred in trypanotolerant N′Dama cattle. Characterization of bloodstream *T. congolense*, *T. evansi*, and *T. vivax* sialidases showed pH optima of 6, 5.5, and 6.5, respectively, and a common optimum temperature of 37°C (26, 115, 116). Sialidases can, moreover, be considered in the context of alterations other than anemia and are known to desialate thrombocytes, leukocytes, and brain cells as well (115). In the first case, subsequent phagocytosis of altered platelets could result in thrombocytopenia. This lesion is clinically important in all ATs (33), but a real hemorrhagic syndrome is particularly reported in some *T. vivax* infections, this trypanosome species being long known to present strong sialidase activity (5, 40, 44). Because surface sialic acids of lymphocytes can be resynthesized within a few hours, sialidases are not thought to affect destruction by phagocytosis (65). Sialidase treatment of macrophages, however, has been shown to result in a loss of their binding capacity, which could contribute to trypanosome-induced immune depression (65). To date, no report has been made of treatments using sialidase inhibitors or immunization assays.

In *T. cruzi*, the involvement of a *trans*-sialidase in leukocyte apoptosis has been reported (102). However, *trans*-sialidase activity has not been found in any bloodstream form of African trypanosomes.

The Brain-Blood Barrier and CNS Lesions. The role of parasite soluble products in AT-induced CNS disturbances has been considered over the last few years. The involvement of parasite products has been proposed both in the passage across the blood-brain barrier (BBB) and in the induction of lesions through apoptosis.

The involvement of sVSG has been suggested in BBB passage due to its ability to increase the expression of proinflammatory cytokines (TNF, interleukin-6 [IL-6], IL-8, and NO) and adhesion molecules (intracellular adhesion molecule 1, E-selectin, and vascular adhesion molecule-1) shown in cultured endothelial cells (47). In addition, human bone marrow endothelial cell layers (an in vitro model for BBB) have been shown to increase the permeability coefficient when cultured with live *T. brucei gambiense*, parasite culture supernatant, or sVSG (47). In this regard, the facilitating role of gamma interferon (IFN-γ) in CNS invasion by *T. brucei brucei* has been well-described and must be considered (87).

Changes in BBB permeability, however, are commonly accepted to be multifactorial, and the roles of other parasite products, such as proteases (CPs), phosphatases, endotoxins, procyclin, or cytokine-like factors, in this process have not been ruled out (18, 68). A crucial role for CPs was first suspected from the greater ability of *T. brucei gambiense* to cross the BBB in comparison with *T. brucei brucei*, which was shown to express lower CP levels (110). CPs have indeed been shown to play a crucial role in this phenomenon in a human bone marrow epithelial cell model, with CP inhibitors abrogating epithelium crossing by *T. brucei gambiense* and CP-enriched fractions enhancing it (111). OPs from *T. brucei brucei* and *T. congolense* have also been presented as potential BBB disruptors through the cleavage of atrial natriuretic factor, this hormone being the ligand of high-affinity receptors localized on cells of the BBB, which constitutes the end organ of atriopeptin control for water balance in the CNS (68).

Procyclin (a surface protein expressed in procyclic forms of trypanosomes), and also procyclin derivatives, has been identified as a factor inducing apoptosis (trypanosome apoptotic factor) in mouse and human brain vascular endothelial cells, being produced by *T. brucei brucei* bloodstream forms (148). The involvement of trypanosome apoptotic factor is thus proposed not only in the entry of parasites into the CNS through endothelial cell apoptosis but also in immune evasion, targeting immune cells, and in the induction of extensive lesions in the cerebellum and brainstem. Such lesions have been found to occur during peak parasitemia in mice infected by *T. brucei brucei* (147) and have also been shown to occur in mice inoculated intraperitoneally with *T. brucei brucei* culture supernatant, suggesting an early role for this factor in pathogenesis

(148). Apoptosis has also been described in brain endothelial and microglial cells, which are both constituents of the BBB, when exposed to cerebrospinal fluid from patients in the early hemolymphatic stage (stage 1) or late encephalitic stage (stage 2) of AT (46). Microglial cells display macrophage-related functions and constitute an example of an immune target for African trypanosome apoptosis-inducing factor. Interestingly, apoptosis depends on different mechanisms in endothelial and microglial cells, the latter being more sensitive to sFasL apoptosis induction and occurring only during stage 2, while endothelial apoptosis has been found in both stages and was proven not to involve BBB disruption (46).

Lymphopenia and Apoptosis. T-lymphocyte unresponsiveness observed in AT is thought to result from a depression of IL-2 and IL-2 receptor production, which are induced by suppressor macrophages (84). In addition to T- and B-cell unresponsiveness, however, true lymphopenia is reported at the onset of the first parasitemia wave during early infection by *T. congolense*, *T. vivax*, and *T. cruzi* (39, 77, 86, 89). In sheep, *T. evansi* infection has been shown to provoke a decrease in $CD4^+$ T cells with an increase in $CD8^+$ T cells in the most susceptible individuals, while opposite modifications have been shown in more resistant individuals, suggesting that $CD4^+$ lymphocyte depletion is one of the cornerstones of parasite survival (123). Recently, parasitemia-associated B-cell apoptosis was shown in a murine model of *T. brucei brucei* infection (128). This parasite-induced B-cell dysfunction was, moreover, shown to disable the host's capacity to raise a long-lasting specific protective antiparasite antibody response and to abrogate a vaccine-induced protective response to nonrelated pathogens, which is a classical feature of AT (54, 55, 124, 136).

In *T. cruzi* infection models, the parasite has been shown directly to induce lymphocyte death through apoptosis (36). Apoptosis induction has been shown to affect $CD4^+$ T cells in particular (70–73, 85). Specific factors have been implicated, such as HSP70, *trans*-sialidases, and a protein called Tc52, sharing functional and structural properties with the thioredoxin and glutaredoxin family (20, 21, 85, 102). B-cell apoptosis in *T. cruzi* infection has also been described (162, 163).

Peptidase Inhibitors. African trypanosomes possess protease inhibitors, which modulate endogenous enzyme activity. Initially described in *T. cruzi*, a family of CP inhibitors has been named chagasin, but the inhibitors are also referred to as inhibitors of cysteine peptidases (ICPs). ICPs are distinct from the cystatin family, which is present in mammals and plants but absent in kinetoplastids (1). To date, few studies concerning ICPs in AT are available. A recent study involving ICP-deleted *T. brucei brucei* parasites highlighted the importance of CPs for their virulence and their ability to delete parasites, inducing higher parasitemia (140). However, as cystatins or cystatin-like molecules present immunomodulatory properties, ICPs should constitute a promising research field for AT (159).

Complement-Activating Factors. Complement occupies a central role in trypanosomiasis pathogenesis, of which hypocomplementemia is known to be a characteristic feature both in humans and animals (50, 109, 137, 153). Abnormal complement activation has long been known to be linked to trypanosome-released products (31, 108, 110, 142). These early studies characterized two complement-activating factors, called CAF-T in *T. congolense* (and *T. lewisi*) and SCAF in *T. cruzi*. Both components were shown to be nonproteinaceous, and the molecular mass of SCAF was 23 kDa. More recently, anticomplement properties have been found in different parasite factors, constituting an evasion strategy from complement-mediated lysis. In *T. cruzi*, these identified factors are T-DAF (C3 convertase decay accelerating factor, homologous to mammalian DAF), gp160, gp58/68, a complement regulatory protein (CRP), and gp63 surface protease (160). gp63 surface protease is also present in African trypanosomes and *Leishmania* spp., in which it was originally identified as a protein involved in cell invasion (37).

A recent review was dedicated to another *T. cruzi* complement-modulating factor: calreticulin (TcCRT), a 45-kDa calcium-binding protein (41). Thought to cause classical pathway inhibition through interaction with the C1q complement component, TcCRT provides a plausible explanation for the known inability of classical pathway activation to result in efficient *T. cruzi* lysis. As a calreticulin ortholog is present in African trypanosomes (GenBank accession number Tc00.1047053509011.40) (16), a similar role could also be suspected.

The role of complement is conceived of as much wider than just immune defense through opsonization or cell lysis. Complement components are also involved in tissue regeneration, growth factors for T and B cells, and in hematopoiesis and inflammation (58). Blood parasites might thus not only be resistant to complement system attack but might also use some of its properties to their own benefit.

OTHER CANDIDATES: MYTHS AND CONTROVERSIES

Trypanosome Lymphocyte Triggering Factor and IFN-γ. The trypanosome lymphocyte-triggering factor (TLTF) is a 185-kDa protein which was first found to be released by *T. brucei brucei* (120). Since its discovery in *T. brucei brucei*, TLTF has been shown to also be produced by *T. brucei rhodesiense*, *T. brucei gambiense*, and *T. evansi* (10, 112). It has been proposed that TLTF mediates the production of IFN-γ by T cells, which would in turn act as a growth factor for the parasite and stimulate TLTF production (11, 53, 120, 121). IFN-γ-induced TLTF production has, moreover, been shown to be variable between parasite species, with trypanosomes with a better productive response being characterized by better proliferation and a longer infection time (112).

In fact, this immunomodulatory function of TLTF is highly controversial, as is the role of IFN-γ as a parasite growth factor; many independent efforts to reproduce these results are known to have failed. Isolation of the TLTF gene and production of a 54-kDa recombinant protein have allowed the determination of its localization in vesicles near the flagellar pocket (154). Some more recent studies have highlighted the role of TLTF, now called trypanin, as a component of the flagellar dynein regulatory complex (129, 130). As such, trypanin was shown to be necessary to parasite survival and multiplication, probably through its role in cell motility, with incorrect cell division resulting from impeded motility (25, 129, 130). While many researchers would restrict trypanin to its cytoskeletal function, some authors continue to mention it as an immunomodulatory cytoskeleton-associated protein (18, 158).

B-Cell Mitogen. Polyclonal B-cell activation (PBA) is a major immunosuppressive event in a variety of pathogens, as it is

believed to divert the specific response against the parasite (133). The production of nonspecific or even autoreactive immunoglobulin M (IgM) could furthermore be associated in AT with anemia and lesions in kidneys, in articulations, or in the CNS (57, 61, 156). However, while research into *T. cruzi* has yielded several identified candidate parasite mitogens, among which TcPA45 (a proline racemase) is considered a vaccine candidate (43, 93, 94, 133), in AT this phenomenon is still poorly described. Nevertheless, interesting similarities between PBA in Chagas' disease and AT have been raised. These include the description of a cell cycle arrest both in *T. cruzi* and in *T. brucei brucei* infection models and the importance of $CD5^+$ B cells in nonspecific IgM production in *T. cruzi*, *T. evansi*, and *T. congolense* infections in mouse, sheep, and cattle, respectively (27, 52, 93, 138, 162). In AT, early studies showed that PBA resulting from stimulation by *T. brucei rhodesiense* and *T. brucei brucei* extracts was mediated by spleen macrophages or T cells (118, 139). However, there is an obvious lack of information regarding PBA for different African trypanosomes. In this regard, the report of a direct mitogenic effect of *T. brucei brucei* and *T. congolense* extracts on rabbit normal lymphocytes in vitro is quite intriguing, while in the same study, no direct effect was observed in mouse, rat, or guinea pig lymphocytes (83). Some level of importance of the animal model used to investigate this point of pathogenesis should not be ruled out. Recently, Namangala et al. (106) revived the hypothesis of a B-cell mitogen-like molecule from a *T. congolense* murine model.

ANTIDISEASE TREATMENTS

Vaccination Trials. In AT, the only example available of an "antidisease vaccine" is a cattle immunization strategy using congopain (7). Immunization resulted in a statistically significant beneficial effect on anemia and immunosuppression during infection. However, this protective effect proved to be too limited to be practical, and other antidisease vaccine candidates are needed to enhance efficacy.

For *T. cruzi*, immunization trials (as a protective or therapeutic vaccine) based on a cysteine protease or a *trans*-sialidase have been mentioned (30, 76, 161).

Although this does not constitute an antidisease vaccine, interesting results have been obtained in protection against *T. brucei brucei*, *T. brucei rhodesiense*, *T. evansi*, and *T. congolense* through the immunization of mice using tubulin-rich preparations or microtubule-associated proteins (12, 66, 74, 132). Microtubule-associated proteins are a family unique to trypanosomes, binding subpellicular microtubules one to another and to the plasma membrane (131).

Immunomodulatory Treatments. The important role of GIP as a pathogenesis-inducing factor has led to trials consisting of the treatment of mice with GIP molecules before infection (145). These trials were successful in reducing weight loss, liver damage, acidosis, and anemia during infections by *T. brucei*. This reduction in pathology was associated with a reduced TNF production and an increased level of IL-10, along with the expression of alternatively activated macrophage markers. This protective action of GIP treatment was not dependent on B cells and immunoglobulin production and, therefore, cannot be considered as a vaccination, in contrast to the case of GIP treatments in malaria. Quite strikingly, these GIP treatments were also efficient in pathology alleviation, particularly of anemia, in *T. congolense* and *T. evansi* infection models, in which TNF has been shown not to play the same role in pathogenesis in general and in acute anemia in particular (15, 105). This points out that the role of GIP in pathogenesis must not be considered to be solely through the overproduction of TNF.

Despite the controversial role of TLTF as an immunomodulatory factor, in vivo neutralization trials in *T. brucei brucei*-infected mice have been briefly mentioned (11). Those authors showed that repeated injections of a monoclonal antibody previously shown to impede in vitro triggering of IFN-γ production by TLTF resulted in a significant suppression of parasite growth and prolonged survival.

As far as the involvement of immune imbalance in the pathogenesis of AT is concerned, direct treatment with immunomodulatory molecules can be considered, as illustrated by the early report of the beneficial effect of an injection of immunostimulants prior to or at trypanosome inoculation (103). Another trial consisting of the treatment of *T. congolense*-infected sheep with levamisole was also conducted but was not successful (17).

In this regard, several studies aimed at elucidating immunopathology of AT have made use of diverse tools to neutralize detrimental immune pathways or to enhance beneficial ones. Briefly, one example reported the treatment of *T. congolense*-infected mice (chimeric SCID mice possessing a bovine immune system) with anti-CD40 antibodies in an attempt to promote correct global immunoglobulin production and a switch to IgG, as these last two factors have been described as characteristics of bovine trypanotolerance (52).

Given the known central involvement of TNF in *T. brucei* pathogenesis, countering this response could be an interesting option. In this regard, the shedding of soluble TNF receptor 2 (P75) in the murine model has proved to correlate with pathogenesis reduction (81). Thus, different TNF-α inhibitors (monoclonal antibodies, such as Infliximab or Adalimumab, or of type P75, such as Etanercept) could be proposed for human AT, as these are already registered for the treatment of human inflammatory diseases. Nevertheless, this track should not be considered economically practical in breeding stocks.

Recently, TGF-β use in *T. congolense*-infected mice has also been reported in the context of the development of alternative treatments (106). A triple intraperitoneal injection of human recombinant TGF-β1 (days −5, −1, and +3 relative to infection) resulted in a significant reduction in early anemia, a reduction in the first parasitemia peak, the absence of acute splenomegaly, and finally a delayed mortality. However, after the first parasite wave, no effect of the treatment could be observed, the protection being thus limited to the early stages, delaying pathogenesis. Interestingly, similar results have been obtained for murine malaria (122). TGF-β1 was shown to operate in a very transient way, possibly through an enhancement of the role of NK cells in the first response to infection. Taking this into account and given its very narrow therapeutic range and the side effects of a potential long-term treatment, TGF-β1 should not be considered as constituting a viable treatment on its own. Nevertheless, immunomodulatory intervention appears to be an interesting alternative which deserves further investigation. Immunomodulatory cocktail protocols,

which would help the host to manage correctly its own inflammatory response, could thus be envisaged.

A last treatment trial using heparin in *T. brucei gambiense*-infected rats can also be cited in this category (113). In this study, it was shown that the significant inhibition of parasite growth upon heparin treatment probably resulted from an increase in serum levels of high-density lipoprotein and haptoglobin, which are known to possess trypanocide properties. Heparin thus acted as an enhancer of natural immunity.

Anemia and EPO. Treatment of anemia in *T. congolense*-infected mice was also recently tried through injections of recombinant human erythropoietin (5,000 U/kg of body weight) (150). This treatment was successful at impeding anemia development in the acute phase, in contrast with a pentamidine treatment. Survival rates were dramatically improved in EPO-treated mice: all untreated animals died by day 9 of infection, while 100% of EPO-treated mice survived until day 20 and 50% survived until day 40. These authors proposed the use of such a treatment in conjunction with classical trypanocide treatments, as their mechanisms are obviously different. Interestingly, in the trypanotolerant N′Dama cattle, Epo transcript levels in the kidney during *T. congolense* infection have been shown not to be statistically different from those in infected Boran cattle (149). By contrast, Epo receptor transcript levels in bone marrow were downregulated in the Boran cattle in comparison with the N′Dama. This was attributed by the authors to the increased IFN-γ response in the Boran breed.

BBB Crossing and Minocycline. Preventing BBB passage is of great importance in human AT, as the arsenical treatment presently available for late neurological stages presents high toxicity, and even mortality (5%). Based on the similarities between the mechanisms underlying BBB crossings by trypanosomes and leukocytes, the use of minocycline (a tetracycline derivative) has been proposed in AT, given the efficacy of this pleiotropic molecule in experimental allergic encephalitis (88). This treatment showed remarkable efficacy in a murine model (87) and could thus be proposed for use in human or domestic animals in conjunction with other trypanocide or antidisease treatments.

SUMMARY AND CONCLUSION

As parasite strains resistant to chemotherapy are being disseminated around the world, new methods will be needed to improve our control of AT. From this point of view, the "antidisease strategy" is of major interest. This strategy implies a need for accurate knowledge regarding host-parasite interactions so that targets may be located at the level at which chemotherapy or vaccination can act. Important achievements have already been reported and opportunities for research remain vast. Promising progress in *Trypanosoma* spp. genomics, proteomics, and comparative pathology studies using the mouse model will certainly bring forth exciting new pathways and challenges for research in the near future.

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