



## Immunology and immunopathology of African trypanosomiasis

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### ABSTRACT

Major modifications of immune system have been observed in African trypanosomiasis. These immune reactions do not lead to protection and are also involved in immunopathology disorders. The major surface component (variable surface glycoprotein, VSG) is associated with escape to immune reactions, cytokine network dysfunctions and autoantibody production. Most of our knowledge result from experimental trypanosomiasis. Innate resistance elements have been characterised. In infected mice, VSG preferentially stimulates a Th 1-cell subset. A response of  $\gamma\delta$  and CD8 T cells to trypanosome antigens was observed in trypanotolerant cattle. An increase in CD5 B cells, responsible for most serum IgM and production of autoantibodies has been noted in infected cattle. Macrophages play important roles in trypanosomiasis, in synergy with antibodies (phagocytosis) and by secreting various molecules (radicals, cytokines, prostaglandins,...). Trypanosomes are highly sensitive to TNF- $\alpha$ , reactive oxygen and nitrogen intermediates. TNF- $\alpha$  is also involved in cachexia. IFN- $\gamma$  acts as a parasite growth factor. These various elements contribute to immunosuppression. Trypanosomes have learnt to use immune mechanisms to its own profit. Recent data show the importance of alternative macrophage activation, including arginase induction. L-ornithine produced by host arginase is essential to parasite growth. All these data reflect the deep insight into the immune system realised by trypanosomes and might suggest interference therapeutic approaches.

**Key words:** Trypanosome, African trypanosomiasis, immunology, macrophage, lymphocytes, nitric oxide, cytokine, autoantibodies.

### INTRODUCTION

Sleeping sickness or human African trypanosomiasis (HAT) is an endemic parasitic disease exclusively located in intertropical Africa where it is transmitted by the tsetse fly or *Glossina*, its unique vector (Vickerman 1985). The new taxonomy tools used in African trypanosomes (isoenzyme charac-

terisation, DNA analysis) have allowed scientists to separate the *Trypanosoma brucei* clade in several subspecies. Two are infective for humans: *T.b. gambiense*, and *T.b. rhodesiense*. These extracellular parasites are injected into humans by the bite of infected tsetse fly..

The inoculation of trypanosomes into their mammalian hosts triggers a series of events involving, at first, innate immunity and, secondarily, spe-

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sific immunity. The latter requires an efficient presentation of parasitic antigens, activation of T and B cells implying specific antigen receptor recognition, and the development of effector cells and molecules. These mechanisms are highly regulated by multiple signals delivered through a large number of receptors transduced across the plasma membrane and processed. During co-evolution with their hosts, trypanosomes have learnt to cope with host immune systems, by penetrating, diverting, and altering the numerous steps leading to the generation of an effective immune response. Major modifications of immune systems have been observed in trypanosomiasis: lymphadenopathy, splenomegaly (up to thirty times the normal size) with destruction of lymphatic tissue architecture and hypergammaglobulinemia. However, their effectiveness is limited as, most of the time, parasites cannot be eliminated and immunopathological phenomena, which induce tissular alterations, appear.

One of the major characteristics of trypanosomes is the presence of the Variant Surface Glycoprotein (VSG) which covers nearly all the membrane of trypanosomes in mammals and is the predominant surface antigen of African trypanosomes. VSG constitutes an important molecular interface between trypanosomes and the host immune system (Figure 1). VSG prevents trypanosome lysis by complement alternative pathway, and, above all, enables them to avoid the specific immune response via the phenomenon of antigenic variation (trypanosomes sequentially express antigenically distinct VSG). VSG also has several effects on immune elements such as induction of autoantibodies and cytokines, in particular tumour necrosis factor (TNF)- $\alpha$  (Tachado and Schofield 1994, Okomo-Assoumou et al. 1995a, Magez et al. 2002). Other trypanosome components and soluble factors, such as a trypanosome-released triggering factor (TLTF) which triggers interferon (IFN)- $\gamma$  production by T cells, are also involved in modulation of the immune system by acting on the synthesis of immune elements (Olsson et al. 1991, Vaidya et al. 1997) (Figure 2). Furthermore, increased levels of circulating endotoxins

are a feature of human and experimental trypanosomiasis. These endotoxins, potent immunomodulatory molecules, participate to the immune disorders observed in trypanosomiasis. (Nyakundi et al. 2002). Elaboration of escape mechanisms to host immune defences and induction of parasite growth factor production are well developed by trypanosomes. In a recently discovered escape mechanism, host arginase induction, trypanosomes decrease immune response efficiency and increase the production of L-ornithine, an essential growth factor (Gobert et al. 2000, Vincendeau et al. 2003).

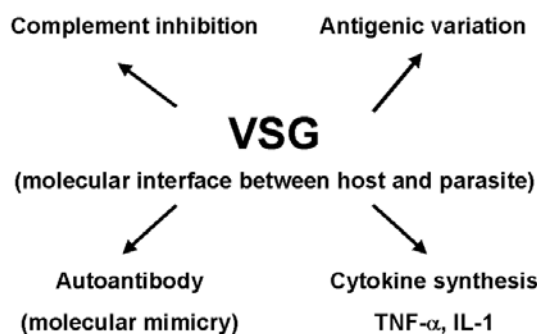


Fig. 1 – Variable surface glycoprotein (VSG), the major surface component of trypanosomes, is also released in host fluids. VSG induce resistance to complement lysis, escape to specific immune response, persistent cytokine production, autoantibody synthesis by molecular mimicry with host tissues.

Understanding of the immune response was recently advanced by the discovery of the T and B subpopulations and, especially, of the T helper (Th) subsets, as well as the cytokines synthesised by each Th1 and Th2 subset. These factors control different aspects of the immune response, in peculiar the synthesis of nitric oxide, which is probably involved in several steps in the immune mechanisms. The role of  $\gamma\delta$ T cells should also be taken into account as they have been implicated in other parasitic diseases such as malaria and leishmaniasis.

Most of the data concerning African trypanosomiasis have been obtained in animal diseases or experimental animal models. Few studies have

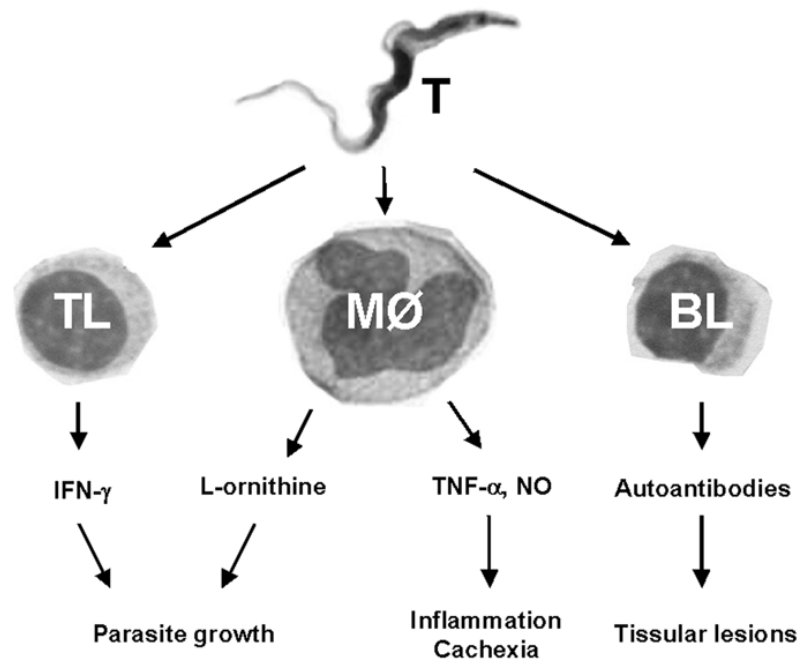


Fig. 2 – Trypanosomes induce secretion of various components from immune cells. Besides their trypanocidal effects, these molecules are also involved in deleterious mechanisms for host tissues and/or favour parasite growth. (T: trypanosome; MØ: macrophage; TL: T lymphocyte; BL: B lymphocyte)

concentrated only on the immunology of HAT. Results obtained from animal diseases or experimental models can be investigated in human trypanosomiasis using adapted means. Genetic analysis of resistance and susceptibility to infection in inbred and congenic animal strains form the basis for research into equivalent genes in humans. Introduction of double-stranded RNA (ds RNA) into parasites induces potent and specific gene silencing a phenomenon called RNA interference (RNAi) and is a valuable tool to investigate trypanosome gene functions. The recent knowledge of the entire genome of *T. brucei* is an essential breakthrough to investigate immunology and immunopathology of HAT (Berriman et al. 2005).

#### CLINICAL SYMPTOMATOLOGY OF HAT

After a painful tsetse bite, the chancre (from several millimetres to centimetres) represents the initial le-

sion at the bite site, characterised by local erythema, oedema, heat, tenderness and a lack of any suppuration. Trypanosomes are present in the inflammatory tissues. The chancre disappears within 2 or 3 weeks. The disease evolves in two distinct successive phases determining its two pathological stages (Molyneux et al. 1996, Dumas and Bisser 1999). Within a few days after the tsetse bite, the patient enters the haemolymphatic stage I of the illness.

#### STAGE I, THE HAEMOLYMPHATIC STAGE

Clinical signs appear very early. Intermittent fever develops as a consequence of the successive waves of invasion of the blood by the trypanosomes. Adenopathies, splenomegaly, or even hepatological signs mark the invasion of the reticulo-endothelial system. Skin eruptions or trypanides are commonly observed. Severe pruritus with scratching skin lesions becomes unsupportable for the patient.

Cardiovascular alterations are less prominent, especially in the Gambian form. Irregular febrile episodes are accompanied by headaches, malaise, exhaustion, anorexia, extreme thirst, muscle and joint pains, pruritus, anaemia, rash and often deep hyperesthesia (the sign of the key of Kerandel). The lymph nodes are generally rubbery and mobile, painful at the beginning. Palpation of the subclavicular region (Winterbottom sign) is an important part of the diagnostic procedure in the Gambian form. Any adenopathy accompanied by fever should evoke the diagnosis of sleeping sickness in patients from endemic areas. Later, pruritus generalises. Oedema of the face and extremities appears early.

Few minor neurological and endocrine disorders may reveal the precocity of central nervous system (CNS) involvement, long before any detectable changes occur in the cerebrospinal fluid (CSF). Daytime somnolence or night-time insomnia may already be reported and electroencephalographic (EEG) tracings may reveal abnormalities. Psychiatric signs, with the alternation of irritability, changes in personality or mood affecting the daily and professional life of the patients, constitute often the first manifestation of the disease. The endocrine syndrome is marked by a permanent feeling of coldness, lack of appetite or in contrast hyperphagia, polydipsia and impotence, amenorrhea or infertility, indicative of vegetative and sexual disturbances.

#### STAGE II, THE MENINGOENCEPHALITIC STAGE

The meningoencephalitic stage appears slowly and insidiously over a period of months or years depending on the trypanosome. However, the clinical signs remain reversible for a long time with treatment attesting to the predominance of potentially reversible inflammatory lesions over irreversible demyelinating lesions. The general signs of the haemolympathic stage do not completely disappear: spikes of fever (but sometimes hypothermia), adenopathies and splenomegaly, cardiovascular manifestations, endocrine disturbances and typical pruritus.

The development of the neurological symptoms is progressive. As neurological signs occur already in stage I, biological criteria are the only means to confirm CNS invasion. The threshold criteria, which are commonly used, are based on CSF examination: more than 5 cells/ $\mu\text{L}$  and/or the presence of trypanosomes.

A wide variety of symptoms are encountered. The main symptoms from which sleeping sickness was named are daytime somnolence and nocturnal insomnia, the patients being "sleepy by day and restless by night". The sleep-wake cycle disturbances are accompanied with either or many of the following symptoms: headaches, sensory disturbances with diffuse superficial or deep sensations (muscle and bone hyperesthesia, either spontaneous or provoked; hyperpathia), presence of primitive reflexes (palm-mental reflex, sucking reflex), exaggerated deep tendon reflexes, psychiatric disorders (confusion, mood swings, agitation, aggressive behaviour, euphoria, absent gaze, mutism, indifference), and tremor (fine and diffuse without any myoclonic jerk at rest or during movement). Pyramidal alterations revealed by a Babinski sign can also be observed along with alterations in muscle tone, numbness or sensory deficit.

An abnormal number of monocytic cells is observed in the CSF. The early neurological symptoms correlate with the widespread meningeal inflammation, which occurs in both forms of HAT. The selective CNS locations explain in part the principal clinical neurological signs. Sleep-wake disturbances may result from invasion of the median eminence by parasites, which also accounts for neuroendocrine dysfunctions with the involvement of the suprachiasmatic nuclei. Disorders of the sleep-wake cycle are accompanied by a state of apathy in the patient, the loss of muscle tone especially in the neck muscles and a drooping of the eyelids. Extrapyramidal symptoms signal the involvement of the striatum. Deep sensory disturbances with hyperpathia may result from the involvement of the thalamus and the early invasion of posterior spinal roots.

Apart from the disruptions of the circadian

rhythm of the sleep-wake cycle, other biological rhythms are disturbed, such as body temperature, cortisol and prolactin or growth hormone secretion. The invasion of the subthalamic and hypophyseal regions account for the persistence of ad-endocrine disturbances such as impotence, amenorrhea or infertility and the development of disturbed sensations of hunger and thirst, with often hyperphagia and polydipsia in contrast to the poor general state of malnutrition of the patients. At the terminal phase of the disease, CNS demyelination and atrophy are accompanied with disturbances in consciousness and the development of dementia with incoherence, incontinence and epileptic fits. The patient dies in a state of cachexia and physiological misery.

#### GENETIC CONTROL OF TRYPANOSOMIASIS

The study of inbred and congenic mouse strains has contributed greatly to our understanding of the genetic regulation of infectious diseases. A number of genes control infections of various pathogens by acting at the level of innate susceptibility, or at the level of acquired immunity. They may or may not be linked to the major histocompatibility class (MHC) locus H2.

Inbred strains of mice differ in their susceptibility to infection with *T. congolense*, as judged by duration of survival following infection. Balb/c and A/J mice were the most susceptible and C57BL/6 the most resistant (Morrison et al. 1978). The existence of inbred susceptible and resistant strains of mice has made it possible to study the inheritance and mechanisms of host resistance. Resistance of mice to African trypanosomes is genetically determined. The control of resistance has been considered as dominant (Greenblatt et al. 1983) or recessive (De Gee et al. 1988). The use of different inbred mouse strains and trypanosome clones may explain this result (discrepancy).

In *T. b. rhodesiense*-infected mice, survival was not correlated with the height of the first peak of parasitemia, but a strong negative correlation between the second peak of parasitemia and survival

time was noted (Seed and Sechelski 1995). However, in *T. congolense*-infected mice, the efficiency of clearance of the first peak of parasitemia was correlated with the survival period. Three loci influencing resistance of mice to *T. congolense* infection have been recently reported on chromosomes 5, 17 and 1 (Kemp et al. 1997).

African cattle trypanosomiasis, mainly due to *T. congolense* and *T. vivax*, causes anaemia and weight loss, leading to death. Some African cattle breeds (N'Dama) are, however, able to live and be productive in endemic areas and are considered to be trypanotolerant. Trypanotolerance is genetically controlled and is an innate character, but can be increased by repeated infections. Trypanotolerant cattle are not refractory to trypanosome infections but limit proliferation of trypanosomes. Parasite counts are lower than in trypanosensitive cattle (*Bos taurus*, *B. indicus*). This resistance depends on the nutritional, physiological and stress conditions of the animal. Besides, studies of other factors in relation to host defence and survival, and especially cytokine production, have revealed that the ability to produce IL-4 plays a role in the susceptibility to *T. brucei* infection (Bakhiet et al. 1996). Recently, mouse strain susceptibility to trypanosome infection has been correlated with an increase in host arginase production (Duleu et al. 2004).

Little is known about the effect of genetic polymorphism on infectious diseases in humans. Identification of human homologues for the murine genes controlling resistance and susceptibility to pathogens is in progress. Family studies should also be performed. Our knowledge of human trypanotolerance is based on reported cases (Lapeyssonnie 1960) and results from immunological screening (Lemesre et al. 1988, Authié et al. 1993). Subjects with a positive CATT (card agglutination trypanosomiasis test) were asymptomatic whereas the presence of blood parasites was observed. Moreover, although many Bantou people from Mbomo foci in the Congo were infected with *T. b. gambiense*, none of the pygmy population was infected. This effect, present before infection and unrelated to antibody

production, is dependent on innate immunity factors. Moreover, in a recent study in the HAT focus of Sinfra (Côte d'Ivoire), single nucleotide polymorphisms within TNF- $\alpha$  and interleukin (IL)-10 promoters and genes were associated with susceptibility to HAT (Courtin et al. 2006).

### INNATE IMMUNITY

#### NATURAL IMMUNITY

Normal human sera injected into *T.b. brucei*-infected mice caused a dramatic reduction in parasitemia (Laveran and Mesnil 1912). This phenomenon was not reproduced with the human trypanosome strains *T.b. gambiense* and *T.b. rhodesiense*. Trypanolytic factors (TLF) contained in normal human serum were identified as high-density lipoproteins (Rifkin 1978). Recently, two TLFs have been characterised in human serum. The first one (TLF1) belongs to a subclass of high-density lipoproteins and is inhibited by haptoglobin. In contrast, the second factor, TLF2, has a much higher molecular weight and does not appear to be a lipoprotein. Probably, the main trypanolytic effect is due to TLF2, which is not inhibited by haptoglobin (Raper et al. 1996). The trypanocidal effect of cape buffalo serum has been attributed to xanthine oxidase (Muranjan et al. 1997). Recently a trypanosome lysosomal protein (SRA) was found to be associated with resistance to normal human serum. SRA is a truncated form of VSG and interacts with serum apolipoprotein L-I in the parasite lysosome (Vanhamme and Pays 2004).

#### CHANCRE

The local response in the skin corresponds to the first protection developed by the host. Following inoculation of *T. brucei* into mammalian hosts, by the tsetse fly, a local skin reaction is induced by trypanosome proliferation and appears a few days after inoculation. In efferent lymphatic vessels, trypanosomes have been detected in lymph 1-2 days before the chancre. Their number declined during development of the chancre (6 days) and later in-

creased. They are detected in the blood 5 days after inoculation. In *T. congolense*-infected sheep, neutrophils predominate in the early days and then T and B lymphocytes infiltrate the chancre. Later, T lymphocytes predominate, especially CD8<sup>+</sup> T cells (Mwangi et al. 1990). An early response due to an increase in CD4<sup>+</sup> and CD8<sup>+</sup> T cells was revealed by flow cytometry in the afferent lymph draining the chancre. As the chancres regressed there was an increase in lymphoblasts and surface immunoglobulin bearing cells (Mwangi et al. 1996). During this first stage, trypanosomes expressed Variable Antigen Types (VATs) found characteristically in the tsetse fly, which changed after few days. An antibody response specific to these VATs appeared in the lymph and then in the plasma (Barry and Emery 1984).

#### COMPLEMENT

Both in humans and animals, complement activation by two pathways is detected in HAT. The alternative pathway, independent of specific antibodies, was studied by the induction of trypanosome lysis (*T. congolense* and *T.b. brucei*) observed after the addition of fresh serum. Serum could induce trypanosome lysis only on uncoated VSG trypanosomes, as observed during the cycle of this parasite (procyclic forms). However, the appearance of VSG on parasites prevents trypanosome lysis by this alternative pathway (Ferrante and Allison 1983). For another strain of *T.b. gambiense*, it was demonstrated that the alternative pathway was incompletely activated without generation of the terminal complex (C5-C9) able to induce membrane lysis (Devine et al. 1986). The classical pathway, mediated by specific antibodies against trypanosomes, was also described and could be involved in parasite clearance by antibody-mediated lysis and/or opsonisation. The coated stages of *T.b. brucei* are lysed by antibodies with activation of complement by the classical pathway. Nevertheless, during these complement activations, the appearance of soluble fragments, including C3a and C5a anaphylatoxins and the C567 complex, could

induce, on the one hand, the chemotactism of neutrophils and monocytes and, on the other hand, the release of amines involved in vasoconstriction and an increase in vascular permeability participating in the initial inflammatory response in the chancre. Immune complexes can also activate the complement. These immune complexes are constituted by antibodies specific to trypanosomes (e.g. anti-VSG antibody) leading to a rapid elimination of complement-fixing immune complexes (Russo et al. 1994) or by autoantibodies (see below), such as rheumatoid factor or anti-nucleic acid antibodies. These immune complexes with complement activation are also involved in some adverse effects, especially in tissue damage mediated by immune complex deposits (Nielsen 1985), such as thrombosis and glomerular involvement (Bruijn et al. 1988, van Velthuysen et al. 1994).

#### NATURAL KILLER CELLS

Natural killer (NK) cells have been identified as an important defence mechanism against tumour cells and intracellular pathogens, especially viruses. They are considered to belong to the lymphocyte lineage and have functions in both innate and acquired immune responses. NK cells lyse extracellular parasites. NK cells from *T. cruzi*-infected mice have been shown to exhibit significant activity against trypomastigotes of *T. cruzi* (Hatcher and Kuhn 1982).

NK cells secrete cytokines and especially IFN- $\gamma$  and TNF- $\alpha$ , which play major roles in trypanosomiasis and are regulated by cytokines which can activate or inhibit NK cell functions. NK cells also participate in the initiation of the inflammatory response, through the synthesis of chemokines.

In *T. brucei*-infected mice, NK activity was not modified in the early stages of infection, but was severely reduced from day 9 onwards (Askonas and Bancroft 1984). By contrast, NK cells were activated in mice infected with a natural extracellular trypanosome (*T. musculi*) and their critical role was demonstrated by the effects of their depletion by antiserum against asialo GM1 or their activation by

polycytidylic copolymer (Albright et al. 1997).

#### T CELLS

Initial studies have evidenced alterations in T cell functions in trypanosomiasis, both *in vivo* and *in vitro* (Mansfield and Wallace 1974) (Figure 2). Histological examination revealed a massive B cell expansion in the lymph nodes and spleen, which replaced the thymus-dependant area in *T.b. brucei* TREU 667-infected mice. These changes were seen within 7 days post-infection and persisted for at least 70 days. Moreover the role of T cells in controlling infection was not clear (Askonas and Bancroft 1984).

Trypanosome antigen-specific T cell response was difficult to identify. In several studies, a transient proliferative T cell response to trypanosome antigens was noted in the first days of the infection followed by an absence of response (Gasbarre et al. 1980). The kinetoplastid membrane protein 11 of African trypanosomes is a potent stimulator of T lymphocyte proliferation (Tolson et al. 1994).

In *T. b. brucei*-infected mice, an increased proliferation of T cells was noted in the first days of infection in spleen and bone marrow, T blasts disappeared very rapidly. In *T. congolense*-infected cattle, antigen-specific proliferation of T cells was obtained with more or less difficulty according to the antigen, the T cell population and the time used. However, a strong trypanosome-specific T cell proliferation occurred in infected cattle following treatment (Emery et al. 1980, Lutje et al. 1995).

Most T cells in humans and mice bear T $\alpha\beta$  antigen receptors. These cells possess surface markers, which allow the discrimination of CD4<sup>+</sup> T cells (helper T cells) and CD8<sup>+</sup> T cells (cytotoxic T cells). The knowledge of T cell subsets has been deeply modified by the discovery of two subsets of T helper cells, Th1 and Th2 cells. Th1 cells expressing a functional T cell response directed to VSG are generated in *T.b. rhodesiense*-infected mice. VSG specific T cells were found predominantly in the peritoneum. These cells did not pro-

liferate but made a substantial IFN- $\gamma$  and IL-2 cytokine response (Schleifer et al. 1993). The cellular phenotype of VSG-responsive T cells (CD4<sup>+</sup> CD3<sup>+</sup>) indicates that the VSG appear to preferentially stimulate a Th1 cell subset during infection.

Analysis of lymphocyte subsets in regional lymph nodes of *T. congolense*-infected N'Dama (trypanotolerant) and Boran (trypanosusceptible) were performed by flow cytometry. In both breeds, a significant decrease in the percentage of CD2<sup>+</sup> and CD4<sup>+</sup> T cells was observed, associated with an increase in the percentage of CD8<sup>+</sup> T cells, B cells and  $\gamma\delta$ T cells. VSG and two invariant antigens (33 kDa cysteine protease and 66 kDa antigen homologous to immunoglobulin heavy chain binding protein hsp70/Bip) induced *in vitro* proliferation and synthesis of IL-2 and IFN- $\gamma$  (Authié et al. 1992, Boulangé and Authié 1994, Lutje et al. 1995). No significant differences in the *in vitro* proliferation of lymph node cells to VSG, Concanavalin A (Con A) or hsp 70/Bip were observed between the two breeds. However, IFN- $\gamma$  production in response to Con A was higher in Boran at 35 days post infection.

Human and mouse immune systems contain few  $\gamma\delta$ T cells, in marked contrast to those of ruminants (Hein and Mac Kay 1991). Functions of  $\gamma\delta$ T cells remain largely unknown. Involvement of  $\gamma\delta$ T cells in malaria and leishmaniasis has been observed (Rosat et al. 1995, Rzepczyk et al. 1997). A proliferative response of CD8<sup>+</sup> T cells and  $\gamma\delta$ T cells from trypanotolerant N'Dama to an antigen complex containing immunodominant epitopes was observed whereas a quasi absence of response was observed in trypanosusceptible Boran. The role of this  $\gamma\delta$ T cell response in parasite resistance remains unclear. So,  $\gamma\delta$ T cells, as CD4<sup>+</sup> or CD8<sup>+</sup>, do not proliferate when stimulated with soluble VSG *in vitro* (Flynn and Sileghem 1994). It would be interesting to determine the role of cytokines synthesised by  $\gamma\delta$ T cells.

Indeed, although specific T cells do not act on trypanosomes in the same way as the cytotoxic T cells in several infectious diseases such as viral infections, they markedly modify immune re-

sponses, especially by the secretion of cytokines. They greatly modify functions of B cells (antibody synthesis, isotype switch) and macrophages (antigen presentation, effector mechanisms).

## B CELLS

In African trypanosomiasis, the main feature is a dramatic increase in immunoglobulin (Ig) levels (especially IgM), including trypanosome-specific antibodies and non-specific Ig production induced by cytokine activation of B cells. Some of these antibodies are also raised against autoantigens, corresponding to non-specific polyclonal activation of B-cells producing natural autoantibodies and also to antigen-driven antibodies induced by molecular mimicry (Figure 2). DNA from *T.b. brucei* stimulated B cell proliferation (Shoda et al. 2001). In *T.b. brucei*-infected mice, B lymphocytes display an aberrant activation phenotype (Sacco et al. 1994).

Antibodies specific to trypanosomes are induced by several parasite antigens, including variant and invariant VSG epitopes, as well as membrane, cytoplasmic and nuclear antigens, through T-dependent and T-independent pathways (Reinitz and Mansfield 1990). Antibodies directed against trypanosome VSG components appeared in sera and their binding to the surface coat of the trypanosomes was able to induce a decrease in parasitemia, both in the blood and extravascular spaces, specifically by immune lysis of parasites and their destruction by the Kupffer cells in the liver. Only heterologous antigenic variants (<0.1%) remain to repopulate the blood and tissues. Parasites are eliminated due to VSG-specific IgM (appearing at high levels, 3-4 days after infection). In contrast, VSG-specific IgG does not seem to be involved in the destruction of trypanosomes, as they appeared after the disappearance of this VAT population. Another induction of antibodies, linked to the new VSG epitopes, appeared in sera and also contributed to decrease the new VAT-specific population. The VAT-specific antibodies therefore decreased to low levels, whereas antibodies, belonging predominantly



to the IgM class specific to invariant epitopes, remained at high levels. During infection, B cell non-specific stimulation was enhanced as T-independent B cell responses to the VSG successive parasitemias. In contrast, specific trypanosome B cell response, depending on T cell regulation, was depressed. Several factors may contribute to this immunosuppression. Macrophages may become unable to present antigens to T cells (by defects in antigen processing and association of epitopes with MHC Class II) and produce immunosuppressive factors as nitric oxide (NO), prostaglandins (PG), and cytokines. An increase in immunosuppressive cytokines, such as  $\text{INF-}\gamma$  and transforming growth factor (TGF)- $\beta$ , was also detected during infection. However, TGF- $\beta$  is known to inhibit the production of IL-4, IL-5, IL-6, the major cytokines implied in B cell proliferation and differentiation (Fargeas et al. 1992).

Several autoantibodies are detected during African trypanosomiasis. High levels of polyclonal Igs were a marked feature of HAT. The specificity of these Igs is frequently characterised against a large range of autoantigens. Autoantibodies were directed against red blood cells (Kobayakawa et al. 1979), liver and cardiolipids (MacKenzie and Boreham 1974), nucleic acids: DNA and RNA (Kobayakawa et al. 1979, Hunter et al. 1992b), intermediate filaments (Anthoons et al. 1986) and rheumatoid factors (Kazyumba et al. 1986). Autoantibodies directed against components of CNS myelin have also been reported. They are specific for the major glycosphingolipids of myelin, the galactocerebrosides, and were detected in sera from both experimentally infected animals (Jauberteau et al. 1991) and patients from the Ivory Coast (Amevigbe et al. 1992). Other autoantibodies directed against not yet characterised proteins have been described in HAT patients (Asonganyi et al. 1989) as well as antibodies directed at myelin basic protein in experimentally infected animals (Hunter et al. 1992a). Other antibodies were raised against an epitope containing L-tryptophan, a precursor to the neurotransmitter serotonin, (Okomo-Assoumou et al. 1995b)

or recognised some neuronal components of the cytoskeleton, neurofilament proteins. In some cases, these autoantibodies (anti-galactocerebrosides and anti-neurofilaments) are associated with the neurological stage of the disease and their detection in sera and CSF could contribute towards defining the neurological involvement of HAT (Courtioux et al. 2005). *In vivo* demyelination has been produced by purified antibodies to galactocerebroside (Saida et al. 1979). There are several hypotheses for the origin of these antibodies. They may be induced by a non-specific stimulation of B cells producing natural autoantibodies (Arneborn et al. 1983, Mortazavi-Milani et al. 1984). In other cases, antigen-driven autoantibodies are specific to epitopes of the causative infecting agent with molecular mimicry to self antigens, inducing a cross-reactivity to intermediate filaments (Dales et al. 1983, Fujinami et al. 1983, Davies 1997) as demonstrated for anti-neurofilament and anti-galactocerebroside antibodies which recognised respectively a flagellar component and a proteolipidic epitope of trypanosomes, and epitopes expressed by neurones (Ayed et al. 1997, Girard et al. 2000).

A subpopulation of B cells, identified by the expression of high levels of surface Igs and of CD5 in humans and Ly-1 in mice is responsible for most serum IgM (Kipps 1990). These CD5 cells produce autoantibodies, and antibodies to thymus-independent antigens. In cattle infected with *T. congolense*, a dramatic increase in these cells (more than four times the control value in blood) was measured and correlated with increases in serum Igs and in the absolute number of B cells (Naessens and Williams 1992). An induction of these CD5 B cells (directly by parasite products or indirectly through the cytokine network) could account for the alteration in immunoglobulin synthesis and antibody production observed in trypanosomiasis.

#### MACROPHAGES

Mononuclear phagocytes play a key role in all steps of immune response in the inflammatory phase, as

antigen presenting cells, in specific immunity, in synergy with antibodies and cytokines. They also can be involved in immunosuppressive and immunopathological phenomena. Quantitative, biochemical and functional changes of mononuclear phagocytes are observed in trypanosomiasis. In *T. brucei*-infected mice, histological examination showed a marked expansion in macrophages of the liver, spleen and bone marrow. The Kupffer cells in the liver increased in number and were often found in mitosis. The cells contained abundant phagolysosomes (vacuolated cytoplasm). An increase uptake of intravenously injected sheep red blood cells was also noted.

Macrophages are highly sensitive to environmental factors, especially microorganisms, microorganism-derived products and cytokines. A clear reduction in mannose receptors, Fc receptors, C3b receptors (Mac-1) and F4/80 occurs by day 4 post *T. b. brucei* infection. The expression of MHC Class II molecules (Ia antigens) was reported to have increased in *T. b. brucei*-infected mice and decreased in *T. b. rhodesiense*-infected mice. The antigen-presenting function was reported to be unmodified in *T. b. brucei*-infected mice and defective in *T. b. rhodesiense*-infected mice. Macrophages react to stimuli by adapted response. They secrete many factors with various functions. They synthesise cytokines and effector mediators.

Macrophages may play an important role in protection against trypanosomes, particularly in the presence of homologous antiserum. The immunological clearance of [75Se]-methionine-labelled *T. brucei* in mice has been conducted to investigate the respective roles of antibodies, macrophage activation and complement in the removal of circulating parasites. The clearance was largely accomplished by antibody-mediated hepatic phagocytosis. C3 is necessary for the full opsonic activity present in murine clearance in passively immunised mice (MacAskill et al. 1980). These *in vivo* studies extend previous studies on the *in vitro* phagocytic function of macrophages in the presence of immune serum (Takayanagi et al. 1992).

As the existence of receptors for the Fc region of IgM on the macrophage membrane is still controversial, the role of IgM antibodies on trypanosome phagocytosis in the absence of complement remains unlikely. Receptor-mediated phagocytosis is enhanced during infection. It is possible that trypanosomes phagocytosed through receptors (C3b receptors, Fc receptors, etc.) or after destruction by complement-mediated lysis trigger macrophage suppressor activity, although the participation of soluble factors or another cell types cannot be ruled out.

Furthermore, macrophages from *T. b. brucei*-infected mice are able to synthesise reactive oxygen intermediates (ROI) after triggering by phorbol myristate acetate. Oxygen-derived species are among the most toxic products produced by macrophages. Trypanosomes are highly sensitive to these species, and in peculiar to hydrogen peroxide and hypochlorous acid, synthesised during phagocytosis.

Macrophages from trypanosome-infected mice also synthesise reactive nitrogen intermediates (RNI). Trypanosomes are highly sensitive to the cytostatic/cytotoxic effects of these compounds (Vincendeau and Daulouède 1991, Vincendeau et al. 1992). They are highly reactive radicals with short half-lives, which can react together to form potent and more stable effector molecules able to act on distant targets such as extracellular parasites. We have recently shown that *T. b. gambiense* are highly sensitive to S nitroso-compounds, which are new effector molecules synthesised by activated human macrophages *in vitro* (Mnaimneh et al. 1997). Nitrosylated compounds could represent new effector molecules with a potent effect on targets distant from macrophages. In a recent study DNA from *T. b. brucei* have increased macrophage production of IL-12, TNF- $\alpha$  and NO (Shoda et al. 2001).

Macrophages are also active in secreting PGs which modulate lymphocyte and macrophage functions. During a *T. b. brucei* infection, the ratio of PGE2/PGF1a is reversed, with an overproduc-

tion of PGE2 (Fierer et al. 1984). Macrophages are involved in immunosuppressive mechanism, and VSG can also inhibits macrophage functions (Flynn and Sileghem 1991, Schleifer and Mansfield 1993, Collier et al. 2003).

Macrophages respond to, and synthesise, a large number of cytokines. The production of IL-1 is increased in *T.b. brucei*-infected mice, but this increase may be due to release rather than synthesis (Sileghem et al. 1989). In murine macrophages, VSG induces IL-1 and TNF- $\alpha$  synthesis. Human monocytes can also be induced by trypanosomes and secreted factors from trypanosomes to express TNF- $\alpha$  RNA transcripts and secrete TNF- $\alpha$  in culture supernatants (Daulouède et al. 2001).

Classical and alternative states of macrophage activation are observed in trypanosomiasis (Figure 2). Classical activation precedes alternative activation in murine trypanosomiasis. However, both activation states are expressed in these mice. By inducing alternative macrophage activation, trypanosomes induce host arginase which both decrease trypanocidal nitrosylated compound synthesis and increases L-ornithine production (Gobert et al. 2000). L-ornithine is the first step of polyamine synthesis, essential for parasite growth and trypanothione synthesis (Vincendeau et al. 2003).

#### CYTOKINES AND CHEMOKINES

A profound dysregulation of the cytokine network is observed in trypanosomiasis. The first evidence of overproduction of TNF- $\alpha$ /cachectin was shown in *T.b. brucei*-infected rabbits. (Rouzer and Cerami 1980). TNF- $\alpha$  is known to induce fever, asthenia, cachexia and hypertriglyceridemia. High levels of TNF- $\alpha$  are associated with the presence of patent inflammatory signs in the early phase of human trypanosomiasis and of major neurologic signs in the late phase (Okomo-Assoumou et al. 1995a). A persistently increased serum TNF- $\alpha$  level could contribute to the hypergammaglobulinemia observed in trypanosomiasis because the role of TNF- $\alpha$  on activation, proliferation and differentiation of B cells

has already been shown (Roldan et al. 1992). Nevertheless, TNF- $\alpha$  participates in the mechanisms leading to trypanosome elimination: TNF- $\alpha$  acts indirectly in a cascade of events leading to cell activation or directly on parasites due to its cytotoxic properties (Lucas et al. 1994). Initial control of parasitemia in *T.b. brucei*-infected mice was diminished by the injection of anti-TNF- $\alpha$  antibodies (Lucas et al. 1993). VSG can trigger TNF- $\alpha$  production by macrophages, which are the cells, which produce the most of this molecule. Moreover, TNF- $\alpha$  production can be stimulated by IFN- $\gamma$ . IFN- $\gamma$  and TGF- $\beta$  can be produced by CD8 T cells activated by TLTF released by *T.b. brucei* (Vaidya et al. 1997). TGF- $\beta$  has immunosuppressive effects. An interesting fact is that IFN- $\gamma$  stimulates parasite growth (Olsson et al. 1991). The binding of epidermal growth factor (EGF) on *T.b. brucei* receptors favoured parasite growth and was one of the first cytokine-parasite interactions noted (Hide et al. 1989). All these data show that by interfering with the cytokine network and by using cytokines as growth factor, trypanosomes can completely modify the effector functions of the immune system. The effects of cytokines could also be completely different according to the presence of co-stimulators and the time period during which they are produced in trypanosome infected animals.

Chemokines also play essential roles in infectious disease control. They induce cell recruitment and activation. They induce adhesion molecules on cells of the immune system, which can bind to various cells, mainly endothelial cells, which express adapted ligands (Hickey 1999). Cytokines and chemokines can also be involved in neurological disorders (Sorensen et al. 1999). So, TNF- $\alpha$  has been reported to contribute to the pathophysiology of cerebral malaria. Mice chronically infected with *T.b. brucei* develop inflammatory lesions of the CNS after treatment with subcurative doses of a trypanocidal agent (Hunter et al. 1991). Chemokines favour macrophage and lymphocyte recruitment in CNS of *T.b. brucei*-infected animals. The activity of these cells in precise and selective ar-

eas of CNS might induce alterations leading to various disorders, such as sleep and endocrine disorders (Lundkvist et al. 2004, Buguet et al. 1993, 2001).

The presence of TNF- $\alpha$  RNA transcripts in the CNS of these mice suggests that TNF- $\alpha$  production could play a role in these lesions. Also, TNF- $\alpha$  and other cytokines contribute to the generation of somnogenic molecules such as IL-1 (Pentreath 1994). In a recent study an intra cerebral infusion of soluble type I TNF- $\alpha$  receptor reduced trypanosome-induced neurodegeneration (Ning et al. 2003). High levels of plasmatic IL-10 are also found in human trypanosomiasis. A number of aspects deserve further investigation: the study of all the various cytokines and soluble cytokine receptors, the possible existence of membrane or soluble cytokine receptors synthesised by the parasite, and the interaction and modulation of all these elements. Cytokines have been shown to play an essential role in the synthesis of NO, whose effects on several features of immune response have been observed over the past few years.

#### NITRIC OXIDE

Nitric oxide is a short-lived diatomic free radical synthesised from L-arginine by NO synthase (NOS). Calcium-dependent constitutive NOS (cNOS) release small amounts (picomoles) of NO within a short time, whereas calcium-independent inducible NOS (iNOS) release high levels (nanomoles) of NO for a long time. Expression of iNOS in macrophages, neutrophils, hepatocytes, endothelial cells and epithelial cells is regulated at transcription level by a number of agents, including microbial products and cytokines. *In vitro*, murine cells produce large amounts of NO after exposure to a combination of stimuli: lipopolysaccharide (LPS), IFN- $\gamma$ , IL-1, TNF- $\alpha$  etc. Human monocytes treated by IL-4 express CD23 antigen. The crosslink of CD23 induces iNOS expression, the release of NO and various other molecules (IL-6, TNF- $\alpha$ , oxygen radicals, lipid mediators).

Nitric oxide is involved in the inflammatory

response mediated by endotoxin, cytokines or physiochemical stress. NO produced by cytokine-activated macrophages is important in host defence and plays a crucial role in controlling infections *in vivo*. The role of NO and cytokines has been studied in detail in mice infected by intracellular parasites as *Leishmania*. In this murine model, IFN- $\gamma$  synthesised by Th1 cells leads to iNOS activity, whereas IL-4 and IL-10 synthesised by Th2 cells have a suppressive effect.

NO or other nitrogen intermediates can also react with the oxygen intermediates and form peroxynitrite and hydrogen radicals. Moreover, NO can form nitrosylated compounds which is able to transport and liberate NO on targets distant from NO producing cells. Nitrosylated compounds can not only act on extracellular parasites, but also modify parasite antigens and host cell function. These compounds may have various effects (parasite killing, alteration of tissue functions such as neurotransmission, etc.) according to their localisation (spleen, liver, peritoneum, CNS, etc.). By selective inhibition of Th1 cells, NO exerts a negative feedback effect. The altered production of NO, induced by dysregulation of the cytokine network, may lead to alteration of immune response and may also be involved in pathophysiological mechanisms. Nitrotyrosine, a marker of peroxynitrite formation, and iNOS are immunodetected in the brains of *T.b. brucei*-infected mice. Nitrotyrosine staining is associated with the appearance of neurological signs (Keita et al. 2000).

In HAT, nitrite production is increased at first. NO can also be stored as nitroso compounds. These NO-adducts are indirectly detected, as they induce the appearance of antibodies directed to nitrosylated antigens (Semballa et al. 2004). However, in trypanosome-infected mice, a decrease in plasmatic L-arginine leads to a decreased NO production. L-arginine is consumed by arginase, which synthetises L-ornithine and urea. L-ornithine is the precursor of polyamines and trypanothione. By inducing arginase, trypanosomes bypass NO production and benefit growth factor production. Arginase induc-

tion by parasites might be considered as a new strategy elaborated by parasites to escape host defence and benefit growth factors.

#### IMMUNOSUPPRESSION

The increased susceptibility of *T. gambiense*-infected patients to secondary infections was pointed out in the initial observations and reports of the Sleeping Sickness Commission (Low and Castellani 1903). Cellular immunity (skin tests to PPD, *Candida* or streptococcal antigens and sensitisation with DNCB) and humoral immunity (response to the H antigen of *Salmonella typhi*) were depressed in patients with HAT (Greenwood et al. 1973). In a recent study, no statistical difference was found between the prevalence of HIV infection in HAT patients and controls (Meda et al. 1995).

Immunosuppression was also observed and investigated in experimental trypanosomiasis and trypanosome-infected cattle (Ilemobade et al. 1982). In these models, immunosuppression was attributed to polyclonal B cell activation as well as the generation of suppressor T cells and suppressor macrophages. General B cell activation was noted in trypanosomiasis (hypergammaglobulinemia and a large increase of B cells in the spleen, as well as the presence of numerous Mott cells in cerebral spinal fluid and plasma cells in perivascular infiltrates), whereas specific antibody response to trypanosome antigens were reduced (Sacks and Askonas 1980).

A marked suppression of antibody response to *Brucella abortus* was reported in cattle infected with *T. congolense* (Rurangirwa et al. 1983). *Trypanosoma evansi* infection in sheep delayed and depressed the increase in total cell and lymphoblast output from a lymph node draining the site of a *Pasteurella haemolytica* vaccine administration. These reduced outputs may limit the dissemination of antigenic specific cells (Onah et al. 1997).

Cells, cytokines and prostaglandins have been studied in order to know their contribution, alone or in synergy, and with or without parasitic elements, to immunosuppression mechanisms. Try-

panosome membrane fragments have been found to mimic the immunosuppressive effects of living parasites (Clayton et al. 1979). A deficient production of IL-2 and of IL-2 receptor expression has been shown in several models (Alcino and Fresno 1985, Sileghem et al. 1989). The roles of macrophage-derived factors, especially prostaglandins and IFN- $\gamma$  secreted by CD8<sup>+</sup> T cells in the suppression of IL-2 receptor expression on CD4<sup>+</sup> and CD8<sup>+</sup> T cells, were also shown (Darji et al. 1993). Besides its action on the Th1 subset, rather than the Th2 subset, NO also acts on other elements, favouring immunosuppression.

#### IMMUNOINTERVENTION

The resistance of mice to African trypanosomes can be increased non-specifically by immunostimulants such as Calmette-Guérin bacilli and *Propionibacterium acnes* (Murray and Morrison 1979, Black et al. 1989). These immunostimulants are considered to activate macrophages. *P. acnes*-treated macrophages inhibited *T. brucei* growth *in vitro* (Black et al. 1989).

An acquired resistance has been observed in trypanocide-treated cattle. In a cohort study in Zaire during the 10 year observation period of adults previously diagnosed and treated for HAT, the risk of a second episode of HAT was greatly reduced compared to the risk of a first episode in previously undiagnosed adults (Khonde et al. 1995). Induction of protective immunity by vaccination is an important goal to control infectious diseases. However, a vaccine must be very effective and not only delay development of the disease, but also include a large number of antigenic variants. A major aim is to identify antigen(s) that elicit a protective immune response in trypanosomiasis. Identifying molecules inducing durable protection may lead to their production as recombinant antigens. Nucleic acid vaccines represent a new promising approach. They are able to induce all the elements of the specific immune response unlike killed microorganisms or defined protein. Studies using dead

or living trypanosomes, soluble-released antigens, purified VSG and irradiated parasites have shown that protection is restricted to the VSG-specific epitopes (Pays 1995). Strategies using invariant antigens (particularly those in the flagellar pocket) may be very worthwhile (Olecnick et al. 1988). Furthermore, in experimental murine trypanosomiasis, vaccination based on glycosylphosphatidyl (GPI) anchor of VSG can prevent TNF- $\alpha$  associated immunopathology and decrease disease severity (Magez et al. 2002). TNF- $\alpha$  associated immunopathology may also be prevented by selective inhibitors of macrophage functions (Mamani-Matsuda et al. 2004).

#### CONCLUSION

The knowledge of host and parasite genomes and all immune response elements can help in understanding immune mechanisms (natural and acquired) developed in trypanosomiasis. These mechanisms are triggered starting with the first contact of hosts with trypanosomes and chancre formations. One of the initial questions which needs to be addressed is the influence of parasite inoculation, and especially of *Glossina* saliva elements, on the modulation of both innate and specific immune response of hosts. Studies have shown that tick saliva contains innate immunity inhibitors (complement, NK cells, etc.), reduces macrophage cytokine elaboration, and impairs the earliest stages of specific immunity (Wikel 1996). At the onset of specific response, IL-12 plays a major role in T cell shift towards a Th1 response. It would be interesting to investigate its role, as well as all the elements composing the cytokine network. Indeed, the presence of parasite molecules interacting with these elements, which indicates that a parasite can deeply penetrate the host immune system, might lead to paradoxical and perverse effects on immune response.

Studies done on transgenic knock-in and knock-out mice have produced many major findings in the understanding of infectious diseases. It has been shown, for instance, that the disruption of

IL-4 gene in *T.b. brucei*-infected B 10.Q mice alters the control of parasitemia and the production of anti-VSG antibodies, though shortening their life expectancy (Bakhiet et al. 1996). The use of different gene promoters/enhancers may contribute to defining the role of a transgene in every type of tissue. The various immune effector mechanisms found to be efficient in controlling trypanosomiasis (antibodies, complement, phagocytosis, TNF- $\alpha$ , NO, etc.) might be effective on one organ, but ineffective elsewhere. Moreover, the chronology of each state of activation of a cell might be tightly regulated to allow an efficient immune response and avoid immune disorders. New data from genome, transcriptome and proteome analysis might help in understanding the sequence of all events involved in resistance/susceptibility to trypanosome and in disease development. Further research on appropriate immunological means, associated with chemotherapeutic agents, might be useful in curing chemotherapy-resistant trypanosomiasis.

#### RESUMO

Modificações importantes no sistema imune são observadas na tripanosomíase Africana. Essas reações imunológicas não protegem e estão envolvidas em distúrbios imunopatológicos. O principal componente de superfície (glicoproteína variante de superfície, VSG) está associado à evasão das respostas imune, às disfunções da rede de citocinas e à produção de autoanticorpos. Muitos de nossos conhecimentos resultam da tripanosomíase experimental. Componentes da imunidade inata estão sendo caracterizados. Em camundongos infectados, a VSG estimula preferencialmente células Th1. Uma resposta de  $\gamma\delta$  e células T CD8 aos antígenos do tripanossoma foi observada em gado tripanotolerante. Um aumento em células B CD5, responsável por IgM sérica e produção de autoanticorpos, foi observado no gado infectado. Os macrófagos desempenham importantes funções na tripanosomíase, em sinergismo com anticorpos (fagocitose) e pela secreção de várias moléculas (radicais, citocinas, prostaglandinas). Tripanossomas são altamente sensíveis ao TNF- $\alpha$ , espécies reativas de oxigênio e nitrogênio. O

TNF- $\alpha$  também está envolvido em caquexia. O IFN- $\gamma$  atua como um fator de crescimento do parasita. Esses vários componentes contribuem para a imunossupressão. Os tripanossomas usam os mecanismos imunes para seu próprio benefício. Dados recentes mostram a importância da ativação alternativa de macrófagos, incluindo a indução pela arginase. A L-ornitina produzida pela arginase do hospedeiro é essencial para o crescimento do parasita. Todos esses dados mostram o envolvimento no sistema imune realizado pelos tripanossomas e sugerem a interferência de métodos terapêuticos.

**Palavras-chave:** Tripanossoma, tripanosomíase Africana, imunologia, macrófago, linfócitos, óxido nítrico, citocina, autoanticorpos.

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