Global Advanced Research Journal of Medicine and Medical Science (ISSN: 2315-5159) Vol. 4(5) pp. 218-230, May, 2015
Available online http://garj.org/garjmms/index.htm

Review

Immunology and immunopathology of African Trypanosomiasis

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Accepted 28 May, 2015

Major modifications of immune system have been observed in Human 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 characterized. In infected mice, VSG preferentially stimulates a Th1-cellsubset. A response of γ δ and CD8T cells to trypanosome antigens was observed in trypanotolerant cattle. An increase in CD5B cells, responsible for most serum IgM and production of auto antibodies 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 etc.). Trypanosomes are highly sensitive to TNF-α, reactive oxygen and nitrogen intermediates. TNF-α is also involved in cachexia. IFN-γ 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 realized by trypanosome sand might suggest interference therapeutic approaches.

Keywords: Trypanosome, Human African trypanosomiasis, immunology, macrophage, lymphocytes, nitricoxide, cytokine, auto antibodies.

INTRODUCTION

Sleeping sickness or human African trypanosomiasis (HAT) is an endemic parasitic disease exclusively located in inter tropical Africa where it is transmitted by the tsetse fly or *Glossina*, its unique vector (Lundkvist 2004). The new taxonomy tools used in African trypanosomes (isoenzyme characterization, DNA analysis) have allowed scientists to separate the *Trypanosomabrucei* clade in several sub species. Two are infective for humans: *T.b. gambiense*, and *T.b. rhodesiense*. These extra cellular 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, secondly, specific 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 effect or cells and molecules. These mechanisms are highly regulated by multiple signals delivered through a large number of receptors transduced across the plasma

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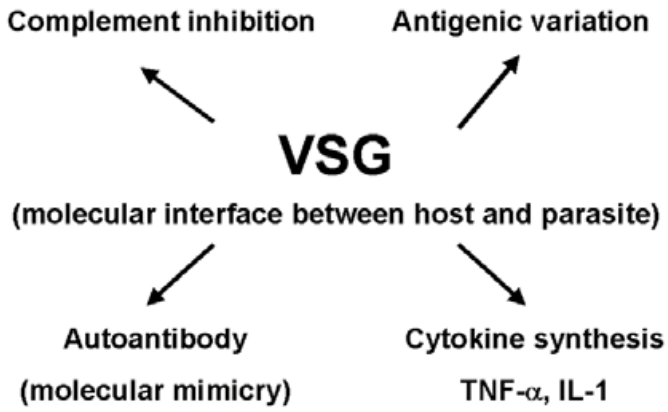

Figure 1. Variable surface glycoprotein (VSG), the major surface component of trypanosomes, is also released in host fluids. VSG induce resistance to complement lysis, escape to specific immune response, persistent cytokine production, auto antibody synthesis by molecular mimicry with host tissues.

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 immuno pathological 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 pre-dominant surface antigen of African trypanosomes. VSG constitutes an important molecular interface between trypanosomes and the host immune system (Figure1). 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 auto antibodies and cytokines, in particular tumournecrosis factor (TNF) α (Magez *et al*., 2002). Other trypanosome components and soluble factors, such as a trypano-some-released triggering factor (TLTF) which triggers interferon (IFN)-γ production by T cells, are also involved in modulation of the immune system by acting on the synthesis of immune elements (Askonas BA 2003) (Figure 2). Furthermore, increased levels of circulating endotoxins area 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 defenses and induction of parasite growth factor production are well developed by trypanosomes. In a recently discovered escape mechanism, hostarginase induction, trypanosomes decrease immune response efficiency and increase the production of L-ornithine, an essential growth factor (Vincendeau *et al*., 2003).

Understanding of the immune response was recently advanced by the discovery of the T and B sub populations and, especially, of the Thelper (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 γ δ 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 concentrated only on the immunology of HAT. Results obtained from animal diseases or experimental models can be investigated in human trypanoso- miasis using adapted means. Genetic analysis of resistance and susceptibility to infection in inbred and congenic animal strains form the basis for re-search 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).

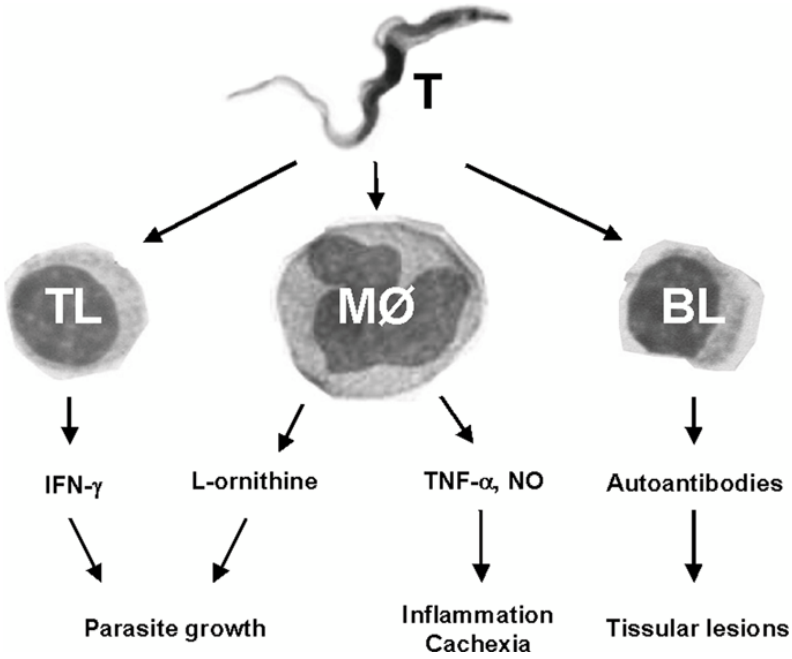

Figure 2. Trypanosomes induce secretion of various components from immunecells. Besides their trypanocidal effects, these molecules are also involved in deleterious mechanisms for host tissues and/or favour parasite growth. (T:trypanosome; Mø:macrophage; TL:Tlymphocyte; BL:Blymphocyte).

CLINICAL SYMPTOMATOLOGY OF HAT

After a painful tsetse bite, the chancre(from several millimetres to centimetres) represents the initial lesion at the bite site, characterised by localerythema, 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 (Dumas and Bisser, 1999). Within a few days after the tsetse bite, the patient enters the haemolymphatic stage of the illness.

STAGEI, 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 eruptionsortrypanides 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 sub clavicular region (Winter bottom 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, pruritusgener- alises. 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). Day time 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 cold-ness, 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 at testing to the predominance of potentially reversible inflammatory lesions over irreversible demyelinating lesions. The general signs of the haemolymphatic stage do not completely dis-appear: spikes off ever (but sometimes hypother-mia), 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/µLand/ or the presence of trypanosomes.

A wide variety of symptoms are encountered. The main symptoms from which sleeping sickness was named are day time 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 deeptend on reflexes, psychiatric disorders (confusion, mood swings, agitation ,aggressive behavior, euphoria, absent gaze, mutism, indifference), and tremor (fine and diffuse without any myoclonic jerkat 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 wide spread 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 neuro-endocrine 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. Extra- pyramidal 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 sub thalamic and hypophyseal regions account for the persistence of ad-endocrine disturbances such as impotence, amenorrheaorin-fertility 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, in continence and epileptic fits. The patient dies in a state of cachexia and physiological misery.

GENETIC CONTROL OF TRYPANOSOMIASIS

The study of inbred and congeneic 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 histo compatibility 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/cand A/Jmice were the most susceptible and C57BL/6 the most resistant (Duleu *et al*., 2004). 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 (Berriman *et al*., 2005) or recessive (DeGee *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 be-tween 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, 17and 1 (Kemp *et al*., 1997).

African animal 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 (*Bostaurus*, *B. indicus*). This resistance depends on the nutritional, physiological and stress conditions of the animal. Besides, studies of other factors in relation to host defense 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 (Courtin *et al*., 2006). 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 them urine genes controlling resistance and susceptibility to pathogens is in progress. Family studies should also be performed. Our knowledge of human trypano-tolerance is based on reported cases (Authie *et al.,* 1999) and results from immunological screening (Authié *et al*., 2001). Subjects with a positive CATT (card agglutination trypanosomiasis test) were a symptomatic where as the presence of blood parasites was observed. Moreover, although many Bantou people from Mbomofoci 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 (Côted'Ivoire), single nucleotide polymorphisms within TNF-αandinterleukin (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 (Albright *et al.,* 2002). 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 *et al.,* 1997). Recently, two TLFs have been characterized 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 (Rifkin *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 apolipo protein L-lintheparasitelyzosome (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 tsetsefly, a local skin reaction is induced by trypanosome proliferation and appears a few days after inoculation. In efferent lymph aticvessels, try-panosomes have been detected in lymph 1-2days before the chancre. Their number declined during development of the chancre (6days) and later increased. 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 $CD8^+$T cells (Mwangi *et al*., 2000). A nearly response due to an increase in $CD4^+$and $CD8^+$T cells was revealed by flowcytometry in the afferent lymph draining the chancre. As the chancres regressed there was an increase in lympho blasts and surface immunoglobulin bearing cells (Mwangi *et al*., 2003). During this first stage, trypanosomes expressed Variable Anti-gen Types (VATs) found characteristically in the tsetsefly, which changed after few days. An antibody response specific to these VATs appeared in the lymph and then in the plasma (Barry and Emergy, 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 in-duce trypanosome lysis only on uncoated VSG trypanosomes, as observed during the cycle of this parasite (procyclicforms). However, the appearance of VSG on parasites prevents trypanosome lysis by this alternative pathway (Ferrante and Allison, 2001). For another strain of *T.b. gambiense*, it was demonstrated that the alternative pathway was in-completely activated without generation of the terminal complex (C5-C9) able to induce membrane lysis (Mwangi *et al*., 2003). The classical path- way, mediated by specific antibodies against trypanosomes, was also described and could be involved in parasite clearance by antibody-mediated lysis and/ oropsonisation. 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 (Ferrante and Allison, 2001) or by auto antibodies (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 (van Velthuysen *et al*., 1994).

NATURAL KILLER CELLS

Natural killer (NK) cells have been identified as an important defense mechanism against tumour cells and intracellular pathogens, especially viruses. They are considered to belong to the lymph ocyteline age and have functions in both innate and acquired immune responses. NK cells lyse extra-cellular 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-γ and TNF-α, which play major roles in trypanosomiasis and are regulated by cytokines which can activate or in hibit 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 anti serum against asialo GM1 or their activation by polycytidyliccopolymer (Albright *et al*., 2002).

T CELLS

Initial studies have evidenced alterations in T cell functions in trypanosomiasis, both *invivo* and *in vitro* (Wallace *et al.,* 1974) (Figure 2). Histological examination revealed a massive B cell expansion in the lymph nodes and spleen, which replaced the thymus - dependant are ain *T.b. brucei* TREU 667- infected mice. These changes were seen within 7days 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 (Lutje et al., 1995).

Most T cells in humans and mice bear T α β antigen receptors. These cells possess surface markers, which allow the discrimination of $CD4^+$ T cells (helper T cells) and $CD8^+$ Tcells (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 proliferate but made a substantial IFN-γ and IL-2 cytokine response (Schleifer *et al*., 1993). The cellular phenotype of VSG-responsive T cells ($CD4^+$ $CD3^+$) 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 $CD2^+$ and $CD4^+$ T cells was observed, associated with an increase in the percentage of $CD8^+$T cells, B cells and γ δ Tcells. VSG and two invariant antigens (33 kD a cysteine protease and 66 kD a antigen homologous to immunoglobulin heavy chain binding protein hsp70/Bip) induced *invitro* proliferation and synthesis of IL-2 and IFN-γ (Lutje *et al*., 1995). No significant differences in the *invitro* proliferation of lymph node cells to VSG, Concanavalin A(Con A) or hsp70/Bip were observed between the two breeds. However, IFN-γ production in response to Con A was higher in Boran at 35 days post infection.

Human and mouse immune systems contain few γδ T cells, in marked contrast to those of ruminants (Hein and MacKay, 1991). Functions of γδ Tcells remain largely unknown. Involvement of γδ T cells in malaria and leishmaniasis has been observed (Rzepczyket al., 1997). A proliferative response of $CD8^+$ T cells and γδ T cells from trypanotolerant N'Damatoan antigen complex contain in gimmunodominantepitopes was observed where as a quasi absence of response was observed in trypanosusceptible Boran. The role of this γ δ T cell response in parasite resistance re- mains unclear. So, γδT cells, as$CD4^+$or$CD8^+$, do not proliferate when stimulated with soluble VSG *invitro* (Sileghem, 1999). It would be interesting to determine the role of cytokines synthesized by γ δ 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 responses, especially by the secretion of cytokines. They greatly modify functions of B

cells (antibody synthesis, iso type switch) and macrophages (anti-gen presentation, effect or 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 auto antigens, corresponding to non-specific polyclonal activation of B-cells producing natural auto antibodies 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 (Semballa *et al*., 2004).

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 (Mansfield, 2002). 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 extra vascular 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, 3-4days 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 de- crease 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 de- pressed. Several factors may contribute to this immunosuppression. Macrophages may become un- able to present antigens to T cells (by defects in antigen processing and association of epitopes with MHCClassII) and produce immunosuppressive factors as nitric oxide (NO), prostaglandins (PG), and cytokines. An increase in immunosuppressive cytokines, such as INF-γ and transforming growth factor (TGF)-β, was also detected during infection. However, TGF-β 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*., 1999).

Several auto antibodies are detected during African trypanosomiasis. High levels of polyclonal Igs were marked feature of HAT. The specificity of these Igs is frequently characterized against a large range of auto antigens. Auto antibodies were directed against red blood cells (Kobayakawa *et al*., 1979), liver and cardiolipids (Ayed *et al., 1998*), nucleic acids: DNA and RNA (Hunter et al., 1992b), intermediate filaments (Anthoons *et al*., 2004) and rheumatoid factors (Kazyumba *et al*., 2001). Auto antibodies 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 (Kobayakawa *et al*., 1999) and patients from the Ivory Coast (Amevigbe *et al*., 2006). Other auto antibodies directed against not yet characterized proteins have been described in HAT patients (Asonganyi *et al.,* 2003) 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-tryptophancursor to the neuro transmitter serotonin, (Arneborn, 2002) or recognised some neuronal components of the cyto skeleton, neuro filament proteins. In some cases, these auto antibodies (anti-galactocerebrosides and anti-neuro filaments) 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). *Invivo* demyelinisation has been produced by purified antibodies to galactocerebroside (Kobayakawa *et al*., 1999). There are several hypotheses for the origin of these antibodies. They may be induced by anon-specific stimulation of B cells producing natural auto antibodies (Arneborn *et al*., 2002). In other cases, antigen-driven auto antibodies are specific to epitopes of the causative infecting agent with molecular mimicry to self antigens, inducing across-reactivity to intermediate filaments (Davies, 1997) as demonstrated for anti-neuro filament and anti-galactocerebroside antibodies which recognised respectively a flagellar component and a proteolipidic epitope of trypanosomes, and epitopes expressed by neurones (Girard *et al*., 2000).

A sub population 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, 1998). These CD5 cells produce auto antibodies, 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 immune-pathological 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 phagolyso somes (vacuolated cytoplasm). An increase uptake of intravenously injected sheep red blood cells was also noted.

Macrophages are highly sensitive to environ- mental factors, especially microorganisms, micro- organism-derived products and cytokines. Aclear reduction in man nose receptors, Fc receptors , C3bi 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 effect or mediators.

Macrophages may play an important role in protection against trypanosomes, particularly in the presence of homologous anti serum. 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 fullopsonic activity present inmurine clearance in passively immunised mice (MacAskill *et al*., 1990). These *invivo* studies extend previous studies on the *invitro* 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 phorbolmyristateacetate. Oxygen-derived speciesare 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 *et al*., 1992). They are highly reactive radicals with short half-lives, which can react together to form potent and more stable effect or molecules able to act on distant targets such as extracellular parasites. We have recently shown that *T.b. gambiense* are highly sensitive to Snitroso-compounds, which are new effect or molecules synthesised by activated human macrophages *in vitro* (Mnaimneh *et al*., 1997). Nitrosylated compounds could represent new effect or 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-α 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 over production of PGE2 (Fierer *et al.* 1984). Macrophages are involved in immune-suppressive mechanism, and VSG can also inhibits macrophage functions (Coller *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-α synthesis. Human monocytes can also be induced by trypanosomes and secreted factors from trypanosomes to express TNF-αRNA transcripts and secrete TNF-α 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 hostarginase which both decrease trypanocidalnitrosylated 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 over production of TNF-α/cachectin was shown in *T.b. brucei*-infected rabbits. (Rouzer and Cerami, 2001). TNF-α is known to induce fever, asthenia, cachexia and hypertrigly ceridemia. High levels of TNF-α 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 (Bakhiet *et al*., 2002). A persistently increased serum TNF-α level could contribute to the hypergammaglobulinemia observed in trypanosomiasis because the role of TNF-α on activation, proliferation and differentiation of B cells has already been shown (Roldan *et al*., 1998). Nevertheless, TNF-α participates in the mechanisms leading to trypanosome elimination: TNF-α acts indirectly in a cascade of events leading to cell activation or directly on parasites due to its cytotoxic properties (Lucaset al. 1994). Initial control of parasitemia in *T.b. brucei*-infected mice was diminished by the injection of anti-TNF-α antibodies (Lucas et al., 1993). VSG can trigger TNF-α production by macrophages, which are the cells, which produce the most of this molecule. Moreover, TNF-α production can be stimulated by IFN-γ. IFN-γ and TGF-β can be produced by CD8T cells activated by TLTF released by *T.b.brucei* (Vaidya *et al*., 1997). TGF-β has immuno suppressive effects. An interesting fact is that IFN-γ stimulates parasite growth (Olssonet 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 effect or 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 ex- press adaptedligands (Hickey, 1999). Cytokines and chemokines can also be involved in neurological disorders (Sorensen et al., 1999). So, TNF-α 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 sub curative doses of a trypanocidalagent (Hunter et al., 1992b). Chemo- kines favour macrophage and lymphocyte recruitment in CNS of *T.b.brucei*-infected animals. The activity of these cells in precise and selective areas of CNS might induce alterations leading to various disorders, such as sleep and endocrine disorders (Lundkvist *et al*., 2004).

The presence of TNF-α RNA transcripts in the CNS of these mice suggests that TNF-α production could play a role in the selesions. Also, TNF-α and other cytokines contribute to the generation of somnogenic molecules such asIL-1 (Pen-treath, 1994). In a recent study an intra-cerebral infusion of soluble type ITNF-α 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 highlevels (nanomoles) of NO for a longtime. Expression of iNOS in macro-phages, neutrophils, hepatocytes, endothelial cells and epithelial cells is regulated at transcription level by a number of agents, including microbial products and cytokines. *Invitro*, murine cells produce large amounts of NO after exposure to a combination of stimuli: lipopolysaccharide (LPS), IFN-γ, IL-1, TNF-α etc. Human monocytes treated by IL-4 express CD23 antigen. The cross link of CD23 induces iNOS expression, the release of NO and various other molecules (IL-6, TNF-α, oxygenradicals, lipidmediators).

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-γ 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 per- oxynitrite 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 neuro-transmission.) 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 immuno detected 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. This NO-adducts are indirectly detected, as they induce the appearance of antibodies directed to nitrosylated antigens (Semballa *et al*., 2004). However, in trypanosome-infectedmice, a decrease in plasmatic L-arginine leads to a decreased NO production. L-arginine is consumed by arginase, which synthetics L-ornithine and urea. L-ornithine is the precursor of polyamines and trypanothione. By inducing arginase, trypanosomes by pass NO production and benefit growth factor production. Arginase induction 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 (Khonden *et al., 1998*). Cellular immunity (skin tests to PPD, *Candida* or streptococcal antigens and sensitization with DNCB) and humoral immunity (response to the H antigen of *Salmonellatyphi*) 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*., 1998).

Immunosuppression was also observed and investigated in experimental trypanosomiasis and trypanosome-infected cattle (Ilemobade *et al*., 2000). In these models, immunosuppression was attributed to polyclonal B cell activation as well as the generation of suppress or 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), where as specific antibody response to trypanosome antigens were reduced (Sacks and Askonas, 1999).

A marked suppression of antibody response to *Brucellaabortus* was reported in cattle infected with *T.congolense* (Rurangirwa *et al*., 1993). *Trypanosomaevansi* infection in sheep delayed and depressed the increase in total cell and lymphoblast output from a lymphnode draining the site of a *Pasteurellahaemolytica* vaccine administration. These reduced outputs may limit the dissemination of antigenic specific cells (Onah *et al*., 1999).

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. Trypanosome membrane fragments have been found to mimic the immunosuppressive effects of living parasites (Alcino *et al*., 1998). A deficient production of IL-2 and of IL-2 receptor expression has been shown in several models (Sileghem *et al*., 1999). The roles of macro- phage-derived factors, especially prostaglandins and IFN-γ secreted by $CD8^+$ T cells in the suppression of IL-2 receptor expression on $CD4^+$and $CD8^+$T cells, were also shown (Darji *et al*., 2000). Besides its action on the Th1 subset, rather than the Th2 subset, NO also acts on other elements, favouring immune-supression.

IMMUNOINTERVENTION

The resistance of mice to African trypanosomes can be increased non-specifically by immuno-stimulants such as Calmette-Guérin bacilli and *Propionibacteriumacnes* (Murray and Morrison, 1979, Black *et al*., 2001). These immunostimulants are considered to activate macrophages. *P. acnes*- treated macrophages inhibited *T.brucei* growth *in vitro* (Black *et al*., 200).

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*., 1998). 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 micro organisms 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 (Kipps *et al.,* 1998). Strategies using invariant antigens (particularly those in the flagellar pocket) may be very worthwhile (Olecnick *et al*., 1998). Furthermore, in experimental murine trypanosomiasis, vaccination based on glycosylphosphatidyl (GPI) anchor of VSG can prevent TNF-α associated immunopathology and decrease

disease severity (Magez *et al*., 2002). TNF-α associated immuno-pathology may also be prevented by selective inhibitors of macrophage functions (Mamani-Mat- suda *et al*., 2004).

CONCLUSION

The knowledge of host and parasite genomes and all immune response elements can help in under-standing 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 need 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 *et al.,* 2002). At the onset of specific response, IL-12 plays a major role in T cell shift to-wards 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 B10. Q mice alters the control of parasitemia and the production of anti-VSG antibodies, though shortening their life expectancy (Bakhiet *et al*., 2002). The use of different gene promoters/enhancers may contribute to defining the role of a trans gene in every type of tissue. The various immune effect or mechanisms found to be efficient in controlling trypanosomiasis (antibodies, complement, phagocytosis, TNF-α, NO, etc.) might be effective on one organ, but ineffective elsewhere. Moreover, the chronology of eachs tate of activation of a cell might be tightly regulated to allow an efficient immuneresponse 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 y to trypanosome and in disease development. Further research on appropriate immunological means, associated with chemo- therapeutic agents, might be useful in curing chemo-therapy-resistant trypanosomiasis.

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