ELSEVIER

Microbes and Infection 4 (2002) 999–1006

Infection

www.elsevier.com/locate/micinf

Forum in Immunology

VSG-GPI anchors of African trypanosomes: their role in macrophage activation and induction of infection-associated immunopathology

Stefan Magez *, Benoît Stijlemans, Toya Baral, Patrick De Baetselier

Laboratory of Cellular Immunology, Free University of Brussels/Flemish Interuniversity, Institute for Biotechnology, Paardenstraat 65, 1640 Sint Genesius Rode, Belgium

Abstract

African trypanosomes express a glycosylphosphatidyl inositol (GPI)-anchored variant-specific surface glycoprotein (VSG) as a protective coat. During infection, large amounts of VSG molecules are released into the circulation. Their interaction with various cells of the immune system underlies the severe infection-associated pathology. Recent results have shown that anti-GPI vaccination can prevent the occurrence of this pathology. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Trypanosomes; GPI; VSG; TNF; Pathology

1. Introduction

African trypanosomes are protozoan parasites that cause disease in man and livestock. Among the large variety of trypanosome species found, only two species, i.e. *Trypanosoma rhodesiense* and *T. gambiense*, are infectious to man. These parasites are distributed throughout numerous subSaharan countries and cause human sleeping sickness, a deadly disease against which the human immune system is unable to mount a protective response. Annually, about 300,000 individuals are estimated to attract trypanosomosis, while more than 50 million people are believed to be continuously exposed to the danger caused by *T. rhodesiense* and *T. gambiense* parasites. Although none of the other trypanosome species present in Africa cause a direct risk for humans, livestock infections can have indirect devastating effects on public health. Indeed, because of the infections in cattle, pigs, sheep, and goats by parasites such as *T. congolense* and *T. evansi*, a major problem at the level of protein availability for man occurs in regions where the tsetse fly (*Glossina* genus) or other hematophagic flies can serve as parasite vectors. For example, the presence of the *Glossina* vector alone already makes at least 10 million square kilometers of potential African grazing land unsuitable for livestock breeding, and places at least one-third of the African cattle herd at risk of trypanosomosis. Due to this vast geographic distribution of the disease vector, and the large number of potential vertebrate hosts which can carry different trypanosome species, complete eradication of trypanosomosis is a virtually impossible mission. With regard to the control and eradication of human sleeping sickness, a number of drug treatments have been developed in order to cure ongoing infections. A severe problem that occurs here is that most of the drugs used, especially those that serve as treatment during the neuropathological late stage of the infection, show significant toxicity. As such, the treatment in itself can be lethal in a considerable percentage of patients. Moreover, it is clear that drug treatment per se can offer only a limited solution in regions where trypanosomes are considered to be endemic. In the end, only the development of an immune intervention strategy would provide a useful alternative to a long-lasting total eradication effort. However, until now, no such strategy has been available due to the complex life cycle of the trypanosome and its capacity to continuously evade the host immune system by a mechanism of antigenic variation of its surface coat.

2. African trypanosomes and the immune system

As all African trypanosomes multiply predominantly in the bloodstream, they need to be able to survive a long-term exposure to the immune system of their mammalian host. Hence, coevolution has resulted in the appearance of

* Corresponding author. Tel.: +32-2-359-03-58; fax: +32-2-358-03-59.
E-mail address: stemagez@vub.ac.be (S. Magez).

PII: S1286-4579(02)01617-9

well-balanced growth regulation systems, allowing the parasite to survive sufficiently long without killing its mammalian host, in order to ensure an effective transmission of the species. Such a system involves the glycosylphosphatidyl inositol (GPI)-anchored variant surface glycoprotein (VSG), which is the major surface antigen and contributes as a protective coat for the parasite [1]. During the ascending parasitemia, the majority of the dividing parasites (e.g. long slender forms) belong to the same antigenic type, called the homotype, expressing each 10^7 densely packed identical VSG molecules on their surface. A peak of parasitemia is reached when these long slenders differentiate into nondividing short stumpy forms, which have a relatively short in vivo half-life of 24–36 h, and release VSGs in the circulation upon degeneration [2]. These degenerating parasites not only allow the host to develop an antibody response to the homotype, but also confront the immune system with periodic challenges of vast amounts of parasite-derived GPI molecules that are now known to be responsible for induction of inflammatory responses. Together, these events result in elimination of the major variable antigen type (VAT), and allow spontaneously arising minor VATs or heterotypes to continue to multiply, giving rise to a new peak of parasitemia. Although an effective anti-VSG response allows the host to regularly eliminate excessive numbers of parasites through phagocytosis of opsonized parasites [3], resistance and survival time of different mouse strains cannot be directly correlated to the antibody response. Indeed, combined results from experimental studies using different trypanosome strains in both resistant and susceptible mice as well as their F1 descendants showed that the ability to produce antibodies to the first variant antigen population is inherited as a dominant trait, while survival time during trypanosomosis is inherited as a recessive trait [4]. Important to keep in mind here is the fact that experimental rodent infection models do not serve as good models for human trypanosomosis as concerns the cause of death. Indeed, while in case of experimental infection in mice, animals can suffer from high parasitemia ($>10^8$/ml blood) and severe infection-induced anemia during the entire course of infection, death itself most often results from a sudden loss of parasite growth control, leading to the presence of extremely high numbers of parasites in the blood and most organs. In contrast, in human trypanosomosis, the number of parasites present in the circulation and organs always remain low ($<10^5$/ml blood), and the occurrence of a meningoencephalitis infection stage leading to coma, rather than uncontrolled parasite growth, will be the cause of death.

Apart from the analysis of events involved in actual parasitemia control and control of survival of trypanosome-infected mammals, it is clear that investigation of the mechanisms of induction of infection-associated immunopathology is crucial as well. In this context, analysis of the immunological background of trypanosomosis-associated immunopathology at the level of cytokine involvement so far has shown that during *T. brucei* infections, modulation of IL-2 and IFN-γ secretion occurs, in combination with the increased production of the inflammatory cytokines IL-1, IL-6 and TNF. In man, in particular, the infection-associated production and systemic presence of TNF is believed to be related to fever induction and to the severity of trypanosomosis-induced pathology. Indeed, the presence of a general state of inflammation in the brain and the occurrence of the neurological signs during the late-stage meningoencephalitic phase of the infections are both TNF-related [5], and the high incidence of mortality, following anti-trypanosomosis treatment could be TNF-related as well [6]. In mice, this hypothesis is experimentally supported by the finding that TNF mRNA in the brain of *T. brucei*-infected mice is increased after treatment with anti-trypanosomal drugs [7]. Further evidence for the detrimental role of TNF during trypanosome infections comes from the finding of its involvement in induction of trypanosomosis-associated immune suppression [8,9] as well as anemia and cachexia [10]. Finally, it is interesting to mention here that it was the search for the cachexia-inducing factor, responsible for the inhibition of lipid clearance from the blood of *T. brucei*-infected rabbits, which gave rise to the isolation of cachectin, later shown to be identical to TNF [11]. Since the discovery of TNF, it has become clear that increased TNF production is not a unique feature of trypanosome infections, but is associated with a number of other protozoan parasitic diseases as well, including Chagas' disease, leishmaniasis, schistosomosis, and malaria. As such, over the last 15 years, a great effort has been made to identify the parasite components involved in TNF induction.

In the case of trypanosomosis, from the start, major attention in the search for a TNF-inducing component has been focused on the GPI-linked variant surface glycoprotein VSG. This molecule represents 10% of the total trypanosome protein content, and on every peak of infection, the host is confronted with the release of an enormous quantity of these molecules [12]. For example, during chronic infections in mice, parasitemia peaks occur with an interval of about 7 d, often reaching levels of 10^7 to 5×10^8 parasites/ml of blood. As parasites are covered with 10^7 molecules of VSG, and as clearance of these peaks can occur in as little as 24 h, the immune system is regularly confronted with the sudden release of up to 4×10^{15} molecules of VSG, or an equivalent of 200 μg of this parasite compound. Naturally, together with exposure to the glycoprotein moieties of VSG, the immune system is confronted with equal molar quantities of parasite-specific GPI moieties as well.

Before considering the role of the different VSG moieties in the induction of trypanosomosis-associated pathology, it is interesting to note that, just as in the case of the discovery of TNF, it was again through the study of African trypanosomes that the GPI anchoring method for membrane proteins was discovered [13]. The molecular structure of

trypanosome GPI was resolved later [14,15], showing the presence of a trypanosome-specific galactose modification on the molecule. Based on the VSG C-terminal GPI processing signal, a VSG classification has been proposed, placing all VSGs in three separate groups [16]. Interestingly, the results from the C-terminal classification turned out to correspond to a second classification system in which the number of N-linked carbohydrate binding sites served as selection criteria. In this classification system, group I VSGs encompass a collection of molecules carrying a single conserved glycosylation site, about 50 amino acid residues from the C-terminus [17]. In contrast, group II VSGs have a first conserved glycosylation site five or six residues from the C-terminus as well as an additional, second less conserved glycosylation site [18]. Finally, group III, thus far, is represented only by one single trypanosome variant, i.e. *T. brucei* MITat 1.5. While this VSG variant is characterized by the presence of three glycosylation sites, it is also unique in the sense that it is the only VSG described so far that lacks the trypanosome-specific galactose modification of the VSG-GPI anchor.

Taking all the data available today, it is clear that the trypanosome-specific galactose-modified GPI anchor is the only moiety common to all VSGs, with the exception of the MIT at 1.5 that shares the GPI core but lacks the galactose modification. Further, as GPI VSG represents 10% of the total trypanosome content and as the immune system is periodically confronted with the presence of vast quantities of both released VSG and membrane-anchored VSG-GPI molecules, the trypanosome GPI was a prime target in the investigation of trypanosomosis-associated pathology and TNF induction.

3. VSG cleavage from the trypanosome surface occurs through phospholipase C activation

As described above, VSG molecules are anchored into the plasma membrane of the trypanosome by means of a GPI anchor. Surface molecules, anchored via GPI-like structures are present on all eukaryotic cells. However, while mammalian cells express up to 10^5 copies of GPI-like anchored molecules per cell, parasitic protozoa, including trypanosomes, express up to 2×10^7 copies of GPI anchor and/or GPI-related glycolipids per cell [19]. In the case of trypanosomes, one of the advantages of GPI-type anchors is the fact that it makes it possible to liberate VSG as a soluble molecule by means of a phospholipase C (PLC) enzyme, abundantly present in the parasite [20]. When VSG is cleaved by a PLC, the dimyristoylglycerol compound (DMG) of the GPI anchor is left in the membrane while VSG is released in a soluble form (sVSG) carrying the glycosyl-inositol-phosphate moiety (GIP) of the GPI. As PLC-released molecules all have the same distal structure, this epitope has been called the cross-reacting determinant or CRD [21]. Polyclonal antibodies against this epitope have been generated and can be used to discriminate soluble VSG molecules and membrane bound VSG, independently of their antigenic type.

Recently it has been shown that during trypanosome infections, large amounts of sVSG are liberated into the circulation [12]. Moreover, in living trypanosomes, sVSG is present in the flagellar pocket [22]. As it is known that PLC activation in trypanosomes can be induced by different forms of environmental stress [23], PLC null mutant trypanosomes were generated and used for the study of the importance of this enzyme activity in parasite development, virulence, and infection-associated immune modulation. First, a null mutant was obtained in the *T. brucei* EATRO 1125 strain. While this parental strain is know to cause an infection that results in death of the mice after several weeks, mice infected with the null mutant parasite are characterized by reduced parasitemia development, reduced pathology occurrence and prolonged survival [24]. Interestingly, while these parasitemia modifications were observed in a number of different mouse models (C57Bl/6, BALB/c and CBA/Ca), they were more recently found to be absent in C3H/HeN mice that are hypersusceptible to trypanosome infections and infection-associated pathology. These combined data indicate that while the PLC enzyme itself is nonessential for parasite survival or VSG switching, the activation of the trypanosome PLC gene influences specific host immune responses to the infection. As such, it seems that the actual cleavage of the VSG-GPI anchor through PLC activity results in the generation of new parasite epitope that have a direct influence on the immune activation stage of the host and result in the induction of inflammatory responses that are linked to infection-associated immunopathology. This conclusion is supported by the recent findings obtained with a second PLC null mutant parasite that was generated in the hypervirulent 427 *T. brucei* parasite strain, that fails to be controlled by any host mediated growth regulation system, and kills infected mice within 96 h. During this short exponential parasitemia, no proper induction of any infection controlling specific host immune response occurs, and as expected, deletion of the PLC gene from this parasite strain was found to have no effect on the host mediated control of virulence [25].

Having observed that cleavage of the VSG-GPI anchor correlates with the induction of an enhanced inflammatory response during chronic trypanosome infections, the role of PLC activation on the interaction between trypanosomes and the immune system has been analyzed in detail. It was found that attenuation of *T. brucei* by removal of the PLC gene is associated with reduced immunosuppression, increased production of Th2 lymphokines, an enhanced IgG1 anti-trypanosome response and the induction of an alternatively activated macrophage population [26,27]. While these results clearly indicate that the capacity of trypanosomes to cleave GPI-VSG using PLC activity has a direct influence on the steering of the infection-associated immune response, it remains a matter of debate as to how type 1

versus type 2 responses contribute to the control of experimental trypanosome infections. Indeed, in IL–4-deficient mice, *T. brucei* parasitemia control was found to be unaltered as compared to wild-type mice [28]. In contrast, IFN-γ-deficient mice showed drastically reduced survival when infected with pleomorphic trypanosomes, suggesting the need for an efficient type-1-mediated immune response during the early stage of infection [29,30]. Finally, when IL-10-deficient mice were infected with *T. brucei* parasites, the first peak of parasitemia was properly controlled, but mice succumbed to infection-associated pathology within 2 d of parasite elimination [30]. The latter observation very closely resembles the findings recorded during the analysis of *T. cruzi* infections in IL-10-deficient mice, where, in the absence of IL-10, mice were found to rapidly succumb due to a lack of inflammation control resulting in massive TNF induction and the occurrence of septic shock [31].

Together, it is clear that under environmental or immunological stress conditions, African trypanosomes are capable of activating an endogenous PLC enzyme that is responsible for cleavage of the VSG-GPI anchor and release of sVSG into the circulation. From the currently available data, it seems that this cleavage results in the generation of new trypanosome epitopes that are involved in type 1 immune activation, characterized by the production of increased inflammatory cytokines, including TNF. As such, it is clear that the analysis of the direct involvement in macrophage activation of trypanosome GPI and its PLC-cleaved products, i.e. GIP and DMG, has become crucial for the understanding of the occurrence of trypanosomosis-associated immunopathology and inflammation.

4. Two distinct moieties of the VSG-GPI are involved in the induction of TNF-production, and of infection-associated LPS hypersensitivity

By the time the first reports were published on the role of TNF in trypanosome infection [32], it had already been shown that also in the case of malaria infections, this cytokine played a crucial role. Moreover, it also had been proposed by then that the presence of a phosphoinositol-containing malaria toxin could form the basis for TNF induction by *Plasmodium* [33]. Around the same time, a comparative study of the TNF-inducing capacity of both *Plasmodium*- and trypanosome-derived "toxins" showed that GPI-anchored molecules present in both fractions were involved in the regulation of TNF expression [34]. As such, knowing that the GPI-anchored VSG makes up 10% of the total protein content of the trypanosome, and the fact that at regular time intervals, huge amounts of soluble VSG are released into the host blood circulation, this molecule became a prime target in the investigation of trypanosomosis-associated TNF induction.

As outlined above, in comparison to mammalian GPI anchors, the structure of the VSG-GPI is unique in that it contains a branched galactose side-chain modification [15]. As such, due to the fact that African trypanosomes are extracellular parasites, releasing vast amounts of soluble VSG into the circulation during processes of parasite degradation [3,12], the immune system of an infected mammalian host is regularly confronted with the presence of (i) circulating sVSG that carries the galactose-modified glycosyl-inositol phosphate (GIP) core, and (ii) parasite membrane fractions that contain the dimyristoylglycerol (DMG) lipid moiety of the GPI. Although it is clear that GPI-linked macrophage modulation occurs in all protozoan parasite infections, this situation is markedly different from the immune stimulation by, for example, the malaria GPI toxin or *T. cruzi* GPI-linked molecules where (i) numbers of extracellular parasites are several orders of magnitude lower, (ii) release of vast amounts of soluble cleaved GIP-linked molecules has not been reported, and (iii) in the case of malaria GPI, the VSG-specific GIP galactose modification is absent. As such, when analyzing the TNF-inducing capacity of trypanosome VSG, the roles of the GIP and DMG moieties have been studied separately, leading to the following conclusion [35] (Fig. 1): while trypanosome-derived galactose-modified GIP is the minimal moiety needed for the optimal induction of TNF production by IFN-γ activated macrophages, the presence of excessive amounts of DMG during chronic trypanosome infections can lead to macrophage overactivation and induction of LPS hypersensitivity of the infected host. Given the fact that accumulation of LPS has been described to occur in the serum of chronically infected mice [36], these findings indicate that both GIP and DMG are involved in VSG-triggered TNF production and trypanosomosis-associated pathology, respectively, through direct and indirect induc-

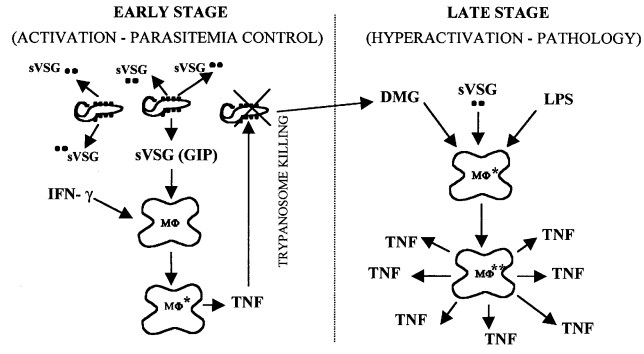

Fig. 1. The role of different VSG-GPI moieties in infection-associated macrophage activation and TNF production during African trypanosomosis. During early stage parasitemia, soluble VSG that carries the GPI carbohydrate core (sVSG-GIP) is released from the parasite surface and is involved in the induction of TNF-production by activated macrophages (Mϕ*). During the successive waves of the infection, parasites are killed and macrophages are confronted with the presence of the lipid moiety of the VSG-GPI anchor (DMG), that by itself can further activate macrophages and render these cells hypersensitive to LPS stimulation. Due to the ongoing infection, LPS levels increase in the circulation, causing excessive TNF production by the sVSG/DMG hyperactivated macrophages (Mϕ**), leading to the occurrence of TNF-mediated trypanosomosis-associated immunopathology.

tion pathways. Interestingly, analysis of TNF induction using a bovine trypanosomosis model has recently confirmed the findings presented above. Indeed, in this model, it was found that sVSG by itself was capable of inducing TNF production by IFN-γ-primed macrophages in the absence of the GPI-DMG anchor [37].

When it comes to the signal pathways involved in, respectively, VSG-GIP and VSG-DMG-triggered macrophage activation, all the available information is derived from experiments in which thyoglicolate-elicited PECs have been used in combination with MIT at 1.5-derived VSG-GPI compounds [38,39]. Although the used VSG is characterized by the exceptional lack of the VSG-specific galactose GPI modification, conclusions drawn from the results might be valid for signals triggered by regular VSGs as well. Results showed that GIP and DMG trigger two different signal pathways in macrophages, resulting in the respective activation of either protein tyrosine kinase or protein kinase C. Using an in vitro stimulation model, it was furthermore confirmed that the protein kinase C activation pathway triggered by DMG could be substituted by IFN-γ activation [40]. Given the fact that during trypanosome infections IFN-γ is induced [29], it seems that DMG-mediated macrophage activation is only of secondary importance during the first peaks of infection. This hypothesis is supported by the finding that in IFN-γ-deficient mice, TNF induction as well as TNF-associated signs of pathology are significantly reduced during trypanosome infections, not withstanding the fact that parasitemia levels themselves are increased and as such, both the amounts of released VSG-GIP and DMG are increased as well [30].

The fact that TNF itself is a key mediator in trypanosomosis-associated immunopathology has long been suggested and has been confirmed using TNF-deficient mice [9]. As concerns trypanosome components involved in the induction of TNF, it seems clear from the data described above that the galactose-modified GIP moiety present on released soluble VSG is a key factor. However, in the context of the TNF induction potential of factors derived from other protozoan parasites, the intrinsic activity of VSG has been questioned. Indeed, when the in vitro TNF induction capacity of sVSG is compared to the activity of GPI mucins from intracellular *T. cruzi* parasites, it is clear that its activity is at least 100- to 1000-fold lower [41]. However, when considered in a physiological context (i.e. in vivo), this might not be surprising. In the case of trypomastigote-derived GPI mucin, the optimal concentration for inducing TNF production was found to be in the 1–10 nM range, corresponding to physiological parasite loads in vivo. Optimal in vitro TNF induction by VSG, on the other hand, is obtained in the low μM range. Taking into account the fact that, during an ongoing trypanosome infection in mice, up to 200 μg of VSG can be released into the circulation within 24 h, the observed dose response can be considered as physiologically relevant as well.

One important feature of GPI-triggered TNF induction that is seen in *T. brucei*-triggered TNF induction as well as *plasmodium*- and *T. cruzi*-triggered TNF induction is the fact that a parasite-specific carbohydrate modification at the level of the GIP core seems to be crucial for optimal activation. Indeed, while for sVSG, the presence of a trypanosome-specific galactose modification is required for optimal activity, GPIs from *plasmodium* require the presence of a distal fourth mannose residue [42]. When TNF induction by the highly potent *T. cruzi* tGPI mucin is considered, it is interesting to note that, in this case, both a distal fourth mannose residue as well as a GIP-linked galactose modification are present [43].

Finally, as to the question through which surface interaction parasite-derived GPI-anchored molecules initiate signal transduction in activated macrophages, the answer is still awaiting full elucidation. While in the case of *Plasmodium* GPI-triggered activation, most recent data show that neither membrane insertion nor endocytosis is involved [42], activation of Toll-like receptor-2 seems to be essential for induction of TNF by *T. cruzi* derived GPI [44]. Regarding TNF induction by sVSG, thus far, data are lacking concerning possible receptor involvement. Incidentally, we have recently found that though the mannose-6-phosphate receptor (MPR46) is upregulated during experimental trypanosome infections, there is no link between the extent of this event and the occurrence of TNF-mediated pathology (unpublished data). As such, analysis of the mechanism involved in GPI-mediated macrophage activation and TNF production requires further investigation.

5. Vaccination with GPI reduces trypanosomosis-associated pathology and prolongs survival

Looking back on the discovery of GPI-linked TNF induction, it is clear that the finding of a phospholipid containing moiety as part of the TNF-inducing malaria toxin was a first milestone [45]. However, before that, it had already been shown that antibodies against exoantigens from malaria could abrogate their ability to induce TNF [46], and the idea of an anti-disease vaccination strategy based upon the generation of anti-TNF-inducing compounds had been proposed as well. The generation of polyclonal and later monoclonal antibodies with phosphatidylinositol specificity that neutralized malaria-triggered TNF induction further supported this idea [47,48]. Moreover, the initial discovery of infection-induced antibodies in serum collected in malaria endemic areas that block the TNF-inducing capacity of malaria toxin [49], and the more recent confirmation of the anti-GPI nature of these antibodies [50], further substantiate the idea that an anti-disease vaccination strategy would be a very efficient tool to attenuate severe GPI-induced pathologies. Finally, the generation of monoclonal antibodies that block GPI-triggered signal transduction [39,40], and the finding by Schofield et

al. (reported at www.BioMedNet.com, News August 2001) that administration of anti-GPI monoclonal Ab to malaria-infected mice can abrogate the symptoms of cerebral malaria and prevent death, give further support for this possibility. However, despite all these findings, thus far, no successful active vaccination strategy has been reported that can actually prevent the occurrence of GPI-induced immunopathology during a parasitic infection. Quite recently, we have obtained evidence that a VSG-GPI-based vaccination strategy can indeed prevent TNF-associated immunopathology in trypanosome-infected mice (manuscript in preparation). While cross-protection experiments against various trypanosome infections as well as cerebral malaria are ongoing, our results have already indicated the virtual absence of anemia in VSG-GPI-vaccinated trypanosome-infected mice. Coinciding with the inhibition of pathology, we have observed a significant prolongation of survival time of the infected mice. Since the induced protection was found to be VSG-type independent, the results indicate that a GPI-based anti-disease vaccine for trypanosomosis might be efficacious. Currently, we are investigating in detail the immunological basis for this protective anti-disease response, paying special attention to the regulation of the activation status of the macrophages involved in VSG-mediated TNF release. In addition, one point that will have to be addressed during the functional analysis of this vaccination strategy is the problem of induction of memory. Thus far, we have no data on how long a persistent anti-VSG-GPI memory can be induced, but taking into account the T-cell-independent nature of anti-malaria toxin antibodies [46], one might expect only a short-term response in laboratory animals. However, in the case of individuals or animals that are living in an endemic trypanosomosis area, this problem could possibly be overcome by the regular natural challenge with small doses of VSG-GPI, even of nonpathogenic *T. brucei* origin. In fact, this might then mimic the situation of man in endemic malaria areas where, as mentioned above, the occurrence of elevated anti-GPI antibody titers, possibly due to regular natural challenges, seems to coincide with resistance to clinical malaria attacks [50].

6. Conclusion

African trypanosomosis is a protozoan parasitic disease marked by severe immunopathology and systemic inflammation. Due to stimulation by the trypanosome-specific VSG-GPI anchor, excessive macrophage activation results in systemic TNF overproduction. A key element in this process is the repetitive challenge of the immune response with vast amounts of the galactose-modified VSG-GPI-carbohydrate core. This cleaved GPI epitope is presented to the immune system during the successive waves of parasitemia, when soluble VSG is released from the parasite surface by activation of an endogenous PLC enzyme. An important step in this process, however, is that infection-associated TNF induction requires a macrophage priming signal. This is provided by the induction of IFN-γ during the early stage of infection, and can be enhanced by the presence of the GPI lipid anchor during later stages of the infection. As such, both the carbohydrate and the lipid moieties of the VSG-GPI anchor can play distinct roles in the final induction of TNF-mediated trypanosomosis-associated immunopathology. Based on our most recent results, it is clear that a GPI-based anti-vaccine strategy might represent an optimal way of preventing infection-associated immune complications during experimental trypanosomosis. While under laboratory conditions such a vaccine does not result in the induction of any direct anti-trypanosomal activity, it does allow the infected host to survive with low levels of parasitemia in the absence of excessive inflammatory responses. As such, once optimized for field use, this type of vaccine strategy could be a useful tool in the fight against trypanosomosis, as it would enable reducing the pathological complications of human sleeping sickness as well as cutting economic losses due to pathological complications in livestock infections.

. Biblio non appelée

[33]

Acknowledgements

The Trypanosome Research Project at the Free University of Brussels is funded by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, The Belgian National Fund for Scientific Research (NFWO-No. 6.0325.95) and an Interuniversity Attraction Pole Program, financed by the Belgian state, Diensten van de Eerste Minister—Federale diensten voor wetenschappelijke, technische en culturele aangelegenheden. S.M. is a Postdoctoral Fellow of the Foundation of Scientific Research-Flanders (NFWO).

References

[1] E. Pays, L. Vanhamme, M. Berberof, Genetic control for expression of surface antigens in African trypanosomes, Annu. Rev. Microbiol. 48 (1994) 25–52.

[2] S.J. Black, R.S. Hewett, C.N. Sendashonga, *Trypanosoma brucei* surface antigen is released by degenerating parasites but not by actively dividing parasites, Parasite Immunol. 4 (1982) 233–244.

[3] W.L. Dempsey, J.M. Mansfield, Lymphocyte function in experimental trypanosomiasis. V. Role of antibody and mononuclear phagocyte system in variant-specific immunity, J. Immunol. 130 (1983) 405–411.

[4] A.L.W. De Gee, R.F. Levine, J.M. Mansfield, Genetics of resistance to the African Trypanosomes. VI. Heredity of resistance and variable surface glycoprotein-specific immune responses, J. Immunol. 140 (1988) 283–288.

[5] M.C. Okomo-Assoumou, S. Daulouede, J.L. Lemesre, A. N'Zila-Mouanda, P. Vincendeau, Correlation of high serum levels of tumor necrosis factor-alpha with disease severity in human African trypanosomiasis, Am. J. Trop. Med. Hyg. 53 (1995) 539–543.

[6] R. Lucas, S. Magez, E. Bajyana Songa, A. Darji, R. Hamers, P. De Baetselier, A role for TNF-α during African trypanosomiasis: involvement in parasite control, immunosuppression and pathology. 51st Forum in immunology, Res. Immunol. 144 (1993) 370–376.

[7] C.A. Hunter, J.W. Gow, P.G.E. Kennedy, F.W. Jennings, M. Murray, Immunopathology of experimental African sleeping sickness: detection of cytokine mRNA in the brains of *Trypanosoma brucei brucei*-infected mice, Infect. Immun. 59 (1991) 4636–4640.

[8] A. Darji, A. Beshin, M. Sileghem, H. Heremans, L. Brys, P. De Baetselier, In vitro simulation of immunosuppression caused by *Trypanosoma brucei*: active involvement of gamma-interferon and tumor necrosis factor-alpha in the pathway of suppression, Infect. Immun. 64 (1996) 1937–1943.

[9] S. Magez, M. Radwanska, A. Beschin, K. Sekikawa, P. De Baetselier, TNF-α is a key mediator in the regulation of experimental *Trypanosoma brucei* infections, Infect. Immun. 67 (1999) 3128–3132.

[10] M. Sileghem, J.N. Flynn, L. Logan-Henfrey, J. Ellis, Tumor necrosis factor production by monocytes from cattle infected with *Trypanosoma* (Duttonella) *vivax* and *Trypanosoma* (Nannomonas) *congolense*: possible association with severity of anaemia associated with the disease, Parasite Immunol. 16 (1994) 51–54.

[11] B. Beutler, D. Greenwald, J.D. Hulmes, M. Chang, Y.-C.E. Pan, J. Mathison, A. Cerami, Identity of tumor necrosis factor and the macrophage-secreted factor cachectin, Nature 316 (1985) 552–554.

[12] D.M. Paulnock, S. Coller, Analysis of macrophage activation in African trypanosomosis, J. Leukoc. Biol. 70 (2001) 142–148.

[13] M.L. Cardoso de Almeida, M.J. Turner, The membrane form of variant surface glycoproteins of *Trypanosoma brucei*, Nature 302 (1983) 349–353.

[14] M.A.J. Ferguson, M.G. Low, G.A.M. Cross, Glycosyl-sn-1,2-dimyristyl phosphatidylinositol is covalently linked to *Trypanosoma brucei* variant surface glycoprotein, J. Biol. Chem. 260 (1985) 14547–14555.

[15] M.A.J. Ferguson, S.W. Homas, R.A. Dwek, T.W. Rademacher, Glycosyl-phosphatidylinositol moiety that anchors *Trypanosoma brucei* variant glycoprotein to the membrane, Science 239 (1988) 753–759.

[16] M.A.J. Ferguson, S.W. Homans, The membrane attachment of the variant surface glycoprotein coat of *Trypanosoma brucei*, in: K.P.W.J. Mc Adam (Ed.), New Strategies in Parasitology, Churchill-Livingstone Press, London, UK, 1989, pp. 121–140.

[17] S.E. Zamze, E.W. Wooten, D.A. Ashford, M.J. Ferguson, R.A. Dwek, T.W. Rademacher, Characterization of the asparagine-linked oligosaccharides from *Trypanosoma brucei* type-I variant surface glycoproteins, Eur. J. Biochem. 187 (1990) 657–663.

[18] S.E. Zamze, D.A. Ashford, E.W. Wooten, T.W. Rademacher, R.A. Dwek, Structural characterization of the Asparagine-linked oligosaccharides from *Trypanosoma brucei* Type II and Type III variant surface glycoproteins, J. Biol. Chem. 266 (1991) 20244–20261.

[19] M.A.J. Ferguson, J.S. Brimaombe, S. Cottaz, R.A. Field, L.S. Güther, S.W. Homans, M.J. McConvill, A. Mehlert, K.G. Milne, J.E. Ralton, Y.A. Roy, P. Schneider, N. Zitzmann, Glycosyl-phosphatidylinositol molecules of the parasite and the host, Parasitology 108 (1994) s45–s54.

[20] J.A. Fox, M. Duszenko, M.A.J. Ferguson, M.G. Low, G.A.M. Cross, Purification and characterization of a novel glycan-phosphatidilinositol-specific phospholipase C from *Trypanosoma brucei*, J. Biol. Chem. 261 (1986) 15767–15771.

[21] S.E. Zamze, M.A.J. Ferguson, R. Collins, R.A. Dwek, T.W. Rademacher, Characterization of the cross-reacting determinant (CRD) of the glycosyl-phosphatidylinositol membrane anchor of *Trypanosoma brucei* surface glycoprotein, Eur. J. Biochem. 176 (1988) 527–534.

[22] M. Geuskens, E. Pays, M.L. Cardoso de Almeida, The lumen of the flagellar pocket of *Trypanosoma brucei* contains both intact and phospholipase C-cleaved GPI-anchored proteins, Mol. Biochem. Parasitol. 108 (2000) 269–275.

[23] S. Rolin, J. Hanocq-Quertier, F. Paturiaux-Hanocq, D. Nolan, D. Salmon, H. Webb, M. Carrington, P. Voorheis, E. Pays, Simultaneous but independent activation of adenylate cyclase and glycosylphosphatidylinositol-phospholipase C under stress conditions in *Trypanosoma brucei*, J. Biol. Chem. 217 (1996) 10844–10852.

[24] H. Webb, C. Carnall, L. Vanhamme, S. Rolin, J. Van Den Abbeele, S. Welburn, E. Pays, M. Carrington, The GPI-phospholipase C of *Trypanosoma brucei* is nonessential but influences parasitemia in mice, J. Cell Biol. 139 (1997) 103–114.

[25] S. Leal, A. Acosta-Serrano, Y. Morita, P. Englund, U. Böhme, G. Cross, Virulence of *Trypanosoma brucei* strain 427 is not affected by the absence of glycosylphosphatidylinositol-phospholipase C, Mol. Biochem. Parasitol. 114 (2001) 245–247.

[26] B. Namangala, P. De Baetselier, L. Brys, B. Stijlemans, W. Noël, E. Pays, M. Carrington, A. Beschin, Attenuation of *Trypanosoma brucei* is associated with reduced immunosuppression and concomitant production of Th2 lymphokines, J. Infect. Dis. 181 (2000) 1110–1120.

[27] B. Namangala, P. De Baetselier, W. Noël, L. Brys, A. Beschin, Alternative versus classical macrophage activation during experimental African trypanosomosis, J. Leukoc. Biol. 69 (2001) 387–396.

[28] L.R. Schopf, H. Filutowicz, X.J. Bi, J.M. Mansfield, Interleukine-4-dependent immunoglobulin G1 isotype switch in the presence of a polarized antigen-specific Th1-cell response to the trypanosome variant surface glycoprotein, Infect. Immun. 66 (1998) 451–461.

[29] C. Hertz, H. Filutowicz, J.M. Mansfield, Resistance to the African trypanosome is IFN-gamma dependent, J. Immunol. 161 (1998) 6775–6783.

[30] B. Namangala, W. Noël, P. De Baetselier, A. Becshin, Relative contribution of Interferon-gamma and Interleukine-10 to resistance to murine African trypanosomosis, J. Infect. Dis. 183 (2001) 1794–1800.

[31] C. Hölscher, M. Mohrs, W.J. Dai, G. Köhler, B. Ryffel, G.A. Schaub, H. Mossmann, F. Brombacher, Tumor necrosis factor alpha-mediated toxic shock in *Trypanosoma cruzi*-infected interleukin 10-deficient mice, Infect. Immun. 68 (2000) 4075–4083.

[32] S. Magez, R. Lucas, A. Darji, E. Bajyana Songa, R. Hamers, P. De Baetselier, Murine tumour necrosis factor plays a protective role during the initial phase of the experimental infection with *Trypanosoma brucei*, Parasite Immunol. 15 (1993) 635–641.

[33] C.A.W. Bate, J. Taverne, J.H.L. Playfair, Detoxified exoantigens and phosphatidylinositol derivatives inhibit tumor necrosis factor induction by malarial exoantigens, Infect. Immun. 60 (1992) 1894–1901.

[34] S.D. Tachado, L. Schofield, Glycosylphosphatidylinositol toxin of *Trypanosoma brucei* regulates IL-1α and TNF-α expression in macrophages by protein tyrosine kinase mediated signal transduction, Biochem. Biophys. Res. Com. 205 (1994) 984–991.

[35] S. Magez, B. Stijlemans, M. Radwanska, E. Pays, M.A.J. Ferguson, P. De Baetselier, The glycosyl-inositol-phosphate and dimyristoylglycerol moieties of the glycosylphosphatidylinositol anchor of the trypanosome variant-specific surface glycoprotein are distinct macrophage-activating factors, J. Immunol. 160 (1998) 1949–1956.

[36] V.W. Pentreath, Endotoxins and their significance of murine trypanosomiasis, Parasitol. Today 10 (1994) 226–228.
[37] M. Sileghem, R. Saya, D.J. Grab, J. Naessens, An accessory role for the diacyglycerol moiety of variant surface glycoprotein of African trypanosomes in the stimulation of bovine monocytes, Vet. Immunol. Immunopathol. 78 (2001) 325–339.
[38] L. Schofield, S. Novakovic, P. Gerold, R.T. Schwarz, M.J. Mc Conville, S.D. Tachado, Glycosylphosphatidylinositol toxin of *Plasmodium* up-regulates intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin expression in vascular endothelial cells and increase leukocyte and parasite cytoadherence via tyrosine kinase-dependent signal transduction, J. Immunol. 156 (1996) 1886–1896.
[39] S.D. Tachado, P.G.M.J. Mc Conville, T. Baldwin, D. Quilici, R.T. Schwarz, L. Schofield, Glycosylphosphatidylinositol toxin of plasmodium induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signal pathway, J. Immunol. 156 (1996) 1897–1907.
[40] S.D. Tachado, R. Mazhari-Tabrizi, L. Schofield, Specificity in signal transduction among glycosylphosphatidylinositol of *Plasmodium faciparum*, *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp, Parasite Immunol. 21 (1999) 609–617.
[41] I.C. Almeida, M.M. Camargo, D.O. Procopio, L.S. Silva, A. Mehlert, L.R. Travassos, R.T. Gazinelli, M.A. Ferguson, Highly purified glycosylphosphatidylinositols from *Trypanosoma cruzi* are proinflammatory agents, EMBO J. 19 (2000) 1476–1485.
[42] M. Vijaykumar, R.S. Naik, D.C. Gowda, Plasmodium falciparum glycosylphosphatidylinositol-induced TNF-alpha secretion by macrophages is mediated without membrane insertion or endocytosis, J. Biol. Chem. 276 (2001) 6909–6912.
[43] M.M. Camargo, I.C. Almeida, M.E.S. Pereira, M.A. Ferguson, L.R. Travassos, R.T. Gazinelli, Glycosylphosphatidylinositol-anchored mucin-like glycoproteins isolated from *Trypanosoma cruzi* trypomastigotes initiate the synthesis of proinflammatory cytokines by macrophages, J. Immunol. 158 (1997) 5890–5901.
[44] M.A. Campos, I.C. Almeida, O. Takeuchi, S. Akira, E.P. Valente, D.O. Procopio, L.R. Travassos, J.A. Smith, D.T. Golenbock, R.T. Gazinelli, Activation of toll-like receptor-2 by glycosylphosphatidylinositol anchors from protozoan parasites, J. Immunol. 167 (2001) 416–423.
[45] K. Taylor, C.A.W. Bate, R.E. Carr, G.E. Butcher, J. Taverne, J.H.L. Playfair, Phospholipid-containing toxic malaria antigens induce hypoglycaemia, Clin. Exp. Immunol. 90 (1992) 1–5.
[46] J. Taverne, C.A.W. Bate, D.A. Sarkar, A. Meager, G.A.W. Rook, J.H.L. Playfair, Human and murine macrophages produce TNF in response to soluble antigens of *Plasmodium falciparum*, Parasite Immunol. 12 (1990) 33–43.
[47] C.A.W. Bate, J. Taverne, H.J. Bootsma, R.C. Mason, N. Skalko, G. Gregoriadis, J.H.L. Playfair, Antibodies against phosphatidylinositol and inositol monophosphate specifically inhibit tumor necrosis factor induction by malarial exoantigens, Immunology 76 (1992) 34–41.
[48] C.A.W. Bate, D. Kwiatkowski, A monoclonal antibody that recognizes phosphatidylinositol inhibits induction of tumor necrosis factor alpha by different strains of *Plasmodium falciparum*, Infect. Immun. 62 (1994) 5261–5266.
[49] C.A.W. Bate, D. Kwiatkowski, Inhibitory immunoglobulin M antibodies to tumor necrosis factor-inducing toxins in patients with malaria, Infect. Immun. 62 (1994) 3086–3091.
[50] R.S. Naik, O.H. Branch, A.S. Woods, M. Vijaykumar, D.J. Perkins, B.L. Nahlan, A.A. Lal, R.J. Cotter, C.E. Costello, F. Ockenhouse, E.A. Davidson, D.C. Gowda, Glycosylphosphatidylinositol anchors of *Plasmodium falsiparum*: molecular characterization and natural elicited antibody response that may provide immunity to malaria pathogenesis, J. Exp. Med. 192 (2000) 1563–1575.