Immunological reactions in response to apicomplexan glycosylphosphatidylinositol

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Apicomplexan protozoa are a phylum of parasites that includes pathogens such as Plasmodium, the causative agent of the most severe form of malaria responsible for almost 1 million deaths per year and Toxoplasma gondii causing toxoplasmosis, a disease leading to cerebral meningitis in immunocompromised individuals or to abortion in farm animals or in women that are infected for the first time during pregnancy. The initial immune reactions developed by the host are similar in response to an infection with Plasmodium and Toxoplasma in the sense that the same cells of the innate immune system are stimulated to produce inflammatory cytokines. The glycosylphosphatidylinositol (GPI) anchor is the major carbohydrate modification in parasite proteins and the GPIs are essential for parasite survival. Two immediate GPI precursors with the structures ethanolamine phosphate-6(Man α1-2)Man α1-2Man α1-6Man α1-4GlcN-PI and ethanolamine phosphate-6Man α1-2Man α1-6Man α1-4-GlcN-PI are synthesized by P. falciparum. Two main structures are synthesized by T. gondii: ethanolamine phosphate-6Man α1-2Man α1-6(GalNAcβ1-4)Man α1-4GlcN-PI and ethanolamine phosphate-6Man α1-2Man α1-6(Glcα1-4GalNAcβ1-4)Man α1-4GlcN-PI. This review describes the biosynthesis of the apicomplexan GPIs and their role in the activation of the host immune system.

Keywords: apicomplexa/GPI/immunology/Plasmodium falciparum/Toxoplasma gondii

Apicomplexan parasites

Apicomplexan protozoa are a phylum of parasites that includes pathogens causing important human and farm animal diseases. They are named for their cell apex that contains a number of organelles (rhoptries, micronemes, conoid and apical polar ring), important for their invasion and development within host cells. The apicomplexa have acquired during their evolution the apicoplast, a chloroplast-derived organelle with an algal origin. Apicomplexan parasites are surrounded by the pellicle, a structure consisting of the plasma membrane and the closely apposed inner membrane complex that consists of flattened vesicles.

Apicomplexans undergo haploid and diploid stages during their life cycle. In many cases, their life cycle is distributed between two hosts, an intermediary host and a definitive host. Rapidly multiplying haploid stages of the parasites cause the acute and deleterious symptoms of infection. In addition to asexual mitotic proliferation, these parasites differentiate into gametes that make zygotes after fusion in the respective definitive hosts. Plasmodium gametogenesis and fertilization occur in the mosquito intestine whereas the sexual part of the life cycle of Toxoplasma gondii takes place in the intestinal epithelia of cats. The apicomplexan protozoan parasites replicate within the cells of their host and thus are obligate intracellular parasites. The active merozoites (in the case of T. gondii called tachyzoites) released by lysis of the host cell must invade new host cells in order to stay viable. Parasite replication occurs within the host cell in a so-called parasitophorous vacuole, derived from the host cell by invagination of the plasma membrane during entry, which is modified by the parasite (pictures of intracellular P. falciparum and T. gondii on Figure 1).

Although Toxoplasma infects about 25% of the world population (Jones et al. 2003), immunocompetent individuals show no or very mild symptoms of the disease. On the other hand, immunocompromised individuals (e.g. patients who have AIDS, undergo organ transplantation or cancer chemotherapy) develop serious diseases such as cerebral meningitis due to the reactivation of bradyzoites in brain cysts into tachyzoites (Luft and Remington 1992). Another group at risk are women that are infected for the first time during pregnancy. The parasites can cause birth defects or abortion of the fetus (Jones et al. 2001). Infection of farm animals (particularly sheep) with Toxoplasma leads to abortion. Infection with T. gondii occurs by oral route after consumption of meat containing the cysts or by ingestion of oocysts present in cat feces.

Malaria is one of the most common infectious diseases and an enormous public health problem. Four types of the Plasmodium parasite can infect humans; the most serious form of the disease is caused by Plasmodium falciparum, which infects about 250 million people with almost 1 million deaths in...
Immune response to infection with *P. falciparum* and *T. gondii*

*In vivo* studies indicate that the activation of the host innate immunity plays a crucial role in the early resistance against infection and pathogenesis of malaria and toxoplasmosis (Gazzinelli et al. 1994; Day et al. 1999). A systemic inflammatory status could be explained by secretion of large amounts of inflammatory cytokines by host cells (Clark et al. 1981). Clinical studies have shown a correlation between malaria severity and circulating levels of the inflammatory cytokine tumor necrosis factor (TNF-α) in African and Melanesian children or in European adults (Kern et al. 1989; Butcher et al. 1990; Kwiatkowski et al. 1990). These inflammatory cytokines induce a strong production of nitric oxide (NO) through the activation of the inducible NO synthase (Nussler et al. 1992).

The cells of the innate immune system, dendritic cells (DCs), monocytes/macrophages, natural killer (NK) cells, natural killer T (NKT) cells and γδ T cells, play a fundamental role in shaping the adaptive immune response to blood-stage malaria. Both immunopathology and adaptive immune responses are critically dependent on the early events in the host–parasite interaction and the resulting cytokine balance. Early in infection, when parasitemia is still low, infected erythrocytes directly interact with DCs that mature and secrete IL-12 or TNF-α (Seixas et al. 2001). These cytokines activate NK cells to produce interferon (IFN)-γ, which may have a cytotoxic effect on parasite growth via NO production and may activate monocytes and macrophages and enhance phagocytosis of infected erythrocytes (Artavanis-Tsakonas and Riley 2002). Mature DCs and activated monocytes/macrophages may present parasite antigens to naive and primed T cells, respectively, and promote Th1 polarization of CD4+ T cells (Perry et al. 2004). With increasing parasitemia, monocytes/macrophages and DCs may become paralyzed by the ingestion of hemozoin, a by-product resulting from digestion of hemoglobin by the parasite in the red blood cell (Skorokhod et al. 2004). Production of IL-10 by DCs may counteract the initial proinflammatory cytokine cascade and shift the balance towards an antiinflammatory response (Urban et al. 1999). Nevertheless, IL-10 is an important growth factor for B cells and may promote B-cell survival and differentiation, eventually leading to class switch towards IgG1 and IgG3 subclasses (Taylor et al. 1998; Ndungu et al. 2002), the two main cytphilic antibodies that allow opsonization and clearance of merozoites or infected erythrocytes by macrophages and neutrophils. CD4+ T cells as well as B cells are required for the cell-mediated and antibody-dependent mechanisms leading to the control and resolution of infection and to immunity against the clinical syndromes associated with malaria. However, antibody responses against specific antigens are short-lived and dependent on the presence of circulating parasites (Cavanagh et al. 1998; Bull et al. 2002), suggesting that memory and longevity of B- and T-cell responses are perturbed in malaria.

During early stages of mouse infection, *T. gondii* components activate cells such as macrophages, DCs and neutrophils to produce high levels of IL-12 (Gazzinelli, Hienny, et al. 1993), which is responsible for initiation of IFN-γ synthesis by NK cells and for favoring the differentiation of lymphocytes to the Th1 phenotype that is the basis of the protective immunity and control of parasite replication (Gazzinelli, Eltoum, et al. 1993; Hayashi et al. 1996). Thus far, IFN-γ has been shown to be the most crucial cytokine in mediating resistance during acute infection with this protozoan (Gazzinelli et al. 1991). Studies in mice have also demonstrated the important role of TNF-α and generation of reactive nitrogen intermediates as mediators of host resistance.
to early *T. gondii* infection (Denkers and Gazzinelli 1998). Animals deficient in interleukin (IL)-12, IFN-γ and inducible nitric oxide synthase (iNOS) or treated with neutralizing anti-
ytokine antibodies or specific inhibitors of iNOS have an increased morbidity and/or mortality in response to an infection
with *T. gondii* (Suzuki et al. 1989; Gazzinelli et al. 1991; Gazzinelli, Eltoum, et al. 1993; Gazzinelli, Hieny, et al. 1993; Hayashi et al. 1996). During chronic toxoplasmosis, neutralization of either IFN-γ, TNF-α or inhibition of iNOS results in the reactivation of dormant bradyzoites and the development of encephalitis as well as uveitis (Gazzinelli, Eltoum, et al. 1993; Hayashi et al. 1996). Pathology associated with excessive immune stimulation of Th1 responses and high levels of IFN-γ has been also demonstrated during acute infection with *T. gondii* in the mouse model (Liesenfeld et al. 1996). Production of the antiinflammatory cytokine IL-10 is stimulated during infection with *T. gondii*, involved in the downregulating production of IL-12, IFN-γ and TNF-α and associated with exacerbated pathology (Gazzinelli et al. 1996). Humans infected with *T. gondii* have been associated with exacerbated pathology (Gazzinelli et al. 1996; Dieckmann-Schuppert et al. 1996; Garenieux et al. 2008; Luk et al. 2003). Decreased cellular immune responses, including IL-12 and IFN-γ synthesis elicited by *T. gondii* antigens, have been demonstrated in patients coinfected with HIV and have been associated with the development of toxoplasmic encephalitis in humans (Hunter et al. 1996). Patients with congenital toxoplasmosis who develop an ocular disease appear to have decreased cytokine synthesis towards the parasite antigens (Yamamoto et al. 2000). Many studies have been performed to identify the apicomplexan molecules triggering the host immune response and inflammation, and this report focuses on the role of glycosylphosphatidylinositol (GPIs).

**GPIs of apicomplexan parasites**

It has generally been believed that O-glycosylation is the major carbohydrate modification in the intraerythrocytic stage of *P. falciparum* and that the parasite does not possess the machinery required for N-glycosylation. However, later studies have shown that *P. falciparum* has a low N-glycosylation capability, and O-glycosylation is either absent or present at an extremely low level, whereas GPI anchor modification is common and is the major carbohydrate modification in parasite proteins (von Itzstein et al. 2008). N-glycosylation was demonstrated to be quite prevalent in *T. gondii* (Odenthal-Schnittler et al. 1993; Dieckmann-Schuppert et al. 1996; Garenaux et al. 2008; Luk et al. 2008). GPI-anchored proteins dominate the surface of the *T. gondii* tachyzoite (Black and Boothroyd 2000; Lekutis et al. 2001), and GPI biosynthesis is an essential process for viability in *T. gondii* (Wichroksi and Ward 2003). It is also important to note that protein-free GPIs are present at the surface of *T. gondii* (Azzouz et al. 2006). The immunodominant 17-kDa surface antigen (gp15) of *Cryptosporidium parvum*, an apicomplexan parasite that has caused numerous outbreaks of diarrheal illness in humans, is GPI anchored (Priest et al. 2001). The function of the glycoproteins gp40 and gp15 of *C. parvum* in sporozoite attachment to and invasion of host cells appears to be dependent on the presence of multiple O-linked α-N-acetylgalactosamine residues (O’Connor et al. 2003).

**GPIs of *P. falciparum***

*P. falciparum* synthesizes two merozoite surface proteins (MSP-1 and MSP-2) during schizogony. MSP-1 and MSP-2 are estimated to represent two-thirds of the total membrane-associated surface coat. Proteomic analysis identified GPI anchoring on eleven proteins: MSP-1, -2, -4, -5, -10, rhoptry-associated membrane antigen, apical sushi protein, Pf92, Pf38, Pf12 and Pf34 (Gilson et al. 2006). These proteins represent approximately 94% of the GPI-anchored schizont/merozoite proteome and constitute the largest set of GPI-anchored proteins in this organism. Biochemical studies were focused mainly on the asexual, intraerythrocytic stages of *P. falciparum* because of their role in the clinical phase of the disease and also the possibility of their propagation in a cell culture system. Two candidates for putative GPI-anchor precursors to malarial GPI-membrane proteins with the structures (Figure 2A) ethanolamine phosphate-6-Manα1-2Manα1-6Manα1-4GlcN-PI (Pfα) and ethanolamine phosphate-6-Manα1-2Manα1-6Manα1-4-GlcN-PI (Pfβ) were identified and confirmed in an independent investigation (Gerold et al. 1994; Gowda et al. 1997). Only Pfα serves as an anchor for MSP-1 and MSP-2 (Gerold et al. 1996). The GPI membrane anchor precursor Pfα is synthesized and transferred to other, until now uncharacterized, proteins essentially in trophozoite stages (Gerold et al. 1994; Schmidt et al. 1998). Interestingly, the core glycans of nine *P. falciparum* isolates from different geographic regions show a universal core glycan structure (Berhe et al. 1999). Similarly, studies on GPI structure of two rodent parasites (*P. chabaudi* chabaudi and *P. yoelii yoelii*) show that core glycans are also identical to *P. falciparum* GPIs (Gerold et al. 1997; Kimmel et al. 2003). An analysis of the acyl substituent on C-2 of the inositol of the nonlabeled GPIs of *P. falciparum* showed palmitic acid (~90%) and myristic acid (~10%). The diacylglycerol moiety contains predominantly C18:0 and C18:1, minor proportions of C14:0, C16:0, C18:2, C20:0, C22:0 and unidentified acids, with C18:1 and C18:2 at the sn-2 position (Naik et al. 2000). Chemical synthesis of malaria GPIs was performed by several laboratories. A strategy for fully inositol acylated and phosphorylated GPIs was elaborated by the group of Fraser-Reid (Lu et al. 2004), and a highly convergent synthesis of a fully lipidaded GPI anchor of *P. falciparum* was established by the laboratory of Peter Seeberger (Kwon et al. 2005; Liu et al. 2005). A rapid access to the target GPIs in a highly efficient manner in sufficient quantities for the biological studies has been achieved.

**GPIs of *T. gondii***

GPIs of *T. gondii* have been shown to be identical with the “low molecular weight antigen”, identified by the group around Remington (Remington and Desmonts 1990), and elicit an early immunoglobulin M immune response in humans (Erlich et al. 1983; Tomavo et al. 1992). The following structures of *T. gondii* GPIs were identified (Figure 2B): Manα1-2Manα1-6(GalNAcβ1-4)Manα1-4GlcNα-PI and the novel structure Manα1-2Manα1-6(Glcα1-4GalNAcβ1-4)Manα1-4GlcNα-PI.
both with and without terminal ethanolamine phosphate (Striepen et al. 1997). Only *T. gondii* GPIs bearing the unique glucose-N-acetylgalactosamine side branch are immunogenic in humans and are widely distributed among *T. gondii* isolates. Characterization of the phosphatidylinositol moieties shows the presence of a diacylglycerol lipid, whose

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**Fig. 2.** Structures of the GPIs of (A) *P. falciparum* and (B) *T. gondii.*
sn-2 position contains almost exclusively a C18:1 acyl chain. In another study, GPI anchor peptides were isolated from \[^{1}H\]glucosamine labeled SAG1 (P30) major surface protein from *T. gondii* cultivated in Vero cells using protease digestion and phase partitioning. Two glycoforms were characterized by gel filtration and high performance ion exchange chromatography in combination with exoglycosidase treatment. Both forms were shown to have an *N*-acetylgalactosamine side-chain modification bound to the first mannose of the conserved three-mannosyl core. The second glycoform carries an additional terminal hexose linked to GalNAc (Zinecker et al. 2001). Comparison of these structures with free GPI glycolipid precursors characterized in *T. gondii* suggests that core modification of the anchor takes place prior to transfer to the protein. In infected human cells, the pool of *T. gondii* GPs having only GalNAc residue linked to the evolutionary conserved trimannosyl core glycan are competent for transfer to nascent surface GPI proteins, whereas the pool of GPs having an additional Glc linked to GalNAc side branch accumulated at the cell surface as protein-free metabolic end products (Azzouz et al. 2006). A synthesis of a fully phosphorylated toxoplasmal GPI anchor pseudohexasaccharide was achieved by the group around R.R. Schmidt, Konstanz (Pekari et al. 2001).

**GPI**

**GPIs of *C. parvum***

The *C. parvum* 17-kDa antigen GPI anchor is composed of a very basic Manα1,2-Manα1,6-Manα1,4-glucosamine glycan core with an acylated inositol and either a *lyso*-acyl- or a diacyl-glycerol having only C16:0, C18:0 and C18:1 fatty acids. At least for the most abundant diacyl (C16:0, C18:0) form of the GPI, there is only one ethanolamine phosphate per molecule (Priest et al. 2006).

**GPI biosynthesis**

A detailed understanding of GPI synthesis in protozoa is a prerequisite for identifying differences present in biosynthetic pathways of parasites and host cells. The multiple biosynthesis steps were studied in detail in *T. gondii*. As in yeast or mammalian cells, the pathway starts with the transfer of GlcNAC from UDP-GlcNAC to PI, followed by the de-N-acetylation of the GlcNAC and the addition of three mannose residues. The Manα1,GlcN-PI structure is then modified by GalNAc residue linked to the mannose adjacent to glucosamine. This GalNAc-containing intermediate plays two roles: firstly as precursor for free Glc-GalNAc-containing GPs and secondly as precursor for the GalNAc-containing GPs. In contrast to trypanosome GPs, side-chain modification in *T. gondii* GPs takes place before addition to protein in the ER. Therefore, the biosynthesis of the side-chain modifications was studied in an in vitro system prepared from hypotonically lysed *T. gondii* parasites. Radiolabeled glucose-containing GPI precursors were synthesized by *T. gondii* membrane preparations. Synthesis of glucosylated glycolipids was shown to take place only in the presence of exogenous uridine diphosphate glucose with a direct transfer of glucose from uridine diphosphate glucose. Dolichol phosphate glucose is not involved in this step (Striepen et al. 1999). Furthermore, using hypotonically permeabilized *T. gondii* tachyzoites, the topology of the free GPs within the ER membrane was investigated. A significant portion of the early GPI intermediates (GlcN-PI and GlcNAc-P) could be hydrolyzed following PI-PLC treatment, indicating that these glycolipids are predominantly present in the cytoplasmic leaflet of the ER. Permeabilized *T. gondii* parasites labeled with either GDP-[\(^{2}\)H]mannose or UDP-[\(^{6}\)H]glucose showed that the more mannosylated and side chain (Glc-GalNAc)-modified GPI intermediates are also preferentially localized in the cytoplasmic leaflet of the ER (Kimmel et al. 2006). Novel inositol-acylated GPI intermediates were observed and characterized. Inositol acylation of the nonmannosylated GPI intermediate d-GlcNAc1-6-d-myosinositol-1-phosphoryl-sn-lipid precedes mannosylation and is acyl-CoA-dependent. Inositol deacylation of the fully mannosylated GPI intermediate allows further processing, i.e. addition of GalNAc side chain to the first mannose. Progress was made in characterizing glycosyltransferases involved in the biosynthesis of the core glycans of the GPI-anchor, like PIG-M that encodes the mammalian GPI-MT-I, the first mannosyltransferase. PIPiG-M partially restored cell surface expression of the GPI-anchored protein CD59 in Plasmodium deficient mammalian cells and first mannosyl transfer activity in vitro (Kim and Hong 2007). Putative *T. gondii* GPI biosynthetic genes for steps 1 (PIG-A, PIG-C and GPI1), 2 (PIG-L), 3 (PIG-W), 4 (PIG-M), 5 (PIG-V), 6 (PIG-B), 8 (PIG-O and PIG-F) and 9 (PIG8 and GAA-1) and for the generation of Dop-P-Man (DPM1) were identified by searching the *Toxoplasma* Genome for homologues of known *P. falciparum*, human and yeast GPI biosynthetic genes and cloned (Figure 3). No homologous gene for the other mammalian genes implicated in the first step (PIG-H, PIG-P and DPM2) could be found (Smith et al. 2007). Furthermore, the parasite PIPiG-B mannosyltransferase-III is novel in that its signature sequence HKEKHI is unique and only partially conserved as compared to HKEXRF signature motif of mammalian PIG-B enzymes (Basagoudanavar et al. 2007). Therefore, glycoconjugate pathways of apicomplexan protozoa are being unraveled and might represent targets for the development of new drugs. An acyl group modifies the inositol ring of GPs of *P. falciparum*. The preferred donor of this fatty acid at the inositol ring is myristoyl-CoA (Gerold et al. 1997). Inositol acylation is a salient feature of plasmodial GPs and thus might provide a potential target for drug therapy. Another target could also be the dolichol phosphate mannosyl synthase (DPM), which is a key enzyme catalyzing the reaction between dolichol phosphate (Dol-P) and guanosine diphosphate mannosyl (GDP-Man) to generate dolichol phosphate-mannose (Dol-P-Man) (Haselbeck and Tanner 1982; Costello and Orlean 1992). Dol-P-Man is the main mannosyl donor providing not only four mannosyl residues for the synthesis of the lipid-linked precursor oligosaccharide Dol-PP-GlcNAc2Manα1,Glc3 of N-glycosylation of proteins (Hoflack and Kornfeld 1985; Herscovics and Orlean 1993), but also offers three (four in yeast and *Plasmodium*) mannosyl residues for the synthesis of GPs (DeGasperi et al. 1990; Low et al. 1991; Endo et al. 1996). It was shown that the Dol-P-Man synthases from several *Plasmodium* species fall into very different classes, whereby differences between the Dol-P-Man synthases of human and *Plasmodium* species could be exploited in the development of antimicrobial agents (Shams-Eldin et al. 2008). It should be a straightforward strategy to predict what effects a compound will have on the parasite. In yeast and mammalian but not protozoan cells, the first mannoside is modified by phospho-ethanol-
amine at position 2 (Hirose et al. 1992; McConville et al. 1993; Canivenc-Gansel et al. 1998; Sutterlin et al. 1998). The second mannose of mammalian and yeast GPIs can also be modified by phospho-ethanolamine (Ueda et al. 1993). This main difference in the structure explains the immunological reactions induced by apicomplexan GPIs. The considerably higher GPI surface density on protozoan than on mammalian cells (Macrae and Ferguson 2005) is certainly a cause of strong reaction in infected host.

**Immunological reactions to apicomplexan GPIs**

The GPIs exhibit a variety of functions other than the mere anchoring of membrane proteins. Many biological and immunological properties have been shown to be associated with this glycolipid modification. Prior to the study of their biological activities, GPIs must be extracted from the parasites. For this, extraction with organic solvents followed by separation by chromatography on octyl-sepharose, high performance chromatography or thin layer chromatography has been used (Schofield et al. 1996; Debierre-Grockiego et al. 2003; Krishnegowda et al. 2005). The absence of *Mycoplasma* in the cell culture as well as the absence of endotoxins in the GPI preparation must be checked to exclude the possibility that contaminating bacterial compounds give false results. An alternative way would be to use synthetic GPI but only the malarial GPIs have been obtained (Lu et al. 2004; Liu et al. 2005), and to date no study on their biological effects is published. Data were obtained with derivatives slightly or largely different from the natural GPI structures (Schofield et al. 2002; Debierre-Grockiego et al. 2003). Acute infection with protozoan parasites results in the production of high levels of inflammatory cytokines, such as ILs and TNF-α by macrophages, DCs and neutrophils. These cytokines initiate various effector mechanisms that are responsible for the control of parasite growth and pathology. GPI of *Plasmodium* was first shown to act as a parasite-derived toxin by its ability to induce TNF-α and IL-1 production by macrophages (Schofield and Hackett 1993). GPI purified from the variant surface glycoprotein of *Trypanosoma* (a nonapicomplexan protozoa) has similar activities in macrophage activation, and could thus also account for the high level of IL-1 and TNF-α found in trypanosomiasis (Tachado and Schofield 1994). In the same way, we have shown that *T. gondii* GPIs are able to induce the production of TNF-α in macrophages (Debierre-Grockiego et al. 2003). To date no work is published on the biological activity of *C. parvum* GPI, and it is not known if it is able to stimulate cells to produce inflammatory cytokines. The GPI from *Plasmodium* increases secretion of NO and expression of intercellular adhesion molecule-1 (ICAM-1).

**Fig. 3.** Biosynthesis of the GPIs of *T. gondii* and *P. falciparum*. Step 1: generation of acetyl-glucosamine phospho-inositol, step 2: generation of glucosamine phospho-inositol (deacetylation), step 3: generation of glucosamine acyl phospho-inositol (inositol acylation), steps 4, 5 and 6: addition of first, second and third mannoses, step 7: inositol deacylation and addition of acetyl galactosamine on the first mannose (*T. gondii*) or addition of a fourth mannose (*P. falciparum*), step 8: addition of phospho-ethanolamine (EtN-P) to the third mannose, step 9: attachment of the GPI to a protein (action of a transamidase). All genes involved in the biosynthesis of protozoan GPIs are not identified.
Fig. 4. Involvement of the Toll-like receptor (TLR) signaling pathway in the activation of the immune response by apicomplexan GPIs. Apicomplexan GPIs are recognized by toll-like receptor 2 (TLR2) or TLR4 leading to the activation of adaptor molecules. The myeloid differentiation factor 88 (MyD88) by inducing inflammatory cytokines plays a key role in host resistance to infection with parasites. *Plasmodium* GPIs trigger phosphorylation of kinases [extracellular regulated kinase-1 (ERK-1)/ERK-2, MAPK kinases (MKK) and stress-activated protein kinases (SAPK)/p38], while numerous cytoplasmic proteins are phosphorylated on tyrosine residues in response to GPIs of *Toxoplasma*. GPIs of both species induce the nuclear translocation of the transcription factor NF-κB responsible for the expression of inflammatory cytokine genes. *P. falciparum* GPIs are also able to increase the expression of surface adhesion molecules on endothelial cells and to induce apoptosis of rat cardiomyocytes. In contrast, the GPIs of *T. gondii* do not induce apoptosis of various human cell lines. ICAM, intercellular adhesion molecule-1; IκB, inhibitor of NF-κB; IKK, inhibitor of NF-κB-kinase; IL, interleukin; IRAK, IL-1R-associated kinase; MyD88, myeloid differentiation primary-response protein 88; NF-κB, nuclear factor-κB; NO, nitric oxide; TAK, TGF-β-activated kinase; TIRAP, TIR domain-containing adaptor protein; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAF6, TNFR-associated factor 6; TRAM, TRIF-related adapter molecule; TRIF, TIR domain-containing adapter inducing IFN-β; VCAM, vascular cell adhesion molecule-1.
and vascular cell adhesion molecule-1 (VCAM-1) in host cells, which are implicated in the etiology of the cerebral malaria syndrome (Schofield et al. 1996; Tachado et al. 1996). These studies have concluded that GPIs are likely to be the dominant agents responsible for inflammatory cytokine production that these parasites generate. However, a 100-fold higher concentration of the plasmoidal or toxoplasmal GPIs than bacterial lipopolysaccharides is required to induce similar TNF-α levels in macrophage culture (Nemoto et al. 1999; Debierre-Grockiego et al. 2003; Zhu et al. 2005). Although endogenous inflammatory cytokines are important for resistance against T. gondii, an excessive production leads to the death of the host (Hunter et al. 1996). For this reason, the control of the GPI-induced inflammation may be a strategy to reduce pathogenicity and mortality due to toxoplasmosis.

In mammals, the Toll-like receptors (TLRs) are the first instruments of the innate immune system to recognize every known category of microorganisms that cause human diseases. Indeed, TLRs are critical for the recruitment of phagocytes to infected tissue and subsequent microbial killing. To date, 13 TLRs have been described, and for most of them, the microbial molecules that they recognize have been identified. The GPs of Plasmodium and Trypanosoma were shown to exert their inflammatory effects by activation of signaling pathways in host cells mainly through TLR2 (Campos et al. 2001; Krishnegowda et al. 2005) (Figure 4). This pathway involves kinases like p38, and c-Jun N-terminal kinases (JNK1 and JNK2) in macrophages. The expression of host loci implicated in parasite pathogenesis (e.g. TNF-α, IL-1, NO, ICAM) in response to GPs depends on the phosphorylation of these kinases and on the activation of the transcription factor NF-κB (Tachado et al. 1997; Zhu et al. 2005). As the GPs of Plasmodium and Trypanosoma, the GPs of Toxoplasma induce the production of TNF-α in macrophages through NF-κB activation. However, GPs of Toxoplasma, as well as the glycan moiety alone, activate CHO cells via TLR4, while the lipid moiety cleaved from the GPs activate these cells via both TLR4 and TLR2 (Debierre-Grockie, Campos, et al. 2007). Both TLR2 and TLR4 trigger TNF-α production by macrophages exposed to T. gondii GPs. When macrophages were deficient for both TLR2 and TLR4, the production of TNF-α in response to GPs was completely abrogated (Debierre-Grockiego, Campos, et al. 2007). Phospholipases expressed at the surface of macrophages might cleave GPs of T. gondii, and liberated lipid moieties could activate TLR4/7 macrophages through TLR2. This indicates that both TLR2 and TLR4 are involved in the signaling pathway leading to the production of TNF-α by macrophages exposed to T. gondii GPs.

Apoptosis is the process of programmed cell death that may occur in multicellular organisms and is caused by a series of biochemical events that lead to morphological changes (blebbing, changes to the cell membrane with loss of membrane asymmetry, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation etc.). T. gondii renders infected cells resistant to programmed cell death triggered by multiple apoptotic stimuli (Lüder and Gross 2005). On the other hand, increased apoptosis of lymphocytes and granulocytes after in vivo infection with T. gondii may suppress the immune response against the parasite (Wei et al. 2002). T. gondii GPs fail to block apoptosis that was triggered in human-derived cells via extrinsic or intrinsic apoptotic pathways (Debierre-Grockiego, Hippe, et al. 2007). Furthermore, characteristics of apoptosis, e.g. caspase-3/7 activity, phosphatidylinerine exposition at the cell surface or DNA strand breaks, were not observed in the presence of T. gondii GPs (Debierre-Grockiego, Hippe, et al. 2007). These results indicate that T. gondii GPs are not involved in survival or in apoptosis of host cells. In contrast, the GPI extracted from Plasmodium was shown to induce apoptosis in spleen and liver of mice in vivo (Wichmann et al. 2007). The plasmoidal GPI induced direct apoptosis in rat cardiomyocytes by regulating the expression of pro- and antiapoptotic genes (Wennicke et al. 2008). Furthermore, this report indicates that apoptosis was observed in the heart biopsy of a patient infected with P. falciparum who succumbed from cardiac impairment. Thus, the GPI of P. falciparum might play a role in myocardial impairment observed in patients suffering of severe malaria.

A new antidisease concept using the host response to GPs could protect infected hosts from the fatal development of parasitic diseases. On the basis of the sequence of P. falciparum GPI glycan, the nontoxic analog ethanolamine phosphate-6 (Man1-2) Man1-2 Man1-6 Man1-4-glucosamine-α-1-6 myo-inositol-1,2-cyclic phosphate was chemically synthesized, conjugated to carriers and used to immunize mice (Schofield et al. 2002). In this study, it was shown that parasites continue to proliferate; however, toxic activity of GPs was abrogated. Indeed, recipients were substantially protected against malarial acidosis, pulmonary edema, cerebral syndrome and fatality, confirming the hypothesis that GPI is a highly conserved endotoxin of malarial parasite origin. Anti-GPI antibodies also neutralized proinflammatory activity by P. falciparum in vitro. Thus, a nontoxic GPI oligosaccharide coupled to carrier protein is immunogenic and provides significant protection against malarial pathogenesis and fatalities in a preclinical rodent model. Therefore, GPI could serve as the basis for an antidisease vaccine alleviating the most severe symptoms of malaria. In addition, animals treated with intact GPI (encompassing both the lipid and the carbohydrate moiety) of the African trypanosomes (T. brucei) before infection with this parasite were significantly protected against host clinical manifestations of T. brucei-induced pathology like anemia, acidosis, loss of weight and of locomotor activity, without influencing initial parasite development (Stijlemans et al. 2007). In addition, GPI-based treatment resulted in reduced circulating serum levels of the inflammatory cytokines TNF-α and IL-6, increased circulating IL-10 and abrogation of infection-induced lipopolysaccharide hypersensitivity. Thus, this kind of vaccine, which prevents the most severe consequences of the disease (without inhibiting the multiplication of the parasite), is a new strategical concept. It might be the method of choice also for the treatment of toxoplasmosis, which is difficult to control with classical vaccines because of immune evasion mechanisms developed by the parasite (Lüder et al. 1998; Lüder and Gross 2005).

Concluding remarks

The apicomplexan parasites P. falciparum and T. gondii activate the innate immune system leading to an inflammatory response due to the production of cytokines, chemokines and
NO. The GPIs free or linked to surface antigens of these parasites have been shown to be involved in the pathogenicity of protozoan parasites by inducing the production of inflammatory cytokines by macrophages and by eliciting clinical symptoms such as hypoglycemia, acidosis or anemia. Although the GPI biosynthetic machinery is different enough between mammalian and protozoan to represent a target for antiparasite chemotherapy, GPIs may, in addition, be targeted in an antiparasite vaccine to reduce the severity of malaria and toxoplasmosis diseases.

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Conflict of interest statement
None declared.

Abbreviations
DCs, dendritic cells; Dol-P-Man, dolichol-phosphate-mannose; GPI, glycosylphosphatidylinositol; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; NK, natural killer; NO, nitric oxide; PBMC, peripheral blood mononuclear cells; TLRs, Toll-like receptors; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1.

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