Expanding the antimalarial toolkit: Targeting host–parasite interactions

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Recent successes in malaria control are threatened by drug–resistant *Plasmodium* parasites and insecticide–resistant *Anopheles* mosquitoes, and first generation vaccines offer only partial protection. New research approaches have highlighted host as well as parasite molecules or pathways that could be targeted for interventions. In this study, we discuss host–parasite interactions at the different stages of the *Plasmodium* life cycle within the mammalian host and the potential for therapeutics that prevent parasite migration, invasion, intracellular growth, or egress from host cells, as well as parasite–induced pathology.

Malaria control has been a success story in recent years: mortality has roughly halved with expanded access to existing tools like insecticide-treated bed nets and artemisinin combination therapies, but the landscape is concerning. Malaria still caused an estimated 584,000 deaths in 2013 (http://www.who.int/mediacentre/factsheets/fs094/en/). Intensive efforts have reduced the malaria burden but have not eliminated infection in some low transmission areas (Kleinschmidt et al., 2015); in some high-transmission areas, malaria burden increased despite the application of existing tools (Kakuru et al., 2013). Artemisinin-resistant parasites have emerged across Southeast Asia and are poised to enter India (Tun et al., 2015) while insecticide-resistant mosquitoes threaten vector control measures in many areas (Knox et al., 2014). The first malaria vaccine is being considered for deployment by the World Health Organization; however, thus far it only confers partial protection against clinical malaria and no protection against severe malaria in infants (RTS,S Clinical Trials Partnership, 2015). New therapeutic and prophylactic tools are urgently needed.

Past approaches to developing interventions have been largely empirical and used traditional platforms such as small molecule drug screens and vaccines. Many vaccine targets have proved unsuccessful, for reasons that include polymorphisms (Thera et al., 2011), poor immunogenicity, and inadequate understanding of protein function and its role in the parasite life cycle. Furthermore, protective immune mechanisms are complex and poorly understood. Similarly, although many drug candidates have been screened, few have advanced to clinical trials, and frontline therapy for malaria now relies on artemisinins. Small molecule screens have identified

many exciting targets, such as DDD107498 that targets translation elongation factor 2 at multiple parasite stages (Baragaña et al., 2015) and imidazopyrazines targeting phosphatidylinositol-4-OH kinase (McNamara et al., 2013), but the development path has a high failure rate. Efforts at adjunctive therapies have been unsuccessful to date, and in some cases harmful (John et al., 2010). Against this backdrop, we need not only new interventions but also new approaches to identify targets for intervention.

Two recent papers published in *The Journal of Experimental Medicine* (Cha et al., 2015; Zenonos et al., 2015) highlight the possibility of targeting host factors for antimalaria therapy. This has been highly successful in other infections, such as HIV (Lieberman–Blum et al., 2008; Bruno and Jacobson, 2010; Jacobson et al., 2010), but has not so far been investigated for *Plasmodium*. In parallel, new high throughput approaches are rapidly expanding our understanding of host–parasite interactions, and genome-wide association studies are identifying host factors that could influence malaria pathology (Malaria Genomic Epidemiology Network et al., 2015). This review focuses on the potential for therapeutics that exploit host–parasite interactions as a strategy to develop new antimalarials, highlighting recent discoveries that illustrate this approach.
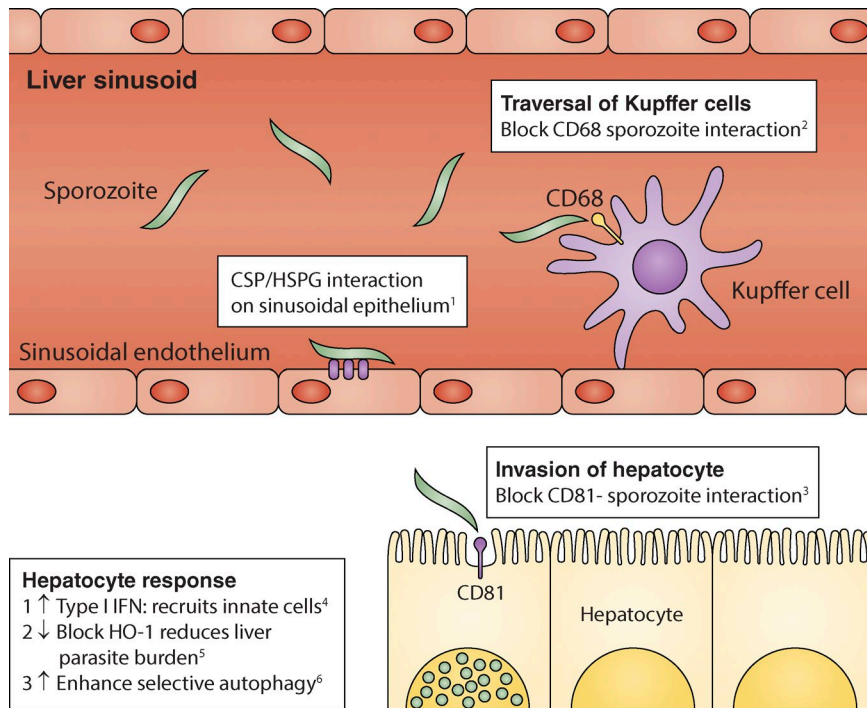
Exo-erythrocytic stages of *Plasmodium*

The exo-erythrocytic stages of *Plasmodium* comprise the sporozoites injected into the mammalian host by the mosquito and the developing forms within the hepatocyte (Fig. 1). These stages of the life cycle in the mammalian host are clinically silent but offer great potential for malaria prevention. *Plasmodium* sporozoites are deposited in the skin when the female *Anopheles* mosquito takes a blood meal.

Figure 1. **Potential points of intervention in the preerythrocytic stages of the *Plasmodium* life cycle.** (1) Frevert et al., 1996; Coppi et al., 2007; (2) Mota et al., 2002; Cha et al., 2015; (3) Yalaoui et al., 2008; Foquet et al., 2015; (4) Liehl et al., 2014; (5) Epiphanio et al., 2008; Sinnis and Ernst, 2008; (6) Prado et al., 2015.

Within minutes, they leave the skin, circulate in the blood, and enter hepatocytes. To exit the blood, sporozoites actively penetrate and traverse Kupffer cells (Pradel et al., 2002; Frevert et al., 2005) and, to a lesser extent, endothelial cells (Tavares et al., 2013). Sporozoites may then traverse several hepatocytes before productively invading a terminal hepatocyte and replicating (Mota et al., 2001). This replicative stage in the hepatocyte leads to a dramatic amplification of parasite numbers, with 10,000 merozoites or more formed from one infected hepatocyte. Transfer from skin to blood, from blood to liver, and subsequent infection of hepatocytes represent the first "bottlenecks" in the life cycle. Interventions that target the underlying host–parasite interactions could be deployed to prevent infection or interrupt transmission (Fig. 1).

One of the first prehepatocyte interactions with the host is between circumsporozoite protein (CSP), the immunodominant *Plasmodium* protein that covers the entire surface of the sporozoite, and heparan sulfate proteoglycans (HSPGs) on sinusoidal endothelium (Frevert et al., 1996; Coppi et al., 2007). Although CSP/liver HSPGs do not appear to be essential for sporozoite invasion (Frevert et al., 1996), they are important for attachment to liver sinusoids and liver arrest. CSP has long been targeted for vaccine development, in part because high titers of antibodies to its peptide repeats can inhibit the invasion of liver cells. Nevertheless, the recently tested RTS,S vaccine containing these repeats has conferred only modest protection against infection, possibly because antibody titers dropped rapidly after vaccination. Targeting the host molecule, HSPG, is a more difficult option, as the level of sulfation of HSPGs seems to determine whether there is productive invasion of hepatocytes or

not (Coppi et al., 2007), and the vital roles that HSPGs play in the liver would preclude their use as targets for intervention.

Traversal through Kupffer cells is thought to activate the sporozoite for invasion (Mota et al., 2002). Using a complex phage display library screen comprising $>10^9$ peptides, Cha et al. (2015) recently identified the host molecule, CD68, as an important surface receptor on rat Kupffer cells for sporozoite entry. CD68 is a member of the scavenger receptor R family, owing to its lysosome-associated membrane protein–like domain and predominant endosomal distribution. It is a highly glycosylated glycoprotein on myeloid cells that binds to low-density lipoproteins. Its property of rapid internalization and recycling could aid in moving sporozoites through the cell. Before targeting host CD68 as a potential therapeutic to prevent traversal through Kupffer cells, we need a better understanding of its role in macrophages. Although CD68 KO mice have no discernible phenotype except enhanced antigen processing for CD4 T cells (Song et al., 2011), the longer-term effects of blocking a low-density lipoprotein receptor are unknown. Importantly, the blocking or loss of CD68 does not completely abolish sporozoite infection in vivo, which is not surprising if some sporozoites can traverse via endothelial cells (Tavares et al., 2013). Although Cha et al. (2015) identified a peptide from the phage display library that bound CD68 and inhibited sporozoite entry, its parasite ligand remains unknown. Techniques such as avidity-based extracellular interaction screening (AVEXIS; Frei et al., 2012; Bartholdson et al., 2013) should make it possible to define the parasite molecules that bind to CD68, which may be more feasible targets for prophylactic intervention.

Another such target is CD81, a member of the tetraspanin 4 family, which can be a coreceptor with the scavenger receptor type B class I (SR-BI) on the surface of host hepatocytes. Both play an important role during sporozoite invasion (Rodrigues et al., 2008; Yalaoui et al., 2008). In a humanized mouse model, monoclonal antibodies to CD81, but not to SR-BI, completely blocked invasion by *Plasmodium falciparum* sporozoites (Foquet et al., 2015), confirming the potential of CD81 as a therapeutic target.

Within hepatocytes, there are likely to be unique host factors and interactions that permit or control parasite development. Different groups using transcriptional profiling have identified changes in several hundred host genes in hepatocytes or HEP-2 cells after infection with *Plasmodium berghei* or *P. falciparum*, with a large proportion from those that regulate immune processes, cell adhesion and communications, metabolism pathways, cell cycle regulation, and signal transduction (Albuquerque et al., 2009; Chattopadhyay et al., 2011). Of particular interest are those host responses in the hepatocyte activated as a defense against the invading parasite. Liehl et al. (2014) described *Plasmodium* RNA as a pathogen-associated molecular pattern capable of activating a type I IFN pathway in the hepatocyte via a cytosolic receptor, *mda5*, which is thought to lead to the recruitment of leukocytes to the infected cell. This innate pathway can reduce the number of parasites that will subsequently emerge to give rise to the erythrocytic stages.

Heme oxygenase 1 (HO-1), a host protein that is up-regulated in *Plasmodium*-infected hepatocytes (Albuquerque et al., 2009), has been shown to promote liver infection, probably by down-regulating chemokines MIP1-α and MCP-1, as well the cytokines TNF and IL-12 in the innate immune response. This may protect the parasite in the hepatocyte from immune-mediated attack (Epiphanio et al., 2008; Sinnis and Ernst, 2008). Blocking HO-1 reduces liver stage parasite burden, but doing so may lead to unwanted effects at the erythrocytic stage of infection. Because of its antiinflammatory properties, HO-1 protects against experimental cerebral malaria (CM) in the mouse *P. berghei* ANKA model (Pamplona et al., 2007). Thus, immunotherapeutics that block HO-1 to attack liver-stage parasites seem an unlikely strategy, unless they might be shown to remove hypnozoites of *Plasmodium vivax* when there is no accompanying blood-stage infection.

Parasites in the hepatocyte exist within the parasitophorous vacuole (PV). The PV is formed by the invagination of host cell membrane during sporozoite invasion, and it is extensively modified by the parasite through the insertion of parasite proteins and the loss of some host proteins. Although several PV membrane resident parasite proteins are known to be essential (e.g., UIS3 and UIS4; Mueller et al., 2005a,b) for parasite development in the liver, we still do not know their function. The fact that they are on this host–parasite interface suggests that they might be involved in some host interaction that is critical for infection. Understanding these interactions might lead to the development of novel strategies to combat the parasite.

The PV can be targeted by selective autophagy host proteins such as light chain 3, ubiquitin, and SQSTM1/p62 and lysosomes (Prado et al., 2015). As the parasite develops, these are lost from the PV membrane, suggesting protection of the parasite from selective autophagy. It is interesting to speculate whether augmentation of selective autophagy, if possible, would enhance the killing of the parasite and whether this could be of use for treatment of the hypnozoite forms of *P. vivax*.

The exciting prospect of obtaining the full life cycles of *P. falciparum* and *Plasmodium ovale* (Soulard et al., 2015) and complete liver-stage development of *P. vivax* (Mikolajczak et al., 2015) in the laboratory, as well as new in vitro systems with primary human hepatocytes to follow *P. falciparum* and *P. vivax* liver-stage infection (March et al., 2013), will really advance our understanding of the exo-erythrocytic stages of the human malarias, aspects of which can presumably be exploited for antimalarial interventions. The complete development of *P. vivax* liver stages, including hypnozoites and persistence in vivo in human liver–chimeric FRG KO huHep mice, will allow us to identify host and parasite molecules involved in the latency and activation of hypnozoites. This could lead to the identification of possible alternative interventions that target the hypnozoite stage of *P. vivax* and *P. ovale* to prevent relapses.

Erythrocytic stages of *Plasmodium*

Disease and death occur during *Plasmodium* erythrocytic stages, making these a priority for new interventions (Fig. 2). Upon completing development within hepatocytes, merozoite progeny are released into hepatic sinusoids as clusters within vesicles, called merosomes (Sturm et al., 2006). Merosome membranes are derived from the host cell, although host proteins are lost (Graewe et al., 2011), and phosphatidylserine display is inhibited (Sturm et al., 2006), perhaps to mask the parasite from immune clearance. Thereafter, the parasite undergoes repeated rounds of erythrocyte invasion, intracellular multiplication, egress, and reinvasion. During this cycle, parasite biomass can reach 10e12–13 organisms per host (Dondorp et al., 2005).

Invasion of erythrocytes has long been an attractive target for vaccines but is increasingly viewed as a potential druggable target. Invasion involves a sequence of events, including initial attachment, reorientation of the apical end of the parasite toward the host cell, formation of a tight junction, and then invasion with concomitant formation of the PV. This is orchestrated through a series of receptor–ligand interactions whose timing is determined by protein release and processing events in conjunction with signaling cascades (Gaur and Chitnis, 2011). For example, the *Plasmodium* homologue of calcineurin, a calcium-regulated protein phosphatase, has recently been shown to play a critical role in merozoite attachment and invasion by regulating specific merozoite–host cell binding interactions (Paul et al., 2015; Philip and Waters,
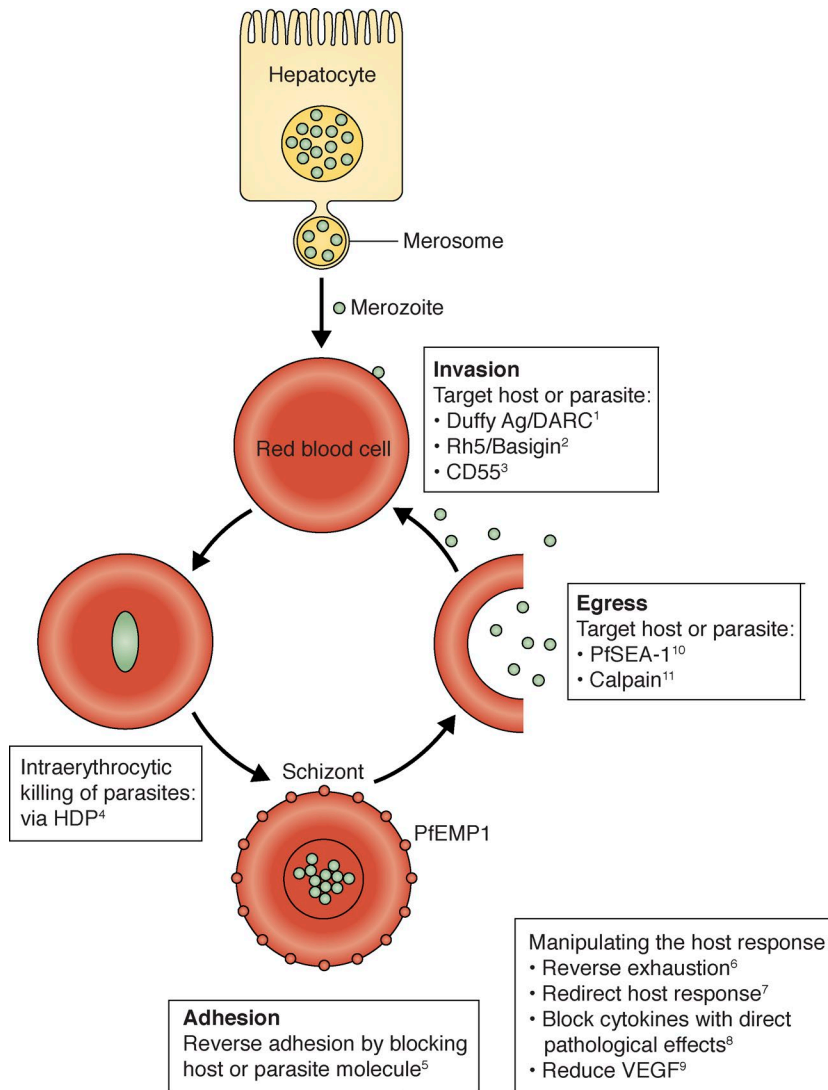
Figure 2. Potential points of intervention in the erythrocytic stages of the *Plasmodium* life cycle. (1) Horuk et al., 1993; Chitnis et al., 1996; (2) Zenonos et al., 2015; (3) Egan et al., 2015; (4) McMorran et al., 2009; Love et al., 2012; (5) Dormeyer et al., 2006; Soerli et al., 2009; (6) Butler et al., 2012; Horne-Debets et al., 2013; (7) Berretta et al., 2011; Abel et al., 2012; (8) Grau et al., 1987, 1989; Engwerda et al., 2002; (9) Epiphanio et al., 2010; Hempel et al., 2014; (10) Raj et al., 2014; (11) Chandramohanadas et al., 2009; Millholland et al., 2011.

2015), as well as roles in other stages of parasite development (Philip and Waters, 2015).

The earliest described host receptor for invasion is the Duffy antigen receptor for chemokines (DARC), a member of the G protein–coupled receptor family, which is required for *P. vivax* to infect erythrocytes (Adams et al., 1990). This requirement explains the paucity of *P. vivax* in sub-Saharan Africa, where the near universal penetration of a mutation in its gene promoter ablates the expression of DARC on the erythrocytes (but not other cells) of ethnic Africans. Individuals lacking DARC on erythrocytes suffer no adverse effects, and although DARC has seven transmembrane domains, as do other G protein–coupled receptor family members, it does not couple G proteins, explaining the absence of downstream signaling (Horuk et al., 1993). These features make the DARC–parasite interaction an attractive target for interventions against *P. vivax* infections. Several studies have shown that the interaction between Duffy binding protein and DARC can be competitively inhibited using chemokines, Duffy binding protein, or the monoclonal antibody to DARC (Horuk et al., 1993; Chitnis et al., 1996), and a modified chemokine inhibits both binding and invasion by the parasite (Lu et al., 1995).

Invasion of *P. falciparum* was thought to be a less tractable target until recently because of multiple redundant pathways and extensive sequence variation in parasite proteins involved in this process. However, nonredundant pathways for *P. falciparum* invasion have been identified. Among the erythrocyte-binding antigens and reticulocyte-binding protein homologue (Rh) proteins, only Rh5 cannot be deleted in any *P. falciparum* strain, suggesting that it is essential for invasion (Baum et al., 2009). Using the AVEXIS assay to screen an erythrocyte ectodomain library, PfRh5 prey interacted with a single bait, basigin. Merozoite invasion is inhibited by soluble basigin, basigin knockdown, or antibasigin antibodies for all parasite strains tested (Crosnier et al., 2013). In a recent study

(Zenonos et al., 2015), a recombinant chimeric antibody against basigin was shown to inhibit the interaction of basigin with PfRh5, thus blocking merozoite invasion of multiple *P. falciparum* strains, which resulted in rapid clearance of parasitemia in an in vivo model. As monoclonal antibodies against basigin have already undergone clinical trials for cancer and graft versus host disease and have been well tolerated by patients (Chen et al., 2006; Xu et al., 2007), targeting this host molecule in *P. falciparum* infections could be an effective and feasible therapeutic approach.

Meanwhile, a forward genetic screen has identified CD55 (decay-accelerating factor) as another erythrocyte surface protein that is essential for merozoite invasion (Egan et al., 2015). Because erythrocytes are not amenable to genetic manipulation, hematopoietic stem cells were exploited to knock down the expression of 42 blood group antigens using RNA interference with shRNA. In a comparison of shRNA abundance, CD55 hairpins were ranked as the most underrepresented in invaded versus control cells. CD55 knockdown reduced parasite invasion significantly, and CD55-null cells from genetically deficient individuals resisted invasion by all *P. falciparum* isolates tested, apparently because merozoites failed to attach properly (Egan et al., 2015). Interestingly, CD55-deficient individuals are rare but hematologically normal, making this molecule an attractive target for intervention. However, because the null phenotype has not been selected nor spread in malaria-exposed populations, the possibilities remain that the antimalarial benefits of CD55 deficiency do not occur in vivo, or that its deleterious effects might be unmasked during malaria episodes or in relation to other conditions that are common in these populations, like polyparasitism and gastrointestinal infections.

Once inside the erythrocyte, the parasite extensively remodels its host cell. In particular, *P. falciparum* exports proteins to the erythrocyte surface that serve as ligands for adhesion to endothelial receptors and thereby mediate parasite sequestration in deep vascular beds. Sequestration is implicated in *P. falciparum* virulence; for example, coma is associated with the burden of parasites sequestered in cerebral vessels (Dorovini-Zis et al., 2011). Treatments that reverse or prevent parasite sequestration might alleviate local and systemic inflammation (Dondorp, 2008) and restore microvascular blood flow, thereby alleviating severe symptoms and promoting parasite clearance in the spleen. Antiadhesion agents could target the cell surface to block the ligand–receptor interaction and hence could be immediately active upon reaching adequate plasma levels.

Parasite adhesion has been ascribed to the *P. falciparum* EMP1 (PfEMP1) family (~60 members) of clonally variant, surface-expressed erythrocyte multidomain transmembrane proteins (Smith et al., 2013). PfEMP1 displays enormous antigenic diversity, making the task to design vaccines or immunotherapeutics against these targets daunting. However, broad immunity against severe malaria develops quickly in early childhood (Gonçalves et al., 2014), suggesting that the

diversity of protective epitopes might be restricted. Similarly, African women develop antibodies against placental parasites and become clinically immune over only a few pregnancies (Fried and Duffy, 1996), and placental parasites uniformly bind chondroitin sulphate A and express a single member of the PfEMP1 family called VAR2CSA (Salanti et al., 2003). A human monoclonal antibody derived from African multigravidae inhibits the binding of several malaria parasite strains to chondroitin sulphate A (Soerli et al., 2009), indicating that antiadhesion molecules directed against parasite surface antigens can be broadly effective.

Conversely, therapies can be designed to mimic the binding motifs of host receptors. For example, a small molecule that mimics an ICAM-1 loop was shown to inhibit the binding of two parasite variants to the ICAM-1 receptor (Dormeyer et al., 2006). Thus, a single adhesion-inhibiting molecule might exhibit cross-strain and multi–PfEMP1 activity for a particular host receptor. A major obstacle for the rational design of antiadhesion therapy has been the identification of the PfEMP1 variants and endothelial receptors responsible for severe malaria. Recent evidence suggests that individual domains or combinations of domains (called domain cassettes), rather than full-length PfEMP1, may be the key virulence factors. Domain cassettes DC8 and DC13 in particular have been associated with severe malaria (Avril et al., 2012; Claessens et al., 2012; Lavstsen et al., 2012). DC8 and DC13 bind to endothelial protein C receptor (EPCR), and parasites collected from children with severe malaria commonly bind to EPCR (Turner et al., 2013). Interestingly, DC8 and DC13 competitively inhibit the binding of activated protein C to EPCR, and recombinant activated protein C has been given to a limited number of individuals suffering from severe malaria with anecdotal success, suggesting a potential approach to adjunctive therapy.

One domain of PfEMP1, DBL1-α, plays a key part in mediating binding to uninfected erythrocytes via glycosaminoglycan heparan sulfate (Vogt et al., 2003) in a process called rosetting. Rosetting has long been thought to contribute to the obstruction of blood flow in the microcapillaries and has also been associated with severe forms of malaria (Kaul et al., 1991). Recently, low anticoagulant heparins such as DFX232 and sevuparin have been demonstrated to block rosetting and have therefore been proposed as possible adjunct therapies for severe malaria (Leitgeb et al., 2011).

Apart from their surface antigens, intraerythrocytic parasites themselves were thought to be inaccessible to host macromolecules such as antibody. However, recent evidence suggests that certain biologics may access and impair intracellular parasites. Platelets can bind infected erythrocytes and kill intracellular malaria parasites (McMorran et al., 2009), leading to speculation that host defense peptides (HDPs) might contribute to parasite control. HDPs have an amphipathic topology and act by binding and aggregating on membranes, leading to permeabilization and disruption. In a screen of different HDPs for antiparasitic activity, human platelet factor

4 (hPF4) was shown to rapidly accumulate in infected, but not uninfected, erythrocytes and to kill malaria parasites by selectively lysing the parasite digestive vacuole (Love et al., 2012). The mechanism by which large molecules like hPF4 entered infected erythrocytes was not clear, but uptake is blocked by preincubation with protamine sulfate, suggesting a role for electrostatic interactions. Selective uptake by erythrocytes infected with viable parasites implies a parasite-derived mechanism or, alternatively, an activated endocytosis-like mechanism, for large molecules such as PF4. A screen of small nonpeptidic mimics of HDPs identified compounds that rapidly lyse digestive vacuoles and kill the parasite without affecting the erythrocyte plasma membrane (Love et al., 2012).

The process of parasite egress from erythrocytes has received increasing attention and may be a potential target for immunotherapeutics. In a differential screen of the *P. falciparum* blood-stage proteome, plasma from Tanzanian children who controlled parasite density during infection preferentially reacted to a large antigen expressed in schizont-infected cells, PfSEA-1 (Raj et al., 2014). Disruption of PfSEA-1 impaired parasite replication, and antibodies to PfSEA-1 decreased parasite replication by arresting schizont rupture. As with PF4, the mechanism by which anti–PfSEA-1 antibodies access intracellular schizonts is not clear, although they were observed inside the erythrocyte in immunolocalization studies with intact nonpermeabilized schizont-infected cells, consistent with the increased permeability of these cells observed in earlier studies (Ahlborg et al., 1996; Goodyer et al., 1997; Bergmann-Leitner et al., 2009).

Several other host proteins have been implicated in parasite egress from the red cell. Host-derived protease calpain is required and is thought to proteolyze the actin cytoskeleton (Chandramohanadas et al., 2009; Millholland et al., 2011). A G-α(q)–coupled host signaling cascade is necessary, promoting protein kinase C (PKC)–mediated loss of the host cytoskeletal protein adducin with cytoskeletal compromise. This leads to a Ca^{2+} influx mediated by the mechanosensitive cation channel TRPC6 and subsequent activation of host calpain (Millholland et al., 2013). Notably, PKC inhibitors have been shown to have antiparasitic activity in mouse models of malaria: mammalian PKC inhibitors demonstrated activity in these models, and an orally bioavailable PKC inhibitor prolonged survival in an experimental CM model.

Manipulating inflammation and immune responses

Host immune responses contribute to the pathogenesis of malaria. Therefore, targeting the pathways involved could lead to therapeutic interventions in severe disease, deviate the immune response away from inflammation, and/or potentiate protective immunity. Immune responses involved in any of these processes are inextricably linked in a highly complex self-regulating immune system, and without fully understanding the dynamics and interactions involved, intervention could be detrimental to the outcome of infection (Frosch and John, 2012). Furthermore, with such diverse dis-

ease manifestations as fever, severe anemia, CM, pregnancy malaria, acute lung injury (ALI), and acute respiratory distress syndrome (ARDS), it is unlikely that a single molecule or cell could be targeted to alleviate all forms of severe malaria. It is very important to determine whether a particular response or biomarker is in the causal pathway. This can be examined in experimental animal models; however, although they can determine causality, they may offer only approximate representations of the different severe malaria syndromes.

Manipulating host responses using antibodies or immune cells has been pioneered in cancer treatment, where immune "checkpoint" therapy has recently become a major component of the treatment repertoire. This approach targets regulatory pathways either to enhance T cell responses or to remove inhibitory pathways that block effective immunity. Most recently, antibodies against surface receptors that inhibit effector T cells such as CTLA-4, PD-1, and Lag3 (Topalian et al., 2012; Powles et al., 2014) have been used successfully (Hodi et al., 2010; Robert et al., 2011; Nguyen and Ohashi, 2015). Blockade of PD-1 can reverse T cell exhaustion and promote T cell effector responses in chronic infections and cancer (Barber et al., 2006; Sakuishi et al., 2010), and blockade of Lag3 in mice results in defective down-regulation of T cell responses (Workman et al., 2004).

Up-regulation of PD-1 and Lag3 on T cells have both been implicated in impeding development of protective immunity in experimental malaria, leading to high parasitemias and chronic *Plasmodium chabaudi* malaria (Butler et al., 2012; Horne-Debets et al., 2013). Blocking or removing these molecules results in better clearance of parasites and elimination of chronic infection. Blockade of CTLA-4, in contrast, has different effects on *Plasmodium yoelii* infections depending on parasite virulence; nonlethal infections in mice are cleared more effectively, whereas treatment increases parasitemia in lethal *P. yoelii* (Lepenies et al., 2007). Would blocking these molecules really be a feasible means of redirecting the host response to eliminate parasites and prevent chronic malaria? One major problem could be the potential for enhancement of potentially pathogenic T cell responses, thereby increasing the risk of severe disease, and the timing of intervention would be hard to define. Furthermore, as a loss of PD-1 results in fewer long-lived plasma cells (Good-Jacobson et al., 2010), blockade of this molecule may impair antibody responses that are thought to be important for long-term control of blood-stage malaria.

Regulatory T (T_{reg}) cells (CD4$^+$Foxp3$^+$CD25$^+$) are key regulators of immune responses. They can suppress tumor-specific responses, and selective antibody depletion of T_{reg} cells is being tested in various tumor settings. Additionally, novel approaches exploiting the different cytokine and metabolic responses of T_{reg} cells are being investigated to try to deviate immune responses away from T_{reg} cells toward dominant effector responses (Nishikawa and Sakaguchi, 2014). Manipulation of T_{reg} cell responses as therapy against malaria is more difficult to envisage. Although T_{reg} cell depletion during vac-

cination augments T effector responses against liver stages of experimental malaria, it does not enhance memory responses (Espinoza Mora et al., 2014), and the role of T_{reg} cells during blood-stage infections is not at all clear. T_{reg} cells in human malaria are sometimes associated with malaria disease or asymptomatic infections and sometimes not (Scholzen et al., 2010; Torres et al., 2014). Similarly, in experimental models, T_{reg} cells can dampen or enhance both clearance of malaria and infection-induced pathogenic responses (Berretta et al., 2011; Abel et al., 2012). With our current state of knowledge, it is not possible to say whether targeting T_{reg} cells would benefit the host, the parasite, both, or neither, or whether such treatment could be effective or even feasible for patients already presenting with severe malaria.

Cytokines or chemokines present in plasma, or the types of effector cells responding to *Plasmodium* antigens, have long been known to associate significantly with severe symptoms of, or protection from, the consequences of blood-stage malaria (Freitas do Rosario and Langhorne, 2012; Crompton et al., 2014; Ioannidis et al., 2014). Although potentially valuable as prognostic biomarkers of the severity or otherwise of malaria, association does not necessarily indicate causation. However, some cytokines such as TNF (Grau et al., 1987), lymphotoxin (Engwerda et al., 2002), and IFN-γ (Grau et al., 1989) have long been known to contribute to the development of severe malaria in experimental models, and blocking these cytokines and/or the pathways leading to their induction in human CM have been proposed as possible therapies. Indeed, if given at the time of infection or before the onset of symptoms in the mouse *P. berghei* ANKA model, experimental CM can be prevented, but not if given once symptoms have started. For human CM, however, intervention can only start when the patient presents with symptoms, and if not already present, CM can develop within a very short time, at which point cytokine blockade may well be too late.

The problems with manipulating cytokines or chemokines for the treatment of severe disease are their multiple potentiating and suppressive effects on the host response. IL-10 is a good example. Despite its importance in controlling immune responses in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, Crohns's disease, and type 1 diabetes, administration of IL-10 has never been very effective and in some cases is detrimental, even though animal model studies show that the removal of IL-10 can exacerbate disease caused by infectious agents (Kugelberg, 2014; Saxena et al., 2015). IL-10, particularly from CD4 T cells, is important for down-regulating host responses and pathology in experimental malaria (Freitas do Rosário et al., 2012; Villegas-Mendez et al., 2013), and its expression in these cells is increased in humans with, or after, acute malaria (Jagannathan et al., 2014). However, IL-10 is produced by many different cells during rodent malaria infection (Freitas do Rosário et al., 2012; Liu et al., 2013), is regulated differently in the various cells (Gabryšová et al., 2014), and is involved in multiple pathways to suppress, or even stimulate, immune responses. It would be necessary to understand exactly what is required to promote the right cells producing IL-10 at the appropriate time and place. With that knowledge, potentiation of endogenous IL-10 in the right location and from the appropriate sources could be explored.

Vascular endothelial growth factor (VEGF) has been proposed as a possible adjunct treatment for some severe malaria syndromes. VEGF is necessary for survival of endothelium and also stimulates the permeability of the blood–brain barrier to allow angiogenesis and oxygenation of the tissues. High levels of circulating VEGF are associated with human and mouse ALI/ARDS and are also elevated in the *P. berghei* model of experimental CM (Epiphanio et al., 2010; Hempel et al., 2014) as well as during chronic inflammatory *P. falciparum* placental malaria (Muehlenbachs et al., 2006). Both inflammation and hypoxia can drive the induction of VEGF, and in mouse models, reduction of VEGF levels by treatment with antiinflammatory agents such as carbon monoxide or erythropoietin reduce ALI/ARDS (Epiphanio et al., 2010). However, to translate this to treating human severe disease, it has to be effective when given after the onset of severe disease. If raised VEGF levels were observed in blood film–positive children, an adjunct therapy such as erythropoietin could be considered in combination with antimalarials.

Concluding remarks

New screening platforms, together with many hypothesis-driven studies, are expanding our knowledge of host–parasite interactions that could be targeted for therapeutic intervention in malaria. More emphasis should be placed on agonists, antagonists, or mimics that exploit these interactions for clinical benefits. Until now, a major limitation in validating such molecules has been the lack of appropriate animal models for *P. falciparum* and *P. vivax*. The recent advances in the development of humanized mice (Mikolajczak et al., 2015; Soulard et al., 2015) for malaria research has the potential to revolutionize the elucidation and efficacy testing of therapeutics against specific molecular pathways in malaria. Together with traditional approaches of vaccine and drug development, an additional focus on parasite and host molecules that are key in the life cycle can replenish our diminishing pharmacopeia.

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