

## Targeting Host Factors to Circumvent Anti-Malarial Drug Resistance

Miguel Prudêncio\* and Maria M. Mota\*

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal

**Abstract:** The most common treatments for infectious diseases target the invading pathogen. The efficacy of such an approach may, however, be countered by the possibility of the development of resistance to a pharmacophore, through mutation(s) in pathogen molecules required for activity. Given the fact that pathogens exploit host factors in order to grow in an otherwise hostile environment, one possible way to circumvent the emergence of resistance is to develop drugs that target non-essential host factors hijacked by the pathogen, rather than the pathogen's own molecules. Such solutions are already being developed for various viral and bacterial pathogens, but much less has been achieved with infections caused by protozoan parasites, as is the case of *Plasmodium*. Here, we highlight recent progress in host target-based anti-viral and anti-bacterial approaches and discuss possible host targets that may be used for anti-malarial interventions. Host molecules that play a role during either the liver or the blood stage of *Plasmodium* infection are outlined and their potential merits as anti-malarial targets are discussed.

**Keywords:** Malaria, *Plasmodium*, host factors, host targets, hepatitis C virus, liver-stage, blood-stage, drug resistance.

### HOST TARGETS AGAINST INFECTION

Drug resistance arguably constitutes the biggest problem faced in the field of infectious diseases today and is a major obstacle to the development of effective strategies to combat infection (see also Noedl H. The need for new antimalarial drugs less prone to resistance. *Curr Pharm Des* 2013; (this issue)). Pathogens, particularly those with an intracellular habitat, exploit and subvert various host factors for survival and growth in an otherwise hostile environment. As such, one possible way to circumvent the emergence of a pathogen's resistance is to develop drugs that target non-essential host factors hijacked by the pathogen rather than the pathogen's own molecules. Indeed, host proteins are generally well conserved, when compared with the genetic variability of many pathogens. Thus, while mutations within a microbial gene can render useless a drug targeting its encoded protein (see also Garcia-Bustos JF, Gambo FJ. Antimalarial Drug Resistance and Early Drug Discovery. *Curr Pharm Des* 2013; (this issue)), the need for the parasite to significantly re-direct its entire infection strategy to compensate for a missing host factor is a much more difficult obstacle to overcome through natural selective variation. As such, novel pharmaceutical agents targeting host factors, either on their own or as part of combination therapies, may provide powerful new weapons to battle the resistance problem as they lie outside the genetic control of the parasite (see also Sullivan DJ. Drug Targets Outside The Genetic Control Of The Parasite. *Curr Pharm Des* 2013; (this issue)). It should be acknowledged that the inhibition of a host factor is an inherently less specific approach than targeting a protein of the pathogenic microorganism. The disruption of important roles of a cellular protein could therefore lead to increased side effects. On the other hand, the host factor approach also offers the potential for accelerated drug discovery and development through synergies with ongoing outputs from studies of different diseases. Host factors needed for a given infection may likely have been the focus of therapeutic development work in other fields, thus opening the possibility of finding anti-infective activities as second medical uses for existing drugs.

### TARGETING HOST FACTORS IN INFECTIOUS DISEASES: PRESENT SUCCESSES AND FUTURE PROMISES

For a long time pathogens were viewed as mere illness-causing intruders. Nowadays, however, researchers study pathogens in the

context that they are organisms optimally adapted to life in the host. As such, many consider that studying the biochemical interactions of hosts and infectious agents may be the key to finding novel strategies to fight the diseases that arise from them. This work involves answering a number of questions such as: how do pathogens obtain nourishment and reproduce in their hosts? How do hosts react to unwanted guests? And how do disease agents manage to circumvent host defenses? Answering these questions inevitably leads to the finding of the critical host molecules that contribute, either positively or negatively, to the establishment of infection, allowing further studies on how to target and manipulate those host factors.

Additionally, researchers have now on hand a wide array of high throughput methods that allow them to search for critical host factors in unbiased ways. Such strategies for identifying potential host targets may include not only analyses of transcriptomic, proteomic or microRNA (miRNA) expression profiles but also screening of small interfering RNA (siRNA) or short hairpin RNA (shRNA) libraries to identify host genes required for pathogen entry or establishment inside host cells. Furthermore, existing pharmacologically active compounds (for example, those with activity against other pathogens or other diseases) can be screened against the infectious agent of interest.

One of the most interesting and successful examples of screening pre-existing drugs against a human pathogen was the finding that cyclophilins, which are targeted by the immunosuppressant cyclosporin A, are required for hepatitis C virus (HCV) replication [1, 2]. Subsequently, cyclosporin A derivatives without immunosuppressive properties were developed as potential antivirals. It has been shown that nonimmunosuppressive analogues of cyclosporin A, such as the compound alisporivir (also known as Debio 025), can bind host cyclophilin A and inhibit HCV replication [2-4]. Alisporivir was found to markedly reduce HCV viremia in human clinical trials when used alone or in combination with pegylated interferon  $\alpha$  [5] and is currently in a phase III trial for the treatment of treatment-naïve HCV genotype 1 patients. Another newly developed drug, celgosivir, an inhibitor of the host enzyme endoplasmic reticulum glucosylase, is currently being evaluated in human trials for efficacy against infection with HCV. Inhibition of this host enzyme results in incorrect folding of HCV envelope proteins and thus in the inhibition of viral assembly and release [6].

In mammals, miRNAs are predicted to control the activity of ~30% of all protein-coding genes, and seem to participate in the regulation of almost every cellular process, including immunological processes such as innate and adaptive immunity [7]. Therefore,

\*Address correspondence to these authors at the Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal; Tel: +351217999509, Fax: +351217999504; E-mail: [mprudencio@fm.ul.pt](mailto:mprudencio@fm.ul.pt), [mmota@fm.ul.pt](mailto:mmota@fm.ul.pt)

it may not be surprising that pathogens also manipulate host miRNAs for their benefit. Indeed, a highly relevant example is the host-encoded antiviral target, miRNA 122 (miR-122) [8]. miR-122 is an abundant liver-specific miRNA which is crucial for efficient HCV RNA replication in cultured Huh7 cells stably expressing HCV replicons [9]. Moreover, a recent study found that, among chronically HCV-infected individuals, intrahepatic miR-122 levels were significantly lower among patients who responded poorly to interferon therapy [10]. Santaris Pharma has developed a locked nucleic acid-modified oligonucleotide (miravirsin or SPC3649) complementary to the 5'-end of miR-122 that resulted in functional inactivation of miRNA-122. Miravirsin was shown to be active in HCV-positive chimpanzees, markedly reducing HCV RNA replication and showing no significant side effects except for a profound decrease in serum cholesterol levels [11]. Moreover, miravirsin-induced antagonism had a potent antiviral effect against HCV genotypes 1-6 *in vitro* [12]. Thus, miravirsin holds promise as a new antiviral therapy with a high barrier to resistance and a tolerable side effect profile. Moreover, functional inactivation of a miRNA to treat an infectious disease represents a truly novel therapeutic paradigm that extends far beyond the HCV or virology fields. A phase II trial of miravirsin in chronically HCV-infected individuals is currently recruiting patients. Most importantly, several other host-targeting agents with very different host targets are presently further upstream in the development pipeline for treatment of HCV. Among those, there are agents that block host molecules required for the different steps in HCV life cycle: entry, replication and assembly/release [13]. As such, there is no doubt that HCV is presently one of the most successful examples on how targeting host molecules may strongly impact an infectious disease.

Nevertheless, HCV is not the only pathogen for which host-targeting therapies are being developed. In HIV infections, the main host co-receptors used by HIV-1 for entry are the chemokine receptors Ccr5 and Cxcr4. The Ccr5 co-receptor is an especially attractive target, because individuals with a natural Ccr5 mutation, which has little apparent impact on immune status or general health, are highly protected against HIV-1 infection [14-17]. Several studies have searched for compounds that block entry of HIV into host cells [18]. One of these compounds, maraviroc, which blocks Ccr5 [19], was identified in a high-throughput screen of a chemical library [20]. Maraviroc has entered Phase II clinical studies [21] and was shown synergistically to augment the anti-HIV effects of current anti-HIV drugs [22].

Quite recently, two teams approached from opposite directions the problem of blocking infection by the filovirus that causes rapid hemorrhagic fever (Ebola) infection—one looked for host factors the virus interacts with while the other started with a small molecule screen and worked backwards to find their targets. Both identified Niemann-Pick disease type C1 (NPC1) as a requisite for cellular entry [23, 24] and both groups have now been reported to be developing small molecules that target the entry protein [25].

Besides viruses, and with the emergence of multidrug resistant (MDR) bacteria and the need to develop new anti-bacterial intervention strategies, targeting host-specific biochemical pathways during bacterial infections has been also investigated. Genome-wide RNA interference of *Drosophila* SL2 cells identified many host factors involved in protection or exploited and subverted by pathogens [26-28]. One of these was the Tim-Tom multiprotein complex, which is involved in the recognition and import of nuclear-encoded proteins to the mitochondria. Depletion of either the Tom40 or the Tom22 components reduced *Chlamydia caviae* infection in mammalian cells [26]. In a different study, forward genetic screens using *Drosophila* fly mutants rather than cell-based screens identified 18 genes important for host resistance to *Listeria monocytogenes* [29] that were not previously identified in cell-based assays [27, 28]. A distinct study has developed kinase inhibitors with antibiotic properties that prevent intracellular growth of unre-

lated pathogens such as *Salmonella enterica* serovar Typhimurium and *Mycobacterium tuberculosis*, and identified their host targets [30]. One of the identified host targets was AKT1, which was shown to be implicated in intracellular survival of those bacteria. An inhibitor of AKT with limited specificity that still showed therapeutic potential as an antibiotic without noticeable side effects in mice was also identified [30]. Since AKT is found activated in many human tumours, several AKT inhibitors are already in human trials as anti-cancer drugs [31]. Thus, this study implies that kinases used as anti-cancer drug targets may yield novel antibiotics that counteract host pathways activated by intracellular pathogens for survival.

Another promising approach is to manipulate key host nutrients essential for bacteria (or possibly any pathogen) growth and replication in order either to use them to deliver a drug to the pathogen or to trick the pathogen into taking up a useless analogue. In this respect, the most used and manipulated host micronutrient is iron. Iron is essential for any living organism and pathogens are no exception. In the context of bacterial infections, it has been definitively established that the competition for iron is critical in the struggle between bacteria and host [32]. While the host has potent defense mechanisms to keep iron away from infectious organisms, invaders have also developed systems to acquire iron in order to survive. Because iron is so important in infection, it has been used in the development of distinct “Trojan horse” strategies to target infections, particularly bacterial infections. In one such strategy, researchers managed to attach highly potent antibiotics to synthetic molecules called siderophores, which act as transport vehicles for iron. In fact, nature has provided examples for siderophore-antibiotics such as albomycins, ferrimycins or salimycins. The occurrence of natural isolates of antibiotics using the siderophore pathway to enter the bacterial cell has prompted synthetic attempts to prepare such “Trojan horses” [33]. Given the siderophore-mediated iron uptake system, this strategy can kill off even bacteria that have developed resistance to multiple drugs because of high concentrations of antibiotic in the bacterium’s cytosol. Interestingly, a recent study has shown that although the antimalarial agent artemisinin itself is not active against tuberculosis, conjugation to a mycobacterial-specific siderophore analogue induces significant and selective antituberculosis activity, including activity against multi- and extensively drug-resistant strains of *M. tuberculosis* [34]. The conjugate also retains potent antimalarial activity. Thus, this “Trojan horse” approach demonstrates that new pathogen-selective therapeutic agents, in which the iron component of the delivery vehicle also participates in triggering the antibiotic activity, can be generated. The result is that one appropriate conjugate has potent and selective activity against two of the most deadly diseases in the world.

On a completely different approach, researchers replaced the active Fe(III) moiety with a metabolically-inactive metal ion such as Sc(III), In(III), or Ga(III) [4, 13, 14] to trick bacteria into taking it up instead of iron [35-39]. Importantly, once inside bacterial cells, these metals cannot function like iron. Recent studies showed that gallium killed microbes, and prevented the formation of biofilms, being effective even against strains of *Pseudomonas aeruginosa* from cystic fibrosis patients that were resistant to multiple antibiotics. Importantly, gallium's action was intensified in low iron conditions, like those that exist in the human body and, in mice, gallium treatment blocked both chronic and acute infections caused by this bacterium [36]. The administration of the metallo-complex desferrioxamine-gallium (DFO-Ga), as well as liposomal gentamicin formulation with gallium metal (Lipo-Ga-GEN), have also proven to be quite efficient in killing *P. aeruginosa* [37, 38].

#### TARGETING HOST FACTORS DURING PLASMODIUM INFECTIONS

*Plasmodium*, the causative agent of malaria, is an apicomplexan protozoan obligate parasite. In the mammalian host, *Plasmodium* is

an intracellular parasite, replicating and developing inside vacuoles, initially inside hepatocytes and then followed by successive cycles of replication and reinvasion of red blood cells. While major advances have been made in terms of looking at host resources as targets against infectious diseases caused by viruses or bacteria (see previous section), much less has been achieved with infections caused by protozoan parasites, as is the case of *Plasmodium*.

#### **The Liver Stage of Infection – Host Molecules as Potential Prophylactic Targets**

*Plasmodium* uses *Anopheles* mosquitoes to be disseminated. Infection of the mammalian host starts when an infected mosquito deposits *Plasmodium* sporozoites in the skin of its host during a blood meal. Sporozoites have the ability to interact with host cells in two distinct ways: they can either migrate through cells, breaching the cell's plasma membrane in the process, or they can productively invade a cell, forming a parasitophorous vacuole in which they will replicate [40]. The ability to traverse cell barriers enables *Plasmodium* sporozoites to reach the liver from their injection site in the dermis. Thus, interfering with this step would be critical for *Plasmodium* infection. However, while several parasite molecules have been described to play critical roles in this process, no host factors have ever been established as important for *Plasmodium* migration through cells. Importantly, however, it has been proposed that host heparan sulfate proteoglycans (HSPGs), which modulate the actions of a large number of extracellular ligands, provide an environmental signal that modulates the behaviour of *Plasmodium* sporozoites. Indeed, sporozoites preferentially migrate in the presence of host HSPGs with low levels of sulfation, whereas contact with cells expressing highly sulfated HSPGs, such as the ones encountered in the liver, triggers productive invasion with formation of a vacuole [41]. Thus, HSPGs seem to be a critical host factor not only to capture *Plasmodium* sporozoites in the liver but also to signal them productively to invade hepatocytes. However, their pivotal role in critical functions of the liver limits their potential for anti-malarial intervention.

Once in the liver, *Plasmodium* sporozoites must successfully invade a hepatocyte in order to replicate into thousands of new parasites, called merozoites, which will then infect red blood cells. Why sporozoites specifically target hepatocytes remains to be established. Still, a possible reason is the fact that hepatocytes are a rich source of nutrients and metabolites, which might be critical for such a high replication rate. Having that in mind we sought to undertake a genome-wide microarray approach to investigate liver-parasite molecular interactions and obtain a time-dependent profile of the transcriptional landscape of murine hepatoma cells infected by *P. berghei* sporozoites [42]. This transcriptional analysis of the host cell response to *Plasmodium* infection and development revealed a coordinated and sequential set of biological events. This study has identified several host genes and pathways with clearly modulated expression profiles as a result of infection and which constitute a repertoire of novel host factors that are prime candidates for intervention strategies [42]. A quite recent study has attempted a similar type of approach with *P. falciparum* sporozoites. The authors have used mRNA from HepG2-A16 cells exposed to freshly isolated *P. falciparum* sporozoites for different periods of time (30-180 minutes) [43]. While *P. falciparum* sporozoites are known to enter these cells, whether this occurs with formation of a parasitophorous vacuole or, instead, by breaching their plasma membrane remains to be established. Thus, this study might be identifying the signals induced in host cells by migration of sporozoites rather than by productive invasion. In any case, the host molecules identified in these two studies may now be used in functional assays to determine whether or not they play key roles during liver stage infection.

#### **Host Cell Kinases**

A wide array of high throughput methods is now available to researchers, allowing them to employ unbiased strategies to search for critical host factors. These include not only descriptive analyses, such as transcriptomic (see above), proteomic or miRNA expression profiles (not yet performed for *Plasmodium* liver stage infections), but also functional screens such as screening of siRNA or shRNA libraries.

Since phosphorylation and dephosphorylation events, enzymatically catalysed by kinases and phosphatases, constitute the most important signalling mechanisms known in eukaryotic cells, it is not surprising that the host kinome is frequently the basis for such types of functional screens. Thus, recently, we have used systematic RNA interference (RNAi) screening selectively to silence the expression of 727 genes encoding proteins with known or putative kinase activity, as well as kinase-interacting proteins, thereby covering the entire annotated kinome. This study showed that down-modulation of the expression of MET, PKC $\zeta$  (PKCzeta), PRKWNK1, SGK2 and STK35 led to a marked decrease in infection of hepatoma cells by *P. berghei*, implicating these host kinases in liver-stage infection by *Plasmodium* parasites and identifying them as potential targets for anti-malarial intervention [44]. Interestingly one of those kinases, MET, has been previously identified in a different study. Indeed, we have previously observed that hepatocyte traversal by sporozoites, which occurs prior to the productive invasion of a final cell [40], activates the parasites for infection [45]. Cell traversal induces the secretion of hepatocyte growth factor (HGF), which then binds to the MET receptor, a tyrosine kinase that acts as a mediator of signals that make the host cell susceptible to infection [46]. Besides, HGF was recently suggested to interact with the promoter region of *Plasmodium* subtilisin-like protease 2 (SUB2), an essential integral membrane serine protease in both *P. falciparum* and *P. berghei* [47]. Genistein, a major component of soybeans, is a known inhibitor of tyrosine kinases [48], an activity suggested to be responsible for this compound's ability to inhibit the intraerythrocytic development of *P. falciparum* and *P. chabaudi* *in vitro* [49, 50]. Stemming from these observations, the effect of genistein during the hepatic stage of *Plasmodium* infection and its potential as a prophylactic anti-malarial were investigated. This study demonstrated not only that genistein inhibits *P. berghei* sporozoite development *in vitro* and *in vivo* but also that the inclusion of genistein as a mouse dietary supplement affects the full course of a malaria infection [51]. This report speculates that the effect of genistein on infection might depend, at least partly, on its activity as a tyrosine kinase inhibitor on the hepatocyte [51]. This would make genistein the first host target-based agent against malaria, and one whose safety profile [52] would make it ideally suited for prophylactic intervention. However, it was recently reported that, unlike *P. berghei*, cell traversal by *P. yoelii* or *P. falciparum* does not lead to the activation of MET [53]. This study highlighted the fact that different *Plasmodium* species have evolved different mechanisms to infect their hosts. Thus, the identification of signalling molecules important for hepatic infection should be broadly applicable to malaria parasites in general, including those infecting humans. In any case, given the wide array of kinase inhibitors available and under development, signaling molecules such as those identified in this first RNAi screen are potential targets for drug-based intervention.

#### **Hepatocyte Membrane and Lipoprotein Pathway Molecules: SR-BI, CD81, and L-FABP**

Another RNAi-based approach was employed systematically to address the effect of down-modulation of the expression of human lipoprotein pathway genes on infection of human hepatoma cells by *Plasmodium* sporozoites. Results from this screen, complemented

by a variety of experimental approaches, identified an important role for scavenger receptor class B type I (SR-BI) during cell invasion by the parasite as well as during the latter's intracellular developmental process [54]. This hepatocyte membrane receptor mediates the traffic of cholesterol to and from lipoproteins, acting as the major receptor for high density lipoproteins (HDL) [55, 56]. SR-BI mediates the selective uptake of cholesteryl esters (CE) from lipoproteins, a process in which lipoprotein-derived CE are transferred to cells without degradation of lipoprotein particles [57, 58]. It is now also recognized as a major player during *Plasmodium* infection of liver cells and a potential host target for prophylactic intervention [54]. A series of small synthetic molecules, termed "blockers of lipid transport" (BLTs), were reported to inhibit the SR-BI-mediated selective uptake of lipids from HDL [59]. Several of these compounds were shown to inhibit *Plasmodium* infection of hepatic cells *in vitro* and BLT-1 inhibits *in vivo* infection of mouse models of malaria infection [54]. It should be noted, however, that SR-BI plays a physiologically crucial function in cells, whose disruption would impair cellular cholesterol uptake, which limits its potential as an anti-malarial target.

The exact mechanism through which SR-BI influences invasion of cells by *Plasmodium* is unknown. It has been proposed that cholesterol uptake by SR-BI at the plasma membrane facilitates reorganization of the hepatocyte membrane, which would involve the transport and/or positioning of CD81 molecules at the cell membrane, facilitating CD81-mediated sporozoite entry [60]. CD81 is a member of the tetraspanin integral membrane protein family, which associate with one another and with other membrane proteins to form specific proteo-lipidic membrane microdomains [61]. Tetraspanins have 4 transmembrane domains that delimitate 3 short cytosolic regions and 2 extracellular domains of unequal size (the small extracellular loop (SEL) and the large extracellular loop (LEL)) [62]. CD81 has previously been identified as an essential receptor for HCV (reviewed in [63]). More recently, it has been shown to be required for human *P. falciparum* and rodent *P. yoelii*, but not *P. berghei*, sporozoite entry into hepatocytes with formation of a parasitophorous vacuole [64]. Presumably, CD81 is not required for *P. vivax* infection, as these sporozoites can infect HepG2 cells, which do not express CD81. Host cell susceptibility to *Plasmodium* sporozoites is differently affected by expression of human CD81 depending on parasite species [65] and on host cell type [66]. Besides, CD81 localization into cell surface tetraspanin microdomains has been shown to depend on cholesterol, supporting a functional link between cholesterol and CD81 during *Plasmodium* sporozoite infection [67]. Nevertheless, the mechanism through which CD81 allows entry of *Plasmodium* sporozoites into hepatic cells is still unknown. It has been shown that hepatocyte permissiveness to *Plasmodium* infection depends on a conserved 21 amino acid stretch on CD81's LEL [68]. However, a GST-fused recombinant CD81 LEL failed to block infection of hepatocytes by sporozoites [64], suggesting that CD81 does not serve as a direct receptor for a *Plasmodium* protein. Instead, it has been suggested that CD81 functions through the regulation of an associated protein that could function as a sporozoite receptor [68]. The identification of CD81 partner molecules will provide insights into the mechanisms by which it supports infection by *Plasmodium* sporozoites and might contribute to the design of inhibitors specifically targeting the CD81-dependent entry step [68].

Besides its putative effect on the organization of CD81-enriched microdomains, SR-BI has also been suggested to activate the expression of liver-fatty acid-binding protein (L-FABP) [60]. L-FABP has been identified as a critical host factor for the development of *Plasmodium* liver stages [69]. It interacts directly with upregulated in sporozoites-3 (UIS3), a parasite protein essential for liver stage development, which constitutes the first identified direct liver stage *Plasmodium*-host cell protein interaction. L-FABP belongs to a large family frequently referred to as the intracellular

lipid binding proteins or iLBPs (reviewed in [70]). The down-regulation of L-FABP's expression in hepatocytes severely impairs parasite growth while its overexpression promotes growth [69]. Structural and biochemical evidence of PFUIS3 (a.a. 130-229) interactions with lipids (phosphatidylethanolamine), with phospholipid liposomes, and with the human L-FABP has been obtained [71]. The latter interaction most likely provides the parasite with a conduit for importing essential fatty acids/lipids and provides a new possible target for inhibiting parasite development within liver cells [71].

In sum, while interfering with molecules such as those described above may be difficult due to their very important physiological functions in the host, a detailed understanding of the biology behind their roles in *Plasmodium* infection may inform strategies to intervene at the host-parasite interface.

### The Role of HO-1 in Liver Infection

As previously mentioned, our study of the transcriptome of hepatoma cells identified several host factors whose expression is modulated throughout infection by *P. berghei* parasites [42]. Among these, the expression of heme oxygenase 1 (HO-1, encoded by *Hmox1*) was found to be up-regulated at all time points assessed [42]. HO-1 is a stress-responsive enzyme that converts the protoporphyrin IX ring of heme into biliverdin, releasing iron (Fe) and producing carbon monoxide (CO) [72]. Interestingly, it has been shown that the induction of HO-1 expression liver stage by *Plasmodium* modulates the host inflammatory response, protecting the infected hepatocytes and promoting the liver stage of infection [73]. This study demonstrated that the abrogation of the expression of HO-1 led to the resolution of liver infection by *P. berghei*-infected mice, making it a possible host target for prophylactic intervention against malaria.

It is important to bear in mind that intervention during the liver stage of infection must be prophylactic unless future research demonstrates that host factors contribute to the establishment, maintenance or re-activation of *P. vivax* hypnozoites.

## THE BLOOD STAGE OF INFECTION – THERAPEUTIC TARGETS

### HO-1 and Blood-Stage *Plasmodium* Infection

While HO-1 seems to play a detrimental role to the host during liver infection by the malaria parasite [73], it plays a clearly beneficial one during the symptomatic blood stage of infection. In fact, HO-1 has been shown to prevent the development of experimental cerebral malaria (ECM) in mouse models of infection [74]. This study demonstrated that ECM incidence was increased by deletion of *Hmox1* and inhibition of HO-1 activity whereas it was decreased by induction of HO-1 and exposure to the end-product of its activity, CO, leading to the suggestion of the controlled use of CO as an anti-malarial therapeutic strategy [74-76]. Very recently, a novel carbon monoxide releasing molecule (CO-RM) (ALF492) was shown to protect mice from severe malaria through controlled CO delivery *in vivo*. The protective effect was shown to induce the expression of HO-1, which contributes to the observed protection [77]. When administered in combination with artesunate, this CO-RM revealed to be an effective adjunctive and adjuvant treatment for ECM conferring protection after the onset of severe disease [77]. Interestingly, HO-1 was also shown to afford protection against non-cerebral forms of severe malaria, such as those associated with hemolytic anemia and hepatic damage [78]. This protection was shown to stem from the anti-oxidant effect of HO-1, which counters the oxidant effect of free heme and thereby affords protection against tumour necrosis factor-mediated apoptosis. Importantly, this cytoprotective effect is mimicked by pharmacological antioxidants such as N-acetylcysteine, which, when administered therapeutically, suppressed the development of hepatic failure in *P. chabaudi*-infected mice [78]. Altogether, while upregulation of HO-

1 promotes the establishment of *Plasmodium* liver infection, HO-1 activation seems to induce tolerance to infection by *Plasmodium* blood stages protecting the host from severe disease. Interestingly, and similarly to what we have described for blood versus liver stages of infection [73], a recent report shows that HO-1 activation occurring during malaria infections impairs resistance to a co-infection by *Salmonella* [79], providing an explanation for the susceptibility to nontyphoid *Salmonella* bacteremia in individuals with malaria, which is a common and often fatal complication of *P. falciparum* infection in sub-Saharan Africa. Importantly, population genetic studies have suggested that genetic variants of the HO-1 gene may contribute to malaria pathogenicity and severity in patients with severe *P. falciparum* malaria [80, 81]. Whether this effect is related to the role that HO-1 plays in the establishment of *Plasmodium* liver stage infection [73] or in the reduced resistance against other co-infections, such as *Salmonella* [79], remains to be established.

### Vascular Endothelial Growth Factor (VEGF)

The wide range of clinical presentations of severe malaria includes, among several other syndromes, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). High levels of plasma VEGF, a critical molecule for vasculogenesis and angiogenesis, were previously found in non-malaria ALI/ARDS patients [82]. More recently, it was shown that high levels of circulating VEGF also correlate with malaria-associated ALI in a rodent model of infection [83]. The host inflammatory response has been postulated to play a major role in the onset of distinct severe forms of malaria infection [84]. In the case of malaria-associated ALI, an uncontrolled inflammatory response of the host to the parasite seems to be responsible for the observed increase in VEGF levels, which in turn results in the VEGF-mediated increase in lung vascular permeability [83]. Interestingly, administration of the anti-inflammatory molecule CO by inhalation [83] or through a CO-RM [77] suppressed the onset of malaria-associated ALI in mice. The identification of VEGF as a critical host factor for the onset of malaria-associated ALI in mice led to the suggestion that interfering *in vivo* with VEGF levels might protect from the onset of this syndrome, in agreement with the observation that treatment of mice with the soluble form of the VEGF receptor (sFLT1) led to a decrease in VEGF levels in circulation, concomitant with protection from malaria-associated ALI [83].

Placental malaria (PM) can occur during pregnancy and can induce changes in placental structure and function, affecting fetal growth and leading to low birth weight [85]. The VEGF pathway has been implicated in maternal-fetal conflict occurring during chronic PM and hypertension in first-time mothers [86]. Another study has reported lower VEGF levels in the syncytiotrophoblasts of PM placentas than in those of asymptomatic controls [87]. More recently, an association has been found between pregnancy outcome during PM and the genotype of infant soluble *fms*-like tyrosine kinase 1 (sFlt1), also known as soluble VEGF receptor 1, which, the authors postulate, may modulate the maternal inflammatory response to PM [88].

Strategies to develop VEGF-targeted therapies are currently being pursued in various fields of biomedical research, ranging from cancer to neovascular age-related macular degeneration. Hence, the application of some of these therapies to malaria patients with critically high VEGF levels is a possibility worth considering.

### Erythrocyte Receptors for *Plasmodium* Invasion

Erythrocyte invasion by *P. falciparum* is central to the pathogenesis of malaria. Up until recently, it was believed that *P. vivax* relies on a single pathway to invade red blood cells (RBCs), using the Duffy blood group antigen as a receptor for invasion [89]. The Duffy antigen is a chemokine receptor on the surface of the RBCs and belongs to the superfamily of G-protein coupled receptors

(GCRs) [90]. The correlation between the Duffy-negative serological phenotype and resistance to *P. vivax* malaria would explain the near absence of this parasite from West Africa, where almost 95% of the population has the Duffy-negative phenotype and is resistant to *P. vivax* malaria [91], an observation bearing important implications in drug or vaccine design [92-94]. However, recent analyses of Duffy blood group polymorphisms in Madagascar [95], Angola and Equatorial Guinea [96], and in Mauritania [97], demonstrated that *P. vivax* is able to infect Duffy-negative patients, which may have a significant impact on *P. vivax*'s current distribution as well as on the development of intervention strategies. Contrary to *P. vivax*, *P. falciparum* has long been described as invading RBCs through multiple, alternative pathways, with significant redundancy [98]. The *Plasmodium* receptors identified on RBCs include glycoporphin A [99], glycoporphin C [100], glycoporphin B [101], and complement receptor 1 [102]. *P. falciparum* has been shown to invade erythrocytes in both a sialic acid (SA)-dependent and an SA-independent fashion, and to be able to switch reversibly between these two modes of invasion [103]. However, none of the known receptor-ligand interactions identified until recently were required in all parasite strains tested. This state of affairs changed with the recent identification of the Ok blood group antigen, basigin, as being essential for erythrocyte invasion across *P. falciparum* strains [104]. Basigin is a receptor for PfRh5, a parasite ligand that is essential for blood stage growth [105]. Soluble basigin, basigin knockdown, or anti-basigin antibodies efficiently inhibited erythrocyte invasion [104]. The essentiality of the interaction between basigin and PfRh5 for the strain-independent parasite entry may therefore provide new possibilities for therapeutic intervention [106].

### Cytoadherence and Sequestration Receptors

Another host-dependent process that plays a fundamental role in the pathogenicity of malaria parasites, particularly *P. falciparum*, is its unique ability to cause infected red blood cells (iRBCs) to adhere to the linings of small blood vessels. This process is termed cytoadherence and the ensuing sequestration of iRBCs is frequently suggested to be a key feature in the pathogenesis of severe malaria (see [107, 108] for reviews). The most widely suggested justification for sequestration in *P. falciparum* malaria is that adhesion of iRBCs to the endothelium allows the parasite to escape peripheral circulation and be cleared by the spleen. Another *Plasmodium* survival advantage is that sequestration in the deep tissue microvasculature provides the parasites with a microaerophilic venous environment that promotes maturation and faster asexual replication. Alternative, but not necessarily exclusive, explanations include sheltering of the iRBCs against destruction by the immune system of the host, enhanced survival of the gametocyte and immunomodulation by inhibition of the maturation and activation of dendritic cells (reviewed in [109]). Recently, a rodent model of malaria was employed to show that, in addition to avoiding spleen removal, other factors related to sequestration may be beneficial for parasite growth [110].

*P. falciparum* erythrocyte-membrane protein-1 (PfEMP-1) is the major parasite ligand involved in cytoadherence. PfEMP-1 is a high molecular weight protein that is inserted in the erythrocyte membrane between 16 and 20 hours after invasion and protrudes out of the knobs on the iRBC [111]. PfEMP1 is encoded by a large and diverse gene family called *var*, which enables PfEMP-1 to undergo antigenic variation, a major virulence factor in malaria (reviewed in [112]). Different host molecules on the surface of the endothelium serve as ligands for different forms of PfEMP-1 and participate in the interactions that mediate cytoadherence of iRBCs. Among the most important ones identified so far are CD36 [113], thrombospondin (TSP) [114], intercellular adhesion molecule-1 (ICAM-1) [115], vascular cell adhesion molecule-1 (VCAM-1) [116], E-selectin (endothelial leukocyte adhesion molecule 1, ELAM-1) [116], platelet/endothelial cell adhesion molecule-1 (PE-

CAM-1/CD31) [117], chondroitin sulphate A (CSA) [118], and hyaluronic acid (HA) [119], the latter two being specifically implicated in placental malaria. Whilst some host ligands appear to be nearly ubiquitous, others seem to be organ- or cell-specific. This specificity has implications in terms of disease severity and adhesion mechanisms. Moreover, sequestration in an organ is likely to involve multiple receptors, and different combinations of specific receptors for adhesion may determine the site at which parasites adhere and accumulate [107]. Although the exact role of each of these molecules in pathogenesis remains largely unclear, the inhibition of iRBC adhesion to certain host receptors has been proposed to bear therapeutic value. Two such examples are given below.

CD36 (encoded by *CD36*), also known as GP88 or platelet glycoprotein IV, is probably the most extensively studied receptor molecule involved in cytoadherence of *P. falciparum*-iRBC. Nevertheless, the role of CD36 in malaria pathogenesis is far from being established. CD36 is ubiquitously expressed in lung, kidney, liver and muscle vasculature, where it can contribute to the sequestration of iRBC [120]. It has been suggested that preferential sequestration of iRBCs by CD36 expressed in non-vital organs such as skin and muscle, as opposed to the brain, could be advantageous for host survival [121]. On the other hand, analyses of *CD36* polymorphisms have yielded contradictory results regarding the role of CD36 in disease severity (reviewed in [122]). The fact that the role of CD36 in the pathogenicity of malaria is still ambiguous has important implications in terms of the development of therapies or vaccines that target the interaction between PfEMP-1 and the CD36 receptor. The *in vitro*-based assumption that adherence to CD36 contributes to disease severity and results in negative clinical outcomes triggered research aimed at interfering with this interaction, which resulted in the identification of several antibodies or peptides that block adhesion of iRBC to CD36 (reviewed in [122]). Further insight into the role of CD36-mediated sequestration in malaria pathogenesis has been gained from studies employing rodent models of infection [123]. A role for CD36 in sequestration of the rodent *P. berghei* parasites was initially established by Franke-Fayard *et al.*, in a report that also showed that ECM-associated pathology is not associated with CD36-mediated sequestration [124]. Cunha-Rodrigues *et al.* later employed bone marrow chimeric mice, expressing CD36 exclusively in haematopoietic or in non-haematopoietic cells, to study the relevance of different CD36 adhesion phenotypes on the pathological course of *Plasmodium* infection. The authors concluded that CD36 plays a dual role in *P. berghei* infection, its expression in macrophages and dendritic cells having a beneficial effect for the host, and its expression in endothelial cells leading to adverse effects [125]. Thus, the question remains: are therapies aimed at blocking CD36 cytoadherence likely to improve the clinical outcome of malaria or will they have the opposite, undesired, effect? On the one hand, the idea of blocking adhesion of iRBC to what is likely to be their highest affinity receptor seems appealing in light of the long-held assumption that cytoadherence is associated with severe malaria. On the other hand, field studies have so far been unable to demonstrate that CD36 adherence is associated with disease severity and some have even suggested otherwise (reviewed in [122]). At present, it is clear that further studies are needed in order to unequivocally ascertain the role of CD36-mediated adhesion in the clinical severity of a malaria infection and the merits of targeting this host factor as a means to fight disease.

ICAM-1, also known as CD54, is widely distributed in endothelial cells including, unlike CD36, those of the brain microvascular system [126]. Unequivocal *in vivo* evidence of the involvement of ICAM-1 in sequestration, mainly in the brain, has been reported [127], leading to the notion that ICAM-1 may play an important role in cerebral malaria. This receptor has also been shown to contribute to the cytoadherence of iRBC within the intervillous spaces

of the *P. falciparum*-infected placenta, supporting a possible role of ICAM-1 in placental malaria [128]. For these reasons, it is generally accepted that ICAM-1 may play an important role in iRBC sequestration in *P. falciparum* malaria and in the severity of disease. However, studies attempting to correlate a high-frequency coding polymorphism in the *ICAM-1* gene of individuals from an area of high malaria endemicity with disease severity yielded contradictory results (reviewed in [122]). Thus, the potential usefulness of anti-malarial therapies aimed at interfering with ICAM-1 sequestration remains questionable. Anti-ICAM-1 monoclonal antibodies have been developed and shown to block adhesion of iRBC and to reduce or even to reverse sequestration (reviewed in [122]). Available data seem to suggest that an approach aimed at blocking adhesion to ICAM-1 may hold therapeutic potential, namely in preventing the development of cerebral malaria. However, only *circa* 10% of the clinical parasite isolates tested adhere to ICAM-1 [129], an observation that raises doubts about the usefulness of anti-ICAM-1 therapies in the field. Therefore, it seems unlikely that targeting ICAM-1-mediated sequestration alone will be of great therapeutic benefit. Nevertheless, given its well-documented association with the development of CM (*e.g.* [130]), ICAM-1 clearly needs to be taken into account in any combined anti-adhesive therapies that may be envisaged.

### Iron and Hepcidin

Infectious diseases induce alterations in the distribution of iron in the human body. Many of these alterations are attributable to the actions of hepcidin, a peptide hormone that is a major regulator of iron metabolism and is thought to play a central role in the anemia of chronic inflammation [131]. Hepcidin inhibits the absorption of iron through enterocytes and impairs iron release by macrophages through degradation of the iron exporter ferroportin [132]. *P. falciparum*-parasitized erythrocytes were shown to up-regulate hepcidin production by peripheral blood mononuclear cells [133]. Increased serum hepcidin concentrations, accompanied by hepcidin-mediated iron delocalisation, were described in children with asymptomatic *P. falciparum* and *P. vivax* parasitemia, in the absence of a marked acute phase response [134]. Intracellular iron sequestration and reduced iron availability have been shown to be beneficial by reducing iron availability to other pathogens [135]. Recent findings have shown that hepcidin also plays a protective role in the protection of mice against malaria superinfection [136]. The notion that hepcidin-mediated iron re-distribution may play an important role during infection by *Plasmodium* is further discussed in [137] and reaffirms the previously suggested need for a careful evaluation iron supplementation programmes in malaria-endemic regions [138]. Conversely, given the essential role of iron during *Plasmodium*'s life cycle, while iron-chelating drugs might appear as tempting strategies to control infection, the potential advantages of such strategies must be carefully weighed against the increased risks of anemia.

### CONCLUSION

Altogether, while current treatments for infectious diseases aim to kill invaders, more creative solutions that take into account the pathogen's needs for host resources are being considered. Obviously the overall success of such approaches also depends on obtaining a global view of humans and microbes using methodologies such as "systems biology", which are still in their infancy. Nevertheless, current tools have already yielded deep insights into the interplay between hosts and infectious agents. Moreover, recent reports on the effects of drugs with known targets in the host cell on *Plasmodium* liver stage infection [139-141] will certainly shed light on novel host-parasite interactions. Altogether we believe that a creative future with important changes in the way diseases are treated in the clinic might become a reality.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflicts of interest.

**ACKNOWLEDGEMENTS**

MP wishes to acknowledge support granted by FCT projects PTDC/SAU-MII/099118/2008 and PTDC/SAU/MIC/117060/2010. MMM acknowledges support by FCT grants PTDC/SAU-GMG/100313/2008 and HMSP-CT/SAU-ICT/0068/2009. This review was written under the scope of EVIMalaR.

**REFERENCES**

- [1] Watashi K, Ishii N, Hijikata M, *et al.* Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. *Mol Cell* 2005; 19: 111-22.
- [2] Inoue K, Umehara T, Ruegg UT, *et al.* Evaluation of a cyclophilin inhibitor in hepatitis C virus-infected chimeric mice *in vivo*. *Hepatology* 2007; 45: 921-8.
- [3] Paeshuyse J, Kaul A, De Clercq E, *et al.* The non-immunosuppressive cyclosporin DEBIO-025 is a potent inhibitor of hepatitis C virus replication *in vitro*. *Hepatology* 2006; 43: 761-70.
- [4] Coelmont L, Kaptein S, Paeshuyse J, *et al.* Debio 025, a cyclophilin binding molecule, is highly efficient in clearing hepatitis C virus (HCV) replicon-containing cells when used alone or in combination with specifically targeted antiviral therapy for HCV (STAT-C) inhibitors. *Antimicrob Agents Chemother* 2009; 53: 967-76.
- [5] Flisiak R, Feinman SV, Jablkowski M, *et al.* The cyclophilin inhibitor Debio 025 combined with PEG IFN $\alpha$ 2a significantly reduces viral load in treatment-naive hepatitis C patients. *Hepatology* 2009; 49: 1460-8.
- [6] Durantel D, Alotte C, Zoulim F Glucosidase inhibitors as antiviral agents for hepatitis B and C. *Curr Opin Investig Drugs* 2007; 8: 125-9.
- [7] Sonkoly E, Stahle M, Pivarcsi A MicroRNAs and immunity: novel players in the regulation of normal immune function and inflammation. *Semin Cancer Biol* 2008; 18: 131-40.
- [8] Jopling CL, Yi M, Lancaster AM, *et al.* Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005; 309: 1577-81.
- [9] Esau C, Davis S, Murray SF, *et al.* miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. *Cell Metab* 2006; 3: 87-98.
- [10] Sarasin-Filipowicz M, Krol J, Markiewicz I, *et al.* Decreased levels of microRNA miR-122 in individuals with hepatitis C responding poorly to interferon therapy. *Nat Med* 2009; 15: 31-3.
- [11] Lanford RE, Hildebrandt-Eriksen ES, Petri A, *et al.* Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010; 327: 198-201.
- [12] Li YP, Gottwein JM, Scheel TK, *et al.* MicroRNA-122 antagonism against hepatitis C virus genotypes 1-6 and reduced efficacy by host RNA insertion or mutations in the HCV 5' UTR. *Proc Natl Acad Sci U S A* 2011; 108: 4991-6.
- [13] von Hahn T, Ciesek S, Manns MP Arrest all accessories--inhibition of hepatitis C virus by compounds that target host factors. *Discov Med* 2011; 12: 237-44.
- [14] Dean M, Carrington M, Winkler C, *et al.* Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* 1996; 273: 1856-62.
- [15] Derdeyn CA, Decker JM, Sfakianos JN, *et al.* Sensitivity of human immunodeficiency virus type 1 to fusion inhibitors targeted to the gp41 first heptad repeat involves distinct regions of gp41 and is consistently modulated by gp120 interactions with the coreceptor. *J Virol* 2001; 75: 8605-14.
- [16] Liu R, Paxton WA, Choe S, *et al.* Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996; 86: 367-77.
- [17] Samson M, Libert F, Doranz BJ, *et al.* Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 1996; 382: 722-5.
- [18] Este JA, Telenti A HIV entry inhibitors. *Lancet* 2007; 370: 81-8.
- [19] Fatkenheuer G, Pozniak AL, Johnson MA, *et al.* Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat Med* 2005; 11: 1170-2.
- [20] Dorr P, Westby M, Dobbs S, *et al.* Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob Agents Chemother* 2005; 49: 4721-32.
- [21] Opar A New HIV drug classes on the horizon. *Nat Rev Drug Discov* 2007; 6: 258-9.
- [22] Nakata H, Steinberg SM, Koh Y, *et al.* Potent synergistic anti-human immunodeficiency virus (HIV) effects using combinations of the CCR5 inhibitor aplaviroc with other anti-HIV drugs. *Antimicrob Agents Chemother* 2008; 52: 2111-9.
- [23] Carette JE, Raaben M, Wong AC, *et al.* Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature* 2011; 477: 340-3.
- [24] Cote M, Misasi J, Ren T, *et al.* Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. *Nature* 2011; 477: 344-8.
- [25] Flemming A Achilles heel of Ebola viral entry. *Nat Rev Drug Discov* 2011; 10: 731.
- [26] Derre I, Pypaert M, Dautry-Varsat A, *et al.* RNAi screen in *Drosophila* cells reveals the involvement of the Tom complex in Chlamydia infection. *PLoS Pathog* 2007; 3: 1446-58.
- [27] Agaisse H, Burrack LS, Philips JA, *et al.* Genome-wide RNAi screen for host factors required for intracellular bacterial infection. *Science* 2005; 309: 1248-51.
- [28] Cheng LW, Viala JP, Stuurman N, *et al.* Use of RNA interference in *Drosophila* S2 cells to identify host pathways controlling compartmentalization of an intracellular pathogen. *Proc Natl Acad Sci U S A* 2005; 102: 13646-51.
- [29] Ayres JS, Freitag N, Schneider DS Identification of *Drosophila* mutants altering defense of and endurance to *Listeria monocytogenes* infection. *Genetics* 2008; 178: 1807-15.
- [30] Kuijl C, Savage ND, Marsman M, *et al.* Intracellular bacterial growth is controlled by a kinase network around PKB/AKT1. *Nature* 2007; 450: 725-30.
- [31] Granville CA, Memmott RM, Gills JJ, *et al.* Handicapping the race to develop inhibitors of the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway. *Clin Cancer Res* 2006; 12: 679-89.
- [32] Nairz M, Schroll A, Sonnweber T, *et al.* The struggle for iron - a metal at the host-pathogen interface. *Cell Microbiol* 2010; 12: 1691-702.
- [33] Roosenberg JM, 2nd, Lin YM, Lu Y, *et al.* Studies and syntheses of siderophores, microbial iron chelators, and analogs as potential drug delivery agents. *Curr Med Chem* 2000; 7: 159-97.
- [34] Miller MJ, Walz AJ, Zhu H, *et al.* Design, synthesis, and study of a mycobactin-artemisinin conjugate that has selective and potent activity against tuberculosis and malaria. *J Am Chem Soc* 2011; 133: 2076-9.
- [35] Ratledge C, Dover LG Iron metabolism in pathogenic bacteria. *Annu Rev Microbiol* 2000; 54: 881-941.
- [36] Kaneko Y, Thoendel M, Olanmi O, *et al.* The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. *J Clin Invest* 2007; 117: 877-88.
- [37] Banin E, Lozinski A, Brady KM, *et al.* The potential of desferrioxamine-gallium as an anti-*Pseudomonas* therapeutic agent. *Proc Natl Acad Sci U S A* 2008; 105: 16761-6.
- [38] Halwani M, Yebio B, Suntres ZE, *et al.* Co-encapsulation of gallium with gentamicin in liposomes enhances antimicrobial activity of gentamicin against *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2008; 62: 1291-7.
- [39] Rasmussen TB, Givskov M Quorum-sensing inhibitors as anti-pathogenic drugs. *Int J Med Microbiol* 2006; 296: 149-61.

- [40] Mota MM, Pradel G, Vanderberg JP, *et al.* Migration of *Plasmodium* sporozoites through cells before infection. *Science* 2001; 291: 141-4.
- [41] Coppi A, Tewari R, Bishop JR, *et al.* Heparan sulfate proteoglycans provide a signal to *Plasmodium* sporozoites to stop migrating and productively invade host cells. *Cell Host Microbe* 2007; 2: 316-27.
- [42] Albuquerque SS, Carret C, Grosso AR, *et al.* Host cell transcriptional profiling during malaria liver stage infection reveals a coordinated and sequential set of biological events. *BMC Genomics* 2009; 10: 270.
- [43] Chattopadhyay R, de la Vega P, Paik SH, *et al.* Early transcriptional responses of HepG2-A16 liver cells to infection by *Plasmodium falciparum* sporozoites. *J Biol Chem* 2011; 286: 26396-405.
- [44] Prudencio M, Rodrigues CD, Hannus M, *et al.* Kinome-wide RNAi screen implicates at least 5 host hepatocyte kinases in *Plasmodium* sporozoite infection. *PLoS Pathog* 2008; 4: e1000201.
- [45] Mota MM, Hafalla JC, Rodriguez A Migration through host cells activates *Plasmodium* sporozoites for infection. *Nat Med* 2002; 8: 1318-22.
- [46] Carrolo M, Giordano S, Cabrita-Santos L, *et al.* Hepatocyte growth factor and its receptor are required for malaria infection. *Nat Med* 2003; 9: 1363-9.
- [47] Liao S, Liu Y, Jung SY, *et al.* Transcriptional activity of *Plasmodium* subtilisin-like protease 2 (Pf-Sub2) 5'untranslated regions and its interaction with hepatocyte growth factor. *Korean J Parasitol* 2010; 48: 291-5.
- [48] Akiyama T, Ishida J, Nakagawa S, *et al.* Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 1987; 262: 5592-5.
- [49] Dluzewski AR, Garcia CR Inhibition of invasion and intraerythrocytic development of *Plasmodium falciparum* by kinase inhibitors. *Experientia* 1996; 52: 621-3.
- [50] Gazarini ML, Garcia CR Interruption of the blood-stage cycle of the malaria parasite, *Plasmodium chabaudi*, by protein tyrosine kinase inhibitors. *Braz J Med Biol Res* 2003; 36: 1465-9.
- [51] Cunha-Rodrigues M, Portugal S, Prudencio M, *et al.* Genistein-supplemented diet decreases malaria liver infection in mice and constitutes a potential prophylactic strategy. *PLoS One* 2008; 3: e2732.
- [52] Morabito N, Crisafulli A, Vergara C, *et al.* Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. *J Bone Miner Res* 2002; 17: 1904-12.
- [53] Kaushansky A, Kappe SH The crucial role of hepatocyte growth factor receptor during liver-stage infection is not conserved among *Plasmodium* species. *Nat Med* 2011; 17: 1180-1; author reply 1.
- [54] Rodrigues CD, Hannus M, Prudencio M, *et al.* Host scavenger receptor SR-BI plays a dual role in the establishment of malaria parasite liver infection. *Cell Host Microbe* 2008; 4: 271-82.
- [55] Rhainds D, Brodeur M, Lapointe J, *et al.* The role of human and mouse hepatic scavenger receptor class B type I (SR-BI) in the selective uptake of low-density lipoprotein-cholesteryl esters. *Biochemistry* 2003; 42: 7527-38.
- [56] Acton S, Rigotti A, Landschulz KT, *et al.* Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 1996; 271: 518-20.
- [57] Stangl H, Cao G, Wyne KL, *et al.* Scavenger receptor, class B, type I-dependent stimulation of cholesterol esterification by high density lipoproteins, low density lipoproteins, and nonlipoprotein cholesterol. *J Biol Chem* 1998; 273: 31002-8.
- [58] Swarnakar S, Temel RE, Connelly MA, *et al.* Scavenger receptor class B, type I, mediates selective uptake of low density lipoprotein cholesteryl ester. *J Biol Chem* 1999; 274: 29733-9.
- [59] Nieland TJ, Penman M, Dori L, *et al.* Discovery of chemical inhibitors of the selective transfer of lipids mediated by the HDL receptor SR-BI. *Proc Natl Acad Sci U S A* 2002; 99: 15422-7.
- [60] Yalaoui S, Huby T, Franetich JF, *et al.* Scavenger receptor BI boosts hepatocyte permissiveness to *Plasmodium* infection. *Cell Host Microbe* 2008; 4: 283-92.
- [61] Hemler ME Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol* 2003; 19: 397-422.
- [62] Boucheix C, Rubinstein E Tetraspanins. *Cell Mol Life Sci* 2001; 58: 1189-205.
- [63] Farquhar MJ, Harris HJ, McKeating JA Hepatitis C virus entry and the tetraspanin CD81. *Biochem Soc Trans* 2011; 39: 532-6.
- [64] Silvie O, Rubinstein E, Franetich JF, *et al.* Hepatocyte CD81 is required for *Plasmodium falciparum* and *Plasmodium yoelii* sporozoite infectivity. *Nat Med* 2003; 9: 93-6.
- [65] Silvie O, Greco C, Franetich JF, *et al.* Expression of human CD81 differently affects host cell susceptibility to malaria sporozoites depending on the *Plasmodium* species. *Cell Microbiol* 2006; 8: 1134-46.
- [66] Silvie O, Franetich JF, Boucheix C, *et al.* Alternative invasion pathways for *Plasmodium berghei* sporozoites. *Int J Parasitol* 2007; 37: 173-82.
- [67] Silvie O, Charrin S, Billard M, *et al.* Cholesterol contributes to the organization of tetraspanin-enriched microdomains and to CD81-dependent infection by malaria sporozoites. *J Cell Sci* 2006; 119: 1992-2002.
- [68] Yalaoui S, Zougbede S, Charrin S, *et al.* Hepatocyte permissiveness to *Plasmodium* infection is conveyed by a short and structurally conserved region of the CD81 large extracellular domain. *PLoS Pathog* 2008; 4: e1000010.
- [69] Mikolajczak SA, Jacobs-Lorena V, MacKellar DC, *et al.* L-FABP is a critical host factor for successful malaria liver stage development. *Int J Parasitol* 2007; 37: 483-9.
- [70] Thompson J, Reese-Wagoner A, Banaszak L Liver fatty acid binding protein: species variation and the accommodation of different ligands. *Biochim Biophys Acta* 1999; 1441: 117-30.
- [71] Sharma A, Yogavel M, Akhouri RR, *et al.* Crystal structure of soluble domain of malaria sporozoite protein UIS3 in complex with lipid. *J Biol Chem* 2008; 283: 24077-88.
- [72] Tenhunen R, Marver HS, Schmid R The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci U S A* 1968; 61: 748-55.
- [73] Epiphanio S, Mikolajczak SA, Goncalves LA, *et al.* Heme oxygenase-1 is an anti-inflammatory host factor that promotes murine plasmodium liver infection. *Cell Host Microbe* 2008; 3: 331-8.
- [74] Pamplona A, Ferreira A, Balla J, *et al.* Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. *Nat Med* 2007; 13: 703-10.
- [75] Pamplona A, Hanscheid T, Epiphanio S, *et al.* Cerebral malaria and the hemolysis/methemoglobin/heme hypothesis: shedding new light on an old disease. *Int J Biochem Cell Biol* 2009; 41: 711-16.
- [76] Pamplona A, Clark IA, Mota MM Severe malaria increases the list of heme oxygenase-1-protected diseases. *Future Microbiol* 2007; 2: 361-3.
- [77] Pena AC, Penacho N, Mancio-Silva L, *et al.* A novel Carbon Monoxide-Releasing Molecule (CO-RM) fully protects mice from Severe Malaria. *Antimicrob Agents Chemother* 2011.
- [78] Seixas E, Gozzelino R, Chora A, *et al.* Heme oxygenase-1 affords protection against noncerebral forms of severe malaria. *Proc Natl Acad Sci U S A* 2009; 106: 15837-42.
- [79] Cunnington AJ, de Souza JB, Walther M, *et al.* Malaria impairs resistance to *Salmonella* through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. *Nat Med* 2011.
- [80] Sambo MR, Trovoado MJ, Benchimol C, *et al.* Transforming growth factor beta 2 and heme oxygenase 1 genes are risk factors for the cerebral malaria syndrome in Angolan children. *PLoS One* 2010; 5: e11141.
- [81] Takeda M, Kikuchi M, Ubalee R, *et al.* Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to cerebral malaria in Myanmar. *Jpn J Infect Dis* 2005; 58: 268-71.
- [82] Thickett DR, Armstrong L, Christie SJ, *et al.* Vascular endothelial growth factor may contribute to increased vascular permeability in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001; 164: 1601-5.

- [83] Epiphanio S, Campos MG, Pamplona A, *et al.* VEGF promotes malaria-associated acute lung injury in mice. *PLoS Pathog* 2010; 6: e1000916.
- [84] Clark IA, Alleva LM, Mills AC, *et al.* Pathogenesis of malaria and clinically similar conditions. *Clin Microbiol Rev* 2004; 17: 509-39, table of contents.
- [85] Umbers AJ, Aitken EH, Rogerson SJ Malaria in pregnancy: small babies, big problem. *Trends Parasitol* 2011; 27: 168-75.
- [86] Muehlenbachs A, Mutabingwa TK, Edmonds S, *et al.* Hypertension and maternal-fetal conflict during placental malaria. *PLoS Med* 2006; 3: e446.
- [87] Boeuf P, Tan A, Romagosa C, *et al.* Placental hypoxia during placental malaria. *J Infect Dis* 2008; 197: 757-65.
- [88] Muehlenbachs A, Fried M, Lachowitz J, *et al.* Natural selection of FLT1 alleles and their association with malaria resistance in utero. *Proc Natl Acad Sci U S A* 2008; 105: 14488-91.
- [89] Miller LH, Mason SJ, Clyde DF, *et al.* The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. *N Engl J Med* 1976; 295: 302-4.
- [90] Neote K, Mak JY, Kolakowski LF, Jr., *et al.* Functional and biochemical analysis of the cloned Duffy antigen: identity with the red blood cell chemokine receptor. *Blood* 1994; 84: 44-52.
- [91] Chitnis CE, Miller LH Identification of the erythrocyte binding domains of *Plasmodium vivax* and *Plasmodium knowlesi* proteins involved in erythrocyte invasion. *J Exp Med* 1994; 180: 497-506.
- [92] Yazdani SS, Shakri AR, Mukherjee P, *et al.* Evaluation of immune responses elicited in mice against a recombinant malaria vaccine based on *Plasmodium vivax* Duffy binding protein. *Vaccine* 2004; 22: 3727-37.
- [93] Ntumngia FB, Adams JH Design and Immunogenicity of a Novel Synthetic Antigen based on the Ligand Domain of the *Plasmodium vivax* Duffy Binding Protein. *Clin Vaccine Immunol* 2011.
- [94] Nobrega de Sousa T, Carvalho LH, Alves de Brito CF Worldwide genetic variability of the Duffy binding protein: insights into *Plasmodium vivax* vaccine development. *PLoS One* 2011; 6: e22944.
- [95] Menard D, Barnadas C, Bouchier C, *et al.* *Plasmodium vivax* clinical malaria is commonly observed in Duffy-negative Malagasy people. *Proc Natl Acad Sci U S A* 2010; 107: 5967-71.
- [96] Mendes C, Dias F, Figueiredo J, *et al.* Duffy negative antigen is no longer a barrier to *Plasmodium vivax*--molecular evidences from the African West Coast (Angola and Equatorial Guinea). *PLoS Negl Trop Dis* 2011; 5: e1192.
- [97] Wurtz N, Mint Lekweiry K, Bogreau H, *et al.* *Vivax* malaria in Mauritania includes infection of a Duffy-negative individual. *Malar J* 2011; 10: 336.
- [98] Cowman AF, Crabb BS Invasion of red blood cells by malaria parasites. *Cell* 2006; 124: 755-66.
- [99] Sim BK, Chitnis CE, Wasniowska K, *et al.* Receptor and ligand domains for invasion of erythrocytes by *Plasmodium falciparum*. *Science* 1994; 264: 1941-4.
- [100] Maier AG, Duraisingh MT, Reeder JC, *et al.* *Plasmodium falciparum* erythrocyte invasion through glycophorin C and selection for Gerbich negativity in human populations. *Nat Med* 2003; 9: 87-92.
- [101] Mayer DC, Coffie J, Jiang L, *et al.* Glycophorin B is the erythrocyte receptor of *Plasmodium falciparum* erythrocyte-binding ligand, EBL-1. *Proc Natl Acad Sci U S A* 2009; 106: 5348-52.
- [102] Tham WH, Wilson DW, Lopaticki S, *et al.* Complement receptor 1 is the host erythrocyte receptor for *Plasmodium falciparum* Pfrh4 invasion ligand. *Proc Natl Acad Sci U S A* 2010; 107: 17327-32.
- [103] Stubbs J, Simpson KM, Triglia T, *et al.* Molecular mechanism for switching of *P. falciparum* invasion pathways into human erythrocytes. *Science* 2005; 309: 1384-7.
- [104] Crosnier C, Bustamante LY, Bartholdson SJ, *et al.* Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. *Nature* 2011.
- [105] Baum J, Chen L, Healer J, *et al.* Reticulocyte-binding protein homologue 5 - an essential adhesin involved in invasion of human erythrocytes by *Plasmodium falciparum*. *Int J Parasitol* 2009; 39: 371-80.
- [106] Douglas AD, Williams AR, Illingworth JJ, *et al.* The blood-stage malaria antigen Pfrh5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. *Nat Commun* 2011; 2: 601.
- [107] Beeson JG, Brown GV Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation. *Cell Mol Life Sci* 2002; 59: 258-71.
- [108] Miller LH, Baruch DI, Marsh K, *et al.* The pathogenic basis of malaria. *Nature* 2002; 415: 673-9.
- [109] Sherman IW, Eda S, Winograd E Cytoadherence and sequestration in *Plasmodium falciparum*: defining the ties that bind. *Microbes Infect* 2003; 5: 897-909.
- [110] Fonager J, Pasini EM, Braks JA, *et al.* Reduced CD36-dependent tissue sequestration of *Plasmodium*-infected erythrocytes is detrimental to malaria parasite growth *in vivo*. *J Exp Med* 2011.
- [111] Kwiatkowski DP How malaria has affected the human genome and what human genetics can teach us about malaria. *Am J Hum Genet* 2005; 77: 171-92.
- [112] Tembo D, Montgomery J Var gene expression and human *Plasmodium* pathogenesis. *Future Microbiol* 2010; 5: 801-15.
- [113] Barnwell JW, Ockenhouse CF, Knowles DM, 2nd Monoclonal antibody OKM5 inhibits the *in vitro* binding of *Plasmodium falciparum*-infected erythrocytes to monocytes, endothelial, and C32 melanoma cells. *J Immunol* 1985; 135: 3494-7.
- [114] Roberts DD, Sherwood JA, Spitalnik SL, *et al.* Thrombospondin binds *falciparum* malaria parasitized erythrocytes and may mediate cytoadherence. *Nature* 1985; 318: 64-6.
- [115] Berendt AR, Simmons DL, Tansey J, *et al.* Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature* 1989; 341: 57-9.
- [116] Ockenhouse CF, Tegoshi T, Maeno Y, *et al.* Human vascular endothelial cell adhesion receptors for *Plasmodium falciparum*-infected erythrocytes: roles for endothelial leukocyte adhesion molecule 1 and vascular cell adhesion molecule 1. *J Exp Med* 1992; 176: 1183-9.
- [117] Treutiger CJ, Heddini A, Fernandez V, *et al.* PECAM-1/CD31, an endothelial receptor for binding *Plasmodium falciparum*-infected erythrocytes. *Nat Med* 1997; 3: 1405-8.
- [118] Rogerson SJ, Chaiyaroj SC, Ng K, *et al.* Chondroitin sulfate A is a cell surface receptor for *Plasmodium falciparum*-infected erythrocytes. *J Exp Med* 1995; 182: 15-20.
- [119] Beeson JG, Rogerson SJ, Cooke BM, *et al.* Adhesion of *Plasmodium falciparum*-infected erythrocytes to hyaluronic acid in placental malaria. *Nat Med* 2000; 6: 86-90.
- [120] Mackintosh CL, Beeson JG, Marsh K Clinical features and pathogenesis of severe malaria. *Trends Parasitol* 2004; 20: 597-603.
- [121] Serghides L, Smith TG, Patel SN, *et al.* CD36 and malaria: friends or foes? *Trends Parasitol* 2003; 19: 461-9.
- [122] Prudencio M, Rodrigues CD, Mota MM The relevance of host genes in malaria. *SEB Exp Biol Ser* 2007; 58: 47-91.
- [123] Franke-Fayard B, Fonager J, Braks A, *et al.* Sequestration and tissue accumulation of human malaria parasites: can we learn anything from rodent models of malaria? *PLoS Pathog* 2010; 6: e1001032.
- [124] Franke-Fayard B, Janse CJ, Cunha-Rodrigues M, *et al.* Murine malaria parasite sequestration: CD36 is the major receptor, but cerebral pathology is unlinked to sequestration. *Proc Natl Acad Sci U S A* 2005; 102: 11468-73.
- [125] Cunha-Rodrigues M, Portugal S, Febbraio M, *et al.* Bone marrow chimeric mice reveal a dual role for CD36 in *Plasmodium berghei* ANKA infection. *Malar J* 2007; 6: 32.
- [126] Adams S, Turner GD, Nash GB, *et al.* Differential binding of clonal variants of *Plasmodium falciparum* to allelic forms of intracellular adhesion molecule 1 determined by flow adhesion assay. *Infect Immun* 2000; 68: 264-9.
- [127] Turner GD, Morrison H, Jones M, *et al.* An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *Am J Pathol* 1994; 145: 1057-69.
- [128] Sugiyama T, Cuevas LE, Bailey W, *et al.* Expression of intercellular adhesion molecule 1 (ICAM-1) in *Plasmodium falciparum*-infected placenta. *Placenta* 2001; 22: 573-9.

- [129] Ho M, White NJ Molecular mechanisms of cytoadherence in malaria. *Am J Physiol* 1999; 276: C1231-42.
- [130] Newbold CI, Craig AG, Kyes S, *et al.* PfEMP1, polymorphism and pathogenesis. *Ann Trop Med Parasitol* 1997; 91: 551-7.
- [131] Andrews NC Forging a field: the golden age of iron biology. *Blood* 2008; 112: 219-30.
- [132] Nemeth E, Tuttle MS, Powelson J, *et al.* Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; 306: 2090-3.
- [133] Armitage AE, Pinches R, Eddowes LA, *et al.* Plasmodium falciparum infected erythrocytes induce hepcidin (HAMP) mRNA synthesis by peripheral blood mononuclear cells. *Br J Haematol* 2009; 147: 769-71.
- [134] de Mast Q, Syafruddin D, Keijmel S, *et al.* Increased serum hepcidin and alterations in blood iron parameters associated with asymptomatic *P. falciparum* and *P. vivax* malaria. *Haematologica* 2010; 95: 1068-74.
- [135] Paradkar PN, De Domenico I, Durchfort N, *et al.* Iron depletion limits intracellular bacterial growth in macrophages. *Blood* 2008; 112: 866-74.
- [136] Portugal S, Carret C, Recker M, *et al.* Host-mediated regulation of superinfection in malaria. *Nat Med* 2011; 17: 732-7.
- [137] Portugal S, Drakesmith H, Mota MM Superinfection in malaria: Plasmodium shows its iron will. *EMBO Rep* 2011; 12: 1233-42.
- [138] Sazawal S, Black RE, Ramsan M, *et al.* Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* 2006; 367: 133-43.
- [139] da Cruz FP, Martin C, Buchholz K, Lafuente-Monasterio MJ, *et al.* Drug screen targeted at Plasmodium liver stages identifies a potent multistage antimalarial drug. *J Infect Dis* 2012; 205: 1278-86.
- [140] Derbyshire ER, Prudêncio M, Mota MM, Clardy J. Liver-stage malaria parasites vulnerable to diverse chemical scaffolds. *Proc Natl Acad Sci USA*; 109: 8511-6.
- [141] Meister S, Plouffe DM, Kuhen KL, Bonamy GM, *et al.* Imaging of Plasmodium liver stages to drive next-generation antimalarial drug discovery. *Science*; 334: 1372-7.