# Malaria Pathogenesis

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In the mosquito–human life cycle, the six species of malaria parasites infecting humans (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale wallickeri, Plasmodium ovale curtisi, Plasmodium malariae,* and *Plasmodium knowlesi*) undergo 10 or more morphological states, replicate from single to 10,000+ cells, and vary in total population from one to many more than $10^6$ organisms. In the human host, only a small number of these morphological stages lead to clinical disease and the vast majority of all malaria-infected patients in the world produce few (if any) symptoms in the human. Human clinical disease (e.g., fever, anemia, coma) is the result of the parasite preprogrammed biology in concert with the human pathophysiological response. Caveats and corollaries that add variation to this host–parasite interaction include parasite genetic diversity of key proteins, coinfections, comorbidities, delays in treatment, human polymorphisms, and environmental determinants.

Pathogenesis, the manner of development of a disease, for a human malaria clinical illness is a complex story that has many players, settings, and potential outcomes. As with any truly successful parasite, the observed outcome of evolution in malaria is the undisturbed transition from mosquito to human to mosquito with little impact on the vector and host. Although impact of malaria can be seen at the individual, community, country, and global level, from the parasite's perspective, a healthy host serving as two blood meals with a bit of fever in between is the norm. In fact, human clinical disease is quite rare relative to the global interaction network of mosquitoes and humans.

The biology of *Plasmodium falciparum* malaria parasites, as measured in vitro, is finite, predictable, and easily experimentally perturbed during the 48-hour life cycle (Bozdech et al. 2003a,b; Llinas and DeRisi 2004). In the mosquito–human life cycle, however, this parasite, along with the other five species infecting humans (*Plasmodium vivax*, *Plasmodium ovale wallickeri*, *Plasmodium ovale curtisi*, *Plasmodium malariae*, and *Plasmodium knowlesi*), undergoes 10 or more morphological states, replicate from a single to 10,000+ cells, and vary in total population from one to many more than $10^6$ organisms (Liu et al. 2011; Cator et al. 2012; Dixon et al. 2012; Mohandas and An 2012; Antinori et al. 2013; Wright and Rayner 2014; Cui et al. 2015; Josling and Llinas 2015; Stone et al. 2015). In addition, all of these parasites (with the exception of *P. knowlesi* in humans) have been exposed for thousands of millennia to the physical, immunological, and more recently chemotherapeutic barriers in mosquitoes and humans, which places tremendous selection

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pressure across the species (Sabeti et al. 2002; Volkman et al. 2007; Bañuls et al. 2013; Perry 2014). It is clearly a finely tuned, well-rehearsed, and deftly executed program.

A similar selection pressure has been placed on humans and resulted in such fascinating evolutionary outcomes as sickle cell disease, hemoglobinopathies, cytokine mutations, and enzyme deficiency, which confer, as a conceptual group, the ability to survive to maturity and reproduction (Bañuls et al. 2013; Perry 2014). Death from malaria at an age less than 6 years (the current most common demographic) cannot be a goal of the parasite (speaking teleologically) and, thus, its occurrence should be cause for concern and investigation. However, the rarity of this event (438,000 out of 214,000,000 clinical cases or ~0.2%) leaves the unfortunate mortality as an aberrant footnote in the overall biology of the species as a whole (WHO 2015). We should not, however, accept even one death from a preventable and treatable disease.

When we turn to the parasite inside the human host, only a small number of these morphological stages lead to clinical disease and the vast majority of all malaria-infected patients in the world produce few (if any) symptoms in the human (WHO 2015). This is a crucial point of the biology that is often missed or ignored by experimentalists and "single-mechanism" focused scientists. Every person who is infected with malaria (regardless of whether or not they show symptoms) has the parasite go through the exact same life-cycle morphological changes and human–parasite interactions. Disease, thus, must be the result of exaggeration of this baseline interaction, which, as mentioned, is beneficial to neither the parasite nor the human. Further evidence for this lies, obviously, within the overall rarity of such events. Moreover, there are relatively few physiological states the parasite can achieve inside the human host—all of this biology is accomplished with a meager 6000 genes, most of which have no known function (Bozdech et al. 2003a,b; Daily et al. 2007; Milner et al. 2012).

Human clinical disease is, thus, the result of the interaction of the parasite preprogrammed biology in concert with the human pathophysiological response. Caveats and corollaries that add variation to this host–parasite interaction include parasite genetic diversity of key proteins, coinfections, comorbidities, delays in treatment, human polymorphisms, and environmental determinants (Goncalves et al. 2014). The final clinical disease result includes a spectrum of fever, anemia, and coma, among many others (Hafalla et al. 2011; Oakley et al. 2011; Grau and Craig 2012).

When one questions, "how do we get rid of cerebral malaria?" (one of the more common causes of death), it is surprising to no one to hear the answer, "reduce the overall burden of malaria disease." This may seem simple but, in fact, is a complex answer. Interventions with rapid drug treatment for anyone with a fever will drastically reduce the burden of mortality (sometimes to zero) in a given location (Clark et al. 2010). The treatment probably not only staves off a prolonged acute disease state (which may be a component of cerebral malaria [CM]) but also provides an antigen source to the immune system to create antibody and other responses that may quiet future infections. This effect, however, only lasts as long as the diversity of the parasite is stable (a result of endemicity) and the drug access continues (a result of infrastructure stability). In a world where eradication is a goal for malaria, the incidence of CM with multiple interventions may decrease or even vanish in the current at-risk population (children less than 6 years of age in sub-Saharan Africa). However, the risk of CM may simply shift to these same children at a later stage (or their children) as a region moves from high endemicity to low endemicity. During this entire process, however, the biology of the parasite will remain relatively stable and, thus, the risk for any of the currently observed diseases states will still exist. How, where, and why these disease states emerge (or vanish) is a product of many factors beyond the parasite, the vast majority of which are within our control.

## UNCOMPLICATED MALARIA

Within the geographic regions where the human population is at risk for malaria infection

(2.5 billion), annually 215,000,000 clinical infections occur for which patients have symptoms and seek medical attention. Patient illness represents, however, a subset of all individuals who have been bitten by infected mosquitoes and a much larger portion of the "at-risk" population would show a positive malaria smear or other diagnostic test if they were screened (asymptomatic infection, true number variable and difficult to estimate) (malERA Consultative Group on Diagnoses and Diagnostics 2011; McMorrow et al. 2011; Laishram et al. 2012; Babiker et al. 2013; Lin et al. 2014; Stone et al. 2015). The exact malaria parasite biology within these two groups is probably very similar with the essential differences being due to the human immune response, number of prior infections, and exposure profile (Doolan and Martinez-Alier 2006; Dzikowski et al. 2006; Marsh and Kinyanjui 2006; De Leenheer and Pilyugin 2008; Punsawad 2013; de Souza 2014; Krzych et al. 2014). The symptoms of malaria infection can only begin in any ill patient with the first liver schizont rupture and release of merozoites into the peripheral circulation—this event is silent for the vast majority of patients who will become clinically ill. As the parasites continue through their asexual life cycle of merozoite reinvasion, trophozoite development, and schizont rupture over 24 to 48 hours, the level of parasitemia parallels the level of human response (i.e., fever, C-reactive protein [CRP], and tumor necrosis factor α [TNF-α]) until the patient crosses a threshold of awareness and "feels ill" (Oakley et al. 2011). Uncomplicated malaria is defined as symptoms present (fever) but no clinical or laboratory signs to indicate severity or vital organ dysfunction (WHO 2015).

Within the human host during an initial infection, macrophage ingestion of merozoites, ruptured schizonts, or antigen-presenting trophozoites in the circulation or spleen leads to release of TNF-α (Chakravorty et al. 2008; Randall and Engwerda 2010). The molecule, along with others in a cascade, is responsible for fever during infection. Other important molecules found during active infection include interleukin 10 (IL-10) and interferon γ (IFN-γ) among others (Clark et al. 2008; McCall and Sauerwein 2010; Freitas do Rosario and Langhorne 2012; Gun et al. 2014; Hunt et al. 2014). In subsequent infections, some degree of antibody production produced by the prior macrophage–T-cell–B-cell axis of the immune system confers additional macrophage activity leading to a more efficient clearance of parasites and production of new antibodies (Wykes and Good 2006; Freitas do Rosario and Langhorne 2012; Krzych et al. 2014; Hviid et al. 2015). As the human host immune system works its way through the continuously presented parasite protein repertoire, additional antibodies develop conferring additional protection.

Uncomplicated malaria is easily treated during each symptomatic episode with antimalarias specific to the parasite and the vast majority of patients easily clear the infection when treated with proper compliance.

### *P. falciparum*

*P. falciparum* (Pf) modifies the surface of the infected red blood cell and creates an adhesive phenotype, which removes the parasite from circulation for nearly half of the asexual life cycle, a unique time frame among the malaria parasites (Grau and Craig 2012). The binding of the infected erythrocytes can occur with endothelium, platelets, or uninfected red blood cells (Fairhurst and Wellems 2006; Kraemer and Smith 2006; Smith et al. 2013). The parasite accomplishes this cytoadherant ("sticky cell") state through the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is the product of *var* gene transcription (Smith et al. 2013). Within a given Pf parasite, there are ~60 copies of the *var* gene, each highly variable and different from the others. These genes represent some of the most diverse within the parasite's genome and within the total parasite population. Their expression is driven by several mechanisms including immune selection pressure and epigenetics. This aspect of the parasites's biology (*var* gene expression) occurs in all infections including asymptomatic and uncomplicated malaria. The potential of this human–parasite interaction to cause disease in humans has a definite

spectrum discussed below (Smith et al. 2013). Regardless of the disease variability, the sequestration (temporary removal of the parasite from circulation through red cell surface binding) of Pf occurs during every human infection for half of the asexual life cycle. Thus, in a low-level infection in which a single mosquito bite has introduced a single brood of synchronous parasites, patients may show negative peripheral blood smears. This may be especially true in the traveler or residents of low-endemicity regions. In highly endemic settings, however, patients are bitten repeatedly and can present with a continuous fever and an accompanying consistently positive blood smear during the first decades of life. As a local immunity to the Pf population evolves in a given host, smears may again drop to very low levels and even become undetectable despite ongoing transmission.

### *P. vivax*

*P. vivax* (Pv) is the most common malaria parasite causing clinical disease outside of Africa (WHO 2015). Unlike Pf, but like all other human malaria parasites, Pv does not show a prolonged period of sequestration during infection (Costa et al. 2011). The parasite is, thus, probably more frequently exposed to clearance by the spleen and more commonly seen on a peripheral blood smear during an infection. One of the unique features of Pv is the red cell preference for reticulocytes and the use of predominantly the Duffy antigen for invasion although not absolutely (Moreno-Pérez et al. 2013; Zimmerman et al. 2013). This leads to a clinical infection with a lower level of parasitemia than is seen in Pf. Because reticulocytes are larger than mature red cells, the infected cells appear larger than the cells around them on peripheral blood smear. Characteristic Schuffner's dots, which are caveola–vesicle structures, are seen in both Pv and *P. ovale* (Udagama et al. 1988). The diagnostic form of Pv is the amoeboid form where the cytoplasm, unique to Pv, has finger-like projections without a typical round-to-oval structure.

Clinically, patients present almost identically to other malaria infections with fever plus a constellation of other possible symptoms. Unlike Pf and *P. malariae* (which have a single liver schizont rupture even shortly after sporozoite invasion), Pv, and Po may "reemerge" when hypnozoites (quiescent forms that last months to years in the liver from a single sporozoite exposure) release merozoites. Thus, the clinical timing of a disease (many months or years after exposure) could be a clue to one of these organisms.

### *P. ovale*

*P. ovale* (Po) was shown to be two distinct species (*P. ovale curtisi* and *P. ovale wallikeri*), which only differ by a shorter latency period in *P. ovale wallikeri* and genetic sequence differences (Oguike et al. 2011). Thus, these two sympatric organisms are impossible to distinguish, present with the same clinical syndrome, and respond to the same therapy. Although their behavior is similar to Pv, Po does not require the Duffy blood group antigen for invasion of red blood cells. On peripheral blood smear, the diagnostic forms of Po are the comet form of the trophozoite as well as the oval appearance of infected red blood cells and the presence of fimbria or finger-like projects of the red cell membrane. The ring, schizont, and gametocyte stages of Po are very similar to Pv.

### *P. malariae*

*P. malariae* (Pm) is the most benign form of malaria infection with several distinct clinical features (Collins and Jeffery 2007; Mueller et al. 2007; Das 2008). Patients have fever every 72 hours during an infection due to the longer parasite life cycle (Collins and Jeffery 2007). The number of merozoites produced with each schizont rupture is lower and, thus, the parasitemias are lower overall in these patient compared with others types of malaria (Collins and Jeffery 2007). This long life cycle and low level of infection leads to a more robust immune response. Thus, Pm is often considered to cause a chronic malaria they may last decades. One unique outcome of Pm is the deposition of immune complexes in the kidneys that can re-

sult in nephritis (Das 2008). On peripheral blood smear, the parasite shows the classic and diagnostic "band" form as well as a schizont with few merozoites and a central pigment globule (golden in color) refer to as a "daisy" form.

Clinically, patients who are ill with malaria symptoms and show forms suggestive of Pm should be evaluated for *P. knowlesi* as well as Pf because the detection of symptoms and/or the likelihood of co-infection is higher than a truly symptomatic Pm patient (Singh and Daneshvar 2013).

### *P. knowlesi*

*P. knowlesi* (Pk) is found in a limited distribution in Malaysian/Indonesian Borneo with cases reported in other southeast Asian countries, including Vietnam, Singapore, Myanmar, Cambodia, Thailand, and the Philippines (Muller and Schlagenhauf 2014). Exposure to mosquitoes that feed on long-tailed and/or pig-tailed macaques (Singh and Daneshvar 2013) is required for transmission as no human-to-human (via mosquito) transmission has been reported. In vitro work has shown that the parasites prefer young red blood cells but can, over time, adapt to infect older human red blood cells, a phenomenon that currently limits rapid spread of the infection beyond the human:monkey milieu (Lim et al. 2013). The disease presents like other malarias with fever/chills and headache with uncommon features like nausea/vomiting, myalgia/arthralgia, upper respiratory symptoms, and jaundice (Muller and Schlagenhauf 2014). Although rare, fatal complications of Pk have occurred and do so with higher frequency than seen in Pv and Pf proportionally (Singh and Daneshvar 2013) owing to the new emergence in humans (zoonosis) and absence of sufficient time for human adaptability. Although Pk is not unique among the nonhuman vertebrate malarias that have been transmitted to humans, the current emergence of a large population distribution of a disease with high mortality has not been previously reported and warrants careful attention (Ta et al. 2014).

## SEVERE MALARIA

### *P. vivax*

During an infection with only Pv and no other comorbidities, death from the disease is exceedingly rare (if not unheard of). However, in the presence of comorbidities, severe disease and fatal outcomes are reported. Because of the relapsing phenotype of the liver, chronic disease can lead to severe anemia and malnutrition, which predispose to coinfections and a poor immune response (Dumas et al. 2009; Anstey et al. 2012; Costa et al. 2012). Like severe Pf and Pk (and any severe infection), the final common pathway can include respiratory distress, hepatorenal failure, and shock (Anstey et al. 2012). Coma has been reported rarely in Pv infection but the cause of this coma is not the same as is seen in Pf (in which parasite sequestration to a high level in the brain is seen in fatal cases).

### *P. knowlesi*

The rate of severe disease in Pk is higher (~8%), proportionally, than is seen in Pf or Pv and has higher mortality (3%) (Antinori et al. 2013). Similar to severe Pf malaria in adults, Pk-severe disease typically presents with the same initial constellation of fever, etc., and progresses in severe disease to include hypotension, respiratory distress, acute renal failure, hyperbilirubinemia, and shock (Rajahram et al. 2012; Antinori et al. 2013). Coma, as is required for a Pf cerebral malaria diagnosis, is not always seen in Pk fatal cases. The "common pathway" of any severe infection (i.e., may be seen in Pf, bacterial sepsis, etc.) is the result of an exaggerated human immune response in the presence of an untreated or delayed in treatment infection and probably not a result of specific mechanisms of the organisms. In fact, Pk as a cause of other morbid conditions (Gram-negative sepsis) has been reported. Pathologically, where Pf shows intense sequestration in the brain along with congestion and possibly brain swelling, Pk has a similar appearance in tissue with a curious lack of ICAM-1 in the brain (Cox-Singh et al. 2010; Menezes et al. 2012). The Pk family of genes homologous to Pf *var* genes is the SICAvars,

which are larger in structure (12 vs. two exons) and quantity (>200 vs. 60) than the *var* genes of Pf (Lapp et al. 2009). The exact mechanism and interactions of Pk with human endothelium to produce sequestration are yet to be elucidated.

## PLACENTAL MALARIA

The Pf parasite can uniquely cause a range of pathological changes in the setting of pregnancy due to the ability of the parasite to sequester paired with the large sink of novel placental molecules such as chondroitin sulfate (CSA). The parasite's PfEMP1 proteins that are products of the *var2CSA* genes bind to CSA as the parasites pass through the placenta, removing them from circulation, whereas non-CSA binding parasites continue circulating. Maternal antibody that has developed to malaria in previous infections appears to destroy the non-CSA binding parasites, whereas the placenta acts as a protected space for propagation. In addition to the direct effects of placental binding, mononuclear cell infiltrates may also be present and in very high numbers. Depending on when the malaria infection occurs during pregnancy, the placenta at examination may show pigment trapped in fibrin (older infection) or parasites and/or mononuclear cells (active infection). In addition to Pf, Pv has been reported to be associated with complications in pregnancy, including anemia, miscarriage, low birth weight, and congenital malaria (Anstey et al. 2012; Costa et al. 2012). The placental pathology in Pv, however, does not show the same degree of parasite or monocyte involvement and remains to be elucidated.

## SEVERE MALARIA ANEMIA

The pediatric population in areas of high transmission is uniquely susceptible to severe malarial anemia (SMA) during the first 2 years of life. When the children present, blood transfusion can be life-saving along with antimalarial drugs. The exact mechanisms of the pathways that lead to SMA are not well understood. The disruption of the immune response of monocytes and lymphocytes in the presence of hemozoin may lead to an inappropriate regulation of erythropoietin (through IL-6, regulated on activation, normal T-cell expressed, and secreted [RANTES], and macrophage inflammatory protein 1s [MIP-1s]) (Perkins et al. 2011). The removal of red cell membrane and red cells completely by the spleen in an accelerated fashion due to the presence of malaria has also been suggested.

## ACIDOSIS

Acidosis is a complex metabolic state with a range of etiologies (Planche and Krishna 2006; Taylor et al. 2012). Within malaria, acidosis is caused by a combination of several factors. The malaria parasite produces *Plasmodium* lactate dehydrogenase (pLDH), which creates lactic acid leading to decreased pH. Respiratory distress is a common feature of severe malaria and, through sequestration, somnolence, and/or brain swelling, direct central suppression of the respiratory centers leads to irregular breathing patterns in the setting of acidosis, which may contribute to the pH imbalance. Supportive therapy to protect the airway and more aggressively rebalance the pH may decrease mortality (Cheng and Yansouni 2013).

## CEREBRAL MALARIA

The unique ability of *P. falciparum* to bind to endothelium produces the clinicopathological syndrome of CM in both children and adults. In highly endemic settings, children under 5 years are at highest risk for the disease with a mortality of 10% to 20%. In low endemic settings, all ages are at risk and mortality can be higher in adults. In the nonimmune population (e.g., travelers, military, etc.), a low level of infection (<1% parasitemia) can result in clinical signs of CM and be life-threatening. The clinical manifestations of CM may start with a typical malaria presentation and quickly (over minutes to hours) degenerate to a comatose state. After exclusion of other possibly causes of coma (e.g., postictal state, hypoglycemia, meningitis, bacterial sepsis, head trauma/cerebral bleed, etc.), a clinical diagnosis of CM can be made, which is

best confirmed by examination of the retinal for signs of malaria retinopathy (Seydel et al. 2015). In any case, the diagnostic pathological feature of the disease—at autopsy—is the presence of *P. falciparum* parasites in greater than 20% of capillaries in the brain by either tissue smear or histological sections (Taylor et al. 2004). Other pathological features that are variably present include fibrin thrombi, ring hemorrhages, discoloration of the brain, axonal injury, and capillary leakage (Dorovini-Zis et al. 2011). Brain vessels will appear congested in all cases with brain swelling more prominent in acute deaths such as African pediatric patients (<48 hours). Multiorgan failure and acute respiratory distress syndrome with diffuse alveolar damage is more common in adult patients, particularly those that have a prolonged course of disease (Hanson et al. 2010, 2014; Medana et al. 2011; Ponsford et al. 2012; Prapansilp et al. 2013; Maude et al. 2014).

The pathobiology of CM is not completely understood but a large body of evidence from both clinical and pathological studies has implicated a series of events and pathways at work within the disease landscape. Endothelial activation to a more "sticky" state is the first step. During the early phases of any malaria infection, macrophage stimulation leads to TNF-α production, the increase of which stimulates display of adherence molecules in the brain endothelium like intracellular adhesion molecule 1 (ICAM-1). Other such stimulations lead to a variety of other up-regulation events as summarized in Figure 1. A large number of malaria parasites can bind, through PfEMP1, to ubiquitous molecules such as CD36 ( platelets and endothelium outside of the brain), which logically explains both the thrombocytopenia of malaria infection as well as the very low incidence of CM compared with all malaria infections. In the African child under 5 years of age, the combination of factors leading to the increase in the "sticky" phenotype is most likely a delay in treatment of a malarial infection paired with a lack of well-developed protective specific antibodies in the setting of poor general health (e.g., malnutrition). For infected individuals outside of this setting, the total lack of immunity drives the disease. The capacity of the human immune system to clear a disease, which relies almost exclusively on macrophage phagocytosis is the macrophage compartment. Antibodies speed this process by increased uptake efficiency. In autopsy studies of children, the spleen, the primary site of clearance of the parasites in most CM patients shows a large burden of malaria pigment and macrophages but no parasites. This suggests that the clearance ability of the spleen is high and efficient. Where the process of clearance breaks down may be in the capacity of circulating monocytes/macrophages to keep up with the sequestration and parasite life cycle (every 48 hours).

## OVERLAP IN SEVERE DISEASE

Severe malaria in African children is often a monosyndromic presentation with little, if any, overt complications beyond coma, anemia, and/or acidosis. Studies suggest that in adult patients, the disease has multiple modalities at play in fatal cases including respiratory, hepatic, renal, and vascular complications. Equivalent studies in pediatric cases suggest that evidence of early changes in these same pathways may be at work; however, the rapid pace from presentation to either death or recovery does not allow these additional processes to manifest. In pediatric patients in Africa, an overlap of SMA, acidosis, and/or CM can occur, which may lead to higher mortality in overlap groups. CM, as there is not current ancillary treatment beyond antimalarias and supportive therapy, remains a key factor in mortality outcomes.

## CONCLUDING REMARKS

Malaria pathogenesis has a broad and narrow context depending on the frame of reference. For fatal disease, the sequestration of Pf in tissues along with up-regulation of cytokines, toxic substances, and a lack of adequate, timely therapy, are key features of the process. For the remainder of malaria infections, the negative aspects of the disease are results of imbalance in the parasite–human interaction for a given species with the exception of Pk, a true zoonosis. As eradication moves from a goal to a ra-

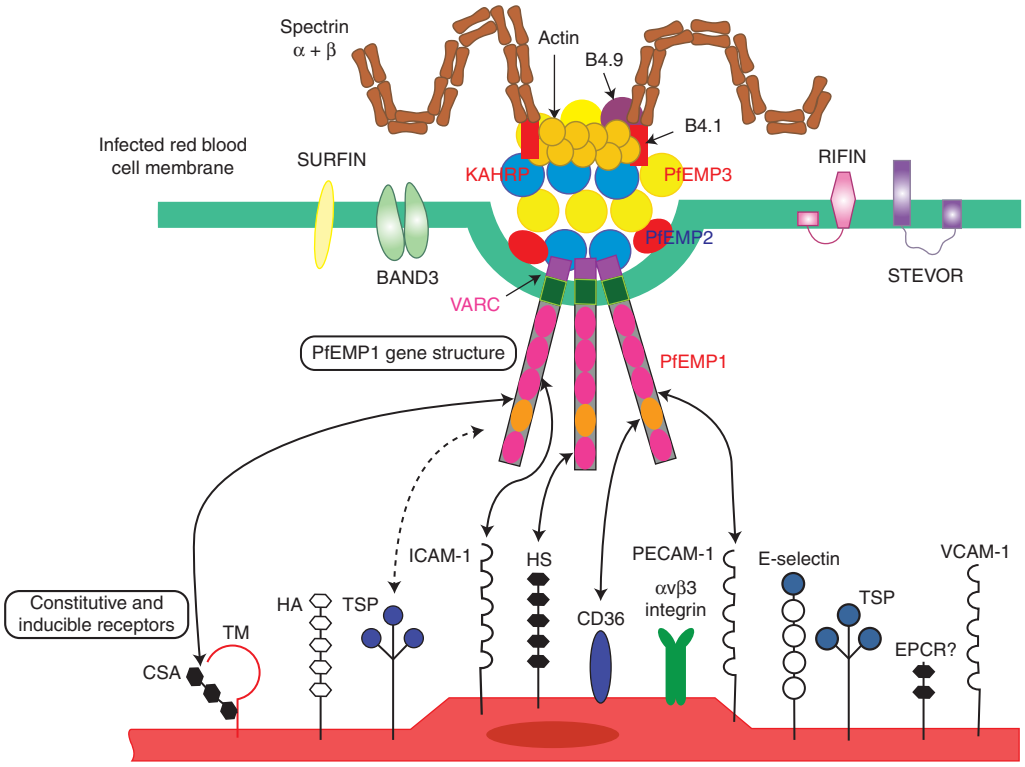


**Figure 1.** A model of the interactions between *Plasmodium* parasites (predominantly from *Plasmodium falciparum*, *top*) and the human endothelium (*bottom*) is shown. The prominent feature is the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) molecules that protrude from the raised knob structures, which in themselves are made of a combination of human and parasite proteins in a tight complex. Surfins, rifins, and stevors from the parasite are also located in the red cell membrane. On the human side, a range of molecules, depending on tissue, are involved infected parasite interactions, including those that are always present on endothelium (e.g., CD36, outside of the brain and on platelets), are present during activation (e.g., intracellular adhesion molecule 1 [ICAM-1]), and are activated during interaction with other molecules (e.g., endothelial protein C receptor [EPCR] and thrombospondin [TSP]). Special tissue situations include chondroitin sulfate (CSA) in the placenta and CR-1 on uninfected red blood cells (which mediates rosette formation).



tional plan of action, a firm understanding of the pathogenesis of malaria in all patient groups is required to not only predict where disease (especially severe disease) will occur but be able to prevent it.

illness with malaria affects the person, their family, and their society and our hope through this review is to bring us many steps closer to an end to malaria for everyone.

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