Malaria: Biology and Disease

Alan F. Cowman,[1,2,*] Julie Healer,[1] Danushka Marapana,[1,2] and Kevin Marsh[3,*]
[1]Walter and Eliza Hall Institute of Medical Research, Melbourne 3051, Australia
[2]Department of Medical Biology, University of Melbourne, Melbourne 3052, Australia
[3]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Headington, Oxford OX3 7BN, UK
*Correspondence: cowman@wehi.edu.au (A.F.C.), kevin.marsh@ndm.ox.ac.uk (K.M.)
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Malaria has been a major global health problem of humans through history and is a leading cause of death and disease across many tropical and subtropical countries. Over the last fifteen years renewed efforts at control have reduced the prevalence of malaria by over half, raising the prospect that elimination and perhaps eradication may be a long-term possibility. Achievement of this goal requires the development of new tools including novel antimalarial drugs and more efficacious vaccines as well as an increased understanding of the disease and biology of the parasite. This has catalyzed a major effort resulting in development and regulatory approval of the first vaccine against malaria (RTS,S/AS01) as well as identification of novel drug targets and antimalarial compounds, some of which are in human clinical trials.

Introduction

Malaria remains a disease of global health importance with 3.3 billion people in 97 countries at risk, leading to an estimated 200 million cases and around 600,000 deaths (WHO, 2015). Six plasmodial species present a significant health threat for humans; *Plasmodium falciparum* is usually considered the most important in terms of deaths, and this Review will focus largely on this parasite. *P. vivax* is a major cause of illness across large parts of the world, and it is increasingly argued that deaths, due to this parasite, have been underestimated (Naing et al., 2014). *P. ovale curtisi, P. ovale wallikeri*, and *P. malariae* are much less common causes of significant disease. Recently the simian parasite *P. knowlesi* has emerged as a local but important cause of disease (including severe disease) in Malaysia and other areas of southeast Asia, where it is predominantly a zoonosis, with no definite evidence of primary human-to-human transmission (Ahmed and Cox-Singh, 2015).

Historically *P. falciparum* has probably exerted greater selective pressure on human evolution than any other pathogen. Despite *P. falciparum's* presence throughout the tropics, the health impact is far from even, with the large majority of the world's parasitized individuals in Asia and south Asia (reflecting the significant human population) and 90% of deaths occurring in Africa, mostly in children (WHO, 2015). Over recent years there have been renewed calls for elimination and eventual eradication of malaria. In this Review, we will outline the major features and recent developments in understanding the biology, epidemiology, and clinical consequences of malaria because they will be critical in developing new approaches for prevention and treatment necessary to tip the balance from control to elimination.

Biology of Malaria

Plasmodium spp. are global pathogens with a complex life cycle alternating between female *Anopheles* mosquitoes and vertebrate hosts that require the formation of unique zoite forms to invade different cell types at specific stages (Figure 1). Once sporozoites enter the host, they infect hepatocytes, and this is followed by the asexual cycle in the blood. Sexual forms that develop during the blood stage are ingested by a feeding mosquito, completing the cycle. In this Review we will concentrate specifically on the stages within the human host, in particular the liver and blood stages.

Infection of the Liver

Plasmodium sporozoites are injected into the host dermis during a blood feed (Figure 1). The fate of these sporozoites is not well understood, but they can take 1–3 hr to exit from this site. Here, they rely on gliding motility, a random process enabling a proportion to reach and penetrate a blood vessel to enter the bloodstream. Those remaining in the skin can be destroyed and are drained by the lymphatics, where a host immune response is generated. The protein Trap-like protein (TLP) plays a role in exit from the dermis, as mutant sporozoites lacking its function display normal gliding motility but cannot enter the circulation. Those that enter the blood stream quickly access the liver by a process known as traversal. This involves crossing the sinusoidal barrier comprising fenestrated endothelial cells and macrophage-like Kupffer cells (Tavares et al., 2013). Proteins required for traversal include SPECT (sporozoite microneme protein essential for traversal) (Ishino et al., 2004), SPECT2 (also known as perforin-like protein 1, PLP1) (Risco-Castillo et al., 2015), CelTOS (cell traversal protein for ookinetes and sporozoites), phospholipase (PL) (Bhanot et al., 2005), and gamete egress and sporozoite traversal protein (GEST). The function of these proteins in cell traversal is not understood, although SPECT2 has a membrane attack complex/perforin-like (MAC/PF) domain, suggesting that it plays a role in punching holes in membranes. Sporozoites traverse through cells by forming a transient vacuole, and SPECT2 and pH sensing is involved in egress from this structure (Risco-Castillo et al., 2015).

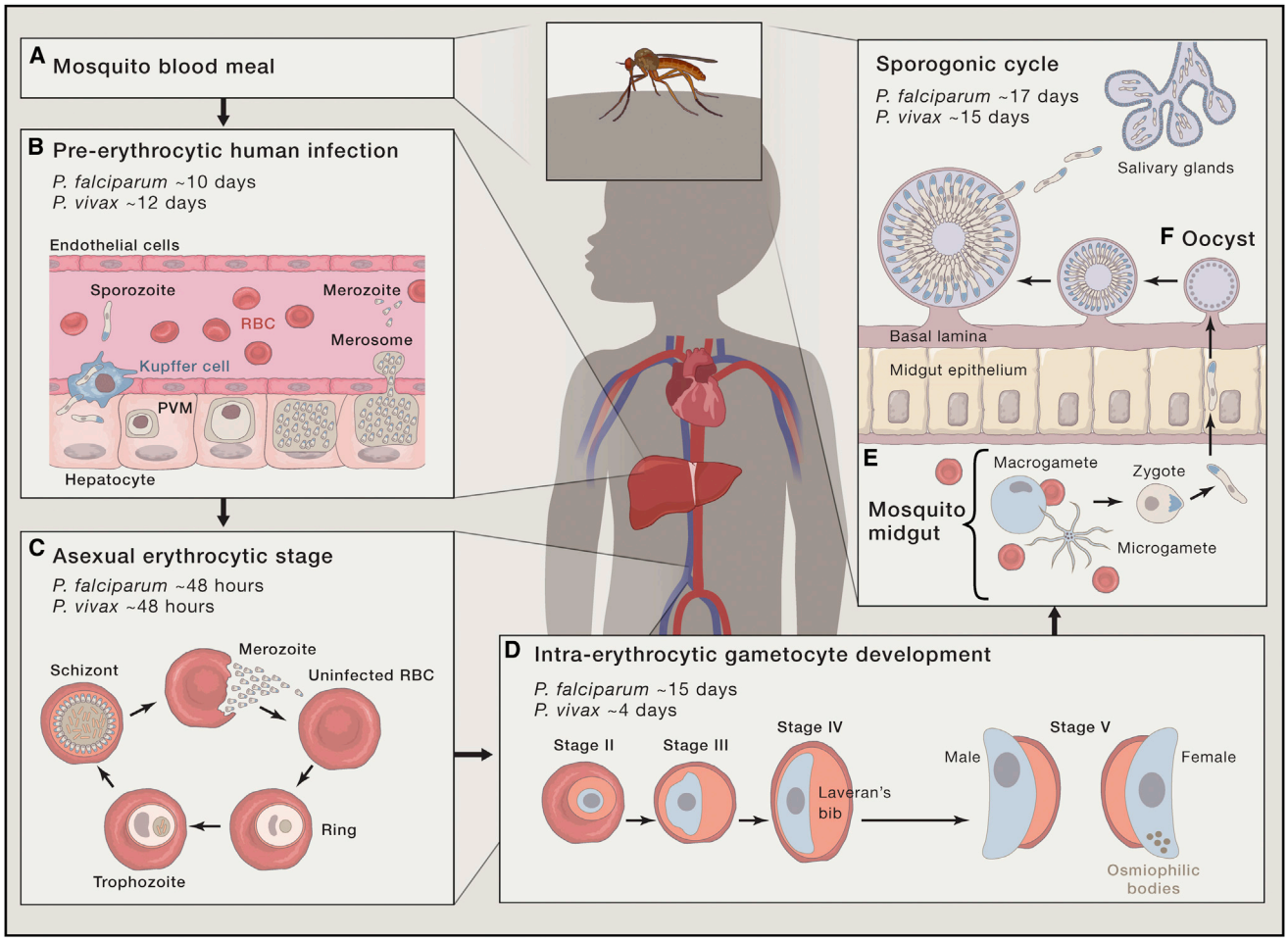

Figure 1. Life Cycle of *P. falciparum*

(A) Malaria infection is initiated with the injection of sporozoites (spzs) into the dermis by a feeding female anopheline mosquito.

(B) The spzs enter the vasculature and are transported to the liver, where they exit the sinusoids through Kupffer or endothelial cells and enter a hepatocyte. Active invasion is preceded by cellular traversal until a suitable hepatocyte is found. They form a PVM and undergo schizogony until tens of thousands of daughter merozoites are released in packets of merosomes into the vasculature.

(C) There they encounter erythrocytes and begin a chronic cycle of asexual schizogony in the bloodstream.

(D) A proportion of asexually reproducing merozoites are reprogrammed to undergo gametocytogenesis.

(E) Within a 15 day period, gametocytes sequester and develop within the bone marrow and, once mature, enter the peripheral circulation for ingestion by a mosquito where they emerge as extracellular male and female gametes in the midgut.

(F) Mating occurs by fusion of micro- and macrogamete to form a zygote transforming over 24 hr into a ookinete that migrates through the mosquito midgut epithelium and encysts to become an oocyst where asexual sporogenic replication occurs.

Motile sporozoites are released into the hemocoel by oocyst rupture and pass into salivary glands where they can be injected into the next human host.

It has been suggested that cell traversal through the sinusoidal barrier is important for infectivity by priming the sporozoite for invasion of hepatocytes, the cells in which sporozoites develop. However, the primary role of sporozoite traversal is crossing the sinusoidal barrier (Tavares et al., 2013). Sporozoites injected into the dermis are in "migratory mode" and upon interaction with hepatocytes convert to "invasive mode." One signal for this switch is recognition of hepatocytes through binding higher sulfated forms of heparin sulfate proteoglycans (HSPGs) activating calcium-dependent protein kinase 6 (CDPK6) (Coppi et al., 2007). The tetraspanin CD81 and scavenger receptor B1 (SR-B1) are human hepatocyte surface proteins required for invasion and formation of a parasitophorous vacuole by *P. falciparum* sporozoites (Rodrigues et al., 2008). In contrast, the hepatocyte receptor EphA2 is not required for hepatocyte invasion but for intra-hepatocytic development by establishment of the parasitophorous vacuole through interaction with parasite proteins p52 and p36 (Kaushansky et al., 2015).

A dense coat covers the sporozoite, and a key protein is the circumsporozoite protein (CSP), consisting of a highly repetitive region and a type I thrombospondin repeat (TSR). Invasion of hepatocytes requires binding of CSP to highly sulfated proteoglycans (HSPGs), activating processing of CSP and removal of the N terminus exposing the TSR domain (Herrera et al., 2015). Subsequent steps involve proteins, including thrombospondin-related anonymous protein (TRAP) and apical membrane antigen-1

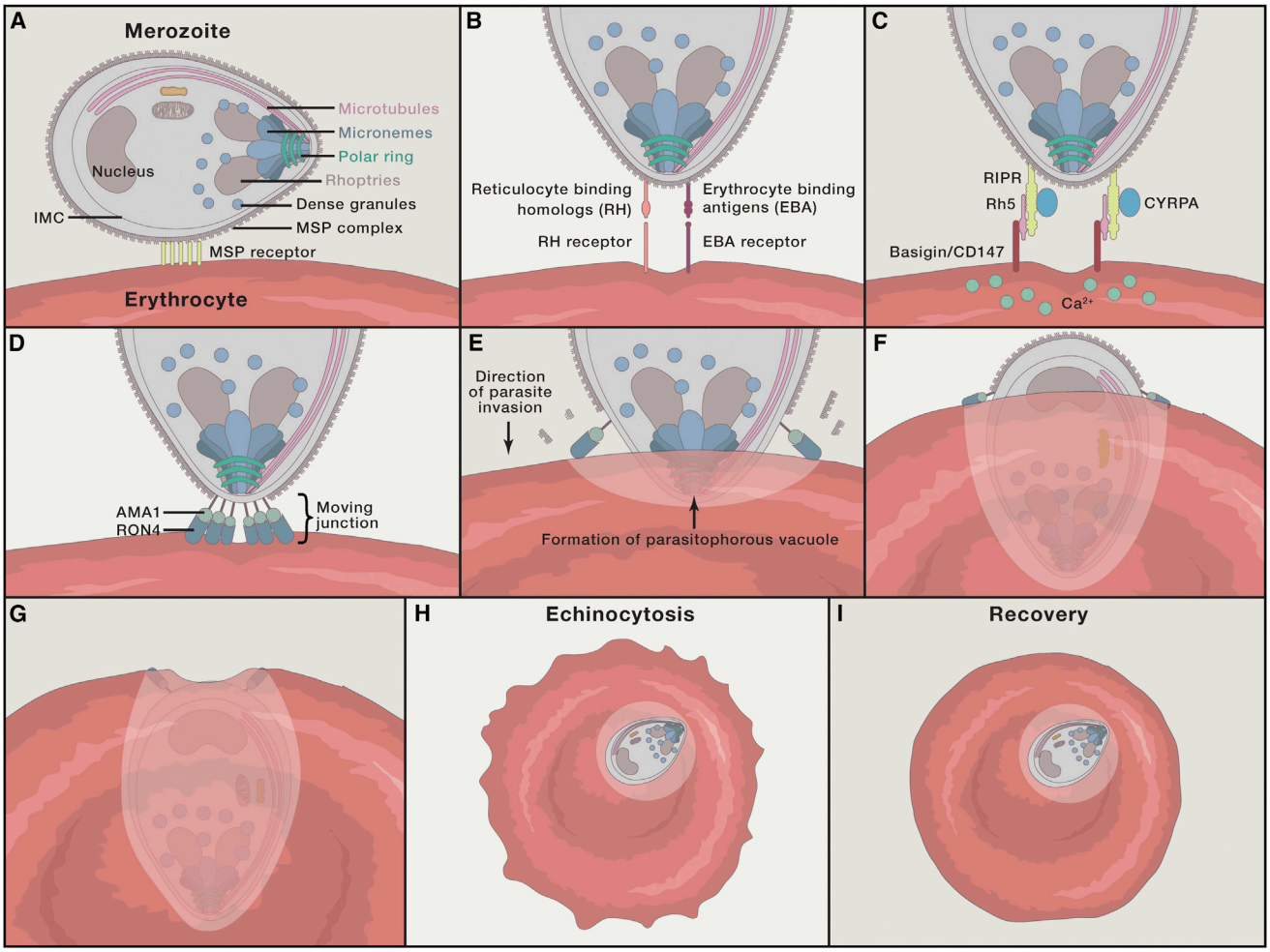

Figure 2. Merozoite Invasion of Erythrocytes

Contact between merozoite and erythrocyte is mediated by low-affinity interactions between red cell and merozoite surface coat proteins (A) wrapping the erythrocyte membrane around the merozoite such that reorientation occurs, bringing the apical end of the merozoite into direct contact with the erythrocyte membrane. Specific ligand-receptor interaction follows (B), mediated by proteins of the EBA and PfRh family members. This initiates downstream invasion events that involve binding of the PfRh5 complex to the host cell receptor basigin (C); this is associated with a calcium flux into the erythrocyte and microneme secretion, allowing deposition of the RON complex into the erythrocyte membrane and then allowing AMA-1 to bind directly to form a moving junction (D). By the reverse mobilization of a merozoite-internal actomyosin complex, the merozoite pushes into the erythrocyte membrane. This is concurrent with discharge of rhoptry contents that contribute to formation of a parasitophorous vacuole membrane (PVM) that will enclose the merozoite (E–G). Upon sealing of the PVM and the erythrocyte plasma membrane, a period of echinocytosis occurs due to loss of water from the erythrocyte cytosol (H). Recovery of erythrocyte homeostasis follows (I).

(AMA-1), with adhesive domains released from the apical organelles (micronemes and rhoptries).

Once hepatocyte infection is established, the sporozoite transforms over the subsequent 2–10 days to a liver stage (LS) or exo-erythrocytic form (EEF), and development culminates in release of up to 40,000 merozoites per hepatocyte into the bloodstream by budding of parasite-filled vesicles called merosomes (Sturm et al., 2006) (Figure 1). Investigation of *P. falciparum* EEF transformation in vivo has been enabled by availability of human liver chimeric mice, providing a crucial window into previously unknown biology (Vaughan et al., 2012). DNA replication begins day 2 post-invasion, and parasites remain within a parasitophorous vacuole membrane into late LS development.

Erythrocyte Invasion

Once released into the hepatic circulation, free merozoites invade erythrocytes in a fast, dynamic, and multi-step process including pre-invasion, active invasion, and echinocytosis (Weiss et al., 2015) that is complete within 2 min (Figure 2). Pre-invasion is the initial interaction of merozoites with erythrocytes, and little is understood about the molecular details of this step. Merozoite surface protein 1 (MSP1) is the major glycophosphatidylinositol (GPI)-associated protein on the merozoite surface (Holder, 1994). MSP1 acts as a platform on the merozoite surface for at least three large complexes with different extrinsic proteins that bind erythrocytes (Lin et al., 2016). Merozoites lacking surface MSP1 can invade erythrocytes, suggesting that it is not absolutely required for invasion (Das et al.,

2015). It is possible that MSP1 is involved in display of proteins involved in evasion of host responses rather than directly in merozoite invasion.

Pre-invasion involves robust interaction between the merozoite and erythrocyte resulting in parasite actomyosin motor-driven deformation of the host cell (Weiss et al., 2015) (Figure 2). This step involves two ligand families of type 1 membrane proteins in *P. falciparum*, the erythrocyte binding-like proteins (EBLs) and *P. falciparum* reticulocyte-binding protein homologs (PfRhs). These protein ligands bind specific receptors including glycophorin A, B, C and complement receptor 1 (CR1). Although the functions of individual members of these families display redundancy, their overall function is essential in *P. falciparum* (reviewed in Tham et al., 2012). PfRh and EBL proteins also play a role in signaling activation of subsequent steps in invasion. Exposure of *P. falciparum* merozoites to low-potassium ion concentrations in blood plasma, after egress from the host cell, leads to a rise in cytosolic calcium levels through a phospholipase C-mediated pathway, triggering release of EBA-175, an EBL family member (Singh et al., 2010). Binding of EBA-175 to its receptor, glycophorin A, triggers release of proteins from the rhoptries. This work provides evidence of the importance of these protein families in sensing and binding to erythrocytes but also signaling downstream invasion events. Similarly, PfRh1 is linked to Ca^{2+} signaling in the merozoite (Gao et al., 2013), and phosphorylation of the cytoplasmic tail of PfRh4 by the *P. falciparum* casein kinase 2 (PfCK2) is required for invasion through the PfRh4-CR1 parasite-host interaction (Tham et al., 2015). Calcineurin is also involved in attachment of merozoites, perhaps through stabilizing the dimerization of EBL and PfRh proteins, as this is critical for host-receptor ligation and signal transduction for subsequent events in invasion (Paul et al., 2015).

Following deformation of the erythrocyte the merozoite appears to reorientate so the apical end abuts the erythrocyte membrane. This involves PfRh5, an atypical member of the PfRh family (reviewed in Tham et al., 2012) as it is not a type 1 membrane protein and it forms a complex with PfRipr (Rh5-interacting protein) (Chen et al., 2011) and CyRPA (cysteine-rich protective antigen) (Reddy et al., 2015). PfRh5 binds the host receptor basigin, which is essential for merozoite invasion (Crosnier et al., 2011). The PfRh5 complex-basigin interaction is associated with a Ca^{2+} influx into the host cell (Volz et al., 2016; Weiss et al., 2015).

Irreversible attachment of merozoites to erythrocytes occurs by formation of a tight junction formed between parasite-derived proteins, primarily AMA1 and the RON complex. The RON complex is deposited in the erythrocyte with RON2 spanning the host membrane and binding to AMA1 on the merozoite surface (Besteiro et al., 2011). Lipid-rich rhoptry contents form the parasitophorous vacuole membrane as the merozoite is propelled into the erythrocyte using force generated by the parasite actomyosin motor (Riglar et al., 2011). After the active invasion phase, fusion of membranes at the posterior end of the merozoite occurs to seal the parasite within the parasitophorous vacuole and erythrocyte. Echinocytosis follows and causes the erythrocyte to shrink and form spiky protrusions. This may be due to Ca^{2+} influx into the erythrocyte during interaction of the PfRh5 complex with basigin (Weiss et al., 2015).

Once erythrocyte infection is established, over the subsequent 48 hr, cell division (schizogony) results in 16–32 merozoites that egress when developed, which results in destruction of the erythrocyte membrane and explosive release of parasites to access new host cells for invasion. The coordinated process of merozoite egress is tightly regulated and involves a number of protein kinases, including the plant-like calcium-dependent protein kinase PfCDPK5 (Dvorin et al., 2010) and cGMP-dependent protein kinase (PfPKG) (Collins et al., 2013). MSP1 has a role in egress of merozoites from *P. falciparum*-infected cells through subtilisin 1 processing on the merozoite surface that activates its ability to bind the erythrocyte membrane protein spectrin (Das et al., 2015).

Transition to Transmission

During rounds of schizogony in the bloodstream, a proportion of parasites undergo a developmental switch initiating commitment to sexual development to form male and female gametocytes. The transmission of malaria from humans to mosquitoes is dependent on development of the sexual stages and this has been recognized as a potential intervention point, either through transmission-blocking drugs or vaccines. Although molecular events around this developmental switch remain elusive, the timing of transition occurs at some point in the previous schizogony cycle, and daughter merozoites from a single schizont-infected cell are committed to develop into either gametocytes or asexual schizonts. Environmental stimuli, such as high parasitemia and exposure to drugs such as chloroquine, are associated with increased conversion to gametocyte production, indicating that parasites can sense their environment. Extracellular vesicles containing protein, RNA, and DNA traffic between parasites in vitro, and this provides a means for cell-cell communication that increases gametocyte production (Mantel et al., 2013; Regev-Rudzki et al., 2013). Epigenetic regulation is critical for control of sexual differentiation, and the transcription factor AP2-G is a master regulator of gametocytogenesis (Kafsack et al., 2014). *P. falciparum* gametocyte maturation is an extended process relative to other species. Once commitment has initiated, it takes 11 days for mature gametocytes that are infectious to mosquitoes to develop. During this time, they remain sequestered within bone marrow (Joice et al., 2014), avoiding splenic clearance until emerging into the peripheral circulation for an unknown time until uptake by a feeding mosquito.

Remodeling of the Host Cell and Immune Evasion

Following invasion, the parasite activates a process of renovation converting a terminally differentiated cell, lacking most organelles, into one in which the intracellular parasite can grow and hide from host responses (reviewed in Boddey and Cowman, 2013). It does this by exporting hundreds of proteins out of the parasite and beyond the parasitophorous vacuole membrane to numerous locations within the parasite-infected erythrocyte (Figure 3). This is dependent on construction of a trafficking network in infected host cells for sorting and moving exported proteins to specific subcellular locations. Key features of this network are Maurer's clefts, membranous structures in the shape of flattened discs that bud from the parasitophorous vacuole after invasion (Grüring et al., 2012). These structures

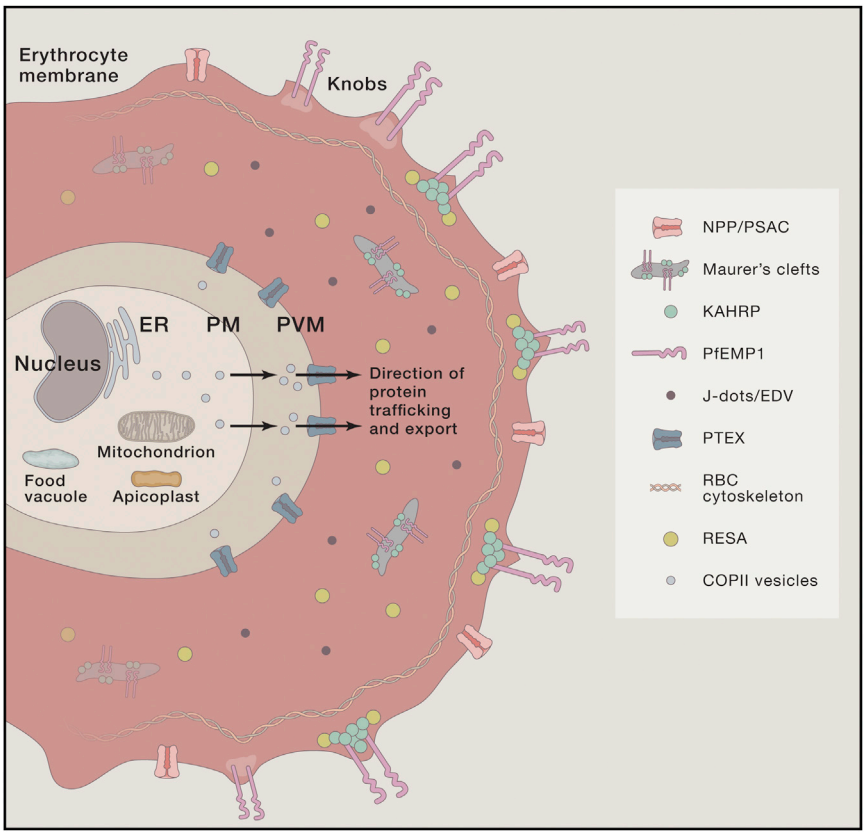

Figure 3. Protein Export-Induced Changes in the Host Erythrocyte

A slice through an erythrocyte infected by *P. falciparum* is shown. Following invasion, the parasite exports hundreds of proteins into the host cell. These proteins travel via a vesicle-mediated secretory pathway from the endoplasmic reticulum (ER), across the PM, and into the PV. At the PVM, a protein translocon (PTEX) mediates export into the host cell. Trafficking within the host cell is mediated by parasite-derived vesicular structures such as EDVs, J-dots, and membranous organelles, the Maurer's clefts. Their destination is the erythrocyte cytosol, cytoskeleton, or membrane. KAHRP induces elevated points at the erythrocyte membrane (known as knobs) from which the major virulence factor PfEMP1 is displayed to the extracellular environment. PfEMP1 mediates cytoadhesion to host cell receptors. Exported parasite proteins induce dramatic restructuring of the erythrocyte cytoskeleton to withstand higher temperatures and shear stress. New permeability pathways (NPP/PSAC) are established at the erythrocyte membrane allowing the parasite to scavenge nutrients and expel waste. Toxic heme is accumulated and stored within the food vacuole of the parasite. Abbreviations: ER, endoplasmic reticulum; PM, parasite membrane; PV, parasitophorous vacuole; PVM, parasitophorous vacuole membrane; PTEX, plasmodium translocon of exported proteins; EDV, electron-dense vesicles; KAHRP, knob-associated histidine-rich protein; RESA, ring-exported surface antigen; PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1.

become tethered to the underside of the erythrocyte membrane at about 20 hr post-invasion. The transmembrane protein MAHRP2 (membrane-associated histidine-rich protein-2) is a component of the Maurer's cleft tether. There are other smaller membranous structures, called EDV (electron dense vesicles) and J-dots, implicated in trafficking of proteins from Maurer's clefts to the host cell membrane (reviewed in Boddey and Cowman, 2013).

The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family is the most extensively characterized of the proteins exported to the surface of the *P. falciparum*-infected erythrocyte. Their display on the erythrocyte surface results in exposure to host antibodies, and the parasite is able to switch expression of antigenically distinct isoforms, a process known as antigenic variation to evade immune destruction. The ability of *P. falciparum* to maintain a chronic infection is largely due to the extreme diversification and tightly regulated expression of PfEMP1 proteins. Through their wide array of adhesive domains they allow parasites to sequester in deep capillary beds thus escaping splenic clearance (Figure 3). The different PfEMP1 proteins have distinct receptor-binding selectivity, and sequestration of *P. falciparum*-infected erythrocytes in the vasculature of different organs including the brain and placenta contributes to the severity of disease and the final outcome (Nunes-Silva et al., 2015). The central role of this protein family in malarial immunity and pathogenesis has recently been reviewed (Hviid and Jensen, 2015).

The Epidemiology of Malaria

Malaria is transmitted in 97 countries in the world. Limits of transmission are set by environmental effects (primarily temperature) on the ability of the mosquito vector to sustain parasite development. Within these limits, transmission level is determined by frequency of contact between infected mosquitos and humans, which is in turn affected by the vector density, their location, and their feeding habits. Transmission level is critical in determining the clinical picture of disease due to malaria in exposed communities. In areas where the risk of infectious bites is low and unpredictable, malaria is said to be unstable. Under such circumstances all ages are susceptible to infection, and in general all infective episodes will result in clinical disease. As exposure rises, eventually transmission becomes more consistent year-to-year, and exposed individuals begin to develop a degree of immunity.

The Relationship between Transmission and Disease Burden

Transmission intensity may range over five log orders from less than one infectious bite a year to many hundreds per day. Transmission intensity is often expressed in terms of the proportion of a population of defined age with parasitemia (usually asymptomatic) at a given time. In stably endemic areas severe disease and death are restricted mainly to young children, with older subjects developing a degree of protective immunity (Figure 4). The higher the transmission the faster immunity is acquired, and this affects the clinical spectrum of disease, with severe anemia dominating in areas of very high transmission, whereas cerebral malaria

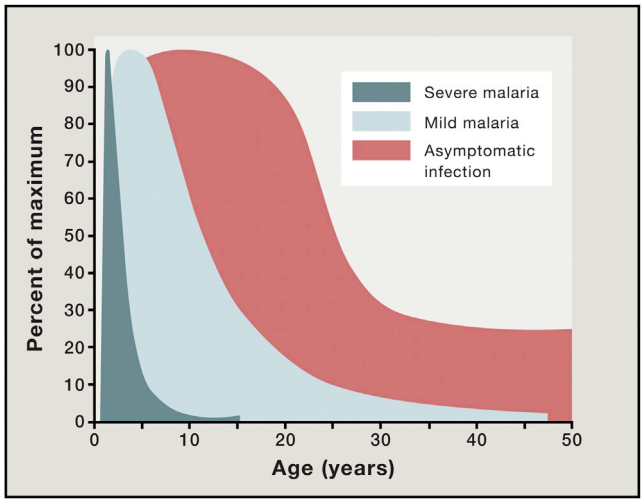

Figure 4. Schematic Illustrating the Development of Immunity to Different Manifestations of Malaria Infection in an Endemic Population

The age distribution of severe, mild, and asymptomatic infections is shown as a percentage of the maximum period prevalence achieved. Adapted from Langhorne et al. (2008).

becomes more dominant under moderate-to-low transmission settings. The relationship between severe malaria and transmission is non-linear (Snow et al., 1997), implying that control measures in a high-transmission setting may lead to marked reductions in transmission with less effect on disease (and by implication deaths) until transmission falls to a critical point, following which reductions in disease may be rapid. This picture is supported by ongoing observations across a number of areas in Africa. Even more important may be the relationship between malaria transmission and all-cause mortality. Long-standing suggestions that exposure to malaria leads to a disproportionately high number of deaths due to other causes (indirect mortality) have recently been given strong support from both epidemiologic studies and the results of direct interventions (Scott et al., 2011).

Naturally Acquired Immunity to Malaria

The typical picture of immunity in a stably endemic population is shown in Figure 4. Risk of severe disease and death is highest in young children and declines rapidly. Susceptibility to non-life-threatening clinical episodes of malaria continues but falls steadily such that by early adulthood febrile episodes are few and mild. Risk of infection continues throughout life. This picture is sometimes interpreted as showing acquisition of immunity to disease ("anti-disease immunity") precedes acquisition of immunity to parasite replication ("anti-parasite immunity"), but this obscures a key aspect of malaria biology: even in a single episode in a naive subject who survives the initial wave of parasitemia, a balance is quickly struck whereby long periods are spent with low-level asymptomatic parasitemia. Thus, even at the time when children are most susceptible to severe disease and death, their *normal* state is to be asymptomatically parasitized. This implies that at this stage immunity is acquired to each parasite isolate that is survived ("strain-specific immunity"), leading to the idea that over time increasing immunity is due either to the acquisition of a repertoire of responses to many different isolates or to the development of a cross-protective response to shared antigenic targets.

Although antigenic polymorphism likely contributes to the relative slowness of acquisition of protective immunity, there is also considerable evidence that malaria infection modulates the host immune response (reviewed in Hviid and Jensen, 2015). Many young children have remarkably short-lived antibody responses to a number of antigens that often fail to boost on re-exposure. The reasons for this are not understood as B cell memory in young children seems to be established and to persist over long periods, even in the absence of continuing infection (Ndungu et al., 2012).

Potential Mechanisms of Acquired Immunity to Malaria

Immune responses could potentially target any stage of the parasite to which humans are exposed. Although highly protective humoral and cellular immune responses can be developed against both the sporozoite and the intra-hepatic schizont in a number of animal model systems and in humans by vaccination approaches (Riley and Stewart, 2013), there is little evidence that pre-erythrocytic responses are important in naturally acquired immunity in humans. Similarly, although humans in endemic areas make antibody responses to sexual stages, and experimental transmission blocking vaccine approaches against these stages are promising (Wu et al., 2015), it is not clear what effect, if any, naturally occurring responses have on transmission.

A large body of evidence, beginning with the direct ability of gamma globulins from immune adults to rapidly control and reduce parasitemia in children (Cohen et al., 1961), supports the idea that naturally acquired immunity is predominantly against blood stages and is antibody mediated. Antibodies could potentially act at two points: against the free merozoite before it invades erythrocytes or against the infected red cell itself. There is clear evidence that responses to both may produce effective protection (Langhorne et al., 2008). In the case of the merozoite such responses could block the ability of the merozite to enter the red cell or lead to cell-mediated destruction of merozoites through a number of mechanisms including opsonophagocytosis, antibody-dependent cellular inhibition (ADCI), or neutrophil-mediated killing. Although its location within the host erythrocyte would seem to offer protection from immune attack, the parasite remodels the host cell surface extensively, exposing an array of altered host cell and parasite antigens. Antibodies against the infected erythrocyte surface could potentially protect by a number of mechanisms (reviewed in Chan et al., 2014), including cell-mediated ingestion or killing, or through blocking cytoadhesion of the infected erythrocyte to endothelial surfaces, making it susceptible to removal in the spleen. Responses to the infected cell surface have been shown to protect from clinical disease, including severe malaria (Bull et al., 1998).

Identifying the antigenic targets for these protective responses has been challenging. For targets on the infected erythrocyte surface the responses to PfEMP1 are most important, but a significant effect of responses to other surface-exposed multigene families cannot be excluded (Chan et al., 2012). So far as the merozoite is concerned, for many years only a handful of cloned merozoite antigens were available for immune-epidemiological

studies, and results were inconsistent between studies (Fowkes et al., 2010). Following the sequencing of the *P. falciparum* genome, several groups have studied much larger numbers of merozoite antigens and identified a number of promising candidates (Osier et al., 2014). By applying the concept of breadth and depth (i.e., combinations of antibody responses to several antigens above a concentration threshold), combinations of responses that appear to give very high levels of protection have recently been identified.

Clinical Features of Disease and Pathogenesis

Malaria infection in a naive individual almost invariably produces a febrile illness. The accompanying symptoms are non-specific and often include rigors, headache, nausea, and muscle pains. If treated with appropriate drugs at this stage the symptoms remit over a few days, though often with considerable exhaustion. In the case of *P. falciparum*, complete treatment will eradicate the infection, and any return of symptoms reflects incomplete treatment, resistance to the drugs used, or new infection. In the case of *P. vivax* and *P. ovale*, subsequent infections may reoccur at intervals as a result of reactivation of the dormant liver-resident hypnozoite stage, unless this is cleared by a prolonged treatment with an 8-aminoquinolone drug. The natural history of untreated or partially treated infection is well known from many thousands of observations made during the period when induced infections with both *P. falciparum* and *P. vivax* were used to treat neurosyphilis (Collins and Jeffery, 1999). Generally, after a period of symptoms of varying severity, illness subsides and parasite densities are controlled at a low level, with symptoms recurring at intervals over the ensuing weeks and months, associated with rises in parasitemia. Successive waves of parasitemia tend to be lower and symptoms less marked until eventually the infection resolves.

Severe Malaria

In a proportion of untreated or partially treated individuals the initial infection is not controlled and progresses to severe or complicated malaria, which may lead to death. The picture of severe malaria varies with both age and transmission level (reflecting the immune status of populations). In Africa, most malaria deaths occur in children and are dominated by three syndromes that can occur separately or in combination: severe anemia, cerebral malaria, and respiratory distress (Marsh et al., 1995). Cerebral malaria is functionally defined as the presence of coma caused by *P. falciparum.* This clinical picture may have a number of causes and is not necessarily synonymous with the histopathological description of cerebral malaria, where there is extensive sequestration of mature-infected parasites in the cerebral micro-vasculature (Taylor and Molyneux, 2015). Respiratory distress in children with severe malaria presents with metabolic acidosis largely reflecting tissue hypoxia. Although acute malaria infection is associated with destruction of both infected and uninfected red cells, severe malarial anemia in young children probably reflects a final ''tipping over'' on a background of chronic anemia in which both immune mechanisms and ineffective erythropoiesis play a role.

In stably endemic areas of Africa severe malaria in older children and adults is very rare because of naturally acquired immunity, and so our understanding of the syndrome in older subjects is based on studies in areas of the world with lower transmission, particularly in Asia. Here, although cerebral malaria, hypoglycaemia, and anemia form part of the picture, severe malaria is more commonly a multi-system disorder often with marked renal and hepatic dysfunction (which are rare in children). Respiratory distress is often due to pulmonary edema, which rarely occurs in children but has high mortality in adults. As previously highly endemic areas of the world experience declines in transmission (and therefore of population immunity), the mean age of severe disease and death is rising, and the pattern of severe disease is changing to that seen in older patients in other parts of the world.

Pathogenesis of Severe Falciparum Malaria

The fundamental features of *P. falciparum* infection that contribute to pathogenesis of severe disease and lead to death are exponential parasite growth, induction of host inflammatory responses, and microvascular obstruction due to adherence of mature parasites to blood vessels and endothelial activation (Wassmer et al., 2015). Exponential parasite growth with greater than 10-fold increase every 48 hr means that high total body parasitemia is achieved quickly. The importance of this may be masked by the phenomenon of sequestration of large parts of the parasite biomass adhering to the linings of blood vessels, meaning that parasite load measured by microscopy is underestimated. Estimates of biomass based on parasite products such as histidine-rich protein (HRP) provide a much clearer picture (Hendriksen et al., 2013).

The exponential phase of intravascular parasite growth triggers an acute inflammatory response. It has been argued that severe malaria arises as a side effect of excessive or poorly controlled responses that have primarily evolved to control acute infection (Cunnington et al., 2013). Systemic levels of some pro-inflammatory cytokines are correlated with increasing severity and death in a number of studies (Kwiatkowski et al., 1990), and IL-1β levels at birth are reported to be predictive of both future IL-1β levels and risk of severe malaria in childhood. A marked pro-inflammatory response probably contributes to the pathogenesis of severe disease in numerous ways, including both systemic and local effects (Cunnington et al., 2013).

Microvascular Obstruction

A central feature of severe malaria due to *P. falciparum* is impairment of tissue blood flow associated with sequestration of mature parasites in microvascular beds. This contributes to both systemic effects such as metabolic acidosis and local end organ damage, most dramatically in the brain. Sequestration and subsequent impaired flow have been directly observed and quantified in a number of tissue beds including the rectal mucosa and retina and correlate closely with clinical outcome, histological features in subjects who succumb to disease, and other prognostic markers such as acidosis (Dondorp et al., 2008).

Sequestration is mediated by modifications of the erythrocyte surface by the insertion and display of variant proteins able to bind receptors on a range of other cells (reviewed in Smith et al., 2013). This includes not only a large number of receptors on endothelial cells (leading to attachment within vessels) but also attachment to uninfected erythrocytes (a phenomenon known as rosetting) and to activated platelets, which leads to the formation of clumps of infected cells. Both rosetting and

clumping likely further contribute to microvascular obstruction and are associated with severity of disease.

Endothelial Activation

Endothelial activation plays a major role in the microvascular pathology of *P. falciparum*. Nitric oxide bioavailability is reduced (Yeo et al., 2014), and levels of angiopoietin 2 raised (Hanson et al., 2015), and these markers correlate with outcome. Endothelial activation also increases the expression of a number of receptors to which the infected erythrocytes can bind. Although systemic effects from a number of cytokines may be important in inducing endothelial changes, the interaction of infected erythrocytes with the endothelium may itself lead to local endothelial activation and thus induce a vicious circle. Recently, identification of endothelial protein C receptor (EPCR) as a ligand for PfEMP1-mediated infected erythrocyte binding provides a potential explanation for how cytoadherence may lead to local endothelial dysfunction and why the brain may be particularly prone to the consequences of sequestration. EPCR is expressed in most vascular beds and plays a central role in endothelial stabilization by promoting protein C activation. Binding of infected erythrocytes to EPCR through the CIDR domain of PfEMP1 blocks protein C activation and leads to highly localized coagulopathy (Turner et al., 2013). It is hypothesized that although EPCR-mediated cytoadherence will take place in many vascular beds, the localized coagulopathy is particularly likely to occur in the brain because of low constitutive expression of EPCR and TM (Moxon et al., 2013). The fact that the PfEMP1 variants able to bind to EPCR are those that have been associated with severe malaria (Turner et al., 2013) provides a potentially important unifying link between parasite and host factors mediating severity.

Interaction with Other Infections

Malaria is particularly a problem of vulnerable communities exposed to multiple and concurrent health risks that often interact. HIV infection is a major risk factor for severe disease and death due to malaria in both children and adults, and malaria in pregnant women is also associated with an increased risk of HIV transmission to the fetus (van Eijk et al., 2007). The most important interactions from a public health perspective are with invasive bacterial disease. In most cases of African children with severe malaria, the prevalence of concurrent invasive bacterial disease is far in excess of that which could occur by chance (Church and Maitland, 2014). Crucially, case fatality is much higher in those with dual infections. A number of mechanisms for the association have been proposed, including translocation of gram-negative organisms across leaky bowel, specific macrophage dysfunction, and functional hyposplenism (Gómez-Pérez et al., 2014). It is estimated that in some areas malaria may be responsible for 50% of all episodes of inflammatory bowel disease (IBD) (Scott et al., 2011), leading to the counterintuitive idea that malaria may cause more deaths indirectly than it does directly. This idea is strongly supported by the fact that massive reductions in all cause childhood mortality over a very short time period concurrent with aggressive malaria control.

Malaria in Pregnancy

In non-immune populations, malaria in pregnancy may be associated with stillbirth and severe disease in the mother with especially high risk of hypoglycemia. In endemic areas, where the populations develop a degree of immunity to malaria, infection leads to maternal anemia and low birth weight of the child (McLean et al., 2015). Although this is sometimes characterized as loss of immunity, this is not strictly the case; rather the presence of the placenta offers a new site for parasite sequestration through the selection of parasites able to bind to chondroitin sulfate A (CSA) on the syncitiotrophoblast (Khunrae et al., 2010). The increased incidence and density of peripheral parasites observed in pregnancy represents spill over from a new "privileged" site in a subject who retains previously effective immune responses. Over successive pregnancies women develop antibodies directed at specific PfEMP1 variants involved in CSA-mediated cytoadherence (Ataíde et al., 2014), and women in later pregnancies are significantly protected against adverse effects of infection.

Severe Malaria due to P. vivax

Historically *P. vivax* has been considered a relatively benign parasite. This idea was supported by the very low incidence of deaths from *P. vivax* in international travelers and minimal or no deaths reported from some geographical areas (Luxemburger et al., 1997). However, this view is increasingly challenged by many studies reporting significant rates of severe disease and death. In children in high-transmission areas the most common reported syndrome of severity is severe anemia, whereas in adults under lower transmission it is multi-organ failure (Rahimi et al., 2014). Two recent comprehensive analyses of published literature (Baird, 2013; Naing et al., 2014) concluded that rates of severe disease and death are broadly comparable in the two parasite species. However, there are substantial difficulties in interpreting many studies: differential diagnosis is often challenging, and hospital-based studies may lead to overestimates of rates (Rahimi et al., 2014). Nonetheless, although more high-quality epidemiological and clinical studies are required, it seems clear that the idea of *P. vivax* being in any sense a benign parasite cannot be sustained.

The fact that *P. vivax* causes significant amounts of severe disease and death (whatever the exact comparative figures) also raises critical questions about the pathogenesis of both *P. vivax* and *P. falciparum*. As *P. vivax* only achieves low parasitemias and does not undergo extensive intravascular sequestration, what does this mean for the idea that biomass and microvascular obstruction are key in *P. falciparum*? There is a dearth of modern autopsy studies in *P. vivax*, but in a recent exception (Lacerda et al., 2012) in 17 deaths attributed to *P. vivax* in Brazil, 13 were considered to be due to the parasite. The most common finding was of respiratory pathology with pulmonary edema and multi-organ failure, though there was no evidence of intravascular sequestration. Recently, Baird has hypothesized that a large part of the biomass may in fact be sequestered in extravascular spaces in hemopoeitic tissue (Baird, 2013). There is an urgent need for high-quality studies of the similarities and differences in both the clinical spectrum and associated pathogenic factors in severe malaria due to *P. vivax* and *P. falciparum*.

Antimalarials, Resistance, and New Drug Targets

Antimalarial drugs have been the mainstay of control and prophylaxis against malaria since the first use of quinine from the cinchona tree. Many drugs were developed against malaria in

the 20th century with the most important being chloroquine and artemisinin. However, the ability of *P. falciparum* in particular to develop resistance to these treatments has threatened their continuing efficacy and raised the importance of combinations as well as developing new drugs and novel targets. In response to the spread of parasite resistance to chloroquine and other antimalarials such as pyrimethamine and sulfadoxine, artemisinin-based combination therapies (ACTs) are now recommended for treatment of uncomplicated malaria, and their use has been an important part of the remarkable decrease in malaria globally. However, there are reports from Cambodia of decreasing sensitivity of *P. falciparum* to artemisinin derivatives where patients had significantly longer clearance times after monotherapy (Dondorp et al., 2009), and this has developed further into decreasing clinical efficacy and potential partner drug resistance. This has become a potential threat to the continued efficacy of ACTs and programs have been developed in an attempt to contain and eliminate artemisinin resistance.

Mechanism of Artemisinin Action and Resistance

Although it is not understood how artemisinin kills the malaria parasite, heme-mediated opening (arising from the degradation of hemoglobin) of the endoperoxide ring in its structure is essential for activity. This results in increased oxidative stress causing parasite death.

Genome-wide association studies and gene candidate-based approaches in *P. falciparum* are revealing potential mechanisms of resistance to artemisinin (Ariey et al., 2014; Mbengue et al., 2015; Mok et al., 2015; Straimer et al., 2015). Genome sequencing of parasites from patients that were treated with ACT and demonstrated reduced parasite clearance rates identified mutations in the gene encoding a K13 propeller protein, suggesting that this protein was an important determinant of artemisinin resistance (Ariey et al., 2014). Insertion of these mutations into the K13 locus conferred increased resistance in specific parasite lines not only showing that this gene was involved in the slow clearance phenotype but also suggesting that it was a complex phenotype in which other genes make a contribution (Straimer et al., 2015). Pfkelch13 is an excellent marker for artemisinin resistance in *P. falciparum;* however, many polymorphisms have been identified in the gene, and it is not clear what role each of these plays in directly conferring artemisinin resistance (Miotto et al., 2015).

Transcriptional analysis of parasites from patients with acute malaria showed that artemisinin resistance was associated with an increase in the unfolded protein response and that parasites exhibited a slow development phase early in the blood stage life cycle, which mitigates the damage caused by artemisinin (Mok et al., 2015). This is consistent with other work showing that artemisinin induced a cell stress response resulting in accumulation of ubiquitinated proteins and increased activity of the proteasome. Resistant parasites have an enhanced cell stress response, resulting in lower levels of ubiquitinated proteins and a delayed onset of cell death. Phosphatidylinositol-3-kinase (PfPI3K) may also be a molecular target of artemisinin and involved in mediating the resistance mechanism (Mbengue et al., 2015). So although there is some tantalizing new information on the mechanism of resistance of *P. falciparum* to artemisinin, there is some inconsistency in current models.

Development of Novel Antimalarials

Currently, there are effective drugs to treat and control both *P.vivax* and *P. falciparum* malaria; however, development of resistance has raised serious concerns about their long-term utility. *P. vivax* and *P. ovale* form dormant liver stages (hypnozoites) that are responsible for a significant number of relapse infections, and currently there is only one drug, primaquine, that provides a radical cure. This is particularly concerning as elimination of malaria is a major global health priority, and the current suite of antimalarial drugs are unlikely to be sufficient for this task. Therefore, in order to progress the malaria elimination agenda it has become important to develop new therapeutics that act broadly to cure the asexual blood stage to alleviate symptoms, clear the liver stage that can result in relapses for *P. vivax* and *P. ovale* malaria, and block transmission. This has resulted in the development of public-private partnerships that have fostered the use of medicinal chemistry-based approaches as well as novel high-throughput drug screens and platforms to identify new chemical entities and targets that are showing promise in preclinical and clinical testing. This has been increased by the availability of the Tres Cantos antimalarial set (TCAMS) of 13,000 compounds that have antimalarial activity (Gamo et al., 2010). From this, Medicines for Malaria Venture (MMV) assembled 400 unique compounds with blood-stage antimalarial activity that have been called the Malaria Box (Spangenberg et al., 2013). MMV, in collaboration with many researchers, have built a research and development portfolio from lead compounds to novel antimalarials currently in clinical trials (see http://www.mmv.org/research-development/interactive-rd-portfolio).

Whole-cell screens of asexual blood stages of *P. falciparum* identified the spiroindolone class of compounds, leading to the candidate (KAE609, cipargamin) currently in clinical trials. Cipargamin and related compounds bind the P-type Na^+-ATPase (PfATP4) expressed on the parasite plasma membrane. This disrupts sodium homeostasis, thus blocking asexual blood-stage development and transmission to the mosquito (Spillman et al., 2013). PfATP4 is a target for a number of chemical classes including the pyrazoleamides (Vaidya et al., 2014). Additionally, a high-throughput screen identified the orally available dihydroisoquinolones resulting in the clinical candidate (+)-SJ733, a potent inhibitor of blood and transmission stages of malaria parasites (Jiménez-Díaz et al., 2014). Dihydroisoquinolones also target PfATP4, confirming that disruption of sodium homeostasis is an excellent target for novel antimalarial drugs.

Whole-cell screening approaches have also identified a 2,6-disubstituted quinoline-4-carboxamide scaffold that led to development of DDD107498 that targets translation elongation factor 2 (eEF2) of *P. falciparum* and is active against multiple life-cycle stages (Baragaña et al., 2015). Additionally, cell-based screening identified the imidazopyrazine class of compounds that target the lipid kinase phosphatidylinositol 4-kinase (PI4K) (McNamara et al., 2013). The imidazopyrazines interfere with the ATP-binding pocket of PI4K, consequently altering the intracellular distribution of phosphatidylinositol 4-phosphate. These compounds inhibit multiple stages of *P. vivax* and *P. falciparum* and, importantly, liver hypnozoites in *P. cynomolgi* (McNamara et al., 2013). They also block mosquito transmission in mouse models of malaria. The clinical candidate KAF156 was derived by screening for compounds

that block development of the *P. falciparum* liver stage, identifying the imidazolopiperazine class of compounds with potent blood- and transmission-stage activity (Kuhen et al., 2014). The exact mechanism of action of this compound class is unknown, but selection of parasite resistance identified the cyclic amine resistance gene (Pf3D7_0321900); however, it is not known whether this is the actual target or a resistance mechanism.

Although whole-cell screening has been successful in identifying new classes of compounds as potential antimalarials, synthetic approaches have also been successful, producing the ozonides, which retain the endoperoxide bridge present in artemisinin. Their mechanism of action is similar to that of artemisinin where the peroxide bond is broken by heme and ferrous iron, producing free radicals that cross-link proteins and other cellular components. Although the mechanism of activation for ozonides appears to be the same as artemisinin, the parasite targets appear to be different. A number of ozonides, including the recently marketed Synriam and OZ439, have been developed and are currently in clinical trials. Additionally, there have been considerable efforts in developing lead candidates against known drug targets. These efforts include the mitochondrial enzyme dihydroorotate dehydrogenase (DHOD) and identification of the triazolopyrimidines, a new chemical class of *P. falciparum* inhibitors. From these DSM265 has emerged, with potent levels of activity in humanized mouse malaria models.

The development of new treatments for the radical cure of *P. vivax* and *P. ovale* is important as the only available treatment is primaquine, which can induce hemolytic anemia in patients with glucose-6-phophate deficiency (reviewed in Ashley et al., 2014). Screening for hypnozoite-active compounds is challenging, but as discussed above, screens have been performed using the *P. cynomolgi*/rhesus model to confirm that the imidazopyrazine KAI407 is active against this dormant form, raising the prospect of new improved treatments against relapsing malaria. The recent development of human liver-chimeric mice that support *P. vivax* infection with hypnozoite persistence is an important technical breakthrough (Mikolajczak et al., 2015). Human liver microscale platforms supporting growth and development of hepatic stages for both *P. falciparum* and *P. vivax* (March et al., 2013) also add considerably to the tools for screening new inhibitors of *P. vivax* and the hypnozoite form. Although it is important that alternatives to primaquine be identified, using low-dose regimens of this drug as well as the 8-aminoquinoline tafenoquine provides potential alternatives (Llanos-Cuentas et al., 2014).

Vaccine Development

There is currently no malaria vaccine available. It is widely accepted that new tools, including vaccines, are required if we are to maintain recently achieved levels of disease control and move toward elimination and eventual global eradication of malaria. Efforts to develop vaccines over the past 30 years have targeted pre-erythrocytic (PE) (sporozoite and liver stages), blood stages, and sexual stages. A fully effective PE vaccine would prevent malaria by stopping establishment of blood-stage infection. Vaccines targeting the replicating asexual blood stages have been considered important to control morbidity and mortality. Vaccines against the sexual stages would interrupt the transmission cycle but not have a direct effect on an established infection in the vaccinee.

As the concept of elimination has gained ground, emphasis in vaccine development has moved toward the PE and sexual stages, predicated on the bottleneck hypothesis. This supposes that attacking the numerical bottlenecks in parasite development—the injected sporozoites prior to liver invasion and mosquito midgut-transmission stages (Figure 5)—will lead to increased vaccine efficacy compared to targeting replicating blood stages that are present in higher numbers and have evolved a variety of immune evasion mechanisms for long-term survival. The problem with this strategy is if a single sporozoite or ookinete breaks through, the pathogenic replicative cycle continues because immunity generated against these targets appears to be strictly stage specific (Felgner et al., 2013). This will become even more important as we move toward elimination. A combination of population loss of naturally acquired blood-stage immunity with waning vaccine efficacy in the context of drug resistance would be a very dangerous situation for rebound of malaria transmission.

The ideal malaria vaccine would be based on conserved targets that induce lifelong sterile protection early in life with as few doses as possible. So far, this has not been achievable. The most advanced clinical candidate is RTS,S/AS01, an anti-sporozoite vaccine. Having progressed through phase III clinical trials involving over 15,000 African children, it is the gold standard against which all future vaccines will be assessed. After a year of follow-up, vaccination with a three-dose series reduced clinical malaria cases by 28% in young children and 18% in infants (Greenwood and Doumbo, 2016). A key finding of these trials is the short duration of protection. Efforts to understand why this is the case are urgently required. A comprehensive study of interaction between malaria parasites and the human immune system and how they co-evolve is another area that will help in designing better vaccine strategies.

The radiation-attenuated, whole-cell sporozoite vaccine (PfSPZ) has delivered sterile protection (no blood-stage development) against homologous challenge (injection with sporozoites of the same parasite strain) in early phase I/IIa clinical trials (Seder et al., 2013). However, this vaccine has major drawbacks, including lack of demonstrated heterologous (cross-strain) protection, high numbers of parasites and intravenous route of delivery required to induce anti-sporozoite immunity in volunteers, and the logistical requirement for a liquid nitrogen cold chain to maintain viability of the vaccine. Other live-cell sporozoite vaccine strategies, such as genetic attenuation of parasites (Spring et al., 2013), which may be beneficial in terms of quality control, and CPS (chemoprophylaxis with sporozoites), where much lower numbers of parasites are needed to induce immunity but which requires chloroquine treatment following each vaccination dose, may have incremental improvements over PfSPZ but all share the fundamental challenge of inducing strain-transcendent protection, a top priority for vaccine development.

A promising strategy may be targeting combinations of antigens expressed on multiple life-cycle stages (Theisen et al., 2014). Assuming we can overcome the issue of longevity of immune responses, the pros of this approach are that addition of a

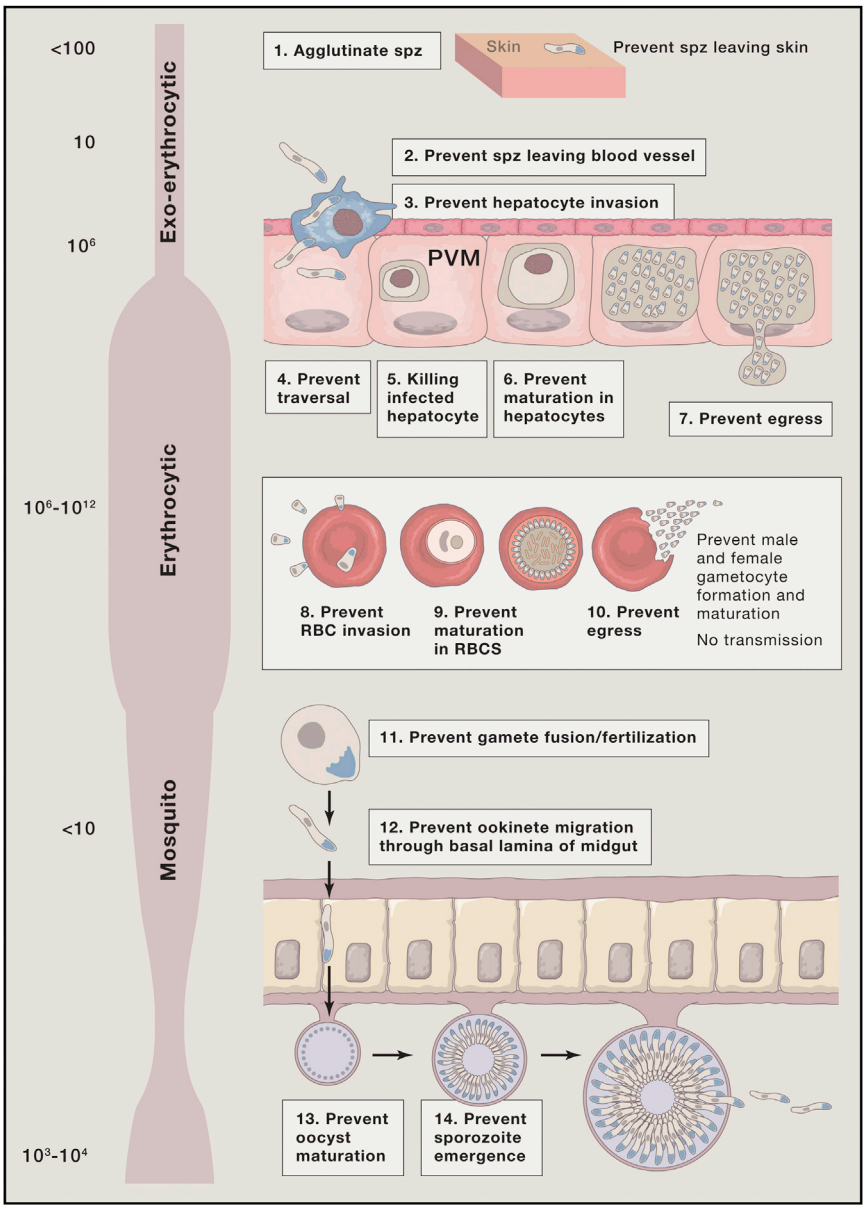

Figure 5. Vaccine-Targetable Processes within the Malaria Life Cycle

A malaria vaccine could potentially intervene at different points in the life cycle. The panel on the left corresponds to parasite population dynamics in individual hosts. Numerical bottlenecks occurring at the points of sporozoite migration through the skin and ookinete penetration of the midgut are considered priority areas for vaccine development.

blood-stage component to an anti-sporozoite target would reduce the risk of morbidity and mortality and provide immune protection while transmission declines, providing insurance against epidemics of severe disease should transmission rebound due to other intervention failures. Leading multi-stage vaccine candidate antigens are CSP from sporozoites (Cohen et al., 2010), PfRh5 on the merozoite (Douglas et al., 2015), Pfs25, 48/45, and 230 on midgut stages, and AMA-1, expressed on both sporozoite and merozoites.

The complexity of *Plasmodium* is the biggest challenge to effective vaccine design. As discussed above, many antigens are highly polymorphic, and those that are conserved may be poorly immunogenic. Interrogation of the genome in combination with high-throughput -omic technologies and immune profiling with sera from patients with clearly defined disease outcomes (Osier et al., 2014) are being used to compile lists of potential new targets. This output combined with functional genomics to identify essential parasite proteins will be a helpful step in ranking the thousands of potential targets for vaccine suitability.

Control and Elimination of Malaria—Historical Perspective and Prospects for the Future

The first half of the 20th century saw remarkable progress in understanding the biology and epidemiology of malaria and applying this to disease-control efforts. The discovery of DDT's insecticidal properties in 1939 provided a powerful new tool for interrupting transmission. In 1955 WHO called for a global campaign of eradication. Much has been written about its failures, most notably in Africa. However, there were also extraordinary successes, and malaria was eliminated from large parts of the world. Perhaps the most important lesson came from the near successes: in some areas enormous reductions were achieved, but the last stages of elimination are by far the hardest, and when progress stalled there was massive rebound. This provides a frightening warning of what we may face if we are incompletely successful in current efforts, especially in areas with inherently high transmission potential.

Few systematic attempts at control were made in Africa, and throughout the 1980s and 1990s the situation grew progressively worse, largely attributed to widespread development of resistance to chloroquine, then the mainstay malaria treatment across Africa. During this period malaria-attributable mortality doubled, and by the end of the twentieth century, malaria in Africa was appropriately described as a disaster, with apparent paralysis of national and international will to tackle the situation. Eventually, however, there was action, and remarkably, between 2000 and 2008 total global spending on malaria control rose 10-fold. Much of this was spent on two key interventions, insecticide-treated bed nets and new approaches to antimalarial therapy based on the combination of artemisinin with partner

drugs. Since 2000 global malaria death rates due to malaria have fallen by 60% and in Africa by 66% (WHO, 2015). However, as is often the case, things are not as simple as they seem. In several well-documented cases it is clear that malaria transmission has been falling steadily over a long time frame, often considerably preceding introduction of new interventions. Increasing investment in control is critically important, but there is much about malaria epidemiology we still do not understand.

Recently there has been renewed interest in the idea of malaria eradication as the goal of global efforts. In 2015 a Global Technical Strategy for Malaria developed by WHO and partners was adopted by the World Health Assembly (WHO, 2015) based on the aim of accelerating efforts with eradication as the long-term goal, but with a realistic appreciation of the enormous barriers to overcome and the threats to current gains, especially the development of artemisinin insecticide resistance. For the vast majority of endemic areas, control of morbidity and mortality is still the first priority and an absolute requisite before elimination can be considered. Few would envisage that current tools will allow eradication of malaria in any reasonable time frame. The hope of elimination and eventual eradication must rest on development of radical new tools, whether it be drugs, vaccines, or genetically modified vectors, and this will require major investment in research to exploit our knowledge of the parasite's basic biology and epidemiology.

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