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Review Series

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The impact of malaria parasitism: from corpuscles to communities

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Malaria continues to exert a tremendous health burden on human populations, reflecting astonishingly successful adaptations of the causative *Plasmodium* parasites. We discuss here how this burden has driven the natural selection of numerous polymorphisms in the genes encoding hemoglobin and other erythrocyte proteins and some effectors of immunity. *Plasmodium falciparum*, the most deadly parasite species in humans, displays a vigorous system of antigen variation to counter host defenses and families of functionally redundant ligands to invade human cells. Advances in genetics and genomics are providing fresh insights into the nature of these evolutionary adaptations, processes of parasite transmission and infection, and the difficult challenges of malaria control.

Evolutionary origin and host preferences of malaria-causing parasites

Most cases of malaria in the world are caused by one of the four species of *Plasmodium* parasites that predominantly infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. Despite efforts over the past century, the global health burden from *Plasmodium* infections remains approximately 250–600 million episodes of malaria each year in Africa, Asia, Oceania, and Latin America (1, 2). Of these episodes, approximately 40% are caused by *P. falciparum*, as are nearly all fatalities attributed to malaria. Another approximately 40% of the episodes of malaria may be caused by *P. vivax*. Although such episodes do not often kill outright, they nevertheless are tremendously debilitating and are responsible for diminished learning, productivity, and economic development. Until recently, *Plasmodium* species native to other primates were thought to only occasionally infect humans. But this picture has now changed with the emergence of *Plasmodium knowlesi*, a natural parasite of macaque monkeys, in human populations of Southeast Asia. Reports from Malaysia, the Philippines, Thailand, and Myanmar suggest that *P. knowlesi* should be considered a fifth species responsible for a substantial prevalence of malaria in humans (3).

Parasites that cause malaria all develop through the same general life cycle, which alternates between the human host and anopheline mosquito (Figure 1). The cycle begins when a *Plasmodium*-carrying female anopheline mosquito takes a blood meal. Sporozoites inoculated into the dermis migrate to the liver where they infect hepatocytes. Over 5–16 days, depending on the *Plasmodium* species, each parasite grows and divides into tens of thousands of merozoite forms that then break out of individual hepatocytes into the blood, where they infect erythrocytes; alternatively, some *P. vivax* and *P. ovale* parasites can remain latent in the liver as hypnozoite forms until they activate and cause relapses of malaria months or years later. Erythrocyte-stage *Plasmodium* parasites undergo repetitive rounds of invasion, growth, and division in one-day (*P. knowlesi*), two-day (*P. falciparum*, *P. vivax*, and *P. ovale*), or three-day (*P. malariae*) periods. Parasite density expands exponentially as a consequence, leading to the symptoms and complications of malaria. *P. falciparum* is particularly dangerous in this phase of the life cycle, as host erythrocytes infected with its mature parasite forms avoid the spleen by seques-

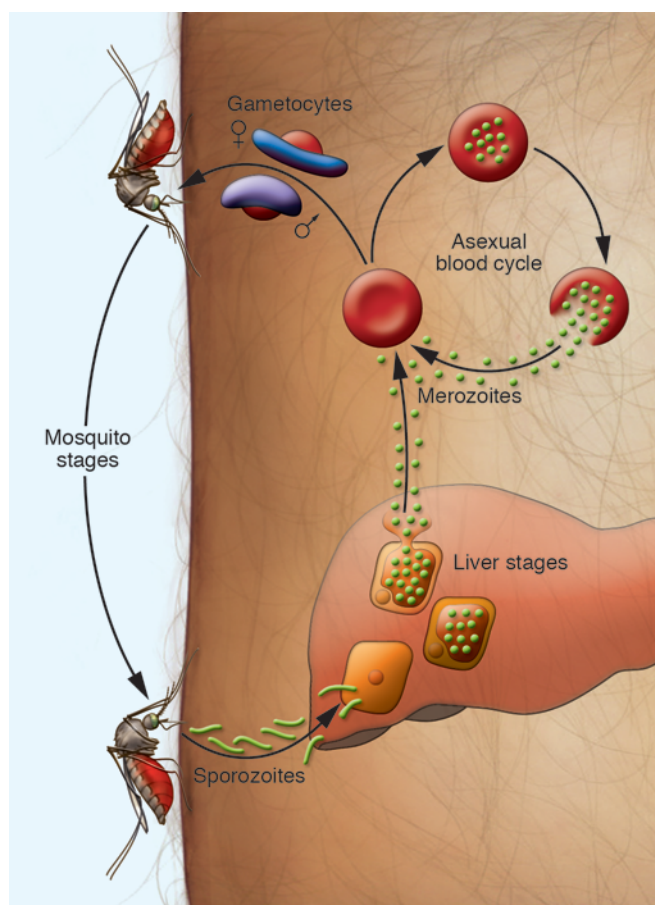
tering in capillaries and microvenules of the brain and other vital organs, a process that is not common with erythrocytes infected by other human malaria parasite species. Bloodstream parasitization can last for months if not treated, during which time some parasites continually leave the asexual replication phase and differentiate into male and female gametocytes. After these sexual stages are taken up by another anopheline mosquito ingesting a blood meal, they mate and infect the mosquito to generate thousands of progeny sporozoite forms that travel to the mosquito salivary glands, where the sporozoites wait, ready to initiate another life cycle.

What are the evolutionary origins of these sinister and highly adaptive parasites, and how did they come to be so successful in their transmission among human populations? *Plasmodium* parasites are members of the large *Apicomplexa* taxon (Figure 2), characterized by an apical complex of rhoptries, micronemes, dense granules, and other organelles specialized for host cell invasion. In addition to malaria, members of this taxon cause cryptosporidiosis, toxoplasmosis, babesiosis, cyclosporiasis, coccidiosis, and theileriosis (Figure 2). Evolutionary relationships among apicomplexan species are evident in the residual genes they carry from a plant plastid genome that was once present in a red algal cell engulfed by an ancient ancestor. The discovery of an algal symbiont of corals that carries a plastid and is closely related to apicomplexans suggested recently that apicomplexan parasitism was probably present in the earliest animals (4, 5). Modern *Plasmodium* species thus may have descended from apicomplexans that interacted with increasingly sophisticated immune systems over hundreds of millions of years (5). Through an evolutionary arms race between parasites and hosts, *Plasmodium* species have come to possess potent defenses to effectively deal with diverse mechanisms of innate and acquired immunity and therefore persist with stubborn success in their animal hosts.

Most of the approximately 200 known *Plasmodium* species infect particular lineages of vertebrates — primates, rodents, birds, and reptiles (6); they either never evolved in or disappeared from other major vertebrate lines. Further, *Plasmodium* species exhibit strong specificity for certain host species (6), with only occasional jumps between host species. One such jump is thought to account for the origin of *P. vivax*, which a recent phylogenetic analysis suggests occurred 53,000–265,000 years ago when an ancestral parasite switched host from macaque monkeys to humans (7). The increasing prevalence of *P. knowlesi* in human populations today may prove to be another example (3).

Conflict of interest: The authors have declared that no conflict of interest exists.

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**Figure 1**

Life cycle of *P. falciparum*. Sporozoites injected by anopheline mosquitoes travel through the dermis and enter the bloodstream to invade hepatocytes. Each infected hepatocyte generates tens of thousands of merozoites, which then break out and reenter the bloodstream to invade erythrocytes. Numerous rounds of asexual reproduction follow, with repeated invasion of erythrocytes every 48 hours. Some parasites in the erythrocytes develop into sexual stage gametocytes, which circulate in the bloodstream and are taken up by female mosquitoes during a blood meal. In the mosquito midgut, gametes emerge from the gametocytes and cross-fertilize (118). The resulting zygote develops into an ookinete that crosses the midgut wall and grows into an oocyst. Mitotic division within the oocyst produces thousands of sporozoites that break out and travel through the hemolymph to the mosquito salivary glands, from which they are injected into a human host.

Recent advances in genetics and genomics make it possible to address key areas of malaria research with new power and detail. Among these are the host-pathogen relationships that support the extremely high burden of *P. falciparum* in Africa and reasons why most parasitized individuals avoid the complications of severe malaria while others succumb. The wealth of data on *Plasmodium* will help us understand the families of antigenically variant but functionally redundant ligands that malaria parasites employ to interact with host cells. As questions in these areas have been difficult to address with previously available experimental tools, approaches that employ information from the rapidly expanding databases will provide invaluable resources for state-of-the-art investigations (8–10). These can

be expected to advance *Plasmodium* biology and support new approaches against malaria.

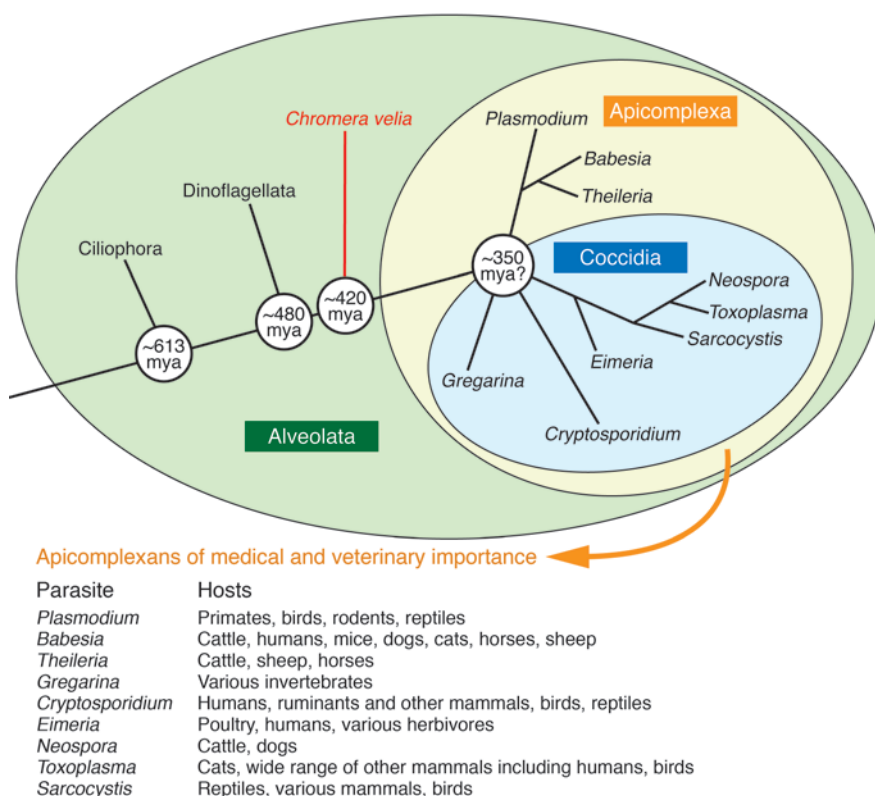
Intense transmission of *P. falciparum* by African mosquitoes

As might be expected of parasites that coevolved with their hosts over millions of years, *Plasmodium* infections in malaria-endemic regions often go unnoticed. Even in Africa, where *P. falciparum* accounts for nearly all deaths from malaria, most bites from infected mosquitoes do not produce clinically apparent disease. Children exposed to such bites typically have outcomes of no infection (about one-half of inoculations), infection with no symptoms (about one-quarter of inoculations), or infection with fever and other symptoms (uncomplicated malaria; about another one-quarter of inoculations). Only in relatively few cases do children develop manifestations of severe malaria, which can include coma, profound anemia, and acidosis-driven respiratory distress (Figure 3A) (11). Many factors determine outcome, including mosquito inoculation rate, sporozoite dose, immunity acquired from previous exposure to *P. falciparum*, parasite virulence, host genetic polymorphisms, nutrition and housing conditions, awareness of the symptoms of malaria, and prompt access to effective treatment.

The prevalence of *P. falciparum* infection is, nevertheless, high enough to kill a million people in Africa each year, where annual death rates among young children are as high as 1%–2% in villages (1, 2, 12). Death rates from malaria were even higher before the availability of modern antimalarial drugs – in past centuries, more than 20% of children may have perished in their first decade of life from malaria in areas of intense *P. falciparum* transmission (13). In these areas, children can be exposed to 700 or more sporozoite-inoculating mosquito bites every year (14) (Figure 3B).

Of the approximately 400 species of *Anopheles* mosquitoes (up to 60 of which can transmit *Plasmodium* species; ref. 15), African mosquitoes, particularly *Anopheles gambiae*, stand out because of their great anthropophily and sheer efficiency of parasite transmission. An example of the alarming potential for African mosquitoes to cause explosive epidemics occurred in the 1930s when they were inadvertently introduced from Africa into Brazil by ship or, less likely, by plane (16). The mosquitoes were able to tentatively colonize riverine and coastline regions north and west of Natal and in 1938 produced the greatest malaria epidemic ever in South America, with over 100,000 cases and 14,000 deaths. The new anopheline infestation was eliminated only after intensive efforts of the Brazilian Malaria Service and the American epidemiologists Fred L. Soper and D. Bruce Wilson. Satisfyingly, reintroduction of these mosquitoes has been successfully averted to this day (16).

In contrast with the experience in Brazil, control of *An. gambiae* and related mosquitoes has proven vastly more difficult in Africa, where the insect populations are highly adapted to their native habitats and have behavioral patterns highly conducive to parasite transmission. This was dramatically demonstrated in the Garki Project of northern Nigeria, 1969–1976. Vectorial capacity, a mathematical expression of the daily rate of indoor and outdoor mosquito contact, was found to be 1000 times the level necessary for maintenance of transmission, putting malaria in the African savanna in a category of its own (17). The Garki Project's intensive control operations, including mass drug administration and comprehensive spraying of domiciles with residual insecticide, were able to reduce vectorial capacity by only 90% (in the absence of insecticide-resistant mosquitoes and drug-resistant parasites) and thus

**Figure 2**

Apicomplexans are obligate intracellular protozoa from ancestors that also gave rise hundreds of millions of years ago (mya) to ciliates, dinoflagellates, and the recently discovered *Chromera velia* (4). Symbiotic relationships of *C. velia* and dinoflagellate species with invertebrates suggest that parasitism developed early in the origin of animals. Depicted is an evolutionary tree of the alveolate protozoans, including *C. velia*, a photosynthetic symbiont of corals (4). The phylogeny of Apicomplexa is not fully resolved, and confirmation of the date of the radiation and branch order awaits further studies. Modified with permission from *Trends in Parasitology* (ref. 119, using information available in refs. 5 and 120).

failed to break transmission. Great reductions in the number of malaria cases were nevertheless achieved. More recently, programs providing antimalarial drugs and insecticide-treated bed nets have produced appreciable reductions in malaria and malaria-associated deaths in other African countries, including Zambia, Rwanda, Tanzania, Kenya, The Gambia, and South Africa (18–22).

In view of the major role of *An. gambiae* as a vector for *Plasmodium* species, a full genome sequence of *An. gambiae*, with analyses of its genes and proteins, was reported in 2002 (23). Insights from these data should contribute to future strategies to reduce the intensity of mosquito transmission of malaria-causing parasites. These insights include new findings on *An. gambiae* systems for sensing hosts, further understanding of which could possibly lead to new interventions to reduce contact between humans and mosquitoes. For example, the identification of odor receptors in the *An. gambiae* genome and the ability to perform functional studies on orthologs in the *Drosophila melanogaster* model led to the recent demonstration that the repellent *N,N*-diethyl-meta-toluamide (DEET) inhibits insect odor receptors (24). Other insights may suggest ways to interfere with blood feeding and insect immune defenses (25). Basic research on insecticide resistance and targets for new insecticides may also be transformed by methods such as genome-wide transcriptional analyses and candidate gene-silencing techniques (25, 26).

Abundant human genome polymorphisms selected by life-threatening malaria

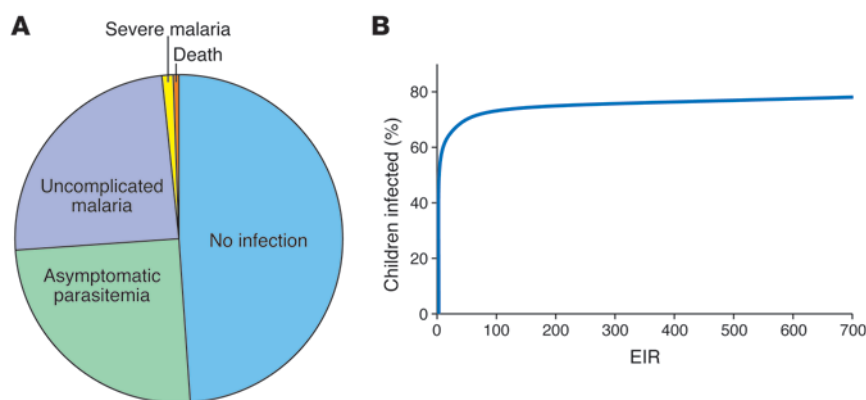
Malaria has exerted great evolutionary pressure on the human genome, resulting in the selection of polymorphisms that can dramatically alter susceptibility of children to life-threatening complications of infection. In 1954, Anthony C. Allison provided important evidence that sickle cell hemoglobin trait (i.e., the presence

of one β -globin gene encoding normal hemoglobin, hemoglobin A [HbA], and one β -globin gene encoding sickle-cell hemoglobin [HbS]) was associated with protection against *P. falciparum* malaria in Kenya (27). Since then, investigators have associated protection against *P. falciparum* and *P. vivax* malaria with a variety of human genetic polymorphisms carried by hundreds of millions of people. While many of these polymorphisms affect the host erythrocyte, others may involve effectors of innate and acquired immunity. Here, we discuss some of these polymorphisms as well as recent insights into their proposed mechanisms of protection.

Erythrocyte polymorphisms and protection against severe malaria

Many of the polymorphisms that protect against severe malaria are found in the genes encoding β -globin and other molecules of erythrocytes, the host cells in which parasites multiply and set up the pathological interactions that cause disease symptoms. Taking advantage of growing human haplotype maps and SNP databases, genome-wide association studies are presently underway to expand our understanding of these and additional polymorphisms associated with reduced malaria severity in geographically disparate populations (28). Various models have been described to account for protection by glucose-6-phosphate dehydrogenase (G6PD) deficiency, α -thalassemia, and other conditions such as the hemoglobinopathies caused by the HbS, hemoglobin C (HbC), or hemoglobin E (HbE) polymorphisms (29–33) (Table 1).

Early models of protection by G6PD deficiency and the hemoglobinopathies often invoked reduced parasite load as the mechanism, even though these models failed to explain important findings from numerous clinical studies performed since the 1950s. In particular, although the polymorphisms underlying HbS, HbC,

**Figure 3**

An. gambiae entomological inoculation rates and infection outcomes in Africa. (A) The clinical outcome of *P. falciparum* sporozoite inoculation by an *An. gambiae* mosquito depends on many factors and can range from no infection to severe malaria and death. Greenwood et al. (121) estimated that in areas of high transmission, for every 400 infectious bites, 200 result in a parasite infection, half of which will develop uncomplicated malaria, with two cases of severe malaria and one death. (B) Relationship between the mosquito entomological inoculation rate (EIR) and the proportion of individuals infected with *P. falciparum* (14). Analysis of combined data from over 90 African communities indicated that 20% of people received 80% of infections and that enormous reductions of existing EIRs would be required to achieve even a modest decrease in parasite prevalence. For example, a two-fold reduction in the EIR, from 200 to 100 infectious bites per year, would reduce parasite prevalence by only 4%, whereas a 100-fold reduction in the EIR, from 100 infectious bites to 1 infectious bite, would reduce parasite prevalence from about 70% to 30%. Part B was generated based on data from ref. 14.

α -thalassemia, and G6PD deficiency confer substantial protection against life-threatening malaria, these polymorphisms often confer less protection against uncomplicated malaria and provide little or no resistance to infection itself (34–37). Further, parasitemias in individuals who carry HbC or have G6PD deficiency show little or no overall difference from parasitemias in control individuals (34, 36, 37). These observations remained difficult to reconcile with models of protection based on reduction in parasite load, as such reductions predict lower parasitemias and decreased risk of both uncomplicated and severe malaria. Protection that occurs differentially against severe malaria and does not depend on reduction

of parasite load requires alternative explanations, one of which we have proposed is at the interface of the parasitized erythrocyte and the microvascular endothelial cell (38).

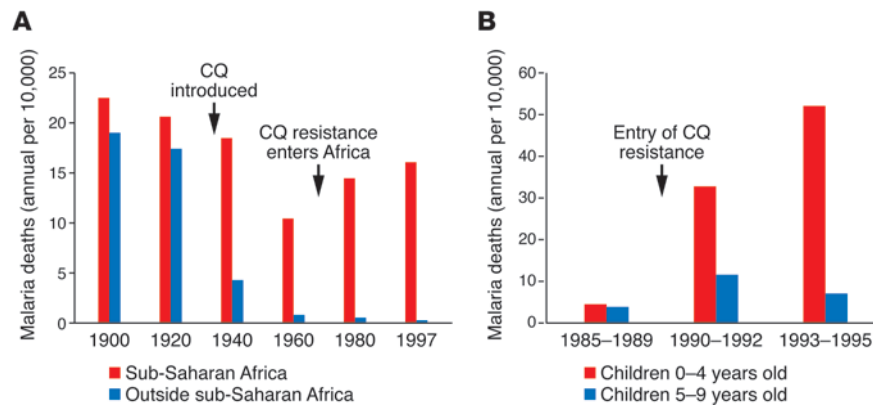
Various forms of severe *P. falciparum* malaria arise from the sequestration of mature parasitized erythrocytes in the capillaries and microvenules of organs such as the brain, heart, lung, liver, kidney, dermis, and bone marrow. In pregnant women, malaria may cause fetal growth restriction, preterm delivery, or fetal loss because *P. falciparum*-infected erythrocytes accumulate in the proteoglycan matrix of the placental intervillous spaces. By sequestering in organs, mature *P. falciparum*-infected erythrocytes are thought to avoid being

Table 1

Hemoglobin and erythrocyte polymorphisms protective against life-threatening *P. falciparum* malaria

Name	Gene affected	Polymorphism	Proposed mechanisms of protection (Reference)
HbS	<i>HBB</i>	$\beta 6$: glutamate to valine	Increased sickling of parasitized erythrocytes, which undergo enhanced clearance by the spleen (124, 125) Reduced erythrocyte invasion and inhibited parasite growth under conditions of low oxygen tension in venous microvessels (29, 126) Enhanced phagocytosis of erythrocytes infected with ring-stage parasites (31) Altered PfEMP-1 display and reduced cytoadherence of parasitized erythrocytes (44) Enhancement of innate and acquired immunity (57)
HbC	<i>HBB</i>	$\beta 6$: glutamate to lysine	Altered PfEMP-1 display and reduced cytoadherence of parasitized erythrocytes (38)
HbE	<i>HBB</i>	$\beta 26$: glutamate to lysine	Unidentified membrane abnormality that renders the majority of erythrocytes relatively resistant to invasion (32)
α -thalassemia	<i>HBA1/HBA2</i>	3.7-kb deletion	Reduced expression of CR1, a receptor that mediates the binding of noninfected erythrocytes to parasitized erythrocytes (127) Increased microerythrocyte count in homozygotes reduces the amount of hemoglobin lost for any given parasite density, thus protecting against severe anemia (128)
G6PD deficiency	<i>G6PD</i>	A376G/G202A (<i>G6PD</i> *(A–))	Enhanced phagocytosis of erythrocytes infected with ring-stage parasites (33)

CR1, complement receptor 1; HBA, α -globin 1; HBA2, α -globin 2; HBB, β -globin.

**Figure 4**

Malaria death rates in Africa fell after the introduction of chloroquine and rose again in the wake of chloroquine-resistant malaria. (A) Malaria death rates in the 20th century. Dramatic reductions in mortality occurred outside sub-Saharan Africa. In Africa, mortality rates declined after the introduction of chloroquine but then rose again with the spread of chloroquine-resistant parasites. (B) Rise in mortality associated with the entry of chloroquine-resistant *P. falciparum* in the village of Mlomp, Senegal. Deaths were primarily among children less than 5 years old, the most susceptible age group in highly malaria-endemic areas. CQ, chloroquine. Part A was generated based on data from ref. 106. Part B was reproduced with permission from Elsevier (107).

destroyed by the spleen (39). Sequestration can also be compounded by direct binding of noninfected erythrocytes to parasitized erythrocytes (rosetting) and platelet-mediated clumping of parasitized erythrocytes (autoagglutination) (40, 41). Inflammatory cells, thrombin deposits, activated tissue factor, upregulated endothelial receptors, and proinflammatory cytokines have all been associated with sequestered *P. falciparum*-infected erythrocytes and contribute to circulatory impairment, metabolic stress, and tissue compromise in individuals with severe malaria and malaria of pregnancy (42, 43).

How might heterozygous states of HbS (sickle-cell trait) or HbC reduce the effects of parasitemia and provide differential protection against severe malaria? In recent studies, the endothelial cell binding of parasitized erythrocytes containing either HbS or HbC was found to be reduced relative to the binding of parasitized erythrocytes containing HbA (38, 44). Further, this reduced binding to endothelial cells correlated with decreased surface levels and altered distribution of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), the major variable cytoadherence protein expressed by the parasites on their host erythrocytes (38, 44). PfEMP-1 display may therefore be altered sufficiently by these hemoglobinopathies to reduce endothelial cell activation, but not to prevent sequestration, so that a substantial fraction of parasitized erythrocytes still avoid being carried away to the spleen. The benefits of reduced endothelial activation, including reduced upregulation of adhesion molecules, reduced activation of tissue factor and thrombin, decreased amounts of proinflammatory cytokines, and decreased recruitment of platelets and monocytes, would be expected to ameliorate the consequences of local and systemic inflammation.

Molecular-genetic investigations have shown that HbS was selected independently at least five times in Africa, Arabia, and India, that HbC arose from at least two independent foci in West Africa and Thailand, and that different genetic forms of α -thalassemia exist in Asia and Africa (45, 46). The independent selection of HbS, despite the deleterious nature of the homozygous HbS state (i.e., sickle-cell disease), provides strong evidence that vigor-

ous forces of natural selection were exerted on these polymorphisms. Oxidative instability and hemoglobin denaturation may underlie the effects of HbS and HbC on PfEMP-1 display; these effects may involve elevated levels of membrane-bound hemichromes (oxidized, denatured hemoglobin molecules) binding to the inner aspect of the erythrocyte membrane and interfering with the trafficking and anchoring of PfEMP-1 (38, 44).

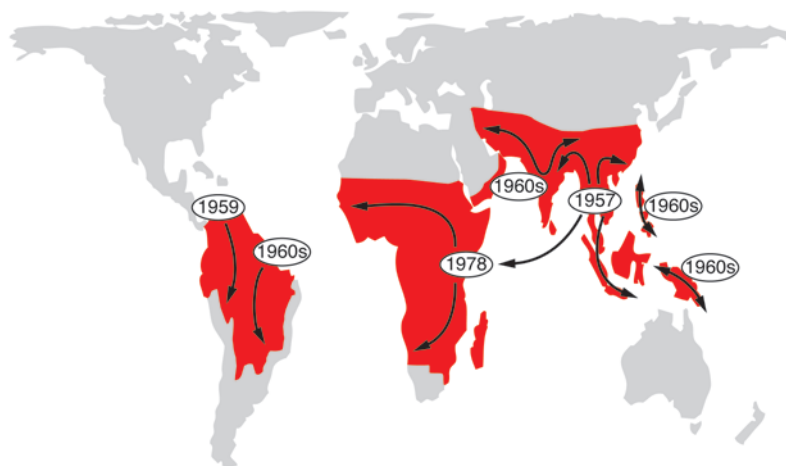
Polymorphism mitigating placental inflammation in malaria of pregnancy

Inflammation caused by parasitized erythrocytes in the placenta is associated with intrauterine growth restriction, miscarriage, and premature delivery (47, 48). Mothers in their first pregnancy are particularly susceptible to these complications, even if they have preexisting immunity as a result of previous *P. falciparum* infection. A substantial part of this susceptibility may be attributed to the expression of PfEMP-1 forms that bind placental chondroitin sul-

fate A (CSA) and are antigenically new to the immune systems of women who have not previously been pregnant (49–51). Less frequent complications in subsequent pregnancies are thought to be explained by acquired immunity to CSA-binding PfEMP-1 molecules (52, 53). The importance of placental inflammation in malaria of pregnancy was recently reinforced by data suggesting that polymorphisms affecting expression of the FMS-like tyrosine kinase 1 (*FLT1*) gene, which encodes a receptor for VEGF (which can act as a proinflammatory cytokine), are under natural selection in this condition. In a study of Tanzanian mothers and infants (54), maternal genotypes of relatively increased *FLT1* expression (as a result of homozygosity for short dinucleotide repeat polymorphisms in the 3' untranslated region [i.e., SS dinucleotide repeat genotypes]) were associated with prior fetal losses in first-time mothers with placental malaria, and SS genotypes in the newborns of first-time mothers were linked to higher occurrences of low birth weight. Placentas delivered with the SS newborns also showed greater inflammation, as judged by histological findings and elevated levels of transcripts encoding IFN- γ and immunoglobulin heavy chains. Relatively decreased levels of VEGF-FLT1 signaling associated with genotypes containing long dinucleotide repeat polymorphisms (i.e., SL and LL genotypes) thus might confer increased fetal fitness in utero by reduced maternal inflammatory response to placental *P. falciparum* infection. Interestingly, a soluble form of FLT1 (sFLT1) that binds VEGF and antagonizes FLT1 signaling has been shown to be elevated in preeclampsia (55), raising the possibility of selection by malaria in the evolution of preeclampsia (54).

Variations of innate and acquired immunity modulate malaria susceptibility

In malaria-endemic areas of Africa, very young children are most susceptible to the severe manifestations of malaria and carry the greatest burden of mortality (56). After about 5 years of age, African children become less susceptible to severe malaria, although

**Figure 5**

Chloroquine-resistant *P. falciparum* spread from at least six known origins. Chloroquine resistance entered Africa in the late 1970s, having originated in Southeast Asia. PfCRT molecules from the individual foci all contain a key K76T amino-acid replacement but are distinguished by different patterns of accompanying codon polymorphisms and chromosome haplotype polymorphisms. In addition to five foci previously depicted (109), the map indicates recently described parasites in India and Iran that probably spread from an additional focus of chloroquine resistance (122, 123). Figure reproduced with permission from ASM Press (109).

they can continue to suffer episodes of debilitating febrile illness. By adolescence, they can be continually infected with parasites but have only mild symptoms or none at all.

Multiple effectors of the innate and adaptive immune systems are involved in the development of “disease-controlling” immunity, which may be accelerated in the presence of such polymorphisms as those underlying HbS and α -thalassemia (57, 58). Enhanced phagocytosis of mutant erythrocytes containing parasites in their early growth stages (ring stages) may also contribute to protection in children with HbS (31). Variations in the prevalence of severe *P. falciparum* malaria among ethnic populations of West Africa have been associated with polymorphisms in the interferon regulatory factor-1 gene and a functional deficit of Tregs (59–61). While the role of Treg cells in malaria protection is unknown, they have been shown to affect in vitro T cell proliferative responses to *P. falciparum* antigens (61).

A growing body of evidence suggests that TNF activity may be involved in the immune control and disease manifestations of *P. falciparum* malaria. Infected erythrocytes may induce TNF release from monocytes through cytoadherence interactions or by the release of parasite-derived factors. For example, *P. falciparum* glycosylphosphatidylinositol, which is released upon schizont rupture, signals through TLR2 and TLR4 on monocytes to induce TNF production (62). While TNF is believed to help control parasitemia, it also induces fever during malaria episodes and may aggravate cerebral malaria by upregulating the expression of ICAM-1 and other leukocyte adhesion receptors on brain microvascular endothelial cells (63). TNF levels are highest in children with severe malaria and fatal outcomes (64, 65).

Gene promoter polymorphisms that increase TNF expression (66, 67) accelerate parasite clearance and symptom resolution (68, 69); they can also enhance the inflammatory consequences of infection and susceptibility to severe malaria and death (70, 71). TNF production during malaria episodes may also be up- or downregulated by polymorphisms that either alter the signal-transducing domains of TLR2 and TLR4 or modify the function of components of their downstream signaling cascades. Common *TLR4* polymorphisms (encoding Asp299Gly and Thr399Ile substitutions) have been associated in African populations with severe malaria in children (72) and with maternal anemia and the delivery of low-birth-weight newborns (73), but the effects of these polymorphisms on TLR4 function are not known. *TLR1* and *TLR6* polymorphisms have also been associated in Brazilian populations with the development of uncomplicated

malaria (74). Another polymorphism in the gene encoding MyD88 adapter-like protein (MAL; also known as TIRAP), the adaptor protein that conveys TLR2- and TLR4-initiated signaling events, has been associated not only with protection against malaria, but also with protection against pneumonia, sepsis, and tuberculosis (75). This *MAL* polymorphism attenuates TLR2-mediated signaling, suggesting that several TLR polymorphisms might have also evolved to dampen the production of TNF during episodes of malaria.

The mechanisms by which individuals acquire immunity to malaria are not well understood. Although T cell- and antibody-mediated responses develop against a number of parasite antigens during infection, no immune response has been found to be superior to patient age and previous parasite exposure as a correlate of protective immunity. In an example of the importance of age-associated protective immunity, older children in Mali treated with chloroquine were better able to clear chloroquine-resistant *P. falciparum* parasites than their younger counterparts (76). The influence of previous parasite exposure is evident in a retrospective analysis of 1940–1963 data from patients who were infected with *P. falciparum* to treat their tertiary syphilis (the parasites produced what were believed to be curative fever spikes) (77). The infections featured declining parasitemias in successive waves, characteristic of sequential populations of parasites expressing different surface antigens on their host erythrocytes (see the following section). Furthermore, when individuals who cleared an infection were reinfectd by either the same or a different *P. falciparum* strain, most developed parasitemia and fever — although the fever tended to be less intense and parasitemia tended to be lower than during the initial infection (78, 79). These and related findings from the literature (80–82) suggest that prior experience with malaria leads to the development of immunity that controls the severity of disease rather than that which prevents the development of symptoms or appreciably dense parasitemias.

Diverse mechanisms for immune system evasion and erythrocyte invasion

Antigen switching — a process by which *P. falciparum* parasite populations change expression from one PfEMP-1 variant-encoding gene (*var* gene) to another — creates distinct waves of parasitemia that must be chased by different antibody responses in the course of bloodstream infection. These waves, along with those produced from additional *P. falciparum* infections, can cause recurrent fevers that may not be cleared for many months or years but are impor-



tant components of the malaria experience that leads to eventual acquisition of disease-controlling immunity. Field studies have associated this immunity with the ability of serum to recognize the diverse PfEMP-1 molecules of heterologous parasite strains. As children grow and expand their antibody repertoire (83–85), they become susceptible to fewer and fewer *P. falciparum* strains. Genome and expression analyses have shown that the PfEMP-1 family has a vast capacity for sequence diversification (86, 87), and some PfEMP-1 variants may cause more severe clinical outcomes than others (88, 89). Structural constraints on the extracellular-binding domains of PfEMP-1 may restrain the ability of different variants to escape recognition by cross-reactive antibodies, thus allowing some degree of protection between strains (90, 91).

A primary determinant of host specificity and virulence for all malaria-causing parasites is their ability to invade the host erythrocyte. *P. falciparum*, perhaps more than any other *Plasmodium* parasite of humans, uses a variety of ligand-receptor interactions to invade erythrocytes through redundant or alternative pathways (92, 93). Two families of *P. falciparum* ligands illustrate this adaptability: first, *P. falciparum* erythrocyte-binding antigen 175 (PfeBA-175) and its paralogs in the family of PfeBA and *P. falciparum* erythrocyte-binding ligand (PfeBL) proteins, including PfeBA-140 (also known as BAEBL), PfeBA-181 (also known as JESEBL), and PfeBL-1 (also known as EBA-1); and second, proteins that, based on their homology to *P. vivax* reticulocyte-binding proteins, are termed *P. falciparum* reticulocyte-binding protein homologs (PfrRH) and include PfrRH1, PfrRH2a, PfrRH2b, PfrRH4, and PfrRH5 (93). Several members of these families are sialic acid-dependent, which means they bind neuraminidase-sensitive receptors on erythrocytes, whereas other members are considered sialic acid-independent because they can bind to erythrocytes that are either deficient in various glycoproteins or stripped of their sialic residues by neuraminidase (92). Point polymorphisms in the genes encoding PfeBA-181 and PfeBA-140 modulate receptor binding and may change the specificities of these proteins for different receptors on human erythrocytes (94–96). In the PfrRH family of ligands, upregulation of PfrRH4 has been linked through microarray and transfection studies to the ability of a *P. falciparum* line to switch from sialic acid-dependent to sialic acid-independent invasion (97, 98). More recently, a point polymorphism in the gene encoding PfrRH5 was linked to the ability of a *P. falciparum* line to infect the erythrocytes of *Aotus nancymae* monkeys and cause severe malaria in these primates (99). PfrRH5 molecules containing this polymorphism bound to a neuraminidase-sensitive receptor on *Aotus* and human erythrocytes, whereas PfrRH5 from a parasite that could not infect *Aotus* bound to no receptor on the monkey erythrocytes and to a neuraminidase-resistant receptor on human erythrocytes (99). Invasion pathways can thus be determined by the erythrocyte surface receptors to which PfrRH family members bind, in contrast with a recent proposal that PfrRH ligands must interact with various PfeBLs to determine invasion pathway specificity (8).

P. vivax stands apart from *P. falciparum* in its lack of a family of proteins analogous to PfEMP-1 and its restriction to reticulocytes expressing Duffy blood group antigens. These observations imply mechanisms of survival in the host that are significantly different from those used by *P. falciparum*. *P. vivax* does not depend on sequestering for survival and is therefore usually not associated with microvascular obstruction and regional inflammation, two pathological features of *P. falciparum* malaria. Rather, mature *P. vivax* parasites are thought to circulate through the spleen and avoid destruction by

other means, possibly by mechanisms that include increased (rather than decreased) deformability of the infected host erythrocytes (100). The restriction of *P. vivax* to reticulocytes appears to involve a family of binding proteins (PvRBPs) that target surface receptors of these host cells (8, 101). This restriction is in addition to Duffy blood group-antigen selectivity. The high prevalence of individuals who have lost Duffy blood group antigen from their erythrocytes accounts for the rarity of *P. vivax* in West sub-Saharan Africa (102). An independent origin of Duffy blood group antigen-negativity has been found in Papua New Guinea (103), where the heterozygous state is associated with reduced prevalence of *P. vivax* infection (104).

The genome sequence information that is now available from *P. falciparum*, *P. vivax*, and *P. knowlesi* opens opportunities to understand the many contrasting features of erythrocyte invasion, pathogenesis, and immune system evasion associated with these species (8–10). Only *P. vivax* shows strong restriction to reticulocytes. *P. vivax* and *P. knowlesi*, but not *P. falciparum*, utilize the Duffy blood group antigen for erythrocyte invasion. While the *var*-encoded PfEMP-1 antigens of *P. falciparum* share some characteristics with *P. knowlesi* SICA-*var* antigens (105), no antigen family with similar characteristics has been identified in *P. vivax* (8). On the other hand, the *P. vivax* *vir* and *P. knowlesi* *kir* members of the *Plasmodium* interspersed repeats (*pir*) gene superfamily have features that show closer relationships to each other than to the more distantly related *P. falciparum* *surfin* and *Pfmc-2tm* families of genes (8). Much work remains to clarify the roles of these and other multicopy gene families.

The fall of chloroquine and rise of artemisinins

The introduction of the antimalarial chloroquine and the pesticide dichloro-diphenyl-trichloroethane (DDT) toward the end of World War II brought dramatic new power to malaria control programs (15). Optimism ran high that these chemicals could be used to eliminate malaria, and in 1955, the WHO launched a campaign to eradicate the disease. While that goal proved unattainable, the campaign achieved regional successes and reduced malaria incidence in many areas of the world. In Africa, where the enormity of *An. gambiae* transmission and futility of large-scale DDT-spraying programs were recognized, chloroquine was so cheap, effective, and easy to use that it soon was provided everywhere in massive quantities. Death rates from malaria among children in Africa dropped and, in the 1970s, approached half the level of the prechloroquine years (Figure 4A) (106). However, chloroquine-resistant *P. falciparum* entered Africa in the late 1970s and began to spread rapidly under drug pressure. Morbidity and mortality consequently resurged (107) (Figure 4); in the late 1990s, death rates from malaria across large areas were again approaching the levels of prechloroquine years.

The former efficacy of chloroquine against *P. falciparum* created one of the greatest global health impacts of any antimicrobial drug ever discovered. As resistant parasites spread from their original foci across malaria-endemic regions (Figure 5), treatment failures provided a clear demonstration of how easily a drug can be rendered ineffective when tremendous numbers of pathogens with rapid reproduction cycles are put under widespread selection pressure. Chloroquine-resistant *P. falciparum* is one of many examples in a growing list of drug-resistant organisms that have evolved within the short span of just a couple of human generations, including multidrug-resistant *Mycobacterium tuberculosis* and other highly antibiotic-resistant bacteria (108) that increasingly threaten our world's communities and hospitals.



Genetic linkage studies have shown that chloroquine resistance in *P. falciparum* is determined by polymorphisms in the gene encoding a molecule termed PfCRT, a transporter that, in modified form, promotes drug efflux from parasite digestive vacuoles (reviewed in ref. 109). Strains of *P. vivax* that are highly resistant to chloroquine have been documented in recent years in Oceania and parts of Asia, and cases of *P. vivax* malaria unresponsive to chloroquine treatment have also been reported in South America; but these forms of resistance seem to involve a mechanism independent of polymorphisms in a *P. vivax* ortholog of PfCRT (110). Resolving the mechanism of *P. vivax* chloroquine resistance will require fresh investigative approaches, as many of the critical laboratory culture and genetic methods available for *P. falciparum* are not available for *P. vivax*. Mapping a critical locus of the resistance mechanism may be possible through the use of genome-sequence data and SNPs in genome-wide surveys of *P. vivax* samples that differ in drug responses.

The problem of chloroquine resistance emphasizes the necessity of discovering new replacement drugs through research. It also emphasizes the importance of preserving current antimalarial drugs by effective stewardship, assurance of open and up-to-date sources of information, appropriate education, and use of antimalarial drugs in combinations, rather than as monotherapies that are more prone to select resistant strains. Effective control of antimalarials — including halting their counterfeit and black market distributions — in some cases can promote the return of drug-sensitive parasite strains to regions that have overwhelming levels of drug-resistant malaria. Recent studies from Malawi showed that a policy that removed chloroquine as an available treatment in 1993 was followed by repopulation of the region with chloroquine-sensitive strains by 2001 (111). A similar but slower return of chloroquine-sensitive strains has also been observed in Hainan, People's Republic of China, where use of the drug against *P. falciparum* malaria was suspended in 1979 (112).

Today, artemisinin-based combination therapies (ACTs) are the leading replacements for chloroquine, but costs to consumers must still be reduced if these drug combinations are to be as available and affordable as chloroquine once was. The artemisinin derivatives, discovered and developed in the 1970s by research teams in the People's Republic of China (113), result in rapid parasite clearance and clinical responses, making them especially valuable for the treatment of severe malaria. All artemisinin derivatives remain effective in Southeast Asia, although there are worrisome concerns about delayed parasite clearance times in areas of Cambodia and Thailand (114, 115). It is therefore critical to protect the artemisinins by stopping monotherapy and using them only in combination with effective partner drugs in ACTs. Unfortunately, parasite strains are often already resistant to partner drugs (e.g., lumefantrine, amodiaquine, mefloquine, and sulfadoxine-pyrimethamine) in the first generation ACTs recommended by the WHO. Second generation ACTs con-

taining more effective partner drugs (e.g., piperaquine) are now being developed and deployed.

Perspective

Global action against malaria is receiving great boosts from new coalitions and funding, notably reflected in the Global Malaria Action Plan created in 2008 by the Roll Back Malaria partnership and backed by the United Nations (<http://www.rollbackmalaria.org/gmap/>). Widespread deployment of readily available ACTs, long-lasting insecticide-treated bed nets, indoor residual spraying with insecticides, and programs of intermittent presumptive therapy that reduce the number of malaria episodes in children and pregnant women will have important effects on the burden of disease in malarious regions. Indeed, in results reminiscent of the impact of chloroquine across Africa in the 1960s and 1970s, today's improved access to drug combination treatments and long-lasting insecticide-treated bed nets is producing appreciable reductions in deaths from malaria in a number of African countries. As long as resistance to ACTs and insecticides remains in check, renewed efforts with current tools should be able to provide better than the approximately 50% reduction of mortality achieved in the period of chloroquine's greatest efficacy. Drug and insecticide resistance are bound to spread, however, and discoveries from a solid foundation of biological understanding will be vital to effective malaria control in the future.

Beyond current efforts to reduce the burden of disease, the much more difficult battles to sustain these reductions and eliminate transmission will require taking on the vectorial capacities of mosquitoes such as *An. gambiae* and the immense source of highly adaptable parasites they transmit from asymptomatic individuals. Success in these battles will be achieved only by lasting breaks in the links of parasite transmission between humans and mosquitoes. While new technologies such as antiparasite vaccines (116) promise to be useful in this regard, it is clear that much more than technological innovation is required. Malaria promotes poverty, and poverty promotes malaria (117). Lasting advances against malaria transmission will be rooted in the economic, educational, and social advances that lift populations out of poverty, empower their communities for malaria control, and foster improvements in housing, environment, and health infrastructure that close out opportunities for the parasites to thrive.

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