The pathogenic basis of malaria

Louis H. Miller*, Dror I. Baruch*, Kevin Marsh† & Ogobara K. Doumbo‡

*Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA
†KEMRI-Wellcome Trust Collaboration Programme, Center for Geographic Medicine Research Coast, Post Office Box 230, Kilifi, Kenya (e-mail: kmarsh@kilifi.mimcom.net)
‡Malaria Research and Training Center, Bamako, Mali (e-mail: okd@mrtcbko.malinet.ml)

Malaria is today a disease of poverty and underdeveloped countries. In Africa, mortality remains high because there is limited access to treatment in the villages. We should follow in Pasteur's footsteps by using basic research to develop better tools for the control and cure of malaria. Insight into the complexity of malaria pathogenesis is vital for understanding the disease and will provide a major step towards controlling it. Those of us who work on pathogenesis must widen our approach and think in terms of new tools such as vaccines to reduce disease. The inability of many countries to fund expensive campaigns and antimalarial treatment requires these tools to be highly effective and affordable.

Millions of children die from malaria in Africa every year[1]. But the clinical outcome of an infection in a child depends on many factors (Fig. 1). These factors, often ill-defined, determine the outcome in each child. The top priority must be disease prevention because of the inability of the mothers to access or afford optimal treatment, and the ever-evolving drug resistance. Prevention may be effected through vector control such as insecticide-treated bednets or through the development of antimalarial vaccines.

Malaria: the disease

Over the past 10 years, there have been several key shifts in our understanding of what constitutes severe malaria, and these shifts define the issues in pathogenesis that need to be explored to develop better treatments for sick children. The first shift is the increasing recognition that severe malaria is a disorder that affects several tissues and organs, even when the most marked manifestations may seem to involve a single organ such as the brain. In particular, metabolic acidosis, often profound, has been recognized as a principal pathophysiological feature that cuts across the classical clinical syndromes of cerebral malaria and severe malarial anaemia[2]. It is the single most important determinant of survival and leads directly to a common, but previously poorly recognized, syndrome of respiratory distress[3]. In most cases, this is predominantly (but not exclusively) a lactic acidosis[4]. There are several causes of lactic acidosis in children with severe malaria, from increased production of lactic acid by parasites (through direct stimulation by cytokines) to deceased clearance by the liver; however, most important by far is probably the combined effects of several factors that reduce oxygen delivery to tissues[5].

A key feature of the biology of *Plasmodium falciparum* is its ability to cause infected red blood cells (RBCs) to adhere to the linings of small blood vessels. Such sequestered parasites cause considerable obstruction to tissue perfusion. In addition, in severe malaria there may be marked reductions in the deformability of uninfected RBCs[6,7]. The pathogenesis of this abnormality is not clear, but its strong correlation with acidosis suggests that it may be involved in compromising blood flow through tissues. Individuals affected with malaria are often dehydrated and relatively hypovolaemic[8],

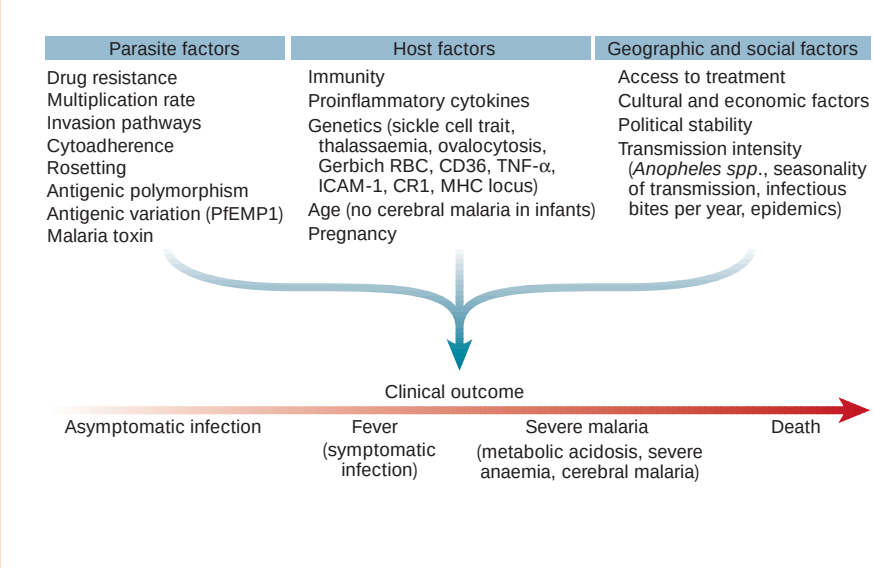

Figure 1 The clinical outcome of malarial infection in an African child depends on many parasite, host, geographic and social factors. These converge in the child to result in a range of outcomes, from an asymptomatic infection to severe disease and death.

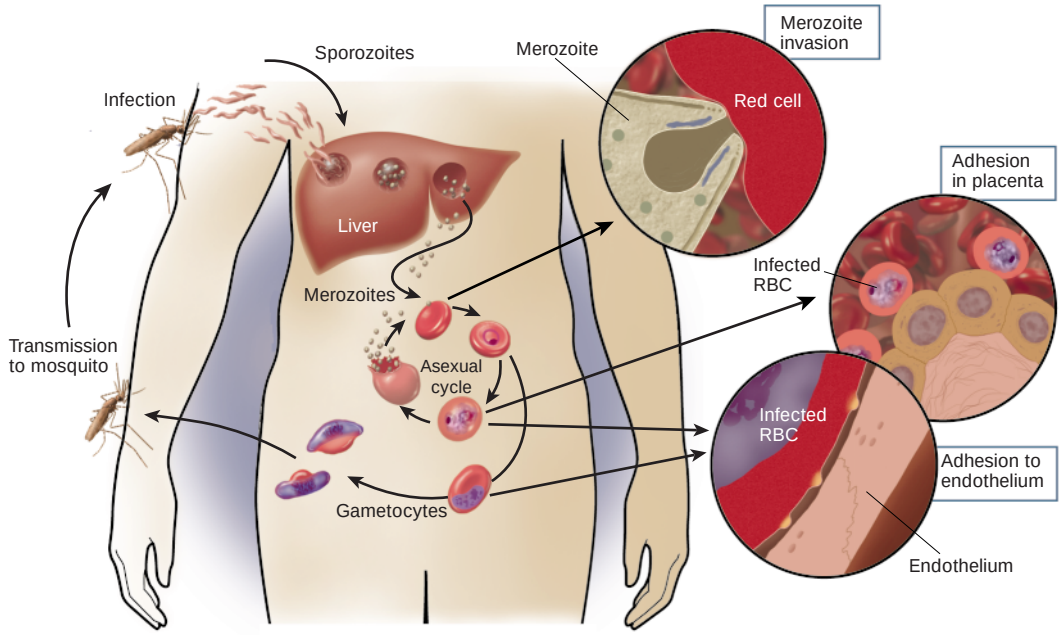

Figure 2 Parasite life cycle and pathogenesis of falciparum malaria. The molecular and cellular events during the parasite life cycle influence the severity of the disease. Disease occurs only as a result of the asexual blood stage after the parasite leaves the liver and begins to invade and grow inside red blood cells (RBCs). All human *Plasmodium* spp. invade by the same mechanism, but *P. falciparum* reaches high parasitaemia because of greater flexibility in the receptor pathways that it can use to invade all RBCs. RBCs infected with *P. falciparum* must bind to endothelium or placenta for the parasite to avoid spleen-dependent killing mechanisms, but this binding also leads to much of the pathology (see Fig. 4).

which potentially exacerbates microvascular obstruction by reducing perfusion pressure. The destruction of RBCs is also an inevitable part of malaria, and anaemia further compromises oxygen delivery.

The second and related shift in our concept of severe malaria is the realization that there is no simple one-to-one correlation between the clinical syndromes and the pathogenic processes. Thus, severe anaemia may arise from many poorly understood mechanisms including acute haemolysis of uninfected RBCs and dyserythropoiesis, as well as through the interaction of malarial infection with other parasite infections and with nutritional deficiencies[9]. For many desperately sick children a simple 'one pathogen/one disease' model is not adequate, as bacteraemia caused by common pathogens may be present with acute malaria and may be a factor in mortality[10,11]. Even the rigorously defined syndrome of cerebral malaria is used to describe children who have arrived at the point of coma through different routes. In many of these children, coma seems to be a response to overwhelming metabolic stress rather than a primary problem in the brain. Such children are often profoundly acidotic and may regain consciousness remarkably quickly after appropriate resuscitation[12], suggesting that cerebral malaria in this instance cannot be a consequence of the classical histologic picture.

Similarly, it has been recognized that a significant proportion of children in coma are, in fact, experiencing covert status epilepticus[13], which responds rapidly to appropriate anticonvulsant therapy. The pathogenesis of this condition is unknown, but again the speed of resolution argues against classical views of pathogenesis. The picture that emerges is one in which many processes lead to a common outcome. These distinctions are much more than academic: they have direct implications for therapy, and they also identify the research issues needed to improve therapy for sick children.

Severe malaria is complex and probably cannot be represented accurately by any single scheme; however, our current understanding of the way in which several key pathogenic processes combine to cause severe disease invokes several basic processes: rapid expansion of infected RBC mass, destruction of both infected and uninfected RBCs, microvascular obstruction, and inflammatory processes that combine to lead to reduced tissue perfusion. This, in turn, may lead to downstream events at a cellular level that further exacerbate the situation.

These general processes, which affect many tissue beds, may also be focused on specific organs in some situations, for instance the brain in cerebral malaria or the placenta during malaria in pregnancy. This could reflect both host-specific factors (for example, an increased likelihood to express particular receptors on cerebral endothelium) and parasite-specific factors (for example, the expression of molecules on the infected RBCs surface that that are particularly suited for binding to certain receptors). In this article, we review the main advances in our understanding of malaria pathogenesis with the hope that these advances will lead to new tools to prevent disease before children become so sick that they need hospitalization.

Although the disease must ultimately be understood in humans, much of our knowledge of pathogenesis depends on studies in non-human species and *in vitro* cultures of *P. falciparum*. The parasitic invasion of hepatocytes and RBCs studied in rodent malarias caused by *Plasmodium berghei* and *Plasmodium yoelii*, and the rhesus malaria caused by *Plasmodium knowlesi*, respectively, have provided insight into these processes. Inflammatory cytokines are often studied in rodent malarias. In addition, these parasite species are important for the screening of drugs and vaccines, including those targeted at human malarias, in New World primates.

Plasmodium life cycle and pathogenesis

Plasmodium falciparum and, to a much lesser extent, *Plasmodium vivax*[14] are the main causes of disease and death from malaria. Mosquitoes inject parasites (sporozoites) into the subcutaneous tissue, and less-frequently directly into the bloodstream; from there, sporozoites travel to the liver (Fig. 2). Evidence indicates that sporozoites pass through several hepatocytes before invasion is followed by

parasite development[15]. The co-receptor on sporozoites that mediates invasion involves, in part, the thrombospondin domains on the circumsporozoite protein and on thrombospondin-related adhesive protein (TRAP). These domains bind specifically to heparin sulphate proteoglycans on hepatocytes in the region in apposition to sinusoidal endothelium and Kuppfer cells[16]. Inside the hepatocyte, each sporozoite develops into tens of thousands of merozoites, which can each invade an RBC on release from the liver. Disease begins only once the asexual parasite multiplies in RBCs. This is the only gateway to disease.

Plasmodium falciparum and *P. vivax* develop over 48 hours in RBCs, producing around 20 merozoites per mature parasite, with each merozoite able to invade other RBCs. A small proportion of asexual parasites converts to gametocytes that are essential for transmitting the infection to others through female anopheline mosquitoes, but cause no disease. Here, the strategy of *P. vivax* differs from that of *P. falciparum*. *P. vivax* develops into gametocytes soon after the release of merozoites from the liver; *P. falciparum* gametocytes develop much later. The early treatment of clinical attacks of malaria by anti-bloodstage chemotherapy for *P. falciparum* also kills the developing gametocytes; *P. vivax* transmits before the symptomatic stage of the disease.

Invasion of RBCs

The sequence of invasion is probably similar for all *Plasmodium* spp. The parasite must engage binding receptors[17] on the RBC, and undergo apical reorientation[18], junction formation[19], and signalling. The parasite induces a vacuole derived from the RBC's plasma membrane and enters the vacuole by a moving junction. Three organelles on the invasive (apical) end of the parasite (rhoptries, micronemes and dense granules) define the phylum Apicomplexa. Receptors that mediate invasion of RBCs by merozoites and invasion of liver by sporozoites are found in micronemes[20], on the cell surface, and in rhoptries. The location of these receptors within organelles may protect the parasite from antibody-mediated neutralization, as the release from apical organelles after contact with the RBC may limit their exposure to antibody.

Identifying the signalling pathways that release organelle contents on contact with a host RBC is a critical issue in parasite biology (Fig. 2). Malaria parasites have intracellular signalling pathways mediated by phosphoinositide, cyclic AMP and calcium-dependent mechanisms. What remains completely unknown is which merozoite surface molecules recognize the RBC surface and then signal the start of the invasion process. Invasion events include releasing essential molecules from apical organelles and initiating the actin–myosin moving junction that brings the parasite inside the vacuole that forms in the RBC. The TRAP protein interacts with skeletal proteins in malaria sporozoites and in *Toxoplasma gondii*[21], but the equivalent molecule for merozoites has yet to be identified.

Both *P. falciparum* and *P. vivax* can cause severe anaemia, but only *P. falciparum* causes the many complications of cerebral malaria, hypoglycaemia, metabolic acidosis and respiratory distress. Certain differences in the biology of the two parasites partially explain the differences in patterns of disease. First, *P. falciparum* can invade a large percentage of RBCs, whereas *P. vivax* is limited to reticulocytes. Similar differences are found between virulent and avirulent *P. yoelii*. Both invade reticulocytes preferentially, but once the reticulocytes are consumed virulent *P. yoelii* can invade all RBCs, leading to higher parasitaemia and death. A comparison between severe and uncomplicated falciparum malaria has suggested a similar pattern of RBC invasion, with the virulent *P. falciparum* invading all RBCs and the avirulent parasites invading only a subpopulation[22].

A second difference is the surprising redundancy of invasion pathways in *P. falciparum* as compared with *P. vivax*. The latter parasite invades only Duffy blood group positive RBCs[23] and is largely limited to reticulocytes. In West Africa, where RBCs are Duffy blood group negative, *P. vivax* has essentially disappeared. The Duffy negative blood group has arisen independently in Papua New Guinea[24] — a region in which *P. vivax* is highly endemic. The limitations of *P. vivax* invasion have led to the discovery of two families of parasite receptors (Fig. 3): first, the parasite molecule that binds to the Duffy blood group system, and homologous Duffy-binding-like (DBL) proteins of *P. falciparum* and *P. knowlesi*[25]; and second, the parasite reticulocyte-binding proteins of *P. vivax*[26], and homologous reticulocyte-binding-like (RBL) proteins of *P. falciparum*[27] and *P. yoelii*[28]. The various members of the DBL and RBL families may recognize different RBC receptors to those of the Duffy blood group or the receptor on reticulocytes. The receptor grouping into DBL and RBL refers to the family of homologous parasite proteins, not the binding specificity on the RBC.

Plasmodium yoelii possesses a large family of RBL genes. Each of the merozoites in a single infected RBC can express a different member of the RBL family[29]. If each has a different RBC-binding specificity, then the parasite has a greater chance for survival. Thus,

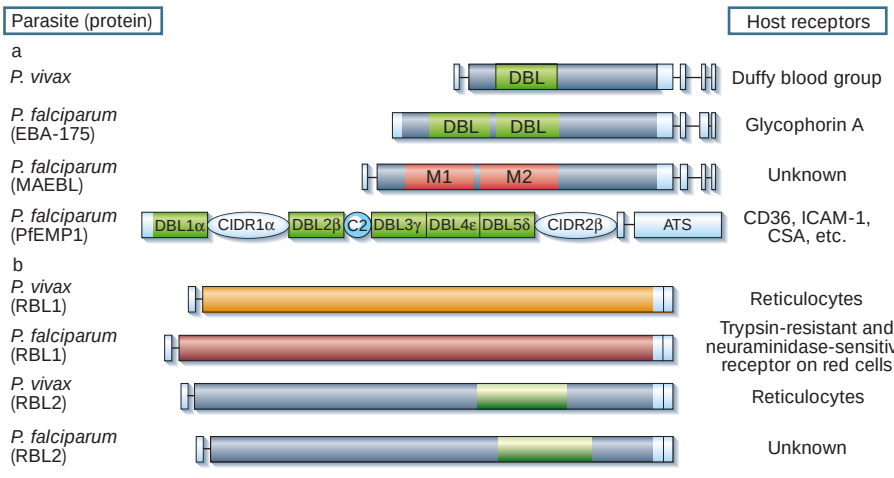

Figure 3 Two families of *Plasmodium* spp. receptors. **a**, The Duffy-binding-like (DBL) family is named after the receptor region of *P. vivax* that binds the Duffy blood group protein on the surface of RBCs. Homologous proteins with a DBL receptor region occur in many *Plasmodium* spp. Whereas *P. vivax* possesses a single DBL protein, *P. falciparum* has several that use RBC receptors other than Duffy, and may in part explain the ability of the parasite to invade all aged RBCs. MAEBL has the same structure as the *P. vivax* protein except that its receptor domain has been replaced by a region that is homologous to another parasite protein. In addition to its function in invasion, DBL is also important as a receptor domain on the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is expressed on the infected RBC surface. See Fig. 4 for the role of PfEMP1 in pathology. **b**, A second family, the reticulocyte binding-like (RBL) parasite proteins, was first discovered in *P. vivax* through its binding to reticulocytes. In *P. vivax*, RBL is present as a RBL1/RBL2 heterodimer. RBL2 has a defined highly homologous region (shaded in green). The receptor domains of the heterodimer remain to be defined, as does the requirement of the heterodimer for binding RBCs in *Plasmodium* spp. other than *P. vivax*.

although the full details of the DBL and RBL families are unknown, these receptors clearly determine much of the flexibility for invasion by the various *Plasmodium* spp. This flexibility, in turn, determines the maximum parasitaemia and disease caused by the various parasites.

Plasmodium falciparum can use its many redundant pathways to invade at equal or reduced efficiency RBCs that lack a particular receptor such as sialic acid[30,31]. Three sialoglycoprotein-dependent pathways involving RBCs and parasite co-receptors have been identified: glycophorin A and the parasite DBL protein EBA-175 (ref. 32); glycophorin C/D and the DBL parasite protein BAEBL[33]; and a trypsin-resistant pathway involving a *P. falciparum* RBL protein[27]. A fourth may involve sialic acid on glycophorin B (ref. 27).

Despite markedly reduced invasion of glycophorin-A-negative RBCs, only glycophorin B mutations occur in Africa. Gerbich RBCs do not express glycophorin D, express an altered glycophorin C and have reduced binding to the parasite molecule BAEBL. Gerbich RBCs are rare in most parts of the world except in the falciparum-endemic regions of Papua New Guinea, where the allele frequency approaches 50% (ref. 34). Such redundancy and alternative pathways are a large advantage to the survival of *P. falciparum* in response to changes in host genetics. The parasite, however, may become less virulent as it adapts to survival in these deficient RBCs.

Studying the DBL and RBL families has begun to yield a molecular understanding of the diverse invasion pathways for *P. falciparum* and other *Plasmodium* spp. Although other parasite proteins on the merozoite surface and in apical organelles have been proposed as receptors[35–37], there is no direct evidence so far. Because invasion is such a complex series of events from RBC binding, to apical reorientation, to entry, it seems likely that several proteins are required for efficient invasion. For example, evidence has suggested that RBC invasion requires the cleavage of a surface protein on the RBC by a parasite serine protease[38]. This parasite enzyme has yet to be identified. Thus, the molecular and cellular events surrounding each step in invasion still remain to be elucidated. Understanding these pathways will give insight into parasite virulence and will facilitate rational vaccine design against merozoite invasion.

Binding of parasitized RBCs to vascular endothelium and placenta

An important difference between *P. falciparum* and other human malarias is the way in which *P. falciparum* modifies the surface of the RBCs so that asexual parasites and gametocytes can adhere to the endothelium and asexual parasites can adhere to the placenta. As a result, only ring forms of *P. falciparum* are found in circulating blood (for review, see refs 39–41). The surface of *P. falciparum* trophozoite- and schizont-infected RBCs is covered with knob-like excrescences that are the contact points with host cells[42]. Adherence protects the parasite from destruction, as non-adherent mature parasitized RBCs are cleared rapidly in the spleen[43].

Attempts to decipher the highly complex and pathogenic adhesion process emphasize how much we have learned and how little we understand. To determine whether and how sequestration can lead to pathogenesis, we should first look at how the parasite sequesters. The *P. falciparum* adhesion process, in which most parasites first tether and then roll, before becoming firmly adherent[44,45], is comparable to leukocyte adhesion. Most host receptors are involved with tethering and rolling but are unable to support on their own firm adhesion under flow[44,46]. Binding to these host receptors is important, however, as it significantly increases adhesion, which may allow the parasite to bind efficiently to the endothelium of various organs[47]. Only two receptors, CD36 and chondroitin sulphate A (CSA), provide stable stationary adherence[44,46].

Parasites sequester themselves in various organs including heart, lung, brain, liver, kidney, subcutaneous tissues and placenta. The various endothelial cells in these organs and syncytiotrophoblasts in placenta express different and variable amounts of host receptors. To successfully adhere to these cells, the parasite can bind to several receptors (ref. 39; and Fig. 4). The adhesion phenotype is not homogenous, and different parasites can bind to variable numbers and combinations of host receptors[48,49]. This variability is believed to affect the tissue distribution and pathogenesis of parasites.

A single parasite protein — *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is expressed at the infected erythrocyte surface — mediates parasite binding to all the various receptors[39–41]. PfEMP1 is encoded by the large and diverse *var* gene family that is involved in clonal antigenic variation and has a central role in *P. falciparum* pathogenesis[50–52]. The multiple adhesion domains located at the extracellular region of PfEMP1 can simultaneously recognize several host receptors. These domains contain variable numbers of different (five types) DBL domains, named for their homology to the DBL domains involved in RBC invasion, and 1–2 cysteine-rich interdomain regions (CIDRs)[52,53]. The binding domains for several host receptors have been mapped to various DBL and CIDR domains[39,54]. The diversity in this gene family is extensive, and numerous *var* genes appear in the parasite population. Although each parasite within an RBC expresses a single *var* gene[55], other *var* genes in its repertoire (out of 50 in its genome) can be expressed up to a rate of 2% per parasite growth cycle[56].

In most cases, the binding to host endothelium does not lead to pathogenesis, as most infections result in malaria that is devoid of complications[57]. What causes the transition from an uncomplicated to a serious infection, such as cerebral malaria, is unclear at present. An intriguing possibility is that the expression of particular binding properties will lead to distinct patterns of sequestration and to pathogenic consequences. One example is the sequestration of infected RBCs in the placenta, which causes premature delivery, low birth weight and increased mortality in the newborn, as well as anaemia in the mother. Parasitized RBCs isolated from placentas have a unique adhesion property that is different from parasites collected from non-pregnant individuals[48,58]. These parasites bind to CSA but fail to adhere to CD36 — the crucial host receptor for sequestration in microvasculature. The apparent dichotomy in adhesion to these receptors has been selected to allow the parasite to sequester not in endothelium but in placenta — perhaps a site of reduced immunity. Indeed, CSA-binding parasites express PfEMP1 with a DBL-γ domain that binds CSA and a non-CD36-binding CIDR1 (refs 59,60). In contrast, CD36-adherent parasites express a PfEMP1 with a CD36-binding CIDR1 (ref. 60).

Sequestration of parasites in the brain may be related to cerebral malaria and may involve the intercellular adhesion molecule 1 (ICAM-1) receptor[41]. Although infected RBCs are bound to brain endothelium at autopsy, it is unknown whether this represents a different distribution of adhesion from that of uncomplicated malaria. An increase in the expression of ICAM-1 in brain endothelium may explain differences in parasite adhesion in cerebral malaria[61,62]. The role of sequestration in other severe complications of malaria remains unclear. Pathogenic connections between adhesion and host receptors are supported by both a nonsense mutation in the gene of the adhesion receptor CD36 that is associated with protection from severe malaria, and the link between complement receptor 1 and ABO blood group antigens and rosetting (the binding of uninfected RBCs)[40,63,64]. Several investigators have suggested that simultaneous binding to multiple receptors might be associated with more severe cases of malaria[65], but specific data are lacking. Some properties, such as rosetting[40] and clumping[66], appear at higher frequencies in cases of severe malaria, but these associations have not been found in all studies and their effect on pathogenesis remains obscure. One possibility is that competition (for adhesion) between parasites drives some of them to develop new adhesion properties and sequester themselves in less desirable locations that lead to pathogenesis.

Although dissecting various individual interactions is a good experimental approach, the outcome of an infection and progression into pathology depend on the specific and dynamic combination of the host and the parasite properties. Clinical disease also changes

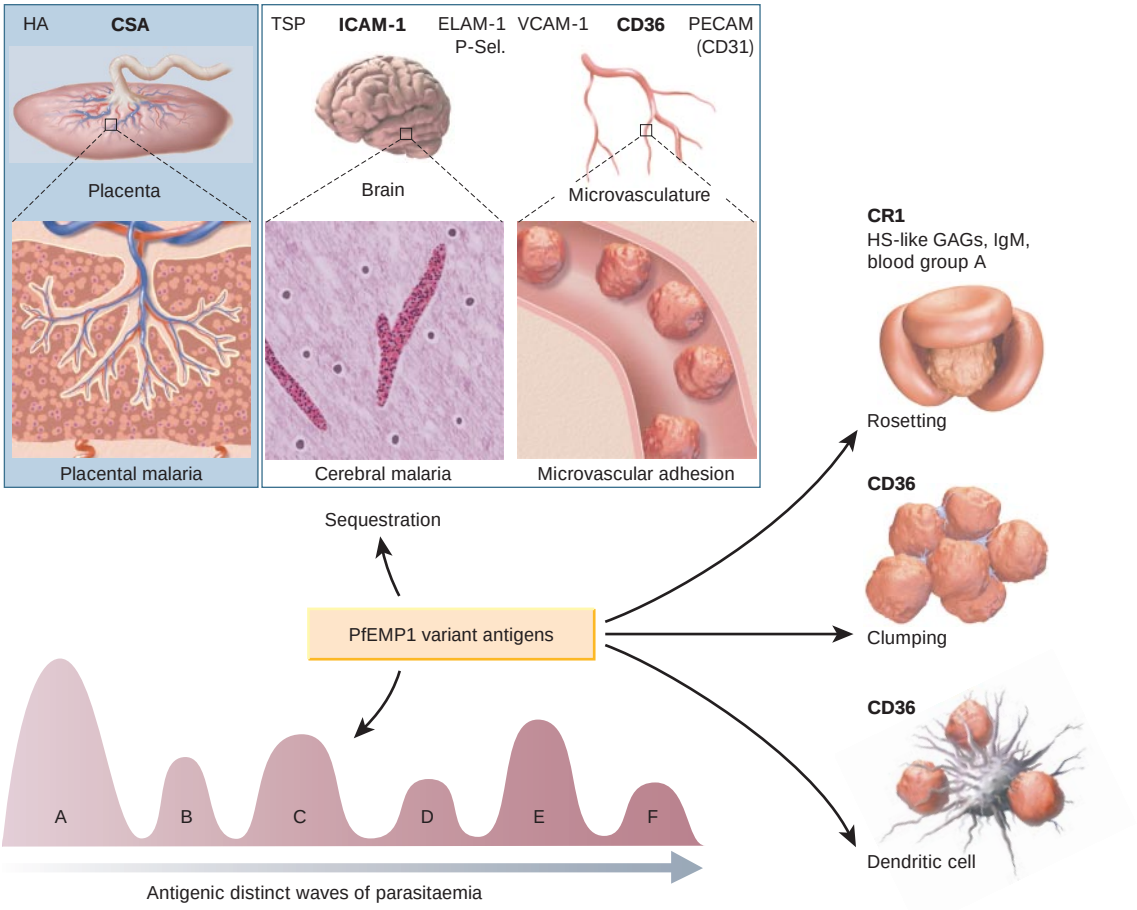

Figure 4 The variant antigen family of PfEMP1 is central to host–parasite interaction and pathogenesis. PfEMP1 expressed on the surface of mature RBCs infected with *P. falciparum* is involved with clonal antigenic variation and can bind to many host receptors through its multiple adhesion domains (Fig. 3). The different properties of PfEMP1 — sequestration for evading spleen-dependent killing and antigenic variation for evading antibody-dependent killing — contribute to the virulence and pathogenesis of *P. falciparum* and are essential for survival of the parasite. Parasite sequestration in the brain and placenta contribute to the complications of cerebral malaria and placental malaria, respectively. Simultaneous binding to several receptors, binding of uninfected erythrocytes (rosetting), and clumping of infected erythrocytes through platelets are associated with the pathogenesis of malaria. Parasite-infected RBCs binding to dendritic cells downregulates the host immune response. HA, hyaluronic acid; TSP, thrombospondin; ELAM-1, endothelial/leukocyte adhesion molecule 1; P-Sel., P-selectin; VCAM-1, vascular cell adhesion molecule 1; PECAM (CD31), platelet endothelial cell adhesion molecule 1; CR1, complement receptor 1; HS-like GAGs, heparin sulphate-like glycosaminoglycans; IgM, immunoglobulin M. Other abbreviations defined in the text.

with age, immunity and transmission rates[57]. Immunity to malaria has a major role in controlling disease and pathogenesis. The properties of PfEMP1 as an adhesion protein (to avoid parasite destruction in the spleen) cannot be separated from its involvement in immune evasion by clonal antigenic variation, which can lead to chronic infection. Even after many exposures, humans are not refractory to malaria parasites but develop clinical immunity that prevents symptomatic disease. This type of immunity limits disease and, although the individual may carry low numbers of parasites, they do not develop into a symptomatic infection[57]. The role of anti-PfEMP1 antibodies in protecting against pathogenic infections is highlighted again in placental malaria. Exposure to parasites that sequester themselves in the placenta during pregnancy induces strain-transcending immunity that stops infected erythrocytes adhering to CSA and may protect the mother and fetus from placental malaria in subsequent pregnancies[67].

During the development of clinical immunity, particularly during early childhood, strain-specific antibodies to PfEMP1 are important in preventing infection with previously encountered isolates[68,69]. This protection can be significant during and after infections with virulent isolates. Bull *et al.*[69,70] have provided evidence for rare and prevalent isolates, and shown that parasites causing severe disease tend to express a subset of variant surface antigens (PfEMP1). Moreover, these isolates were expressed preferentially in children who were less able to recognize (by antibody-mediated agglutination) many isolates. Children exposed once or twice to non-cerebral severe malaria acquire immunity that protects them from this form of the disease[71]. Hence, exposure to pathogenic forms of *P. falciparum* can protect against these parasites, leading to the selection of possibly less virulent parasites in subsequent infections. Despite its variation, regions of PfEMP1 are restricted by function (for example, binding to CD36 or CSA), and these regions may be potential targets for vaccines.

How adhesion progresses to pathology is a principal issue that remains unresolved. Several mechanisms that might cause damage to host endothelium and organs have been proposed, including obstruction of blood flow, and systemic or local production and deposition of pro-inflammatory cytokines (see below). Parasite

adhesion can also affect the endothelium by inducing or blocking signal transduction mediated by host receptors such as CD36. Advances in adhesion research will hopefully provide clues to the mechanism underlying adhesion-related pathogenesis.

The pro-inflammatory immune response and pathogenesis

Antibodies and the pro-inflammatory response protect against the asexual blood stages of some rodent malarias and probably also human malaria. Protection mediated by the pro-inflammatory response may relate to the cytokines tumour-necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), and the release of mediators such as nitric oxide (NO). Clark and Cowden[72] have proposed that mediators, especially NO, are also central to disease. Although it is perfectly logical that these are involved in bone marrow suppression and cerebral malaria, there are no data to prove this as yet. Furthermore, we do not have a animal model in which to study cerebral malaria. One hypothesis suggests that TNF-α induces brain endothelial cells to express ICAM-1 (ref. 73), as vessels in the brain have increased expression of ICAM-1 in cerebral malaria[62]. Although NO has been proposed as the cause of cerebral malaria, there are higher systemic concentrations of NO in uncomplicated malaria than in cerebral malaria[74]. Coma might be caused by local increases in NO in the brain and not by increased concentrations in blood; however, this has not been measured. Indeed, total nitrate plus nitrite levels in the cerebrospinal fluid of children with cerebral malaria are low, and it has been suggested that this may exacerbate NMDA (*N*-methyl-D-aspartate)-mediated neurotoxicity caused by excitotoxins such as quinolinic acid[75].

Data that suggest that a toxin of malarial origin drives the pro-inflammatory response are interesting[76,77], but the physiological significance of this remains to be proved. Evidence that a particular molecule is involved in inducing the pro-inflammatory response has come from an assay measuring the release of TNF-α by macrophages *in vitro*. The isolation of subcellular components from the parasite coupled with this *in vitro* assay led to the identification of the glycosylphosphatidylinositol (GPI) anchor from parasite proteins MSP1 and MSP2 as an inducer of pro-inflammatory cytokines. Antibodies to the GPI anchor are associated with a lack of disease in adults[78], but there is no proof that this is causally related.

Modifications in the immune response to malaria that may not be specific to this disease have been identified. Infection with *P. falciparum* causes apoptosis of mononuclear cells in infected humans[79]. Infecting mice with a rodent malaria to which they had been previously exposed led to the apoptosis of T cells immune to malaria and not those immune to ova, a malaria-unrelated antigen[80]. The cells that were eliminated were pro-inflammatory T cells, which produce IFN-γ and interleukin (IL)-2, but not IL-4. It is unclear whether this is specific to malaria or a more general phenomenon.

Studying genetic differences between populations may inform our understanding of the immune system. Fulanis, an ethnic group in Burkina Faso, have a higher titre of antibodies to many malarial antigens and less disease than two other ethnic groups in the same village who are bitten by equal numbers of infected mosquitoes[81]. The molecular basis is unknown, but the innate immune system may be interacting with the adaptive system to increase antibody titres. The importance of these differences is also highlighted by a study of insecticide-impregnated bednets[82]. The use of insecticide-treated bednets has reduced the infection in Fulanis, but not among other ethnic groups in the same area. Possibly, the higher antibody titres in Fulanis are sufficient to take advantage of the reduction in infectious bites as a result of insecticide-treated bednets.

Perspectives

The clinical outcome of an infection in a child in Africa depends on many factors (Fig. 1). In our attempt to understand disease, we often take a reductionist view and study individual components of the parasite and human in an attempt to identify factors that have a large impact on disease outcome. Such factors can be targets for intervention through the development of new tools such as vaccines. Success in the development and implementation of these new tools will depend on a connection with scientists from endemic countries of Africa who have a better understanding of local customs and are experienced in communicating with the poorest people in villages of Africa. □

1. Snow, R. W., Craig, M., Deichmann, U. & Marsh, K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bull. World Health Organ.* **77,** 624–640 (1999).
2. Marsh, K. *et al.* Indicators of life-threatening malaria in African children. *N. Engl. J. Med.* **332,** 1399–1404 (1995).
3. Taylor, T. E., Borgstein, A. & Molyneux, M. E. Acid–base status in paediatric *Plasmodium falciparum* malaria. *Q. J. Med.* **86,** 99–109 (1993).
4. English, M. *et al.* Deep breathing in children with severe malaria: indicator of metabolic acidosis and poor outcome. *Am. J. Trop. Med. Hyg.* **55,** 521–524 (1996).
5. English, M. *et al.* Acidosis in severe childhood malaria. *Q. J. Med.* **90,** 263–270 (1997).
6. Miller, L. H., Shunichi, U. & Chien, S. Alteration in the rheologic properties of *Plasmodium knowlesi* infected red cells. A possible mechanism of cerebral malaria. *J. Clin. Invest.* **50,** 1451–1455 (1971).
7. Dondorp, A. M., Kager, P. A., Vreeken, J. & White, N. J. Abnormal blood flow and red blood cell deformability in severe malaria. *Parasitol. Today* **16,** 228–232 (2000).
8. English, M. C. *et al.* Hyponatraemia and dehydration in severe malaria. *Arch. Dis. Child.* **74,** 201–205 (1996).
9. Newton, C. R. *et al.* Severe anaemia in children living in a malaria endemic area of Kenya. *Trop. Med. Int. Health* **2,** 165–178 (1997).
10. Berkley, J., Mwarumba, S., Bramham, K., Lowe, B. & Marsh, K. Bacteraemia complicating severe malaria in children. *Trans. R. Soc. Trop. Med. Hyg.* **93,** 283–286 (1999).
11. Prada, J., Alabi, S. A. & Bienzle, U. Bacterial strains isolated from blood cultures of Nigerian children with cerebral malaria. *Lancet* **342,** 1114 (1993).
12. English, M., Waruiru, C. & Marsh, K. Transfusion for respiratory distress in life-threatening childhood malaria. *Am. J. Trop. Med. Hyg.* **55,** 525–530 (1996).
13. Crawley, J. *et al.* Seizures and status epilepticus in childhood cerebral malaria. *Q. J. Med.* **89,** 591–597 (1996).
14. Mendis, K., Sina, B. J., Marchesini, P. & Carter, R. The neglected burden of *Plasmodium vivax* malaria. *Am. J. Trop. Med. Hyg.* **64**(Suppl. 1-2), 97–105 (2001).
15. Mota, M. M. *et al.* Migration of *Plasmodium sporozoites* through cells before infection. *Science* **291,** 141–144 (2001).
16. Frevert, U. *et al.* Malaria circumsporozoite protein binds to heparan sulfate proteoglycans associated with the surface membrane of hepatocytes. *J. Exp. Med.* **177,** 1287–1298 (1993).
17. Chitnis, C. E. Molecular insights into receptors used by malaria parasites for erythrocyte invasion. *Curr. Opin. Hematol.* **8,** 85–91 (2001).
18. Dvorak, J. A., Miller, L. H., Whitehouse, W. C. & Shiroishi, T. Invasion of erythrocytes by malaria merozoites. *Science* **187,** 748–750 (1975).
19. Aikawa, M., Miller, L. H., Johnson, J. & Rabbege, J. Erythrocyte entry by malarial parasites. A moving junction between erythrocyte and parasite. *J. Cell Biol.* **77,** 72–82 (1978).
20. Adams, J. H. *et al.* The Duffy receptor family of *Plasmodium knowlesi* is located within the micronemes of invasive malaria merozoites. *Cell* **63,** 141–153 (1990).
21. Kappe, S. *et al.* Conservation of a gliding motility and cell invasion machinery in Apicomplexan parasites. *J. Cell Biol.* **147,** 937–944 (1999).
22. Chotivanich, K. *et al.* Parasite multiplication potential and the severity of falciparum malaria. *J. Infect. Dis.* **181,** 1206–1209 (2000).
23. Miller, L. H., Mason, S. J., Clyde, D. F. & McGinniss, M. H. The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. *N. Engl. J. Med.* **295,** 302–304 (1976).
24. Zimmerman P. A. *et al.* Emergence of FY*A^{null} in a *Plasmodium vivax*-endemic region of Papua New Guinea. *Proc. Natl Acad. Sci. USA* **96,** 13973–13977 (1999).
25. Adams, J. H. *et al.* A family of erythrocyte binding proteins of malaria parasites. *Proc. Natl Acad. Sci. USA* **89,** 7085–7089 (1992).
26. Galinski, M. R., Medina, C. C., Ingravallo, P. & Barnwell, J. W. A reticulocyte-binding protein complex of *Plasmodium vivax* merozoites. *Cell* **69,** 1213–1226 (1992).
27. Rayner, J. C., Vargas-Serrato, E., Huber, C., Galinski, M. R. & Barnwell, J. W. A *Plasmodium falciparum* homologue of *Plasmodium vivax* reticulocyte binding protein (PvRBP1) defines a trypsin-resistant erythocyte invasion pathway. *J. Exp. Med.* **194,** 1571–1582 (2001).
28. Keen, J. K., Sinha, K. A., Brown, K. N. & Holder, A. A. A gene coding for a high-molecular mass rhoptry protein of *Plasmodium yoelii*. *Mol. Biochem. Parasitol.* **65,** 171–177 (1994).
29. Preiser, P. R., Jarra, W., Capiod, T. & Snounou, G. A rhoptry-protein-associated mechanism of clonal phenotypic variation in rodent malaria. *Nature* **398,** 618–622 (1999).
30. Okoyeh, J. N., Pillai, C. R. & Chitnis, C. E. *Plasmodium falciparum* field isolates commonly use erythrocyte invasion pathways that are independent of sialic acid residues of glycophorin A. *Infect. Immun.* **67,** 5784–5791 (1999).
31. Dolan, S. A., Miller, L. H. & Wellems, T. E. Evidence for a switching mechanism in the invasion of erythrocytes by *Plasmodium falciparum*. *J. Clin. Invest.* **86,** 618–624 (1990).
32. Sim, B. K., Chitnis, C. E., Wasniowska, K., Hadley, T. J. & Miller, L. H. Receptor and ligand domains for invasion of erythrocytes by *Plasmodium falciparum*. *Science* **264,** 1941–1944 (1994).
33. Mayer, D. C., Kaneko, O., Hudson-Taylor, D. E., Reid, M. E. & Miller, L. H. Characterization of a *Plasmodium falciparum* erythrocyte-binding protein paralogous to EBA-175. *Proc. Natl Acad. Sci. USA* **98,** 5222–5227 (2001).
34. Patel, S. S. *et al.* The association of the glycophorin C exon 3 deletion with ovalocytosis and malaria susceptibility in the Wosera, Papua New Guinea. *Blood* **98,** 3489–3491 (2001).
35. Patiño, J. A. G., Holder, A. A., McBride, J. S. & Blackman, M. J. Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. *J. Exp. Med.* **186,** 1689–1699 (1997).
36. Hodder, A. N. *et al.* The disulfide bond structure of *Plasmodium* apical membrane antigen-1. *J. Biol. Chem.* **271,** 29446–29452 (1996).

37. Triglia, T. *et al.* Apical membrane antigen 1 plays a central role in erythrocyte invasion by *Plasmodium* species. *Mol. Microbiol.* **38,** 706–718 (2000).
38. Braun-Breton, C. *et al. Plasmodium chabaudi* p68 serine protease activity required for merozoite entry into mouse erythrocytes. *Proc. Natl Acad. Sci. USA* **89,**9647–9651 (1992).
39. Baruch, D. I. Adhesive receptors on malaria-parasitized red cells. *Baillieres Best Pract. Res. Clin. Haematol.* **12,** 747–761 (1999).
40. Chen, Q., Schlichtherle, M. & Wahlgren, M. Molecular aspects of severe malaria. *Clin. Microbiol. Rev.* **13,** 439–450 (2000).
41. Newbold, C. *et al.* Cytoadherence, pathogenesis and the infected red cell surface in *Plasmodium falciparum*. *Int. J. Parasitol.* **29,** 927–937 (1999).
42. Luse, S. A. & Miller, L. H. *Plasmodium falciparum* malaria. Ultrastructure of parasitized erythrocytes in cardiac vessels. *Am. J. Trop. Med. Hyg.* **20,** 655–660 (1971).
43. Langreth, S. G. & Peterson, E. Pathogenicity, stability, and immunogenicity of a knobless clone of *Plasmodium falciparum* in Colombian owl monkeys. *Infect. Immun.* **47,** 760–766 (1985).
44. Cooke, B. M. *et al.* Rolling and stationary cytoadhesion of red blood cells parasitized by *Plasmodium falciparum*: separate roles for ICAM-1, CD36 and thrombospondin. *Br. J. Haematol.* **87,** 162–170 (1994).
45. Ho, M. & White, N. J. Molecular mechanisms of cytoadherence in malaria. *Am. J. Physiol.* **276,** C1231–C1242 (1999).
46. Rogerson, S. J., Novakovic, S., Cooke, B. M. & Brown, G. V. *Plasmodium falciparum*-infected erythrocytes adhere to the proteoglycan thrombomodulin in static and flow-based systems. *Exp. Parasitol.* **86,** 8–18 (1997).
47. Yipp, B. G. *et al.* Synergism of multiple adhesion molecules in mediating cytoadherence of *Plasmodium falciparum*-infected erythrocytes to microvascular endothelial cells under flow. *Blood* **96,** 2292–2298 (2000).
48. Beeson, J. G. *et al. Plasmodium falciparum* isolates from infected pregnant women and children are associated with distinct adhesive and antigenic properties. *J. Infect. Dis.* **180,** 464–472 (1999).
49. Newbold, C. *et al.* Receptor-specific adhesion and clinical disease in *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **57,** 389–398 (1997).
50. Baruch, D. I. *et al.* Cloning the *P. falciparum* gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell* **82,** 77–87 (1995).
51. Smith, J. D. *et al.* Switches in expression of *Plasmodium falciparum var* genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell* **82,** 101–110 (1995).
52. Su, X. Z. *et al.* The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. *Cell* **82,** 89–100 (1995).
53. Smith, J. D., Subramanian, G., Gamain, B., Baruch, D. I. & Miller, L. H. Classification of adhesive domains in the *Plasmodium falciparum* erythrocyte membrane protein 1 family. *Mol. Biochem. Parasitol.* **110,** 293–310 (2000).
54. Smith, J. D., Gamain, B., Baruch, D. I. & Kyes, S. Decoding the language of *var* genes and *Plasmodium falciparum* sequestration. *Trends Parasitol.* **17**,538–545 (2001).
55. Chen, Q. *et al.* Developmental selection of *var* gene expression in *Plasmodium falciparum*. *Nature* **394,** 392–395 (1998).
56. Roberts, D. J. *et al.* Rapid switching to multiple antigenic and adhesive phenotypes in malaria. *Nature* **357,** 689–692 (1992).
57. Snow, R. W. & Marsh, K. New insights into the epidemiology of malaria relevant for disease control. *Br. Med. Bull.* **54,** 293–309 (1998).
58. Fried, M. & Duffy, P. E. Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. *Science* **272,** 1502–1504 (1996).
59. Buffet, P. A. *et al. Plasmodium falciparum* domain mediating adhesion to chondroitin sulfate A: a receptor for human placental infection. *Proc. Natl Acad. Sci. USA* **96,** 12743–12748 (1999).
60. Gamain, B., Smith, J. D., Miller, L. H. & Baruch, D. I. Modifications in the CD36 binding domain of the *Plasmodium falciparum* variant antigen are responsible for the inability of chondroitin sulfate A adherent parasites to bind CD36. *Blood* **97,** 3268–3274 (2001).
61. Ockenhouse, C. F. *et al.* Molecular basis of sequestration in severe and uncomplicated *Plasmodium falciparum* malaria: differential adhesion of infected erythrocytes to CD36 and ICAM-1. *J. Infect. Dis.* **164,** 163–169 (1991).
62. Turner, G. D. *et al.* An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *Am. J. Pathol.* **145,** 1057–1069 (1994).
63 Pain, A. *et al.* A non-sense mutation in CD36 gene is associated with protection from severe malaria. *Lancet* **357,** 1502–1503 (2001).
64. Rowe, J. A., Moulds, J. M., Newbold, C. I. & Miller, L. H. *P. falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. *Nature* **388,** 292–295 (1997).
65. Heddini, A. *et al.* Fresh isolates from children with severe *Plasmodium falciparum* malaria bind to multiple receptors. *Infect. Immun.* **69**,5848–5856 (2001).
66. Pain, A. *et al.* Platelet-mediated clumping of *Plasmodium falciparum*-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. *Proc. Natl Acad. Sci. USA* **98,** 1805–1810 (2001).
67. Fried, M., Nosten, F., Brockman, A., Brabin, B. J. & Duffy, P. E. Maternal antibodies block malaria. *Nature* **395,** 851–852 (1998).
68. Bull, P. C. *et al.* Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nature Med.* **4,** 358–360 (1998).
69. Bull, P. C., Lowe, B. S., Kortok, M. & Marsh, K. Antibody recognition of *Plasmodium falciparum* erythrocyte surface antigens in Kenya: evidence for rare and prevalent variants. *Infect. Immun.* **67,** 733–739 (1999).
70. Bull, P. C. *et al. Plasmodium falciparum*-infected erythrocytes: agglutination by diverse Kenyan plasma is associated with severe disease and young host age. *J. Infect. Dis.* **182,** 252–259 (2000).
71. Gupta, S., Snow, R. W., Donnelly, C. A., Marsh, K. & Newbold, C. Immunity to non-cerebral severe malaria is acquired after one or two infections. *Nature Med.* **5,** 340–343 (1999).
72. Clark, I. A. & Cowden, W. B. Why is the pathology of falciparum worse than that of vivax malaria? *Parasitol. Today* **15,** 458–461 (1999).
73. Wong, D. & Dorovini-Zis, K. Upregulation of intercellular adhesion molecule-1 (ICAM-1) expression in primary cultures of human brain microvessel endothelial cells by cytokines and lipopolysaccharide. *J. Neuroimmunol.* **39,** 11–21 (1992).
74. Levesque, M. C. *et al.* Nitric oxide synthase type 2 promoter polymorphisms, nitric oxide production, and disease severity in Tanzanian children with malaria. *J. Infect. Dis.* **180,** 1994–2002 (1999).
75. Dobbie, M., Crawley, J., Waruiru, C., Marsh, K. & Surtees, R. Cerebrospinal fluid studies in children with cerebral malaria: an excitotoxic mechanism? *Am. J. Trop. Med. Hyg.* **62,** 284–290 (2000).
76. Schofield, L. & Hackett, F. Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites. *J. Exp. Med.* **177,** 145–153 (1993).
77. Tachado, S. D. *et al.* Glycosylphosphatidylinositol toxin of *Plasmodium* induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signaling pathway. *J. Immunol.* **156,** 1897–1907 (1996).
78. Naik, R. S. *et al.* Glycosylphosphatidylinositol anchors of *Plasmodium falciparum*: molecular characterization and naturally elicited antibody response that may provide immunity to malaria pathogenesis. *J. Exp. Med.* **192,** 1563–1576 (2000).
79. Toure-Balde, A. *et al. Plasmodium falciparum* induces apoptosis in human mononuclear cells. *Infect. Immun.* **64,** 744–750 (1996).
80. Hirunpetcharat, C. & Good, M. F. Deletion of *Plasmodium berghei*-specific $CD4^+$ T cells adoptively transferred into recipient mice after challenge with homologous parasite. *Proc. Natl Acad. Sci. USA* **95,** 1715–1720 (1998).
81. Modiano, D. *et al.* Different response to *Plasmodium falciparum* malaria in West African sympatric ethnic groups. *Proc. Natl Acad. Sci. USA* **93,** 13206–13211 (1996).
82. Modiano, D. *et al.* Baseline immunity of the population and impact of insecticide-treated curtains on malaria infection. *Am. J. Trop. Med. Hyg.* **59,** 336–340 (1998).

Acknowledgements
We thank J. Barnwell for help with Fig. 2 and for sharing data before publication.