Malaria Pathogenesis

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Malaria is a disease caused by repeated cycles of growth of the parasite *Plasmodium* in the erythrocyte. Various cellular and molecular strategies allow the parasite to evade the human immune response for many cycles of parasite multiplication. Under certain circumstances *Plasmodium* infection causes severe anemia or cerebral malaria; the expression of disease is influenced by both parasite and host factors, as exemplified by the exacerbation of disease during pregnancy. This article provides an overview of malaria pathogenesis, synthesizing the recent field, laboratory, and epidemiological data that will lead to the development of strategies to reduce mortality and morbidity.

Malaria, a disease caused by parasites of the genus *Plasmodium*, places a huge burden on human life. Individuals in all continents are potentially at risk, but the greatest suffering falls to people in tropical countries. The degree of endemicity varies between countries and even between different areas in the same country. In regions of very high endemicity, the greatest suffering is borne by children less than 5 years of age, whereas in areas of low endemicity, the disease affects all age groups. The patterns of pathology also differ with changes in the degree of endemicity. In areas of high endemicity, although individuals after 5 years of age continue to harbor malaria parasites, the frequency of disease is greatly reduced. This protection from disease in older children, known as clinical immunity, usually is never reached in regions where there is very low or seasonal exposure to parasites. Pregnant women, even if previously clinically immune, have a significantly enhanced risk for a pathogenic process that has a major effect on the fetus and newborn, particularly during the first pregnancy.

Pathogenesis relates to the various host and parasite factors that are responsible for causing pathology. By understanding the pathogenesis of malaria, we will be better able to develop strategies to prevent the most severe forms of malaria. Currently, prevention of disease is dependent on avoiding contact with mosquitoes or on chemoprophylaxis. Unfortunately, the emergence of drug-resistant parasites in some areas of the world has rendered chemoprophylaxis less effective; consequently, a major question asked of malaria research today is whether it will be possible to develop vaccines to lessen disease.

Our limited knowledge of malaria pathogenesis, to date, has come from the study of both human malaria and animal models. Animal models are of value when it is not possible to conduct particular experiments in humans. Unfortunately, however, there is no truly relevant animal model for studying pathogenic processes caused by *P. falciparum*, the most deadly of the human plasmodia; therefore, observations made in humans are of central importance. In this article, we will document a number of such observations relevant to disease and outline the current understanding of malaria, focusing on its severe forms.

To begin to understand malaria, it is necessary to understand the life cycle of the parasite. The infection commences with the intravenous inoculation of sporozoites by infected mosquitoes. These invade hepatocytes and undergo multiplication for about a week before thousands of merozoites rupture these cells to invade erythrocytes and commence the erythrocytic cycle. Probably only tens to hundreds of hepatocytes are targeted by sporozoites, and this stage, which takes place in the liver, is not responsible for any disease. The pathogenic process occurs only during the erythrocytic cycle. During this stage, there is a huge, periodic amplification of the size of parasite populations that may enhance the probability of differentiation to gametocytes, the stage infectious to mosquitoes. A peculiarity of *P. falciparum* is its ability to adhere to venular endothelium (cytoadherence) of erythrocytes infected with maturing parasites. The parasitized erythrocytes remain attached until merozoites are formed that are released to invade other erythrocytes. Thus, the predominant form seen in the peripheral circulation is the ring-infected erythrocyte, the young form of the parasite. The implications of cytoadherence for parasite survival and for pathology will be discussed later.

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The Immune System: Parasite Survival Strategy and Disease

As a prelude to later sections on malaria pathogenesis, it is helpful to consider the multifaceted nature of the interaction between the host immune system and the parasite. Central to this interaction are cytokines that are released by immunocompetent cells in a highly regulated fashion (*1*). They participate in the control of all immunologically relevant events, whether they concern either activation, proliferation, and subsequent effector functions of recirculating immunocompetent cells or regulation of cells residing in tissues (for example, resident mononuclear phagocytes and endothelial cells). It has been established that cytokines not only participate in the qualitative (for example, antibody isotype switch) and quantitative regulation of the immune response but also participate in many other complex processes such as hematopoiesis and pregnancy.

During the erythrocytic cycle, soluble products of *Plasmodium* spp. known as malarial toxins direct systemic release of proinflammatory cytokines [for example, tumor necrosis factor–α (TNF-α)] which act on many other cellular systems such as endothelium. Equally important are parasite antigens, which stimulate T cells to directly secrete or induce production of cytokines from other cells. Before *P. falciparum* infection, many individuals have *P. falciparum*–reactive T cells, often at high frequency. Such parasite-reactive T cells have probably arisen as a result of antigenic cross-reactivity between environmental organisms and parasite-derived molecules (*2*). Incubation of blood mononuclear cells with parasitized erythrocytes can drive proliferation of these T cells even when the parasitemia is as low as one parasite per microliter of blood.

Because many of these T cells secrete interferon γ (IFN-γ) and other cytokines and can facilitate production of TNF-α by monocytes, they have the potential to be involved in disease pathogenesis. As will be discussed later, both IFN-γ and TNF-α may play roles in dyserythropoietic anemia, and TNF-α may contribute to cerebral malaria as a result of up-regulation of intercellular adhesion molecule–1 (ICAM-1) in cerebral blood vessel endothelium. With respect to TNF-α production, T cells—whether they express γδ (*3*) or αβ T cell receptors—may play as important a role in disease patho-

genesis as that postulated for the direct stimulation of mononuclear phagocytes by malarial toxins.

It is likely that parasite-dependent activation of T cells and mononuclear phagocytes leads not only to disease but also to killing of parasites. Whether all of the cross-reactive T cells, once activated during the primary infection, will remain functional during a persistent infection or until exposure to reinfection deserves to be studied.

The parasite also has other strategies for interacting with the immune system, including the following: (i) antigenic variation (see the section on persistent infections and reinfections); (ii) a still undefined, splenic-dependent regulation of parasite genes encoding structural proteins and adhesive molecules on the erythrocyte surface that are involved in adherence to endothelium (see the section on cerebral malaria); and (iii) low immunogenicity of conserved parasite peptides that are targets of antibodies able to interfere with parasite survival (see the section on persistent infections and reinfections). How low immunogenicity relates to the T and B repertoires and their development during the neonatal period will not be addressed in this review (*4*), although it is a domain that demands further study.

Invasion of Erythrocytes

Two aspects of invasion have a profound effect on pathogenesis: invasion of all erythrocytes or only a subpopulation (for example, reticulocytes), and redundancy in invasion pathways. *Plasmodium vivax* invades only reticulocytes, and *P. falciparum* invades erythrocytes of all ages. The *P. vivax* ligand that binds only reticulocytes has homology to a protein in the mouse malaria *P. yoelii* (*5*). The *P. yoelii* protein is a member of a gene family in *P. yoelii*. Antibodies raised to the *P. yoelii* protein lead to a switch in erythrocyte preference from invasion of all erythrocytes to invasion of only reticulocytes (*6*), and as a result, the maximum parasitemia is lower. This switch converts a lethal infection into a nonlethal one.

One aspect of redundancy is the ability to infect the entire polymorphic human population. *Plasmodium vivax*, which lacks this redundancy, cannot invade erythrocytes of West Africans who are Duffy blood group negative (*7*). The *P. falciparum* ligand homologous to the Duffy binding ligand of *P. vivax* binds specifically to glycophorin A, binding that is dependent on both sialic acid and the peptide backbone of glycophorin A (*8*). In the case of *P. falciparum*, however, there are a number of invasion pathways independent of glycophorin A (*9*). Consequently, erythrocytes missing glycophorin A can be invaded, although at a lower frequency (*10*). An alternative pathway involves glycophorin B (*9*) and may explain the high frequency of glycophorin B–negative erythrocytes in a pygmy population (*9*).

The host has greater success against *P. falciparum* through mutations that do not affect receptors. Melanesian ovalocytosis, a band 3 mutation (*11*) that partially blocks invasion of *P. vivax* and *P. falciparum* (*12*), is protective, presumably through effects on the erythrocyte cytoskeleton. Although the homozygous is lethal during fetal development, the gene has reached frequencies of 16% in Madang, Papua New Guinea (*13*), indicating that—similar to the case of hemoglobin S—the gene gives a high level of protection in the heterozygote.

For many viruses, immunity is heralded by the rise in neutralizing antibodies. In contrast, despite persistent malarial infection over decades, antibodies that block invasion of erythrocytes usually do not appear. For example, immunoglobulin G (IgG) from immune adult Africans that controlled falciparum infections in Thai patients did not block erythrocyte invasion in vitro by *P. falciparum* from these patients (*14*). Why do blocking antibodies not occur? Some of the malaria surface proteins, such as MSP-1, have a high degree of antigenic diversity between clones (*15*). Others may have redundant functions such that selection for deletions under immune pressure has no effect on parasite viability in vivo (*16*). One mechanism of redundancy may be multiple copies of genes with different receptor specificities. The switching on and off of these genes may explain the change in host erythrocyte specificity. A *P. falciparum* clone that was unable to invade neuraminidase-treated erythrocytes was able to invade them normally after selection by growth in neuraminidase-treated cells (*17*). Presumably, a switch in a receptor-binding protein of the merozoite changed the specificity of invasion. Such mechanisms could also lead to immune evasion.

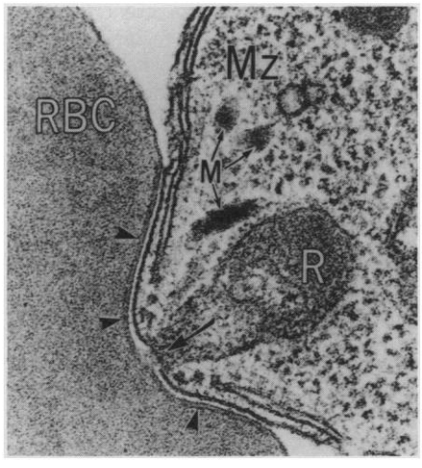

Fig. 1. A merozoite (Mz) invading an erythrocyte (RBC). Two apican organelles, rhoptries (R) and micronemes (M), contain ligands for binding erythrocyte receptors. It is unknown if these ligands are expressed only after the merozoite contacts the erythrocyte or if they are expressed constitutively. The potential concealment of ligands within organelles could limit exposure of parasite molecules to blocking antibodies.

The parasite conceals proteins of invasion within organelles until the merozoite comes into contact with the erythrocyte, when signaling probably leads to their release (Fig. 1). The ligands for binding the Duffy blood group substance on the erythrocyte surface are within an apical organelle, the microneme, and not exposed to antibody on free, viable merozoites (*18*). This limited exposure may explain the unusual finding that a Fab fragment of a monoclonal antibody against a protein in another apical organelle, the rhoptry, blocks invasion more effectively than the bivalent IgG molecule (*19*). Because of the limited exposure to proteins during the release of the malaria parasite from one erythrocyte and its invasion into another, high-titer, high-affinity antibodies may be required to block invasion. The possible low immunogenicity of these target antigens is discussed elsewhere (see the next section on persistent infections and reinfections).

Persistent Infections and Reinfections

The host's susceptibility to a persistent *P. falciparum* infection after a single inoculation and to multiple reinfections is of central importance to both disease pathogenesis and to the parasite's survival strategy. Thus, individuals living in endemic areas, although clinically immune, often remain persistently parasitemic; asexual parasites are continuously switching to gametocytes that are infectious to mosquitoes. In children, this parasite strategy for survival puts the children at continuous risk of disease until the development of clinical immunity.

A single infection persists for a long time

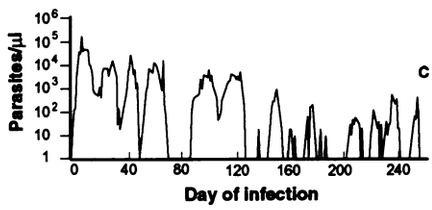

Fig. 2. Fluctuation of *P. falciparum* in the blood after a single infection by mosquito inoculation of parasites. The persistent blood infection is probably because of antigenic variation of a parasite antigen on the surface of infected erythrocytes. The symbol C on day 260 refers to a curative dose of chloroquine (*72*).

and is characterized by periodic peaks and troughs in peripheral parasitemia (Fig. 2). One study found that *P. falciparum* infections could persist for as long as 480 days (*20*). Why is the immune system unable to control such persistent infections? Studies of *P. knowlesi* in monkeys are of prime interest in this context. Recrudescences, periodic peaks in parasitemia, were studied in a monkey after a single infection of *P. knowlesi*, and the parasites during recrudescences were shown to express variant antigens on the surface of infected erythrocytes (*21*). This is referred to as antigenic variation, because cloned parasites express multiple antigenic types (*22*). Thus, through antigenic variation, the parasite is able to evade the immune response directed against these antigens. There is evidence from *P. falciparum* to support the view that antigenic variation accounts for recrudescences (*23, 24*). Cloned parasites in vitro undergo antigenic variation at a rate of approximately 2% (*24*). A study in The Gambia found that postinfection serum from children would agglutinate infected erythrocytes of homologous parasites but not isolates from other children (*25*), suggesting a high level of antigenic diversity. It is unknown in this case, however, if variation between isolates represents polyclonal parasite populations, each clone differing constitutively from the others, or if a clonal population is undergoing antigenic variation.

Deliberate exposure of naïve adults to *P. falciparum* results in chronic recrudescing infections. Rechallenge of these individuals with *P. falciparum* demonstrates that they have developed a degree of protection that is effective against heterologous parasites, although more effective against homologous parasites (*26*). The mechanism of protection against the homologous parasite is not known, nor is the reason for less efficient protection against heterologous parasites. If protection is largely dependent on the acquisition of antibodies to a family of variant antigens, then resistance to both homologous and heterologous parasites might be thought to be equivalent. Because this is not observed, each genotype may express a repertoire of nonoverlapping variants, or other isolate-specific mechanisms of protection may exist.

Whether antigenic variation and highly polymorphic parasite populations account for the slow development of clinical immunity is unknown. Transfer of purified IgG from sera of immune adults results in a rapid drop in parasitemia (*14, 27*). This IgG is able to mediate an antibody-dependent cellular inhibition of parasite growth in vitro but does not block merozoite invasion of erythrocytes (*14*). There is some evidence that the protective effects of IgG from malaria-immune individuals is mediated by cytophilic antibodies (*28*).

The specificities of the protective antibodies remain to be identified. One hypothesis which is supported by some (*25*) but not other studies (*29*) is that adults develop antibodies to conserved parasite epitopes on the erythrocyte surface. Such epitopes present on the erythrocyte surface or on other parasite molecules may not be immunodominant in the native protein and may take many years to induce the production of protective antibodies. An analogous situation may occur in group A *Streptococcus*, where protection is mediated by antibodies to the M protein. The NH_2-terminal, highly polymorphic determinants are immunodominant, but antibodies can be raised to a conserved region with a synthetic peptide as immunogen, and these antibodies can opsonize the bacterium (*30*). Adults living in areas where there is high exposure to *Streptococci* can develop antibodies to the conserved epitope on the M protein, which may contribute to the immunity adults have to *Streptococci* (*31*).

A philosophical question that is often raised is whether we should aim to have a vaccine that induces the mechanisms expressed during development of natural immunity or one that induces protection by means of a different route. It is often argued that, because natural immunity takes so long to develop, other mechanisms indeed should be sought. One way to refocus the question is as follows. Natural immunity, when it does develop, is very effective, at least under conditions of permanent exposure. Attempts to mimic it within a much shorter period of time would seem worthwhile. Identification of conserved epitopes that are targets of protective antibodies should enable design of vaccines that, unlike native parasite proteins, will induce protective antibody responses.

Severe Disease

Fever and chills occur at the time of rupture of erythrocytes containing merozoites that are freed to invade other erythrocytes. These nonspecific signs of malaria are believed to be caused by release of a malarial toxin that induces macrophages to secrete TNF-α and interleukin-1 (*32, 33*), common mediators induced by *Plasmodium* spp. Except for anemia, there are a number of severe complications that are specific to *P. falciparum* infections. In the nonimmune patient, many of the severe complications of *P. falciparum*—such as cerebral malaria, anemia, hypoglycemia, renal failure, and noncardiac pulmonary edema—occur in combination or as isolated complications. For a detailed description of the clinical disease, see (*34*). The pattern of severe disease in the African child differs in that renal failure and noncardiac pulmonary edema do not occur. The pattern is also influenced by the number of infections per year (see below), perhaps by seasonality of infection and by the potential difference in rapidity of onset of clinical immunity when the initial infections occur in adults (*35*). Identification of severe disease within each community is critical for two reasons: the rational design of interventions and the yardstick for effectiveness of any intervention.

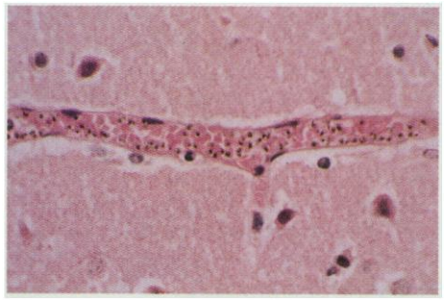

Fig. 3. *Plasmodium falciparum* lining capillaries and venules of the brain of a patient who died of cerebral malaria. [Reproduced from M. Aikawa, Case Western Reserve University, with permission]

Cerebral Malaria

Cerebral malaria causes death in the nonimmune patient and in the African child. High parasitemia is a necessary component in both groups (*36*), but the pattern of associated complications differs (*34*). Cerebral malaria in Africa has a peak incidence in 3- to 4-year-old children in areas of low endemicity where there are few bites by infected mosquitoes per year. In these areas, cerebral malaria is a more common complication of *P. falciparum* malaria (*37*). In contrast, severe anemia is the predominant complication in areas of extremely high endemicity where people are bitten by hundreds of infected mosquitoes per year (for example, in Kisumu, Kenya); cerebral malaria occurs infrequently (*38*). Thus, age of the child and endemicity influence the risk of developing cerebral malaria. Although high parasitemia is a factor in the pathogenesis of cerebral malaria, children in African villages commonly have high parasitemia without evidence of any complications, parasitemias at which patients from nonendemic areas would be at grave risk of death (*39*).

Many theories exist on the pathogenesis of cerebral malaria (*34*), including a role for nitric oxide (*40*), but the one that will be the focus of this discussion is based on the observation that infected erythrocytes line the cerebral capillaries and venules (Fig. 3). One theory is that cytoadherence of infected erythrocytes leads to cerebral anoxia.

The parasite modifies the surface of the infected erythrocyte to enhance its surviv-

al. These modifications lead to adherence of infected erythrocytes to endothelium and to uninfected erythrocytes, the latter phenomenon being referred to as rosette formation (*41*). Knob protrusions appear on infected erythrocytes as the parasite matures and are the areas of contact between the infected erythrocyte and the endothelium. In in vitro culture or in splenectomized animals, the parasite loses the ability to bind endothelium. Such parasites are relatively avirulent in a monkey that has a spleen but reach high parasitemia in splenectomized monkeys (*42*), indicating that endothelial adherence through knobs protects the parasite from clearance mechanisms operating in the spleen.

Host molecules (CD36, ICAM-1, thrombospondin, E-selectin, and vascular cell adhesion molecule–1) have been identified to which the parasitized erythrocytes bind (*41*). Their presence on the surface of microvascular endothelium leads to sequestration of maturing parasitized erythrocytes along venular endothelium (*43*). There are some inconsistencies in the literature as to whether CD36 is expressed on cerebral endothelium in healthy people, but there is general agreement that ICAM-1 is up-regulated during cerebral malaria (*44, 45*). The finding that *P. falciparum*–infected erythrocytes in the *Aotus* monkey are found along endothelium in most organs except cerebral vessels is consistent with the observation that cytoadherence molecules are only expressed during cerebral malaria (*46*). If the hypothesis is correct that up-regulation of endothelial receptors causes binding of infected erythrocytes and this, in turn, causes disease, then what leads to this up-regulation, and why do some children with high parasitemia not suffer from cerebral malaria?

African children with cerebral malaria have circulating TNF-α, and the highest concentrations are associated with the most severe disease (*47*). TNF-α and other cytokines may up-regulate the expression of ICAM-1 on cerebral vessels, leading to cytoadherence of infected erythrocytes. A most intriguing observation is that a malarial molecule may induce the release of TNF-α from macrophages (*48*). Different parasite molecules have been proposed to be responsible, including the glycosyl-phosphatidylinositol (GPI) anchor on a merozoite surface protein (*49*). In addition, extracts of some parasites induce higher concentrations of TNF-α than others (*50*), suggesting that it may be a virulence factor. T cells stimulated by malarial antigens could also induce macrophage release of TNF-α. What is unclear is why the disease occurs in one child and not in another. Antibodies exist that block the effect of the toxin on macrophages (*33*), an observation that has led to the proposal of an anti-disease vaccine. One proposal that remains to be tested is the effect of TNF-α polymorphisms in the African population on the incidence of cerebral malaria (*51*).

Another modification of the surface of infected erythrocytes leads to rosettes of normal erythrocytes around the infected cell in in vitro culture. In a small study in The Gambia, rosettes occurred more commonly with parasites obtained from patients with cerebral malaria than from patients with mild illness with comparable parasitemia (*52*). In addition, plasma factors from the mildly ill patients reversed rosettes; plasma from cerebral malaria rarely reversed rosettes. These data are the first to correlate an in vitro phenomenon and cerebral malaria, raising the possibility that antibodies to a parasite molecule on the erythrocyte surface may prevent cerebral malaria. Molecules involved in rosetting include carbohydrates found on blood group B and A (*53*) and CD36 (*54*). The carbohydrate-binding molecule is parasite isolate–specific in that the rosetting parasites bind predominately to carbohydrates of blood group A or B. How rosettes may be involved in the pathogenesis of cerebral malaria is not obvious. On direct observation of rosette formation in rat mesothelium, rosettes only formed in venules where they are unlikely to cause obstruction. It is possible, however, that rosette formation in vitro may represent an interaction in vivo that occurs with endothelium in addition to normal erythrocytes. A study of cerebral malaria patients in Thailand failed to identify a correlation with rosette formation (*55*), but the pathogenesis in Thailand may differ from that in Africa. There is a need for larger studies of the relation between cerebral malaria and rosette formation in areas of different endemicity.

The parasite molecules involved in cytoadherence and rosette formation have yet to be cloned. Because of their importance to parasite survival and their exposure on the erythrocyte surface for 36 hours, it is possible that they are the parasite erythrocyte surface molecule that undergoes antigenic variation. As clones in culture switch their antigenic type, these switches are associated with loss of the ability to bind to ICAM-1 (*24*). Furthermore, sera from adult Africans that reverse cytoadherence of different isolates of infected erythrocytes are directed against a variant antigen (*56*). As stated above, whether there are common epitopes of low immunogenicity must await identification of the cytoadherence molecules. It is expected that such a vaccine, if successful, would block both the disease and parasite survival.

Another approach to disease prevention would be a vaccine that blocks the parasite molecules that may be responsible for up-regulation of cytoadherent molecules on microvessels of the brain (*50*). If this is the result of a parasite molecule that induces macrophages to release TNF-α, immunization with this may be an anti-disease vaccine, perhaps without affecting parasite growth. Because children in Africa can survive despite high parasitemia, this vaccine may prevent cerebral malaria. One concern about such a vaccine is that the children may not develop fever and other symptoms of malaria caused by TNF-α that alert the parents to seek antimalarial therapy.

Severe Anemia

In low-endemicity areas such as Thailand, the level of anemia correlates with the level of *P. falciparum* parasitemia (*57*). In these patients, the hemoglobin (Hgb) rarely falls below 7 g per deciliter of whole blood. The mechanism appears to involve hemolysis caused directly by the parasite and dyserythropoiesis. Anemia in the African child is sometimes also associated with high parasitemia (*58*). However, among the children who had the most severe anemia (Hgb < 5 g/dl) in western Kenya, half had parasitemia of less than 10,000 per microliter; parasitemia did not predict the risk of death in the severely anemic children (*38*). Severe anemia in the African child begins in infancy and continues through the third year of life (Fig. 4, A and B). It is one of the major causes of death in some areas of Africa, particularly where there is high endemicity (*59*). Because *P. falciparum* infection is common in the areas where severe anemia is a major problem, it raises the question of whether *P. falciparum* is an incidental finding or is the cause of the anemia. The strongest argument that the anemia is caused by *P. falciparum* comes from studies of antimalarial therapy. The hemoglobin of Gambian children receiving weekly chloroquine prophylaxis and a placebo-treated group were studied from birth through 3 years of age (Fig. 4B) (*60*). By approximately 3 months of age, the placebo group had a markedly reduced hemoglobin that returned toward the chloroquine-treated group around 2 to 3 years of age. In a second study, children who had moderate anemia (Hgb of 5 to 7.5 g/dl) were treated with chloroquine or Fansidar in a chloroquine-resistant area; the Fansidar-treated group had a more rapid rise in hemoglobin (*61*). The third observation is the rapid rise in hemoglobin during antimalarial treatment of severely anemic children (*58*). From the data, it is impossible to exclude that other diseases associated with malaria may be contributing to the severe anemia, but *P. falciparum* is a major factor.

The pathogenesis of severe anemia asso-

ciated with low *P. falciparum* parasitemia remains poorly understood. Any explanation of the pathogenesis must explain the curious observation that severe anemia decreases after the age of 3 despite the continued infections in the population and at a time when the incidence of cerebral malaria is increasing. It has been suggested that the anemia associated with low parasitemia may reflect a recent resolving infection, implying that the anemia is, indeed, the result of high parasitemia and the associated hemolytic anemia.

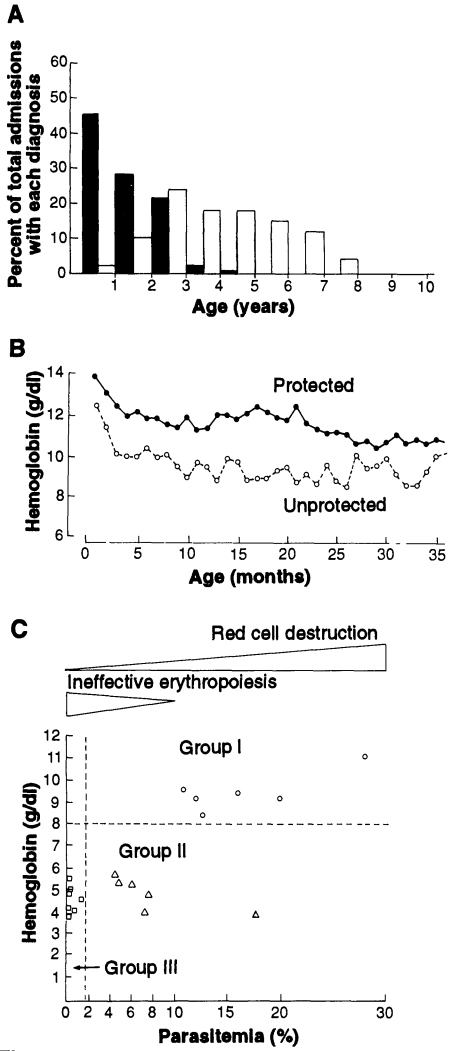

Fig. 4. (**A**) Age distribution of complications of malaria in African children from The Gambia. Severe anemia (dark bars) in malaria patients occurs earlier in childhood than cerebral malaria (open bars). [Modified from Marsh (*37*) with permission] (**B**) The difference in hemoglobin in children treated with antimalarial drugs (protected) or placebo (unprotected) from birth to 3 years of age. Both groups develop anemia, but the *P. falciparum*–infected group (unprotected) has more severe anemia that is evident by 3 months of age. [Reproduced from McGregor *et al.* (*60*), with permission] (**C**) Anemia occurs in association with high parasitemia, but a group exists (group III) that has severe anemia associated with low parasitemia and dyserythropoiesis. [Reproduced from Abdalla *et al.* (*58*), with permission]

In one study of a small number of Gambian children who had severe anemia associated with low parasitemia, dyserythropoiesis was observed in the bone marrow (*58*). The implication of this limited study is that severe anemia may result from the failure of a normal bone marrow response, not primarily from hemolytic anemia (Fig. 4C). Studies are needed to confirm this finding and to determine whether the children had high parasitemia preceding the period of anemia or if the anemia is unrelated to high parasitemia. One possible mechanism may be the immune response to malaria that may induce the release of cytokines within the bone marrow. It has been shown that IFN-γ and TNF-α suppress hematopoiesis (*62*). The finding of an association between severe anemia and a class II human leucocyte antigen (HLA) haplotype could relate to an immune response that causes immunopathology such as dyserythropoiesis (*63*). The importance of innate genetic factors is also consistent with the observation that children who had severe anemia are more likely to have been previously transfused. Alternatively, the innate factors could reduce the severity of infection and, as a result, reduce the frequency of severe anemia.

In the past, one of the feared complications of malaria was blackwater fever—brisk hemolytic anemia and renal failure during quinine therapy. It was discovered during World War II, when quinine was unobtainable in Greece, that the disease disappeared. The mechanism was probably the result of antibodies to quinine causing an immune hemolytic anemia. With the introduction of synthetic antimalarials that replaced the frequent use of quinine for fever in endemic areas, this disease disappeared. As the replacement of quinine by chloroquine had a profound effect on disease, it may be possible to prevent this most severe and common complication when the pathogenesis is defined.

Pregnancy, the Fetus, and the Newborn

There are many studies of the effect of pregnancy on the immune functions in general, but few have focused on immune responses to *P. falciparum*. Such studies must account for the consequences of infection in both nonimmune and clinically immune pregnant women. From limited data (*64*), nonimmune women are more susceptible to severe disease, and fetal loss (abortions and stillbirths) appears to be a consequence of this more severe systemic disease.

It has long been realized that during first and second pregnancies clinically immune women are more susceptible in that they develop higher density parasitemia (*65, 66*). The well-documented consequences are babies of low birth weight and increased mortality. Low birth weight is also associated with the presence of parasites in cord blood (*66*); it remains unknown whether infection is congenital or is introduced around the time of delivery. Intrauterine growth retardation (IUGR, defined as low weight for any point in fetal development) and prematurity cause low birth weight. The discrimination of these two requires precise timing of conception so that developmental markers can be validated within each clinical setting. A study in Malawi found that maternal infection correlated with IUGR, and neonatal infection correlated with prematurity (*66*). Low birth weight is known to increase mortality. The potential short- and long-term effects of IUGR on the health of the infant (for example, anemia), the child, and the adult need to be studied. For example, it has recently been shown that poor maternal nutrition and low birth weight increase the frequency of hypertension later in life (*67*).

During normal pregnancy, the immune system is regulated to ensure that the fetus is not rejected as a foreign allograft. These regulatory pathways rely on peptide and steroid mediators and on placental trophoblasts bathed by maternal blood circulating within the villous space. Thus, intraerythrocytic parasites may multiply in this neovascular bed, interfering with the physiological functions of the placenta (*68*). Indeed, in infected women, the maternal blood bathing the villi is filled with parasite-infected erythrocytes. Unlike infected erythrocytes in other vascular beds, where they are attached to endothelium by knob protrusions, the mechanism by which mature parasites concentrate within the placenta is unknown (*69, 70*). Sequestration in the sinusoidal bed may result from the low pressure and parasite factors (for example, decreased deformability of infected erythrocytes and possibly rosette formation) that block their flow through the postsinusoidal capillaries. Their presence along syncytiotrophoblasts is associated with focal villitis and microfoci of mononuclear phagocytes loaded with pigment (*70*).

These local inflammatory foci and the existence of a peculiar cytokine network (*71*) within and around the chorionic villi may lead to dysfunction of syncytiotrophoblast, resulting in poor nutrition of the fetus. Although differentiating the maternal or fetal origin of these cytokines, as well as their relative proportions within this complex microenvironment, will be a difficult analysis, it might provide insight into the effect of parity in clinically immune women.

Perspective

Until *P. falciparum* is eradicated, the goal must be to reduce the incidence of disease. This will demand that we be attentive to every parameter related to severe disease in different settings. It is through the study of pathogenesis in the laboratory and in the field that methods can be developed to reduce disease in the tropics. The appropriate test of impact on disease reduction can only be carried out after careful consideration of disease patterns in different endemic regions through longitudinal studies.

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