

Review Article

The Impact of HIV Coinfection on Cerebral Malaria Pathogenesis

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Abstract HIV infection is widespread throughout the world and is especially prevalent in sub-Saharan Africa and Asia. Similarly, *Plasmodium falciparum*, the most common cause of severe malaria, affects large areas of sub-Saharan Africa, the Indian subcontinent, and Southeast Asia. Although initial studies suggested that HIV and malaria had independent impact upon patient outcomes, recent studies have indicated a more significant interaction. Clinical studies have shown that people infected with HIV have more frequent and severe episodes of malaria, and parameters of HIV disease progression worsen in individuals during acute malaria episodes. However, the effect of HIV on development of cerebral malaria, a manifestation of *P. falciparum* infection that is frequently fatal, has not been characterized. We review clinical and basic science studies pertaining to HIV and malaria coinfection and cerebral malaria in particular in order to highlight the likely role HIV plays in exacerbating cerebral malaria pathogenesis.

Keywords HIV, malaria, cerebral malaria, coinfection

1 Introduction

Plasmodium falciparum is the most virulent of the malaria species that infect humans, and it is responsible for the majority of morbidity and mortality due to malaria infection. Worldwide, 1.2 billion people are at risk for malaria infection, resulting in 225 million infections and 781,000 deaths in 2010 [198]. The majority of these deaths are due to severe malarial anemia and cerebral malaria (CM) and occur in young children in sub-Saharan Africa, where one in every five childhood deaths is due to malaria [196]. 85% of the world's malaria deaths occur in sub-Saharan Africa. Malaria disproportionately affects poor people with limited access to healthcare, contributing to the perpetuity of poverty in developing countries [197].

Both HIV and malaria have similar geographic distributions, disproportionately affecting people living in sub-Saharan Africa, the Indian subcontinent, and Southeast Asia. Each disease alone causes significant morbidity and mortality, and poses a major threat to public health in these regions. In light of their overlap in global distribution,

there are presumed to be high rates of HIV and malaria coinfection. A review of publications from the late 1980's and early 1990's found no significant difference in incidence or severity of malaria infection between HIV-infected and -uninfected individuals [24]. Many of these were case series or cross-sectional analyses and suffered from selection bias [54]. However, more recent work suggests that the incidence of symptomatic malaria and the severity of illness are increased during coinfection with HIV.

Given the prevalence of both HIV and malaria, even a small effect of coinfection on the severity of clinical disease could have significant public health implications: a mathematical model of HIV and malaria dual infection suggests that the increase in parasitemia seen in HIV results in transmission of malaria to more people, fueling its spread across geographic areas. Additionally, the transient rise in HIV viral load during malaria episodes could result in spread of HIV to additional persons [1].

Areas of the world that are most affected by malaria also carry a heavy burden of HIV. There are 33 million people living with HIV worldwide, with 22.5 million cases in sub-Saharan Africa alone. This results in an estimated overall HIV prevalence of 5% in sub-Saharan Africa, with some countries reporting prevalence rates greater than 25%. While new HIV infections in adults and children have decreased since 2005, there were an estimated 2.5 million children living with HIV in 2007, nearly 90% of whom live in sub-Saharan Africa. It is estimated that 2.1 million deaths in 2007 were due to HIV, of which 1.6 million occurred in sub-Saharan Africa, making HIV/AIDS the number one cause of mortality in that region [176].

HIV infects and depletes CD4+ T lymphocytes and monocytes/macrophages, putting patients at risk for opportunistic infection and malignancy, the major causes of death due to HIV and AIDS. However, it also has effects on the systemic inflammatory response, causing activation and/or apoptosis in a variety of immune cells as well as elevated levels of proinflammatory cytokines and chemokines in lymph nodes and in the circulation. This inflammatory dysregulation may have important

implications for malaria/HIV coinfection, as several pro-inflammatory cytokines have been implicated in the pathogenesis of cerebral malaria, one of the most severe forms of malaria infection.

2 Clinical presentations of malaria

Falciparum malaria has a spectrum of clinical presentations, ranging from asymptomatic parasitemia in people with immunity to severe malarial anemia, placental malaria, cerebral malaria, or multi-organ failure. Cerebral malaria (CM) is one of the most severe manifestations of *Plasmodium falciparum* infection, with an associated mortality rate of roughly 18% despite aggressive treatment [97]. The WHO clinical case definition of CM is peripheral *P. falciparum* parasitemia and unarousable coma (Blantyre coma score of 2 or less in children [120] or Glasgow coma score less than 9 in adults [171]) with no other known cause of coma [203]. The majority of childhood deaths due to CM occur in the first 24–48 hours of central nervous system (CNS) symptom onset. The exact mechanisms of CM are not understood, but it is thought CM and other end-organ damage is mediated through interactions between infected erythrocytes and host receptors on the blood vessel wall, resulting in adherence and sequestration of infected erythrocytes in post-capillary venules, obstruction of blood flow, and subsequent tissue damage [177]. Children and adults who survive cerebral malaria may suffer from long-term developmental and psychological deficits [12, 182].

3 Central nervous system effects of HIV

The neurologic sequelae of HIV range from asymptomatic neurocognitive impairment to minor/moderate cognitive motor disorder and HIV-1 associated dementia. These are commonly referred to as HIV-associated neurocognitive disorders (HAND) and have been shown to affect 23–50% of those infected with HIV-1 [83, 100]. These sequelae have not been evaluated in the context of coinfection with malaria. HIV enters the central nervous system (CNS) soon after peripheral infection resulting in CNS inflammation and damage.

Sub-acute AIDS encephalitis (SAE), now referred to as HIV-associated dementia, is characterized pathologically by cerebral atrophy, diffuse microglial proliferation and focal microglial nodules, multinucleated giant cells and foci of demyelination with associated gliosis [161]. Clinically, individuals with HIV-associated dementia have progressive psychomotor slowing, apathy, memory loss, and difficulty with concentration [137]. In a United States autopsy study from the pre-HAART (highly active anti-retroviral therapy) era, SAE was detected in 26% of adults with AIDS [95]. Despite only 26% having SAE pathology, HIV gp41 was detected by immunohistochemistry in 78% of brains, most frequently in the basal ganglia, brain stem, and deep cerebellar nuclei.

Virtually all adult brains with SAE pathology had detectable HIV gp41 in ramified microglia, and even brains with no significant pathology frequently were HIV gp41 reactive. The intensity of HIV gp41 immunostaining and the number of labeled cells correlated only roughly with the severity/extent of encephalitis as determined by routine histology. Even adult subjects with minimal or no significant brain pathology frequently had detectable HIV gp41. Basal ganglia infarcts, often microscopic, were also documented.

CNS pathology of AIDS in children shares some features with the pathology seen in adults, such as microglial nodules and multinucleated giant cells, but differs with respect to other features. A key difference is that HIV infection in children affects a developing brain, rather than the mature CNS of adults. In the same autopsy study, SAE was detected in 48% of pediatric subjects, higher than that seen in adults (26%). However, HIV gp41 was detected in only 40% of pediatric brains compared with 78% in adults, and when it was detected it was less abundant [95]. In contrast to adults, the most common finding in children was calcification or mineralization of the basal ganglia and frontal white matter. Inflammatory lesions, a hallmark of adult HIV encephalitis, were more common in children older than 1 year [95]. No HIV gp41 was detected in children less than 2 years old, regardless of the histologic findings, while all 4 children over 42 months of age were gp41 immunoreactive [95].

Opportunistic infections of the CNS were uncommon in children [95]. Cerebral white matter was often poorly myelinated, either diffusely or focally in regions of inflammation, possibly related to the effects of HIV on a developing brain. The causes of these differences in pathology are unknown: infants and children may be exposed to lower viral burdens; they may clear the virus more effectively; or they may lack a mature immune system that promotes viral replication.

Neurologic disease in children with symptomatic HIV can manifest with acquired microcephaly, developmental delays, encephalopathy, pyramidal tract signs, and occasionally movement disorders. Clinical courses vary [41, 199]. These differences may be related to age, with development of more progressive disease over time.

In an autopsy series of 14 children with symptomatic HIV who were classified based on their clinical course, brains of subjects with a progressive loss of previously acquired skills showed readily detectable HIV gp41 antigen with immunocytochemistry, whereas those with a plateau neurologic course who failed to acquire additional developmental skills showed little or no gp41 reactivity. All children in the plateau group were 3 years of age or younger, while those in the progressive group ranged up to 6.5 years of age. While gliosis in the deep cerebral white matter was seen in all 14 subjects, it was greater in children with a progressive course than in those who had a developmental plateau. Many

also had involvement of the basal ganglia. Gliosis was also present in HIV-infected children with no detectable HIV gp41 by immunohistochemistry. One explanation for this is that virus is present, but at levels below the detection limit of immunocytochemistry. Alternatively, gliosis may be an indirect effect of systemic infection, resulting from damage to the blood-brain barrier by inflammatory cytokines or toxic metabolites, or it may indicate repression or clearance of HIV in the brain after virus-initiated CNS damage. Most of the comprehensive pathological studies of pediatric HIV CNS disease were performed prior to the development of more sensitive techniques such as quantitative polymerase chain reaction (PCR) and prior to the appreciation that malaria and HIV coinfection might have significant clinical consequences, thus there are many gaps in our knowledge about the effects of HIV in children.

4 HIV-1 subtypes and CNS effects

HIV-1 is genetically diverse and can be classified into several clades or subtypes based on genetic differences in the viral envelope. The prevalence of each clade varies geographically, with clade B being most common in North America and Europe and clades A, C and D predominating in sub-Saharan Africa. The genetic differences between subtypes seem to affect HIV disease progression. African individuals infected with clade D virus have a faster progression to AIDS and higher mortality rates than those infected with clade A virus [6,86,92,183], and in Canada, persons infected with clade B virus had faster rates of CD4+ T lymphocyte decline and progression to AIDS than did those infected with non-clade B virus [89].

There may also be differences in CNS pathophysiology between subtypes, but evidence is at times contradictory. While HIV subtype B-infected persons in the United States and HIV-infected persons in Uganda (where subtypes A and D predominate) had similar rates of HIV-associated dementia (27–31%), Ethiopian individuals infected with HIV subtype C had low rates of neurologic impairment, similar to HIV-uninfected controls [29,146,202]. HIV-infected subjects in the Ethiopian study were healthier than those from the United States and Uganda with a higher mean CD4+ T lymphocyte count, which may have affected the results.

In a study from India where clade C also predominates, there were no cases of HIV dementia, but high rates (60%) of mild to moderate cognitive impairment [71]. Subjects in the study also had higher CD4+ T cell counts than those in the United States and Uganda studies. More recently, Sacktor et al. found that HIV dementia is more common among subtype D-infected individuals than those infected with subtype A in Uganda [147]. The pathophysiology behind these differences is not known, but in vitro and animal studies suggest there are differences in the neurotoxicity of HIV Tat protein among subtypes [21,58,57,140].

5 Observational studies pertaining to HIV and malaria coinfection

The first studies to look for associations between HIV-1 and *P. falciparum* from the late 1980's through the mid 1990's found no significant increases in prevalence or severity of malaria in individuals infected with HIV. Nguyen-Dinh et al. found no association between *P. falciparum* parasitemia and HIV serostatus in pediatric subjects in Zaire [128], and other pediatric studies in Zaire, Malawi and Uganda observed no relation between HIV serostatus and malaria incidence or severity [68,121,169]. Similarly, several studies on adults found no increase in prevalence or severity of *P. falciparum* infection in HIV seropositive individuals [33,99,121,158].

One of the first areas in which HIV was shown to affect malaria disease severity was in pregnancy-associated malaria. In sub-Saharan Africa an estimated 1 million pregnancies are complicated by HIV/malaria coinfection each year [195]. During pregnancy, malaria causes anemia in the mother [16] and can result in placental malaria infection. In placental malaria, parasites sequester within placental tissue resulting in premature birth [7], intrauterine growth retardation (IUGR) [192], and perinatal mortality [7]. In areas of endemic malaria transmission, a woman's risk for placental malaria decreases with each subsequent pregnancy [192], suggesting that protective immunity is acquired during pregnancy. However, Steketee et al. found in a prenatal malaria chemoprophylaxis trial in Malawi that multigravid (third or greater pregnancy) HIV seropositive women were more likely to have placental malaria than HIV uninfected women [163]. At enrollment, HIV seropositive women more frequently had peripheral parasitemia and had a higher mean parasitemia than did HIV uninfected women. Both primigravid (first pregnancy) and multigravid HIV seropositive women had higher geometric mean parasite densities, higher rates of placental malaria, and higher prevalence of umbilical cord blood malaria infection than HIV uninfected women at delivery [163].

Similar results were found in a separate prenatal malaria chemoprophylaxis trial in an area of endemic malaria transmission in Malawi [184]. A study of pregnant women in Kenya found the excess malaria risk in HIV-infected women ranges from 34.6% during the first pregnancy to 50.7% for third or more pregnancies [179]. HIV alters the typical gravidity-specific pattern of malaria in pregnancy, where risk for placental malaria decreases with each subsequent pregnancy, such that HIV-infected multigravid women have a similar risk of placental malaria as do primigravid HIV-infected women [179]. A cross-sectional study of pregnant women in Kenya looked at the association between placental malaria and cord blood parasitemia measured by real-time polymerase chain reaction (RT-PCR), and their association with HIV status [132]. HIV coinfection was associated with a significant increase in

placental parasite density and with cord blood malaria prevalence. The higher the placental parasite density, the higher is the risk of IUGR and of preterm delivery [85]. Thus, HIV's association with an increased risk of placental malaria and higher placental parasite density may potentially result in more cases of IUGR and preterm delivery. Additionally, because there were higher rates of cord blood infection in HIV seropositive mothers [132], there may be higher rates of congenital malaria infection in children born to HIV seropositive mothers.

In placental malaria, parasites sequester by binding to chondroitin sulfate A (CSA). CSA, a sulfated glycosaminoglycan, is expressed in the intervillous space in the placenta and co-localizes with *P. falciparum*-infected erythrocytes [123]. Both placental malaria and HIV infection are independently associated with anemia, low birth weight, prematurity and perinatal mortality [46,144]. HIV/malaria coinfecting women appear to have more severe anemia and low birth weight infants than women singly infected with either HIV or malaria [139]. Women become increasingly resistant to placental malaria over subsequent pregnancies as they develop antibodies against the CSA-binding placental parasite forms [56,142]. HIV decreases the ability of pregnant women to acquire both IgG to variant surface antigens (VSA) expressed on the surface of parasitized erythrocytes and opsonizing antibodies to placental parasites [139], which may account for the alteration in parity-specific susceptibility to placental malaria. The amount of opsonizing antibodies also correlates with degree of immunosuppression among HIV-infected women, as women with fewer than 350×10^6 CD4+ T cells/L had lower levels of opsonizing antibodies than did women with greater than 350×10^6 CD4+ T cells/L.

Pregnancy-associated malaria also affects HIV-related outcomes in women. The relationship between baseline peripheral parasitemia and HIV-related outcomes was studied in Tanzania. In a randomized trial of micronutrient supplementation in HIV-infected pregnant women, peripheral parasitemia was non-linearly associated with HIV viral load at baseline and at times > 90 days after baseline [52]. Even women with low baseline parasitemia versus no parasitemia had higher viral loads at both time points. In women with CD4+ T cells counts ≥ 500 cells/ μ L, any amount of baseline parasitemia predicted an increased rate of AIDS-related deaths [52]. Although there was not strong evidence of an overall association between parasitemia and HIV progression or AIDS-related deaths, the rate of AIDS-related deaths was elevated in women with lower levels of immunosuppression (CD4 T cells > 500 cells/ μ L) with parasitemia, suggesting that malaria may be especially harmful to individuals with higher CD4+ T cell counts.

HIV infection in non-pregnant adults and children is also associated with more frequent and more severe

malaria infection, both in unstable areas of transmission where people are thought to have no malaria immunity and in endemic transmission areas where inhabitants have partial immunity. A case-control study in a malaria-endemic region of Uganda found HIV-infected adults had more frequent clinical malaria, defined as acute fever with malaria parasitemia, than did individuals not infected with HIV [50]. A separate Ugandan cohort study found the incidence of symptomatic malaria in adults to be inversely related to participants' CD4 T cell counts [55].

A significant association between HIV status and severe malaria in adults was found in a malaria-endemic region of Zambia, where HIV prevalence in the community was estimated to be as high as 30% [23]. In this case-control study, severe malaria was defined as peripheral parasitemia and fever with one or more of the following: Glasgow coma scale ≤ 10 , seizures, jaundice, hypoglycemia, hyperparasitemia, renal impairment and cardio-respiratory distress. More than half of adult subjects with severe malaria presented with impaired consciousness, and 22% had convulsions. Ninety three percent (27 out of 29) of subjects with severe malaria were HIV seropositive, versus 15/29 (52%) of those with uncomplicated malaria and 13/29 (45%) with no malaria infection, suggesting that HIV results in more severe malaria disease. However, opportunistic infections such as cryptococcal meningitis or bacteremia can occur concurrently with malaria infection (especially in malaria-endemic areas with high infection rates) and would have gone undiagnosed in this study because blood cultures and cerebrospinal fluid analysis were not performed. A seminal study of fatal pediatric cerebral malaria cases performed by the Blantyre Malaria Project investigators in Malawi showed that up to a quarter of clinical cerebral malaria deaths were due to other causes on autopsy [170]. For that study both blood cultures and cerebrospinal fluid were obtained from comatose children presenting with malaria, and all children with another diagnosis causing coma prior to death were excluded. Thus, the Zambian subjects clinically determined to have severe malaria may have had other diagnoses confounding any imputed association between HIV seropositivity and severe malaria.

HIV-positive serostatus was associated with increased frequency of severe malaria in adults in an urban setting in India with unstable malaria transmission [90]. While estimated population prevalence of HIV infection was 1.8%, prevalence of HIV infection in adults presenting with severe malaria was 11.6% [90]. A South African study in an area of unstable malaria transmission of patients presenting with fever and peripheral parasitemia found that 47% of enrolled HIV-infected adults had severe or complicated malaria versus only 30% of HIV-uninfected adults ($p = 0.003$) [70]. HIV-infected subjects had a higher risk of death compared

with HIV-uninfected subjects (20% vs. 3.8%; $p < 0.001$), and were more likely to present with coma (16% vs. 8%, $p = 0.03$) [70]. No cerebrospinal fluid analysis or blood cultures were performed but similar numbers of HIV seropositive and seronegative subjects received parenteral antibiotics, minimizing bias related to misdiagnosis.

In a separate South African study there was an inverse relationship between CD4+ T lymphocyte count and incidence of severe malaria in HIV-infected adults from areas of unstable malaria transmission. Individuals with CD4+ T lymphocyte counts < 200 cells/ μL had significantly increased risk for severe malaria [32]. Most cases of severe malaria were due to anemia, renal failure and acidosis, rather than cerebral malaria. While this relationship between severe malaria and CD4+ T lymphocyte counts was not seen in study subjects from areas of stable malaria transmission who are thought to be semi-immune to malaria, the median CD4+ T lymphocyte count in malaria non-immune subjects was significantly lower than in semi-immune subjects (134 cells/ μL versus 190 cells/ μL ; $p = 0.007$), possibly influencing the results.

HIV infection does not seem to have a significant impact on treatment response in uncomplicated malaria in individuals with low or moderate degrees of immunosuppression. In a randomized clinical trial of anti-malarial drug regimens in Uganda, HIV-infected adults were more susceptible to new malaria infections after malaria treatment than were HIV-uninfected individuals, but this was not due to recrudescence infection [87]. HIV testing was performed retrospectively and the degree of immunosuppression by clinical staging or CD4+ T lymphocyte counts was not performed. In a clinical trial of uncomplicated malaria in a malaria-endemic region of Zambia, treatment failure was not associated with HIV infection, although HIV-infected subjects with CD4+ T lymphocyte counts < 300 cells/ μL were more likely to experience recrudescence malaria infection than HIV-infected subjects with CD4+ T lymphocyte counts > 300 cells/ μL or HIV-uninfected subjects [180]. This latter finding suggests that severe immune suppression from HIV may affect the ability to clear malaria infection.

Severe malarial anemia (SMA) is one of the primary causes of mortality in children infected with *P. falciparum*. In a prospective study of children presenting with severe malaria in Malawi, HIV prevalence was significantly higher in children with SMA than in children with CM, and HIV-infected children with SMA had a higher prevalence of bacteremia than did HIV-uninfected children with SMA [17]. HIV and malaria coinfecting children in an area of endemic malaria transmission in Kenya had significantly more malarial pigment-containing neutrophils and monocytes (a marker for severe disease), increased rates of SMA and almost a 10-fold greater mortality after 3 months than HIV-exposed (HIV-seropositive with no

detectable virus born to HIV-infected mothers) or HIV-seronegative children [37]. While the risk for SMA was significantly lower in the HIV-exposed vs. HIV-infected children, rates of SMA were significantly higher in HIV-exposed vs. HIV-seronegative children. Another study based in Kenya found that children under the age of 2 who were HIV-exposed or HIV-infected had an increased prevalence of SMA than did HIV-uninfected children [130]. HIV-exposed children in this study had similar rates of SMA as HIV-infected children. These two studies suggest that there may be an effect of in utero HIV exposure on hematologic and/or immunologic development that remains clinically significant during early childhood.

In addition to malarial anemia, CM is a severe complication of infection with *P. falciparum*, with mortality rates of 15–20% despite aggressive medical treatment [22,120]. However, there is little data from observational studies pertaining to incidence or outcomes of CM in HIV coinfecting adults or children. In South Africa, HIV-seropositive children older than 1 year in an area of unstable malaria transmission were more likely to have severe and complicated malaria with more episodes of coma, hypoglycemia, and longer length of stay in hospital than children who were not infected with HIV [69]. A recent pediatric case control study from Uganda found the rate of HIV seropositivity in children presenting with CM to be 9%, significantly higher than in children presenting with uncomplicated malaria (2.3%) or in children presenting for routine care with no malaria (2.5%), although overall numbers of enrolled HIV-infected children were low [79]. HIV-infected children with CM were more likely to have a higher parasite density than HIV negative children. In this study, children with CM were younger (81% were < 5 years old, vs. 68% and 63% in the uncomplicated malaria and no malaria groups, respectively). Because of the known association of CM with younger age, authors performed age-adjusted odds ratios to compare groups. The age-adjusted odds ratio for HIV-infected children to present with CM was 4.98 (95% CI 1.54–16.07, $p = 0.003$) [79].

HIV viremia increases during malaria infection, even with asymptomatic parasitemia [75,94]. In Malawian HIV-infected adults, asymptomatic parasitemia was associated with a 0.25 log increase in HIV viral load, and fever with parasite density $> 2000/\mu\text{L}$ resulted in a 0.89 log increase [94]. Viral load in HIV-infected malaria-treated individuals was still significantly higher at 4 weeks compared to HIV-infected aparasitemic controls [75], but returned to baseline levels by 8–9 weeks after malaria treatment [94]. Given that HIV is more easily transmitted the higher the viral load [59,138], malaria may contribute to HIV transmission in areas where coinfection is common.

In addition to the increase in HIV viral load, a drop in CD4+ T lymphocyte counts is seen during malaria episodes.

In Zambia, symptomatic malaria was associated with a temporary drop in CD4+ T cell counts in both HIV-infected and -uninfected adults [180]. In Uganda, HIV-infected adults were followed for two years and monitored for malaria episodes. In this cohort, malaria infection was associated with a more rapid decline in CD4+ T cell counts over time [113]. Having 2 episodes of malaria in one year resulted in a mean decline of 84 CD4+ T cells/ μ L compared with having no malaria episode, regardless of baseline CD4+ T cell count. Thus, malaria may hasten HIV disease progression.

6 Immune response in malaria

Innate immunity is important for the initial control of blood stage malaria parasites. Innate immunity in malaria involves the production of inflammatory cytokines such as IFN-gamma and TNF-alpha and cells that are directly cytotoxic to blood stage parasites. NK cells are a subset of lymphocytes belonging to the innate immune system that do not express antigen-specific receptors. They are directly cytotoxic to tumor and infected cells, regulate other immune cells through cytokine production, and work in conjunction with antigen-presenting cells [47]. They are among the first lymphocytes to respond to *Plasmodium falciparum* with a strong IFN-gamma response and are important in controlling early parasitemia in murine malaria models [118].

Based on data from mouse studies, immune protection against the liver stages of malaria infection depends mainly on CD8+ T cells recognizing parasite peptides presented on hepatocytes. This causes IFN-gamma and IL-12 production which subsequently stimulates NK cells to produce more IFN-gamma, hindering the development of blood-stage infection [63]. NK cells are found in cerebral vessels prior to the onset of cerebral malaria symptoms in the murine model of cerebral malaria infection, and are later replaced by T lymphocytes [72]. Although they are considered part of the innate response to infection, NK cells are completely dependent on T cells for their IFN-gamma response and thus play a role in adaptive immunity to malaria [110]. HIV infection results in impaired cytolytic activity and dysfunctional cytokine production in NK cells [47], which may affect the innate immune response to malaria. As CD4+ T lymphocyte counts fall due to progressive HIV infection, there may also be a resulting impaired function of NK cells related to adaptive immunity.

Gamma delta T cells are a small subset of T cells that, like NK cells, also bridge the innate and adaptive immune response. They are an even more important source of IFN-gamma during early malaria infection than NK cells [44] and degranulate in response to blood stage parasites, controlling parasite density [36]. Gamma delta T cells are depleted during progression to AIDS through CCR5-mediated effects of HIV [101]. Individuals infected with HIV may thus have higher rates of malaria infection and

increased parasite density, which may fuel the spread of malaria transmission.

Inflammatory (Th1) cytokines such as TNF-alpha and IFN-gamma are important in malaria pathogenesis and in cerebral malaria in particular, as they increase the surface expression of adhesion molecules on endothelial cells, promoting parasite attachment. Activated CD4+ T cells stimulate macrophages to produce TNF-alpha, which leads to cerebral malaria in mouse models [38].

In humans, clusters of cytokines may help discriminate between mild, severe, and cerebral malaria. High levels of IL-12, IL-5, and IL-6 discriminate severe forms of malaria from mild malaria. High IL-1beta levels are associated with cerebral malaria and high IL-12 and IFN-gamma levels are associated with severe malaria [136].

Cerebral malaria occurs early in disease, and is an outcome that reflects the early innate immune response to malaria. However, the frequency of severe malaria, including cerebral malaria, decreases with age and multiple exposures, indicating that the adaptive immune response is important for subsequent protection from the severe sequelae of malaria [5].

With repeated infection, partial immunity develops to symptomatic infection. Antibodies play an important role in this acquired immunity, as has been demonstrated in studies using the transfer of immune sera to non-immune children [111, 145]. One reason for this is the development of antibodies to variant surface antigens produced by *P. falciparum*. Antibody response to *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), a large and polymorphic family of proteins expressed on the surface of infected red blood cells (iRBCs), increases in size and prevalence and becomes more complex with age in regions with high malaria transmission [8]. Immunity to blood-stage parasites is also controlled by CD4+ T cell responses that are independent of antibodies, but this may be due to downstream cytokines, nitric oxide, and/or gamma delta T cells [63].

While monocytes and macrophages play a major role in the innate host defense against malaria, they also mediate the development of parasite-specific cytotoxic lymphocytes and antibodies. Monocytes and macrophages are the main cells responsible for removing parasites from the circulation, phagocytosing iRBCs in the absence of malaria-specific antibodies, possibly via the surface receptor CD36 [164]. In addition, they present malaria antigens to CD4+ T cells, resulting in release of IFN-gamma and mediating the development of an antibody response [164]. The antibodies that develop and protect against blood stage infection do not inhibit parasite growth or red blood cell invasion *in vitro* unless monocytes are present [14, 107]. Antibody-dependent phagocytosis of *P. falciparum* merozoites by monocytes after incubation with immune sera was observed

in vitro [91]. Additionally, the growth rate of *P. falciparum* was significantly inhibited when IgG from immune subjects and purified monocytes from non-immune subjects were added to in vitro cultures [107]. This inhibition was not seen with IgG alone or in combination with platelets, lymphocytes, neutrophils, or splenic macrophages.

Independent of the aforementioned innate and adaptive immune responses to malaria, age-dependent physiologic factors play an important role in disease severity. Severe malarial anemia is more prevalent in children under the age of 2 years regardless of malaria transmission intensity, suggesting that physiologic factors rather than acquired immunity are responsible [141]. CM appears to be more prevalent in slightly older children even in areas of high transmission, which may also be due to age-related immune responses or as a result of immunologic priming from prior malaria infection [5].

Hemozoin (Hz) is a marker for malaria disease severity [78] as well as for CM and death from CM [108]. After schizont rupture, crude Hz is taken up by phagocytes, including macrophages, and it can persist in macrophages for several months. Hz causes significant functional impairment in macrophages, including defects in phagocytosis, a diminished oxidative burst, and decreased protein kinase C activity [115,152]. Uptake of Hz by monocytes and macrophages has been associated with increased cytokine secretion [133,156] and suppression of major histocompatibility complex (MHC) class II antigen presentation [152], which is important for development of an adequate helper T cell response. Expression of ICAM-1 and the integrin CD11c, which are important for cell adhesion and thus T cell stimulation, is also suppressed on the surface of Hz-containing monocytes [152]. In vitro, monocytes fed with Hz alone or with trophozoite-parasitized erythrocytes containing Hz display a long-acting oxidative burst, are unable to degrade Hz, and are unable to repeatedly phagocytose [153]. Hemozoin-containing macrophages in the bone marrow inhibit erythropoiesis, contributing to malarial anemia [160].

HIV-1 infected macrophages serve as a long term stable viral reservoir capable of disseminating virus to tissues [88]. While HIV-1 entry and infection of Hz-containing macrophages is not affected, these cells exhibit significantly diminished HIV-1 replication [42]. Thus, during malaria episodes macrophage viral reservoirs may decrease, but the cells persist and virus remains at low levels. Once the malaria infection is controlled or cleared and hemozoin removed, these macrophages can again be productive sources of HIV.

CCR5 is a chemokine receptor important in leukocyte trafficking. It is expressed on several types of leukocytes, on microglia, neurons and astrocytes in the brain, and in low levels on brain endothelial microvascular cells [11].

It is an important regulator of the inflammatory response to many infections involving the central nervous system [157,185,204], including the neurotropism of HIV [62]. In experimental cerebral malaria, CCR5 mRNA is highly upregulated in brains of mice infected with *P. berghei* ANKA [31,117] and CCR5-deficient mice are less susceptible to the development of cerebral malaria [9]. Additionally, CCR5 expression is increased on intervillous maternal and fetal villous macrophages during placental malaria infection [172]. Because CCR5 facilitates the spread of HIV-1 from cell to cell, the increase in CCR5 seen in both cerebral and placental malaria may increase HIV reservoirs in the brain and placenta. This may in turn facilitate development of HIV-associated neurocognitive disorders (HAND) and increase the risk for mother-to-child transmission of HIV-1. Thus far, clinical studies have not shown a significantly increased risk of mother-to-child transmission of HIV due to placental malaria, and no clinical studies have examined rates of neurocognitive disorders in HIV-infected survivors of cerebral malaria.

7 Immune response to HIV

As with malaria infection, inflammatory cytokines play an important role both in control and pathogenesis of HIV infection. During infection, viral particles are taken up by antigen presenting cells, which are then recognized by CD4+ T cells, causing activation and release of IL-2 and IFN-gamma. These inflammatory cytokines in turn stimulate CD8+ T cells, which control viremia. It is possible that this inflammatory response seen in HIV may compound the effects of adherence and sequestration seen in malaria, by also upregulating adhesion molecules on endothelial cells [60]. Immature dendritic cells are one of the first cell types encountered by HIV in the mucosa during sexual transmission, and they play an important role in transmitting HIV to CD4+ T cells [134]. Once immature dendritic cells encounter HIV, they develop into mature dendritic cells that then stimulate naïve T cells. A subset of mature dendritic cells induce a Th1 response from naïve T cells and show a markedly increased ability to mediate HIV-1 transmission to T cells, which correlates with increased surface expression of ICAM-1 on this mature dendritic cell subset [148].

During malaria infection, iRBCs bind to dendritic cells via CD36. Adherent iRBCs impair the maturation of dendritic cells, and reduce their ability to present antigen and activate T cells [177]. Additionally, differentiation and maturation of hemozoin (Hz)-loaded monocytes to dendritic cells is severely impaired, indicated by decreased expression of MHC class II and co-stimulatory surface markers [159].

Dendritic cells that develop from Hz-containing monocytes display an intermediate maturation phenotype, compared with immature or fully mature dendritic cells. These semi-mature dendritic cells have a lower surface expression

of CCR5 than immature dendritic cells and are less permissive to virus infection. However, these cells exhibit an enhanced transfer of HIV-1 to CD4+ T cells and HIV-1 replication in CD4+ T cells is promoted by contact with these semi-mature, H₂O₂-containing dendritic cells [43]. There does not appear to be a direct effect of H₂O₂ on CD4+ T cells [42].

Dendritic cells also present viral antigens to CD8+ T cells [109], and thus they play an important role in the induction and maintenance of cellular anti-HIV immunity. HIV-exposed dendritic cells also induce apoptosis in CD4+ T cells [103], which may contribute to dysfunction and decreases in CD4+ T cells in HIV-infected individuals. Malaria infection impairs human dendritic cell function in vitro [178] and dendritic cells from malaria-infected mice have impaired antigen-presenting activity [200], possibly affecting both cell- and antibody-mediated immunity to HIV.

CD8+ and CD4+ T cells are not stimulated as effectively after infection with HIV-1, making individuals with HIV more susceptible to most viruses, including HIV [143]. The CD8+ T cell population is expanded in children with HIV [40], as is the activated CD8+ T cell subset [13]. HIV-specific CD8+ T cells are crucial for the development of a protective response against HIV. CD8+ T cells require antigen presentation by dendritic cells in order to differentiate into effector cells [84], where they then have the ability to recognize viral peptides on the surface of infected cells and lyse them. Activated CD8+ T cells may inhibit CD4+ lymphocyte proliferation [40] and in experimental CM they sequester in the brain microvasculature along with other T cells, neutrophils and macrophages [10]. Lethal experimental CM is dependent on these activated CD8+ T cells, which are parasite-specific cytotoxic effector cells [106].

Regulatory T cells (CD4+CD25+FOXP3+), a T cell subset with constitutive immunosuppressive activity, is upregulated during HIV infection. These cells suppress CD4 T-cell proliferation in response to recall antigens and HIV proteins [194]. In human malaria infection, their upregulation correlates with increased TGF- β production (a suppressive cytokine) and more rapid parasite growth [187]. Thus, HIV-induced regulatory T cell expansion could lead to an increase in parasite density during malaria infection.

Additionally, as HIV progresses clinically to AIDS there are effects on both innate and acquired immunity, with progressive loss of T cell responses to common recall antigens [28]. This may in part account for the increased rates of symptomatic malaria seen in cohort studies. HIV-1 has both direct and indirect effects on B cells. HIV directly causes B cell proliferation and polyclonal immunoglobulin secretion [150]. HIV gp120 impairs CD4+ T cells' ability to help B cells, indirectly causing decreased B cell proliferation, polyclonal IgG secretion, and decreased antigen-specific IgG secretion [26]. A major effect of this B

cell dysfunction is a decline in antibody class switching and subsequent increased susceptibility to bacterial infections, as is evident by the increased susceptibility to pneumococcal [74] and cryptococcal [82] infections in people with HIV, which are dependent on adequate antibody responses. Since clearance of erythrocytic forms of *Plasmodium* is thought to be dependent on antibody formation, HIV could impact on all aspects of the immune response to malaria.

HIV infection leads to reduced memory and naïve resting B cell populations, which can result in increases in other infections and decreased response to vaccines [104]. Malaria infection itself is also a risk factor for bacterial infection [154], and HIV is associated with an increased risk of bacterial infection during malaria episodes in children [17,154]. HIV-infected adults receiving antiretrovirals have normal levels of B cells overall but have a decreased memory B cell subset despite treatment, suggesting long-term impairment in the ability to mount an antibody response against coinfections [104].

8 Pathogenesis of cerebral malaria

There are several proposed mechanisms of disease pathophysiology in CM. These mechanisms remain controversial, but it is agreed that parasite sequestration in the brain microvasculature is critical.

One proposed mechanism focuses on the physical consequences of accumulating infected erythrocytes in the brain microvasculature, causing a blockage in circulation of blood and oxygen with subsequent ischemia to brain tissue. In support of this, autopsy studies of children and adults dying from CM have found brain vessels filled with parasitized erythrocytes, sometimes accompanied by thrombosed or ruptured capillaries with surrounding necrosis and hemorrhage [45,162,170]. Others focus on how the host inflammatory response contributes to disease. Pro-inflammatory cytokines such as TNF- α released by cells of the immune system can activate the endothelium, increasing expression of surface adhesion molecules that contribute to sequestration of infected erythrocytes and adhesion of platelets and mononuclear cells. Quantitative PCR of brain tissue obtained at autopsy has shown an increase in TNF- α mRNA in children dying with CM [19], and adults with CM have widespread endothelial activation and express high levels of the adhesion molecule ICAM-1 along the brain microvasculature [175].

Autopsy studies of children dying of cerebral malaria suggest differences in the pathophysiology of disease compared with adults. In children there is evidence of an inflammatory response, with leukocytes and platelets within the cerebral microvasculature and an increase in cerebral vascular permeability [45,66]. In adults, inflammatory cells are seen much less frequently within the cerebral microvasculature or in the perivascular space [175], and

evidence for permeability of the blood-brain barrier is inconsistent [203]. The mechanisms of inflammation and of sequestration in the brain microvasculature are not mutually exclusive, but how significantly each plays a role and how much they overlap is still a matter of debate.

While coma precedes death from cerebral malaria, the exact mechanism of death is unknown. Cerebral edema is often noted in and may be important in disease pathophysiology, but does not seem to be related to coma severity or mortality [119]. CT imaging of 3 Malawian children with retinopathy-confirmed cerebral malaria and coma was significant for edema and obstructive hydrocephalus [135]. However, imaging studies on 7 Kenyan children with cerebral malaria showed no evidence of hydrocephalus [127]. In 26 Kenyan children with cerebral malaria, all had elevated opening pressure of cerebrospinal fluid on lumbar puncture [126]. Direct intracranial pressure monitoring of 23 Kenyan children with calculated cerebral perfusion pressure showed elevated intracranial pressure in all subjects, some with severely elevated intracranial hypertension (> 40 mmHg) and decreased cerebral perfusion pressure (< 40 mmHg) [125]. Mannitol controlled intracranial pressure in children with intermediate levels of intracranial hypertension (20–40 mmHg) but did not stabilize pressure in children with severe intracranial hypertension or decrease mortality [124] and mannitol was associated with a higher risk of mortality in a trial of adults with cerebral malaria and elevated intracranial pressure [119]. Intravenous steroids as an adjunctive measure to decrease brain edema also did not reduce the incidence of death or degree of morbidity in patients with cerebral malaria [76, 189].

9 Experimental cerebral malaria

Plasmodium berghei ANKA infection in susceptible strains of mice (C57BL/6 and CBA) produces a clinical syndrome similar to human CM. There is considerable controversy as to how faithfully this rodent experimental CM model mimics human CM. Mice consistently develop neurologic signs and symptoms, such as partial paralysis, seizures and coma, with relatively low parasitemia on day 6–9 post-infection, and succumb within 24 hours of neurologic symptom onset [73]. These mice have parasites and mononuclear cells containing phagocytosed parasites in the brain microvasculature, with clogging and rupture of vessels. The majority of these mononuclear cells are CD8+, and to a lesser extent, CD4+ T lymphocytes [72, 117]. The endothelium from mice with CM expresses the adhesion molecule ICAM-1, as well as the pro-inflammatory cytokine TNF-alpha [73].

An inflammatory, or Th1-skewed immune response, is seen in both experimental CM and in human disease. In experimental CM, IFN-gamma plays an important role in cerebral sequestration pathology [3, 186]. In human disease,

CD4+ T cells from clinically immune individuals produce little IFN-gamma in response to malaria antigen, whereas peripheral blood mononuclear cells from non-immune individuals exhibit a Th1-skewed immune response with high levels of IFN-gamma [27], potentially upregulating adhesion molecules on endothelial cells leading to sequestration and CM.

CD4+ T cells were initially found to be critical to the development of CM in mice. CBA mice infected with *P. berghei* ANKA that were depleted of CD4+ T cells prior to infection did not develop neurologic symptoms of CM, but did eventually die with severe anemia and high parasitemia [67]. More recently it has been found that while CD4+ T cell depletion early in infection prevents CM, when CD4+ T cells are depleted later in infection and prior to neurologic symptom onset there is no effect on CM development [10]. Development of experimental CM was prevented when mice were depleted of CD8+ T cells regardless of whether it was early or late in infection [10], suggesting that CD8+ T lymphocytes are the effector cells for experimental CM.

TNF-alpha is an important mediator of experimental CM. CBA mice with CM after *P. berghei* ANKA infection have elevated serum TNF-alpha, whereas mice without CM do not [64]. IFN-gamma, which is released by T cells and activates macrophages, also mediates experimental CM. When CBA mice infected with *P. berghei* ANKA were given neutralizing antibody to IFN-gamma, TNF-alpha levels did not rise and incidence of CM decreased [65].

Cytokines, and in particular TNF-alpha, play an important role in some of the pathologic changes seen in human CM. TNF-alpha is found in higher levels in plasma in children with CM than in those with mild disease [96] and brain tissue from fatal CM cases contains both TNF-alpha and IL-1beta mRNA [19]. Additionally, serum TNF-alpha levels are significantly higher in HIV-infected individuals than in individuals without HIV [39]. It is plausible that the higher levels of inflammatory cytokines seen during HIV infection may predispose to the development of CM. While administration of TNF-blocking antibodies prevents development of cerebral malaria in mice [64], it had no effect on mortality in a pediatric clinical trial and was associated with a significant increase in neurologic sequelae [181].

Interferon-gamma-inducible protein (IP-10/CXCL10) is a potent chemoattractant that is stimulated by IFN-gamma and is an important component of the inflammatory response both in experimental CM and in HIV-associated neurocognitive disorders. It is consistently induced in HIV-infected microglia [166], attracting memory T lymphocytes into the central nervous system [93] and stimulating HIV-1 replication in monocyte-derived macrophages and lymphocytes [98]. Microglial nodules seen in HIV encephalitis produce high levels of insulin-like growth factor receptor 2 (IGF2R), which is involved in intracellular HIV replication. When

IGF2R expression is blocked by transfection with IGF2R siRNA, HIV production in microglia is decreased and IP-10 production is decreased [165].

In experimental CM, neutralization of IP-10 and genetic deletion of IP-10 reduced peripheral parasitemia, reduced cerebral intravascular inflammation and protected *P. berghei* ANKA-infected mice from death. This was associated with retention and expansion of parasite-specific T cells in the spleens of infected mice, possibly because these T cells do not migrate to the brain in response to IP-10 [129]. Elevated IP-10 in serum and cerebrospinal fluid has also been identified as an independent predictor for human CM mortality, strengthening the argument that it has a major role in CM immunopathology [4,80]. Immune activation in the brain due to HIV results in increased expression of IFN-gamma and subsequently IP-10, possibly contributing to CM pathogenesis. In experimental and in human CM, microglia generally appear activated, with retracted, stout processes and rounded, enlarged cell bodies [77]. Autopsy studies of adults with CM have shown that microglial activation is widespread in both grey and white matter, and not limited to vessels containing sequestered parasites or to areas of hemorrhage [149]. However, activated microglia also accumulate within and surrounding ring hemorrhages and in the perivascular space of vessels containing sequestered parasitized erythrocytes [81].

10 Sequestration

There have been many molecules and cell surface receptors implicated in the intravascular sequestration associated with severe falciparum infection, both on endothelial cells and on cells circulating within the vasculature. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP-1) is a highly variant, malaria strain-specific protein expressed on iRBCs. It mediates iRBC adhesion to several endothelial receptors. When PfEMP1 binds to endothelial ICAM-1, it increases junctional permeability of the blood-brain barrier and suppresses some aspects of dendritic cell and macrophage activation. PfEMP1 on iRBCs has also been shown to adhere to endothelial cells via CD36, chondroitin sulfate A (CSA), thrombospondin, E selectin, Vascular Cell Adhesion Protein 1 (VCAM-1) and the pan-endothelial marker PECAM-1/CD31 [2,175]. Widespread endothelial activation occurs [175].

CD36, a class II scavenger receptor, was the first receptor involved in adherence to be identified. Expression of CD36 on cell surfaces correlates with the ability to act as a target for cytoadherent iRBCs and likely is the major mechanism of cytoadherence outside the brain. However, it is unclear if CD36 plays a central role in CM sequestration [203] because sequestration occurs even when very low levels of CD36 are expressed within the brain [175]. Franke-Fayard et al. infected CD36 knockout mice with transgenic

luciferase-expressing *Plasmodium berghei* parasites and found that while CD36 is the major receptor for *P. berghei* sequestration, cerebral complications still occurred in the absence of CD36-mediated brain sequestration [53]. The experimental mouse model of CM has limitations pertaining to the study of sequestration, as *P. berghei* ANKA-iRBCs do not express knobs on the cell surface as do *P. falciparum*-iRBCs and the major sequestered cell type in the mouse model is leukocytes, rather than iRBCs.

The role of CD36 in iRBC adherence is of particular interest when one considers HIV/malaria coinfection, as CD36 expression on circulating monocytes is significantly higher in HIV-1 infected individuals compared with healthy controls [114]. This increase in CD36 expression on monocytes has been linked to higher rates of atherosclerosis with HIV [15], but it may also contribute to the inflammatory component of malaria sequestration.

CD36 is also expressed on platelets, which play an important role in CM. Sequestration of parasites within brain vessels is often associated with fibrin-platelet thrombi [45], and platelets accumulate in the microvasculature during pediatric CM even without thrombosis [66]. Platelets have been shown to be essential to the development of experimental CM due to *P. berghei* ANKA infection [167]. In vitro, platelets bind to iRBCs and act as a bridge between iRBCs and human brain endothelial cells, facilitating binding [131,191]. Additionally, platelets are directly cytotoxic to activated human brain endothelial cells in vitro and this effect is amplified by iRBCs, causing increased permeability and endothelial cell death [190].

Platelet/endothelial adhesion molecule 1 (PECAM-1 or CD31) is a glycopeptide expressed only on endothelial cells and intravascular cells such as platelets and monocytes. *P. falciparum* iRBCs adhere to PECAM-1 on endothelial cells via PfEMP1 [25,174]. IFN-gamma increases the binding of iRBCs to endothelial cells via PECAM-1, possibly through redistribution of PECAM-1 from endothelial junctions to the cell surface [174]. During immune surveillance, monocytes transmigrate from the vasculature through the blood-brain barrier into brain tissue via PECAM-1 expressed at endothelial cell junctions [122]. Sera and brain tissue from individuals with HIV encephalitis have elevated levels of soluble cleaved PECAM-1, suggesting that PECAM-1 interactions between endothelial cells and between endothelial cells and monocytes (and possibly between endothelial cells and *P. falciparum*-iRBCs) are altered in HIV infection [48], contributing to changes in blood-brain barrier permeability and enhanced trafficking of HIV-infected monocytes into the brain.

The pro-inflammatory environment that occurs with CM is associated with the release of small fragments of plasma membrane called microparticles. Initially, elevated plasma levels of microparticles from endothelial cells were

found in children during the acute phase of CM [35]. More recently, microparticles shed from activated platelets have been found to bind directly to iRBCs. This adherence is dependent on PfEMP1 variants expressed on the surface of iRBCs binding to CD36 and PECAM-1 present in platelet microparticles [49]. This binding of platelet microparticles to iRBCs increases iRBC cytoadherence to brain microvascular endothelial cells in vitro. Additionally, platelet microparticles are taken up by brain microvascular endothelial cells, causing the endothelial cells to express PECAM-1 and CD36 on cell membranes [49], contributing to the sequestration that is a hallmark of CM. Sequestration may be further enhanced during HIV coinfection via platelet microparticles. HIV-1 Trans-activating factor (Tat) directly interacts with platelets, causing activation and release of platelet microparticles [188].

Expression of Intercellular Adhesion Molecule 1 (ICAM-1) on endothelial cells is upregulated during systemic and local inflammation. Immunohistochemical studies indicate significant co-localization of sequestered parasites in the brain with vascular expression of ICAM-1 [175]. ICAM-1 expression is more widespread and evenly distributed compared with the focal, increased expression of the endothelial surface molecules E-selectin and VCAM-1, both of which are expressed more on medium to large size vessels. E-selectin and VCAM-1 are also associated with sequestered parasites, suggesting that these molecules may also act as sequestration receptors. However, they may not play major roles in sequestration given their overall low levels in the brain vasculature. Because ICAM-1 is widely expressed in brain microvasculature affected by sequestration, it is more likely to be the major molecule responsible for parasite adherence. As with PECAM-1, the soluble forms of ICAM-1 and VCAM-1 are systemically increased during HIV infection [201], potentially making the intravascular environment more prone to sequestration during malaria infection.

Nitric oxide (NO) is a free radical and an important cell signaling molecule that may be protective against severe malarial disease. NO reduces endothelial expression of adhesion molecules like ICAM-1 and VCAM-1 and reduces adherence of iRBCs to endothelial cells [193]. Both NO and the amino acid arginine (the substrate for nitric oxide synthase) are low during symptomatic malaria infection [193], while plasma arginase, an enzyme that converts arginine to ornithine and urea, is elevated in severe malaria. This suggests that less NO is produced, leading to a decrease in vascular dilatation and upregulation of endothelial activation and adhesion molecules [116]. In HIV-positive individuals, increased level of arginase activity correlates with disease severity and HIV viral load [30], suggesting that advanced HIV disease may contribute to sequestration through the nitric oxide pathway.

11 Effects of malaria on the endothelial and BBB during CM

Cerebral malaria is a major cause of death due to *P. falciparum* in children under the age of five. Characterized by coma and/or seizures, it is associated with sequestration of parasitized red blood cells in the brain microvasculature. Brain edema and elevated intracranial pressure are seen, and an ongoing study in Malawi is examining the correlation of MRI changes with the clinical presentation of children with CM [155].

Post-mortem brain samples from children who have died from cerebral malaria show activation of endothelial cells (with upregulation of ICAM-1) and macrophages (with increased macrophage scavenger receptor and sialoadhesin), and disruption of endothelial intercellular junctional proteins [zona occludens 1 (ZO-1), occludin and vinculin] in vessels containing sequestered parasitized red blood cells [18]. While no leakage of plasma proteins such as fibrinogen into the brain parenchyma was seen, pre-mortem cerebrospinal fluid albumin levels were elevated and more recent analysis of pediatric brain tissue has revealed leakage of fibrinogen at sites of parasite sequestration, ring hemorrhages, and vessel thrombosis [45]. Although BBB permeability does appear to be altered during cerebral malaria, disruption of the BBB was also seen in children who died from non-malarial causes, so further studies are needed to determine whether BBB changes are specifically linked to sequestered parasites.

In vitro studies have supported some of these findings. Gillrie et al. showed that parasite sonicates but not intact malaria-infected red blood cells disrupt the endothelial (dermal and pulmonary, not brain) barrier, revealed by discontinuous immunofluorescent staining of endothelial junction proteins, formation of inter-endothelial gaps in monolayers, and loss in total protein content of the tight junction protein claudin 5 and redistribution of ZO-1 [61].

In normal conditions, tightly packed endothelial cells, basement membrane and astrocytes that make up the BBB maintain its functional integrity with help from pericytes, perivascular macrophages and neurons. *P. falciparum* can disrupt this integrity, which is thought to contribute to the mortality associated with CM [34]. The major parasite ligand for adhesion is *P. falciparum* erythrocyte membrane protein (PfEMP-1) mediated iRBC adhesion to ICAM-1, which increases junctional permeability of the BBB and partially suppresses activation of dendritic cells and macrophages [2, 112, 151].

Additionally, it appears that disruption of the blood-brain barrier may be due to specific features of *P. falciparum* isolates [168]. Human umbilical vascular endothelial cells (HUVEC) cultured with malaria isolated from patients with uncomplicated disease had increased mRNA levels of occludin, vinculin and ZO-1. Those cultured

with samples from patients with severe malaria had no change in mRNA levels, and HUVECs cultured with *P. falciparum* from patients with cerebral malaria had decreased mRNA levels of occludin, vinculin and ZO-1, suggesting decreased production of tight junction proteins as a cause of cerebral malaria.

Not only are endothelial junction proteins affected, but so are the endothelial cells themselves. Endothelial cells produce tissue factor, which is important in thrombin formation, when cultured with *P. falciparum*, and brain endothelial cells from patients dying of CM and from those with malaria who died of other causes also showed increased levels of tissue factor [51]. Mature forms of blood stage malaria parasites induce expression of tissue factor by endothelial cells in vitro, and they may facilitate blood coagulation as well.

Endothelial cells upregulate adhesion molecules in response to falciparum malaria. In *P. falciparum* isolates from patients with complicated malaria, binding of iRBCs to human lung microvascular endothelial cells was observed and was primarily mediated through ICAM-1 and chondroitin sulfate (CSA) [173]. Mouse models of cerebral malaria have supported this role of ICAM-1. ICAM-1-deficient C57BL/6 mice infected with *P. berghei* ANKA were protected from mortality due to CM compared with C57BL/6 controls [102]. Additionally, lack of TNF receptor 2 conferred resistance to cerebral malaria in mice, which is important as TNF upregulates ICAM-1 in brain microvascular endothelial cells [105]. Despite the breakdown in tight junctions and adherence of iRBCs to the blood-brain barrier, iRBCs do not escape the vasculature and invade the brain parenchyma.

12 Effects of HIV on the blood-brain barrier

Unlike malaria, HIV is known to infect the brain parenchyma, leading to HIV-associated neurocognitive disorders such as dementia. HIV is thought to enter the brain through the regulated transmigration of HIV-infected mononuclear cells across the BBB, mediated by surface receptors on endothelial cells such as ICAM-1 [20]. Once across the barrier, HIV-infected cells recruit microglia and astrocytes allowing for subsequent infection of these cells and spread of HIV within the CNS. HIV may infect astrocytes at low levels, and it can activate endothelial cells, but it does not infect endothelial cells. As HIV replicates within the CNS, even at low levels, these cells produce inflammatory mediators, including the chemokine CCL2/MCP-1 [20], which can then activate endothelial cells to upregulate surface adhesion molecules.

13 Conclusions

While there have not been extensive clinical studies documenting the effects of HIV coinfection on severe malaria—including cerebral malaria—outcomes, there is growing experimental evidence indicating significant potential for

exacerbation of immunologic and physiologic effects of both HIV and malaria. These in vitro findings highlight the need for more in-depth and prospective studies of individuals with HIV and severe malaria. The inflammatory immune response in the brain due to HIV leads to activation of endothelial cells and breakdown of the blood-brain barrier, potentially increasing susceptibility to and severity of cerebral malaria. Additionally, the dysregulated immune response to pathogens due to HIV infection likely impairs the body's ability to clear malaria infection, leading to high parasitemia and severe anemia. There may also be effects of malaria on HIV-associated neurocognitive decline and malaria may affect long term HIV disease progression, although there is little published on either of these subjects. Future studies of children and adults presenting with cerebral malaria should include HIV testing and staging, and surviving subjects should be followed after discharge to monitor neurocognitive function and HIV progression. If severe malaria does hasten immunologic decline or neurocognitive impairment from HIV, it may be identified as clinical criteria for advanced HIV disease and thus an indication for antiretroviral treatment. HIV and malaria coinfection should be a significant public health and economic concern in sub-Saharan African and Asia, but until more is known of the long term consequences of coinfection there are insufficient data to guide management and improve outcomes in individuals with HIV and malaria coinfection.

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