See related article on page 1306

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Commentary

Cerebral Malaria

A Vasculopathy

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Cerebral malaria (CM), an important disease entity in the developing world, remains the deadliest complication of infection with *P. falciparum*. According to a recent World Health Organization report, half of the world's population remains at risk of acquiring malaria. The majority of the burden of disease occurs in Sub-Saharan Africa, where children under the age of five account for more than 80% of malaria-related deaths. CM is associated with an encephalitic syndrome that includes ataxia, seizures, hemiplegia, and eventually coma and death. These symptoms of severe malaria can progress very precipitously within hours from mild to severe. Indeed, even with successful antiparasitic treatment, residual neurological damage is a common finding in CM. It is estimated that more than 10% to 20% of the children who survive an episode of CM develop long-term cognitive deficits, which can include memory impairment, learning and language impairments, visuospatial and motor deficits, and psychiatric disorders.

The etiology of CM is not entirely understood because clinical studies in humans are not always feasible, and autopsy studies have only given us a limited view of this disease syndrome. In recent years, the increasing availability of computerized axial tomography (CT) and magnetic resonance imaging (MR) scanning has increased our understanding of the pathophysiology of this entity. Multiple mechanisms have been proposed to govern CM pathogenesis, including an intense upregulation of the inflammatory response, hypoxia, hypoglycemia, monocytic and/or red blood cell sequestration, and microvascular dysfunction leading to ischemia. The etiology of CM is likely multifactorial, and these hypotheses are not mutually exclusive.

The mouse model of CM using C57BL/6 mice infected with the ANKA strain of *P. berghei* as described by Cabrales et al[1] in the current issue of this Journal recapitulates many of the features of human CM. Although there has been some discussion as to whether this particular mouse model is a reliable model of human CM, it is now generally acknowledged to be a relevant and practical small animal model for CM. The pathological features of both human CM and the murine model described here and by others include microhemorrhages and vascular occlusion. However, the nature of the vascular occlusion in murine CM differs from that observed in human CM in that the former displays no red blood cell adherence and/or occlusion. Importantly, cognitive dysfunction has been observed in this animal model.[2]

Recently, a number of studies implicate a disruption in the integrity of the cerebral vasculature as an important contributing factor in the pathogenesis of CM. Both human and experimental CM studies are associated with a reduction in cerebral blood flow (CBF), which may be an important factor in the progression to CM. Single photon emission computed tomography (SPECT) in human CM demonstrated marked cerebral hypoperfusion associated with a significant decrease in oxygen saturation and neurological deficits corresponding to the areas of hypo-

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perfusion.[3,4] These abnormalities include decreased or absent perfusion in the capillaries and in larger retinal vessels, intravascular filling defects and leakage of dye material, which is indicative of a breakdown of the blood–retinal barrier, and ischemia.[5] The ischemic changes often correlate with neurological sequelae including seizures, obtundation, and coma.

In the current issue of the Journal, Cabrales et al[1] present substantial evidence for a role for vasoconstriction in the setting of CM and highlight the importance of vascular dysfunction in the pathogenesis of CM. Through the use of intravital microscopy, these authors obtained direct visualization of the pial microvasculature of the brain and correlated vascular dysfunction with progression of CM. Importantly, this disease progression was reversed when the vasculopathy was corrected by the calcium-channel blocker nimodipine.

Previously, it was demonstrated that in the murine model of CM, a reduction in CBF at advanced stages of the disease as measured by MRI/MRA directly correlated with significant decreases in the levels of certain metabolic markers in areas of the brain that were indicative of neuronal damage.[5] Specifically, a decrease in CBF was reported to be associated with a reduction in the ratio of N-acetyl aspartate (NAA) to creatine.[5] NAA has been widely used as an inverse marker of neuronal loss and injury in a variety of pathologies. It is synthesized almost exclusively in neuronal mitochondria, and a decrease in NAA levels usually reflects a mixture of both neuronal loss and recent or ongoing neuronal injury/dysfunction. A reduction in cerebral perfusion has also been associated with damage in the neuron/axon compartment with CM.[5] Conversely, MR spectroscopy studies of mice resistant to murine CM demonstrated no change in CBF or metabolic profile and no central nervous system lesions. These data indicate that alterations in the vasculature are an important component of CM.

In the present report, Cabrales et al[1] demonstrated a clear correlation with neurological deficits such as ataxia, limb paralysis, poor righting reflex, and seizures and the changes in the pial vessels. These deficits appear to be lesion-dependent, as mice with more severe neurological symptoms had a greater degree of vascular constriction and even sustained complete vascular collapse, whereas those with no signs of CM had a minimal decrease in CBF. Importantly, treatment with nimodipine together with the antimalarial agent artemether not only resulted in improved survival but also in a more rapid return to normal neurological function. The authors suggest that the reason for this observation is the partial restoration of CBF in affected mice.

The vasculopathy associated with CM is likely a result of endothelial cell damage, ischemia, activation of vascular cell adhesion molecules, and an associated breakdown in the blood–brain barrier.[6,7] Recently, we have focused on the role of vasoactive compounds in the setting of CM, particularly the 21-aa vasopeptide endothelin (ET-1).[8] Elevated plasma levels of ET-1 and big ET-1 have been reported in patients with *P. falciparum*. This occurs during acute infection and persists days after treatment of malaria. The increase in ET-1 correlates with elevated levels of TNF-α and likely reflects damage to the endothelium. A similar observation has been reported in an experimental CM model. In this same mouse model, there was an increase in the expression of ET-1 and endothelin converting enzyme, the enzyme that is responsible for the synthesis of ET-1 from Big ET-1, as well as increased expression of the endothelin receptors (ET_A and ET_B). This increase in the components of the endothelin pathway was associated with a reduction in CBF and neuronal dysfunction and inflammation.[8] The increase in ET-1 in the brain of mice with CM is consistent with the findings of increased plasma levels of big ET-1 in patients with acute complicated disease. Interestingly, the intraventricular injection of ET-1 has been reported to result in behavioral changes, including barrel rolling, body tilting, nystagmus, clonus, and tail extension. Most of these effects occur at doses that do not cause any changes in CBF.

Low levels of nitric oxide (NO) derived from endothelial nitric oxide synthase (NOS) and neuronal NOS have also been implicated in the vasculopathy of CM, further supporting the observation that vasoconstriction is an integral part in the development of CM. Cabrales et al[1] suggest that microhemorrhages may be important in this disease process because of the alterations in NO levels, which are known to be associated with cerebral hemorrhages.

Using calcium-channel blockers to ameliorate vascular spasm is not entirely novel in experimental cardiovascular disease. For example, Factor et al[9] demonstrated that the administration of verapamil to Syrian cardiomyopathic hamsters ameliorated the vasospasm of the coronary microvasculature, thus resulting in improvement of the cardiomyopathic phenotype. When verapamil was administered early, but not late, in the course of experimental *Trypanosoma cruzi* infection, it prevented the appearance of cardiomyopathy.[10] Furthermore, Tanowitz et al[11] used a cremaster muscle preparation to demonstrate that the *T. cruzi*--associated vasospasm was prevented by the administration of verapamil, similar to the findings in CM in the current report with nimodipine.[1] Nimodipine, the calcium-channel blocker used in the present study, ameliorates the vasospasm accompanying subarachnoid hemorrhage.[1]

Drugs of seemingly disparate classes show some efficacy in experimental CM. For example, Serghides et al[12] recently reported that the peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist rosiglitazone improved the outcome in CM in the mouse model. The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (HMG-CoA reductase inhibitor or statin) atorvastatin has been shown to be highly efficacious when used in combination with artesunate, resulting in a significant decrease in mortality.[13] Erythropoietin has also been reported as effective in ameliorating CM.[14] These agents are used clinically because of their effects on cholesterol metabolism, insulin resistance, and erythropoiesis, respectively. Pertinent to the current study, however, they share with calcium-channel blockers the ability to modulate the repair of microvascular damage.

Damage to the microvasculature, as elegantly demonstrated in the current study, is generally repaired either by replication of local existing endothelial cells, or by bone marrow–derived circulating endothelial progenitor cells (cEPCs), which are stimulated to migrate and incorporate into damaged sites in the microvasculature.[15] Their mobilization, release, and ultimate incorporation into sites of microvascular damage are mediated by the activation of a series of molecules, including stromal cell derived growth factor 1 (SDF-1). The subsequent incorporation of cEPCs into sites of microvascular damage is essential to the maintenance of microvascular integrity. They play a critical role in diseases associated with chronic or extensive damage. Indeed, insufficient or dysfunctional cEPCs and levels of associated factors that effect cEPC mobilization and function are predictive of poor outcomes in diseases associated with microvascular damage such as in cardiovascular disease and stroke[16,17].

PPAR-γ agonists, HMG-CoA reductase inhibitors (statins), erythropoietin and calcium channel blockers have all been reported to modulate the cEPC response. PPAR-γ agonists, which promote the differentiation and mobilization of cEPCs, have been shown to promote revascularization postangioplasty and are being evaluated in the treatment of stroke and ischemia/reperfusion injuries.[18] Statins increase cEPC mobilization, numbers, and functional activity, as well as delay cEPC aging and onset of senescence. These effects are independent of their ability to lower cholesterol and are believed to be responsible for the ability of statins to reduce myocardial and cerebral ischemia and cardiovascular risk.[19] Erythropoietin also increases the number of cEPCs in the bone marrow as well as the peripheral circulation. It improves vascularization in murine models of ischemia and has been shown to be safe and effective in the treatment of stroke patients.[20] Finally, calcium-channel blockers increase cEPC levels, promote their differentiation and migration as well as their vasodilatory effects. They have therefore been proposed as therapeutic agents to enhance microvascular repair by preserving endothelial cell integrity.[21]

A recent study of children with CM in Ghana suggests that the cEPC response to malaria-induced microvascular damage might also play a role in the pathogenesis of CM.[22] Children with CM have lower levels of cEPCs as compared with children with uncomplicated malaria, asymptomatic parasitemic children, children with severe malarial anemia, and healthy controls. In addition, levels of SDF-1 are elevated in children with acute disease (uncomplicated malaria and CM), indicative of host attempts to mobilize cEPCs to sites of microvascular damage. These findings place CM within in the context of current paradigms for microvascular repair mechanisms and suggest that equilibrium exists between malaria-induced microvascular damage and host-mediated repair. This balance is maintained by bone marrow–derived cEPCs, which augment local microvascular repair mechanisms in the brain of *P. falciparum*-infected children.

Acute malarial infection is associated with an increase in SDF-1 levels, leading to mobilization and homing of cEPCs to sites of microvascular damage. Thus, hosts able to maintain the balance between damage and repair do not develop CM. If there are low or insufficient numbers of cEPCs to mediate repair, the equilibrium is lost. CM develops, in part, because of breaches in the integrity of the brain microvasculature. Children able to reestablish equilibrium recover, whereas those that are unable to do so likely will die. As described in other diseases associated with microvascular damage, this equilibrium may be lost either because of the exhaustion of bone marrow progenitor cells or because of cEPC dysfunction stemming from reduced migratory capacity, survival, and/or differentiation.[23] Thus, chemotherapeutic agents such as PPAR-γ agonists, statins, erythropoietin, and calcium-channel blockers that encourage microvascular repair could be effective in the prevention and/or treatment of CM. This is consistent with the present study and highlights the role of the microvascular axis as an important target for adjuvant therapies. The demonstration that vasospasm with post-subarachnoid hemorrhage is ameliorated by nimodipine used in this study supports the notion that the host response to the microvascular damage induced by malaria is similar to that of other diseases involving microvasculature dysfunction. This is supported by current paradigms of microvascular repair, which invoke common repair mechanisms and physiological responses regardless of the initial insult to the vasculature.

As suggested by the authors, nimodipine, which has been used safely and effectively in humans in the treatment of diseases associated with microvascular damage, may be useful as an adjunctive therapy in the treatment of human CM. The authors are understandably cautious regarding the need for further study of such agents and their effects in murine models of CM attributable to the heterogeneity of case presentations of CM and the multifactorial nature of the disease before clinical trials in humans. The pleomorphic effects of these drugs also require a comprehensive study of their mechanisms of action in malaria using enhanced models such as that described by Cabrales et al.[1]

For example, the finding that vasodilatation may precede vasoconstriction as observed in some mice with CM might indicate that calcium-channel blockers could be detrimental depending on the time of administration. Indeed in other microvasculature diseases the potential need for "cocktails" of different agents affecting the microvascular axis has been raised with the possibility of both beneficial and detrimental effects. Optimization of murine models of CM permits the direct study of the effects of these agents before use in humans and therefore is an important advance in the field. However, the apparent safety of several modulators of the response to microvascular damage in recent clinical trials in patients with malaria is encouraging.[24,25]

Finally, it is important to reiterate that in the future, the treatment of CM may involve some form of adjuvant therapy in addition to a potent antimalarial drug. The inclusion of adjuvants, which maintain the integrity of the vasculature, promises to be an important addition to the malaria pharmacopoeia, a point underscored by Carbrales et al.[1]

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