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Rupture and release: a role for soluble erythrocyte content in the pathology of cerebral malaria

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Abstract

Cerebral malaria (CM) is the most severe form of malaria and causes high associated mortality. We propose a multi-step process for CM pathology that is initiated by cytoadhesion of infected erythrocytes to the brain vasculature, followed by rupture and release of contents that complete the disruption of the blood-brain-barrier.

Keywords

Cerebral malaria; cytoadhesion; blood-brain-barrier; *Plasmodium falciparum*; endothelial cells

According to the latest WHO estimates (<http://www.who.int/malaria/media/world-malaria-report-2016/en/>), in 2015 almost half of the world's population was at risk of malaria, there were 212 million new cases and more than 400,000 deaths. Most of the deaths were due to *Plasmodium falciparum* infections, which claimed a child's life every 2 minutes.

Cerebral malaria (CM) is the most lethal complication of *P. falciparum* infections. It affects mainly children under 5 years in sub-Saharan Africa and, to a lesser extent, children and adults in the South-Asia region. The mortality ranges from 15 to 20% and of those who survive, up to 25% develop long-term neurological sequelae.

Brain swelling is strongly associated with death in CM and is considered a major contributor to mortality in children [1]. In adults, brain swelling is less prominent but also an indicator of disease severity [2]. The recent serial magnetic resonance imaging (MRI) scanning of both pediatric and adult CM patients from India demonstrated for the first time that brain swelling mainly results from the loss of cerebral endothelial integrity. Indeed, a strong association between vasogenic edema (i.e. disruption of the blood-brain barrier - BBB) and brain swelling was found in all patients in that cohort, whereas only 5 out of 11 patients showed concomitant ischemia and cytotoxic edema [3]. Although brain endothelial activation and dysfunction are traditionally associated with CM the underlying mechanisms causing BBB disruption during CM are still not well defined.

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Of the 5 species of *Plasmodium* that infect humans, only *P. falciparum*-infected red blood cells (iRBCs) have the unique ability to cytoadhere to the endothelial cells of its host during the asexual life cycle. This is possibly due to the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), a extremely diverse family of antigens that are exported to the membrane of the infected erythrocytes and mediate cytoadhesion to the endothelial cells of different organs including heart, lung, kidney, subcutaneous tissues, placenta and brain [4]. Specific subtypes of PfEMP1 bind to different receptors on endothelial cells. In particular, a subset of PfEMP1 proteins that bind to endothelial protein C receptor (EPCR) are associated with severe malaria [5], highlighting the importance of this binding in the development of pathology. Binding of iRBC to intercellular adhesion molecule 1 (ICAM-1) on brain endothelial cells has also been described in CM, and even dual binding to both EPCR and ICAM-1 of particular PfEMP1 subtypes has been proposed as a potential risk factor to develop CM [6]. Agglutination of infected and uninfected erythrocytes, as well as reduced deformability of the erythrocytes, are also thought to contribute to the obstruction of the blood flow in the capillaries.

It has been proposed that the binding of EPCR to specific PfEMP1 variants not only mediates sequestration, but also allows thrombin-induced barrier disruption in CM [7]. This mechanism would explain the association of expression of these particular PfEMP1 variants with cerebral malaria pathology. Other factors may contribute to the weakening of the brain endothelial integrity, such as high levels of inflammatory cytokines, oxidative stress, hypoxia or acidosis.

A multifactorial cause for CM is suggested by the observation that while all patients who develop CM show cerebral sequestration, there are also definitive evidences for iRBCs sequestration in the brain of some non-cerebral malaria (non-CM) fatal cases. Using electron microscopy techniques, Pongponratn E. *et al.* analyzed the ultrastructure of the brain from 37 CM and 28 non-CM fatal cases. They found that all patients who developed CM show cerebral sequestration, but some patients who did not develop CM had levels of cerebral sequestration comparable to those that developed it. These findings strongly suggest that sequestration of iRBCs in the brain microvasculature is necessary but does not warrant the development of CM [8].

There is substantial evidence suggesting that another mechanism may also contribute to endothelial disruption during cerebral malaria: the rupture of cytoadhered infected erythrocytes in the brain. *In vitro*, rupture of non-adherent *P. falciparum*-iRBCs over a monolayer of human brain endothelial cells (HBMECs) results in the opening of intercellular gaps between cells through specific signaling pathways leading to the activation of β -catenin [9]. Moreover, using a *P. falciparum* line cytoadherent to HBMECs, we and others have observed that the effect over the monolayer after rupture was not different to the effect caused by its non-cytoadherent counterpart [9, 10]. This suggests that the release of intracellular erythrocytic contents triggers endothelial dysfunction. In fact, specific components of the infected erythrocyte have been proposed to contribute to the *P. falciparum*-induced endothelial disruption, including parasite histones, histidine rich protein II and heme [11].

Our hypothesis is that both sequestration and rupture of infected erythrocytes contribute to the impairment of the BBB during CM. We propose a multistep process for CM (Figure 1) where cytoadhesion is a first necessary step that would create a low perfusion environment in the microvasculature of the brain, followed by rupture of the adhered iRBCs within the congested microvessels that would release *Plasmodium*-derived factors directly on the surface of endothelial cells. The reduced blood flow in the obstructed microvessels would also favor that high concentrations of the released parasite-derived materials remain in contact with the endothelium, triggering the disruption of the interendothelial cell junctions.

The specific contribution of these factors to pathology in patients remains unknown and it is possible that the relative importance of these mechanisms may differ in each patient. Interestingly, a post-mortem study of a fatal malaria case caused by *P. knowlesi* showed cerebral pathology very similar to that observed in CM caused by *P. falciparum*, with brain vascular rupture and hemorrhaging [12]. The post-mortem analysis showed no evidence for cytoadherence in the microvasculature as iRBCs were not marginalized but were interspersed with uninfected erythrocytes. However, *P. knowlesi* has the shortest asexual cycle of all *Plasmodium* spp. that infect humans and can reach very high parasitemia levels in a short period of time. In this specific case, the patient developed >10% parasitemia, which probably led to high levels of soluble iRBC-derived factors in the blood, that could have been the cause of the observed impairment of the BBB and brain pathology. In the presence of cytoadhesion, such as in *P. falciparum* malaria cases, these high levels of soluble factors would be easily reached in the brain microvasculature with lower circulating parasite levels, leading to the breakdown of the BBB.

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Figure 1. Sequence of the Proposed Multistep Process of Cerebral Malaria

We propose that **1)** during early infection, iRBC start binding to receptors (e.g. EPCR, ICAM-1, etc.) on host brain endothelial cells through the superfamily antigen PfEMP1. **2)** High levels of inflammatory cytokines are induced in response to infection, inter-endothelial junctions weaken and there is an increase in the expression of endothelial receptors, facilitating the cytoadhesion of more iRBC. The sequestration of high amounts of iRBC and subsequent binding of uninfected erythrocytes and platelets obstructs the microvessels, reducing the blood flow. Binding of iRBC facilitates thrombin-activation of signaling that also contributes to weakening of inter-endothelial junctions. **3)** Rupture of iRBC and release of contents activates β -catenin signaling in brain endothelial cells leading to loss of BBB integrity and edema. **4)** High concentrations of iRBC-derived soluble factors may then further destabilize inter-endothelial junctions, which may lead to leakage of plasma and eventually hemorrhages. Abbreviations: BBB, blood-brain barrier; EPCR, endothelial protein C receptor; ICAM-1, intercellular adhesion molecule 1; RBC, red blood cell; iRBC, infected red blood cell; PfEMP1, *P. falciparum* erythrocyte membrane protein 1.