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Title: Immunological Factors in Cerebral Malaria

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Abstract

Cerebral malaria (CM) is the most severe and fatal complication of *Plasmodium falciparum* infection, with a near 100% mortality rate if untreated. The pathogenic mechanisms of CM, which involve both host and parasite factors, remain incompletely understood. This review examines the pathophysiology of CM, emphasizing the diverse factors implicated in its development. Current evidence indicates that CM involves endothelial cell activation, disruption of the blood-brain barrier (BBB), and dysregulated cytokine production. Key contributors include red blood cells, CD8+ T cells, macrophages, cytokines such as IFNγ, TNFα, and LTα, as well as nitric oxide (NO), ICAM-1, heme, and parasite-derived molecules such as PfEMP1, PfHRPII and EPCR. These factors collectively compromise BBB integrity, promote endothelial activation, and drive the clinical manifestations of CM, thereby increasing its lethality.

Background:

Malaria, a mosquito-borne disease caused by *Plasmodium* parasites, remains a significant global health challenge, particularly in sub-Saharan Africa. While the disease can sometimes be self-limiting without treatment in endemic areas, *Plasmodium falciparum* causes the deadliest form of malaria, making it a critical public health concern. Despite efforts by the World Health Organization (WHO) to eliminate malaria, the disease continues to disproportionately affect low-income countries, with 94% of cases occurring in sub-Saharan Africa in 2022 [1]. According to WHO data, malaria accounted for approximately 249 million cases in 2022, with a staggering 608,000 deaths reported in 2023 [1]. These statistics underscore the significant progress still required to combat the disease effectively. Malaria is the leading cause of morbidity and mortality in impoverished regions, particularly affecting children under five years old. The clinical progression of malaria varies, ranging from uncomplicated to severe malaria, and ultimately to cerebral malaria (CM) in its most fatal form. The severity of the disease is closely linked to the host's immunity, making vulnerable groups such as young children, pregnant women, and immunocompromised individuals particularly susceptible to severe manifestations [2]. Severe malaria is diagnosed based on high parasitemia combined with clinical complications such as impaired consciousness, prostration, multiple convulsions, acidosis,

hypoglycemia, severe anemia, renal impairment, jaundice, pulmonary edema, significant bleeding, or shock [3]. Among malaria complications, CM stands out as the most prevalent and deadly. Without treatment, CM has a 100% fatality rate, while with treatment, the mortality rate remains alarmingly high at 15-25% [4]. Cerebral malaria is characterized by an unarousable coma not attributable to other neuro-pathologies, combined with *P. falciparum* parasitemia. Other pathological signs include ischemic brain injury, and microvascular dysfunction, observed in this study occurred after the formation of vasogenic oedema and was associated with the temporal course of experimental cerebral malaria and a mild clinical severity [5]. Even among children who recover, long-term sequelae such as cognitive and auditory impairments are common [6-9]. The inability to predict which individuals will develop cerebral malaria stems from the incomplete understanding of its mechanisms in humans. However, experimental models using *Plasmodium berghei* in humanized mice suggest that cerebral malaria may have an immune-mediated basis [10]. This review focuses on the immune factors implicated in the pathogenesis of cerebral malaria.

Host factors:

The mechanisms through which *P. falciparum* invades the neurological system and triggers complications remain poorly understood. Current insights are derived from animal studies, particularly those involving *P. berghei*, and clinical observations in children with cerebral malaria. Cerebral malaria results from a combination of host and parasite factors that contribute to the obstruction of brain capillaries [11]. Clinically, children with cerebral malaria often exhibit brain swelling and brain stem herniation, leading to respiratory dysfunction, respiratory arrest, and death [4]. Brain swelling is primarily attributed to increased cerebral vascular permeability, which compromises the blood-brain barrier [12, 13]. Numerous host and parasite-derived factors are critical in initiating endothelial cell activation and disrupting the blood-brain barrier. Key players include host proteins, receptors, cytokines, and parasite-specific factors such as *P. falciparum Erythrocyte Membrane Protein 1* (PfEMP1) and *Histidine-Rich Protein II* (HRPII). These factors act in concert to exacerbate endothelial dysfunction, driving the severe clinical manifestations of cerebral malaria.

Blood Brain Barriers Disruption:

During iRBCs infection by *Plasmodium falciparum* (Pf), the parasite alters their surfaces by presenting variable antigens, including PfEMP1, which facilitate adhesion to endothelial cells [14]. iRBCs are shown to be central to the pathogenesis of CM [15]. Adhesion occurs via three mechanisms including single iRBC cytoadherence to the endothelium, cytoadherence with rosetting, where uninfected RBCs bind to iRBCs to form the rosetting, and cytoadhesion with Clumping**,** where platelets mediate adhesion between iRBCs [16, 17].

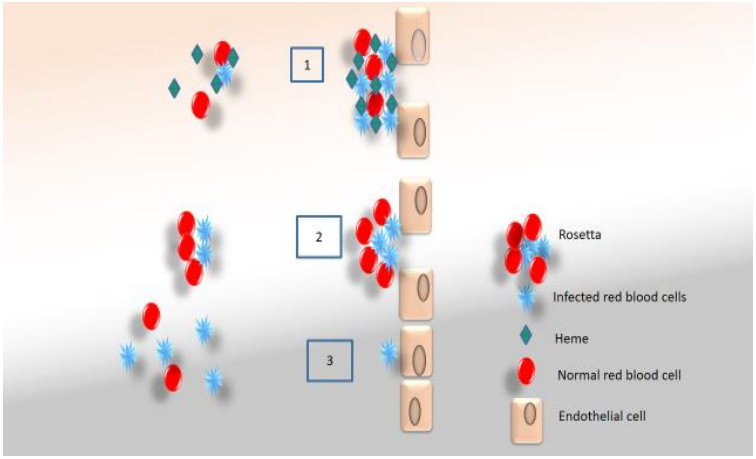

Image 1: types of cytoadherence.
1. Cytoadherence mediated by clumping
2. Cytoadherence madiated by rosetta
3. Cytoadherence madiated by iRBC

In the clumping model of cytoadherence, iRBCs bind to platelets, forming an agglutination or a clump where platelets act as bridges among iRBCs [16]. The adhesion of the clump to the endothelium contributes to the obstruction of the brain microvasculature. [16, 18]. The sequestration of the clump on the endothelium requires the participation of CD36 [19, 20], the globular C1q receptor (gC1qR/HABP1/p32) [21, 22] and P-selectin [23-25].

The sequestration of rosetting to the endothelium typically ensures the onset of BBB disruption. The term 'rosetting' is defined as the spontaneous binding of uninfected red blood cells (RBCs) to infected RBCs. It is established that cytoadherence with rosetting results in the obstruction of brain capillaries, thereby hindering brain blood flow [13, 26]. The rosetting process frequently necessitates the involvement of a cluster of differentiation 36 (CD36), which serves as a rosetting receptor [27]. It can be deduced that rosetting plays an important role in the pathogenesis of cerebral malaria. It has been demonstrated that cytoadherence of rosetting on the endothelium is dependent on the presence of PfEMP1 ligand and the involvement of complement receptor 1 (CR1), as well as heparan sulfate-like molecule. Furthermore, the formation of rosetting is also dependent on the presence of blood group A or B antigens. Indeed, the available evidence indicates that P. falciparum employs its surface protein PfEMP1 to form rosetting with group O RBCs and utilizes RIFINs to form rosettes with group A RBCs [17, 28, 29]. RIFIN is a polypeptide encoded by Plasmodium falciparum and expressed on the surface of infected erythrocytes, playing an important role in iRBC adhesion [30-32].

Conversely, even in the absence of rosetting, single iRBCs are also capable of adhering to the endothelium. It should be noted that the cytoadherence of single iRBCs to the endothelium plays a pivotal role in the activation of endothelial cells, and this process requires the involvement of several molecules. The cluster of differentiation CD36, intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), P-selectin, and thrombospondin (TSP) are well-documented [17]. CD36, also known as FAT, GP4 or GP3B, is found on the surface of numerous animal cells and functions as a receptor for thrombospondin in platelets and a multitude of cell lines. Furthermore, CD36 has been

demonstrated to directly mediate iRBC cytoadherence [33]. It recruits $\alpha_5\beta_1$ integrin to facilitate cytoadherence [34]. Moreover, it functions in conjunction with the ICAM1 molecule to initiate the process of cytoadherence and the activation of endothelial cells [35]. It has been demonstrated that in cases of infection by the protozoan parasite Plasmodium falciparum, endothelial cells are permanently activated, and iRBCs are sequestered in the vasculature. The sequestration of iRBCs functions as a trap for other immune cells It is noteworthy that a considerable number of cells were observed in this trap, which is characterized by a high density of immune cells [36-39]. Moreover, it has been documented that the cerebral vasculature is congested with thrombi containing adherent leukocytes, including CD4 T cells and monocytes [40]. The arrest of large numbers of leukocytes in postcapillary and venules causes significant alterations to the venous blood flow [41, 42], which in turn impairs the endothelial barrier function [15, 41]. In addition to the presence of pathogens and leukocytes, activated astrocytes and microglia cells were observed in the parenchyma in the vicinity of vessels with thrombi [43, 44].

Ligands and receptors :

The aforementioned findings indicate that disruption of the brain-blood barrier is a consequence of the attraction of a multitude of cells within the brain parenchyma. The compromised barrier integrity resulting in the extravasation of plasma proteins and fluids into the perivascular space, leading to vasogenic oedema [24]. The mechanism of BBB dysfunction requires the participation of numerous molecules that function as cell adhesion molecules, which are necessary for the activation of endothelial cells. These include the intercellular adhesion molecule (ICAM-1), the host endothelial protein C receptor (EPCR), and the ligand family of Ephrins (Eph), which have been repeatedly identified in the literature. ICAM-1 is an immunoglobulin (Ig)-like cell adhesion molecule that is expressed by several cell types, including leukocytes and endothelial cells. ICAM-1 plays a role in the arrest and transmigration of numerous leukocytes out of blood vessels into endothelial tissues, as well as in the activation of endothelial cells [45]. This also implies the binding of EPCR to its domain on the PfEMP1 protein [46, 47]. Eph, also known as Eph ligands or Eph family receptors, function as tyrosine kinase receptors (RTKs) [48]. They play a pivotal role in the disruption of the blood-brain barrier (BBB). It should be noted that EphA2 is required for the loss of junction proteins in mouse and human brain microvascular endothelial cells [48]. Moreover, EphA2 is indispensable for the infiltration of CD8+ T cells into the brain and the subsequent breakdown of the BBB in a mouse model of cerebral malaria. Inhibition of EphA2 has been demonstrated to protect against the breakdown of the blood-brain barrier [48].

Cells implication:

A typical response to experimental cerebral malaria in mice is an immune reaction involving T lymphocytes. This has been demonstrated in mice infected with P. berghei, which develop CM. An expansion of T cell clones was observed in the infected mice compared to the non-infected controls.

The observations made during the course of the mice infection demonstrate a significant alteration and compartmentalization of the TCR diversity [49]. Additionally, perivascular T cells have been demonstrated to play a relatively inconsequential role in the process of cytoadherence. They display arrested behaviour specifically during P. berghei ANKA infection [50], despite the brain-accumulating CD8+ T cells exhibiting comparable activation phenotypes during both infections [50]. The number of cells involved in the disruption of the blood-brain barrier (BBB) is unclear. Lymphocytes T, macrophages, neutrophils, and monocytes become trapped in the endothelium, where they are involved in the disruption of the BBB through the adhesion of iRBCs [51]. The cytotoxic effect of the complex Plasmodium-activated CD8+ T cells infiltration on microvasculature endothelial cells in the brain, sometimes followed by CD4+ T cells, represents a noteworthy characteristic of blood-brain barrier disruption in ECM [51, 52]. It was observed that pathogenic CD8+ T cells colocalized with rare apoptotic cells expressing CD31, a marker of endothelial cells, and iRBC within the brain during ECM [15, 50]. The sequestration of CD8+ T cells in the brain at the time when neurological symptoms appear has been linked to mortality in CM, particularly in the absence of phosphatidylinositol 3-kinase γ (PI3Kγ), which plays a role in regulating diverse cellular functions [53]. It has been demonstrated that a deficiency in PI3Kγ results in the loss of the cytotoxic function of CD8+ T cells (Granzyme B expression) and the reduction in the number of CD8+ T cells in the brain. This ultimately results in lethality in experimental CM mice [54].

Cytokines implication:

The physiopathological mechanisms implicated in cerebral malaria development include a disequilibrium in pro- and anti-inflammatory cytokine responses, loss of BBB integrity, and endothelial cell activation.

- CXCL10 (IP-10)

The well-known pro-inflammatory cytokine found in the literature, the chemokine CXCL10 (IFN-γ–inducible protein 10) plays a crucial role in experimental cerebral malaria (ECM) development [55, 56]. This 10-kDa chemotactic chemokine binds to CXCR3 (CD183) to mediate immune responses by recruiting T cells and natural killer (NK) cells [57]. CXCL10 production, triggered by IFN-γ and TNF, is attributed to monocytes and neutrophils. It has been implicated in chronic lung diseases [58, 59], diabetes [60], and pancreatic cancer progression when blocked [61]. Moreover, finding shown that CXCL10 levels correlate with parasite density and infection severity in *P. falciparum* malaria infection [62]. Furthermore, it stabilizes T cell-brain endothelial cell adhesion, contributing to ECM pathogenesis [13]. In fact, high CXCL10 levels promote parasite survival and growth [63]. CXCL10 was independently associated with cerebral malaria (CM) mortality [64]. Conversely, the suppression, neutralization, or genetic deletion of CXCL10 in ECM mice reduces inflammation and prevents cerebral malaria [65, 66].

- Lymphotoxin Alpha:

Among all cytokines in the cascade, lymphotoxin alpha (LTα) is strikingly linked to CM occurrence [67]. This cytokine is believed to be the primary mediator of murine CM. This argument is based on experimental studies showing that LTα-deficient mice do not succumb to CM, while mortality was unavoidable in LTα-expressing mice infected with *Plasmodium berghei* [68]. LTα was shown to inhibit the neurological symptoms of CM. However, unexpectedly, a significant drop in hemoglobin levels and a decrease in RBC count were observed in mice expressing LTα [68]. TNF-α may also play a critical role in the reduction- of hemoglobin levels [69]. Numerous cells are able to produce LTα such as brain endothelial cells and microglial cells as they are radiation-resistant [68, 70, 71]. These findings indicate that LTα is a major cause of death in experimental CM, perhaps in human CM.

- Tumor necrosis factor alpha:

Plasmodium falciparum infection is also associated with increased levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α [67]. It is produced by host cells in response to *P. falciparum* antigen. Higher serum levels of TNF-α have been observed in CM cases compared to other severe non-cerebral forms of malaria in both children and adults [69]. This leads to the conclusion that TNF-α level correlates with infection severity and predicts mortality [69]. Although TNF-α plays a crucial protective role in preventing CM development, excessive levels are linked to fatal outcomes [40, 69]. Conversely, the absence of TNF-α worsens disease progression, as shown in experimental CM, where TNF-α-deficient mice developed neurological symptoms [68]. Findings shown that the negative effects of TNF-α are exacerbated in the presence of interleukin-10 (IL-10) [40].TNF-α also plays a role in nitric oxide (NO) and IFN-γ production in *P. berghei*-infected mice. NO and IFN-γ are essentials in parasites clearance by host immune cells. Nitric Oxide (NO) has been associated with the reduction of inflammatory markers in brain vasculature, mitigating endothelial junction dysfunction and improving blood flow [72]. IFN-γ, released by immune cells such as CD4+ and CD8+ T cells, natural killer cells, and γδ-T cells during malaria infection, is a potent macrophage activator, enhancing their phagocytic activity, which is vital for early parasite control. IFN-γ also induces brain endothelium activation and increases the expression of adhesion molecules [42, 73-75]. Deficiency in TNF-α significantly reduce NO and IFN-γ levels, and thus hinder the immune response against the parasite. [68]. Deficiencies in both NO and IFN-γ have been reported to play significant roles in the development of murine CM [76, 77]. However activated CD8+ T cell-mediated damage is reduced by type I and II interferons, which stimulate neurons to up regulate PD-L1 via the STAT1/IRF1 pathway, initiated by IFN receptors. This mechanism provides a potential pathway to alleviate neuroinflammation during experimental CM [42].

Parasite Factors:

- PfEMP1 and Cytoadhesion

Parasite factors such as *Plasmodium falciparum Erythrocyte Membrane Protein 1* (PfEMP1) play a significant role in the pathogenesis of cerebral malaria (CM). PfEMP1 mediates binding to endothelial cells or facilitates rosetting through endothelial cell receptors, resulting in the sequestration of infected red blood cells (iRBCs) [78-82]. The expression of PfEMP1 determines the cytoadhesive properties of iRBCs and is strongly associated with severe malaria [14]. To evade antibody-mediated immunity, the parasite undergoes antigenic variation by switching between different PfEMP1 variants [83]. PfEMP1 is encoded by the *var* gene family, which contains Duffy-binding-like (DBLα-ζ) domains and cysteine-rich interdomain regions (CIDRα-δ) [84, 85]. Specific *var* gene variants encoding PfEMP1 with CIDRα, β, γ, or δ domains interact with EPCR [86] and are implicated in rosetting [81]. Numerous studies have shown that the *var* gene is associated with severe malaria [87-93], specifically the PfEMP1 protein that binds to the endothelial protein C receptor (EPCR). This binding contributes to severe malaria due to its role in facilitating the sequestration of infected red blood cells (iRBCs) [94-96]. To avoid clearance by the spleen, iRBCs adhere to vessel walls in various organs. This sequestration is àmediated through interactions between the Duffy-binding-like (DBL) domains, cysteine-rich interdomain regions (CIDR), and host molecules such as ICAM-1 and EPCR [83, 86, 97]. The level of PfEMP1-EPCR interaction correlates with the severity of malaria and can lead to cerebral malaria (CM) [93]. Transcript levels of EPCR-binding PfEMP1 variants are significantly higher in children with CM compared to those with severe non-cerebral malaria [96].

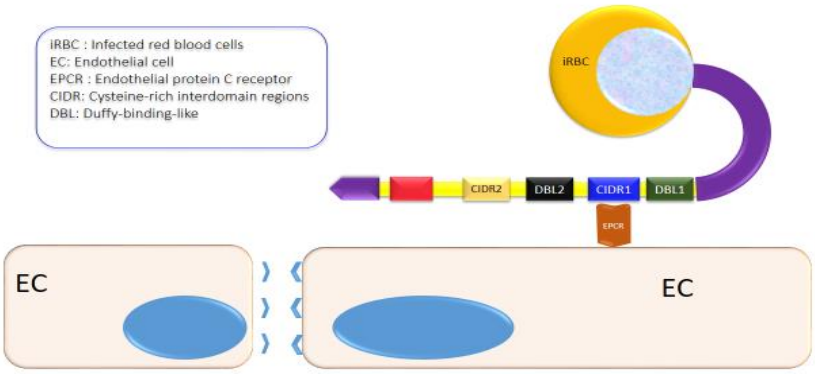

Image 2 : Cytoadherence of PfEMP1 to endothelial cell: the binding of PfEMP1 to endothelial cell (EC) involve host EPCR and the parasite CIDR1. This lead to the activation of EC and the disturbance of blood brain barrier.

- Plasmodium *falciparum* histidin-rich protein II:

In the early stages of *Plasmodium falciparum* (Pf) infection, brain endothelial cells are activated before the sequestration of iRBCs. Although the precise mechanism underlying this activation is unclear, it has been suggested that *P. falciparum* histidin-rich protein II (PfHRPII) may play a pivotal role [98]. PfHRPII has been shown to disrupt the permeability function of the endothelial cell barrier. Additionally, it competitively inhibits the heparin-dependent anticoagulant activity of antithrombin (AT) in a concentration-dependent manner [98]. PfHRPII is secreted by *P. falciparum* during the blood stage of malaria infection, and its high level in plasma has been associated with CM. PfHRPII is demonstrated to induce vascular leakage in the BBB, primarily by activating the endothelial cell inflammasome, which

increases endothelial barrier permeability and compromises the integrity of tight junctions [99, 100]. Moreover, infusion of PfHRPII in experimental models of CM has been shown to increase early mortality in mice [99]. These findings support the claim that PfHRPII is a virulence factor contributing to the severity of cerebral malaria [99]. The virulence of PfHRPII is partly due to its ability to deliver large quantities of heme, a component rich in iron, to endothelial cells. This leads to iron toxicity and the production of reactive oxygen species (ROS), which subsequently activate the NLRP3 inflammasome [98]. Together, these processes result in the loss of BBB integrity and exacerbate cerebral malaria pathology.

Conclusion

Cerebral malaria is a major cause of morbidity and mortality associated with *Plasmodium falciparum* infection, characterized by coma and a high fatality rate despite treatment. The pathogenesis of CM involves the adhesion of iRBCs to endothelial cells, the sequestration of leukocytes and iRBCs at the BBB endothelium that prevent them to play their role to eliminate the parasite at this site. Moreover, the exaggerated increase of pro inflammatory cytokines LTα and TNFα levels are correlated to the lethality rate. These processes, combined with a drop in anti-inflammatory cytokines IFNγ and NO concentration raise the clinical outcomes and mortality imputable to CM, minimize the chance to recover. Key parasite-derived proteins, including PfEMP1, EPCR, CIDR, and PfHRPII, also play pivotal roles in endothelial activation and BBB disruption. Their contributions to immune evasion, cytoadhesion, and vascular damage underscore their critical roles in the development and severity of cerebral malaria. Understanding the mechanisms by which the concentration of LTα and TNFα increase, and IFNγ and NO decrease, will be beneficial for new treatment strategies and vaccine advancement. Furthermore, preventing PfHRPII to target the heme, saving it from hemoglobin degradation will be crucial in anemia prevention and reduce worldwide mortality death tool.

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