Pathophysiology of Cerebral Malaria and Treatment

Subjects: Immunology

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Plasmodium falciparum causes over 90 percent of all malaria infections. Children under the age of 5 years and pregnant women were the most susceptible groups affected by malaria. The World Health Organization (WHO) has characterized malaria as severe and uncomplicated. Delays in the detection and treatment of an uncomplicated infection of *P. falciparum* malaria lead to complications of severe cerebral malaria (CM). CM is usually caused by *P. falciparum*, but *Plasmodium vivax* is rarely responsible for CM complications. CM is a severe neurological complication caused by *Plasmodium falciparum* infection, resulting in high mortality rates. CM is characterized by brain tissue hemorrhage, the accumulation of infected red blood cells and mononuclear cells in brain microvessels, and blood-brain barrier (BBB) disruption.

cerebral malaria pathophysiology cytoadherence

1. Pathophysiology during Cerebral Malaria Progression

Several hypotheses have been reported to explain the mechanism of cerebral malaria development. One such hypothesis affirms that infected RBCs adherence to brain endothelium leads to blockage in microvessels, causing nutrient deprivation and hypoxia in nearby brain tissue. As a result, brain tissues cannot maintain membrane potential, which causes water inflow from extracellular to intracellular compartments, ultimately leading to cell death and tissue damage [1]. According to other groups, the sequestration of infected RBCs and inflammation enhance binding of infected RBCs with non-infected RBCs to form rosette [1] and the adherence of infected RBCs and leukocytes to brain endothelium (**Figure 1**) [2]. Moreover, platelet-mediated clumping of infected RBCs during infected RBCs sequestration [3] causes excess activation of the endothelial wall with pro-inflammatory mediators secretion [4] and an imbalanced release of endothelin-1 and angiopoietin-2 vasoactive mediators [5]. Finally, the brain endothelial cell barrier becomes impaired with leakage in brain parenchyma, resulting in breakage of the BBB.

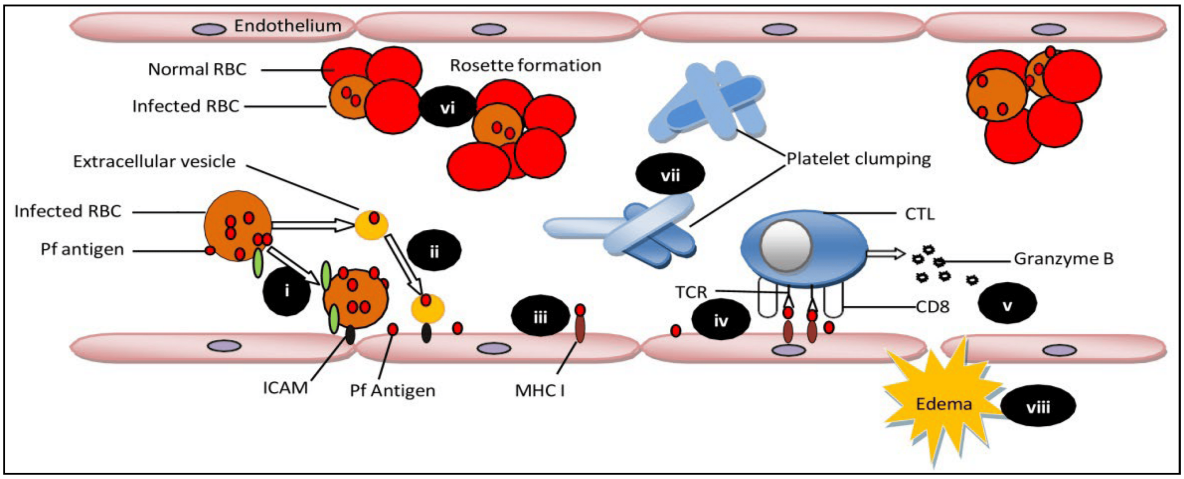

Figure 1. Factors involved in blood-brain barrier breakdown during cerebral malaria.

In addition to acting as a transport system, endothelial cells also facilitate the binding of infected RBCs expressing *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1) linked with CM and act as phagocytic cells. Intercellular adhesion molecule (ICAM) dependent internalization of infected RBCs by endothelial cells results in brain injury and impairment of BBB [6]. After phagocytosis, infected RBCs undergo degradation and exposes host cells to cellular content and toxins [7]. ICAM-1 facilitates the entry of leukocytes in the endothelium by forming ring-like projections [8].

Transfer of Pf antigens from infected RBCs to brain endothelial cells by direct contact (i) or by production of antigen-carrying extracellular vesicles (ii); Pf antigen picked up and expressed by MHC I (iii); interaction of cytotoxic T lymphocytes (CTL) with endothelial cells expressing antigens, through their CD8 and TCR receptors (iv); granzyme B secreted by CTL (v); infected RBCs interact with uninfected RBCs leading to rosette formation (vi); platelet-mediated clumping (vii) disruption of the blood-brain barrier, leading to vasogenic edema (viii) (this figure is adopted and modified from Renia et al. (2020) [9].

In the case of infected RBCs, it is suggested that the mechanism is similar; however, infected RBCs show slower transit than leukocytes, as infected RBCs are deficient in machinery required for motility [8]. Preclinical studies showed that activated protein C (aPC), an anticoagulant serine protease, binds to endothelial protein C receptor (EPCR) and exerts its anti-inflammatory and anti-apoptotic activities, thereby protecting endothelial barrier functions [10][11]. EPCR, expressed at low levels by the brain endothelium, interact with infected RBCs and caused a loss of aPC, leading to a loss of cytoprotection [12].

Consequently, infected RBCs binding with EPCR turn off cytoprotection via the CIDRα1 domain, whereas the ICAM-1 binding DBLβ domain incites binding and phagocytic response by the endothelium and assists cerebral malaria pathology through cerebral swelling and BBB disruption [6]. The link between ICAM-1 and EPCR to cerebral malaria symptoms has been established [6], but the mechanism of pathogenesis is still ambiguous. The activation of endothelial cells is regulated by cytokines such as Tumour Necrosis Factor alpha (TNF-α), Tumour Necrosis Factor beta (TNF-β), and interferon-γ [13]. The upregulation of TNF cytokine was observed in the blood

and the brain cells of human and murine models of cerebral malaria [14]. In vitro TNF exposure induces the upregulation of infected RBCs adhesion factors and endothelial C activation markers, leading to increases in adherence of infected RBCs on human [15] or murine [16] brain microvascular cells (**Figure 2**). Furthermore, the upregulation of endothelial cells activation markers such as E-Selectin, von Willebrand Factor (vWF), ICAM-1, and CD36 all can act as adhesion targets for infected RBCs and contribute to endothelial activation.

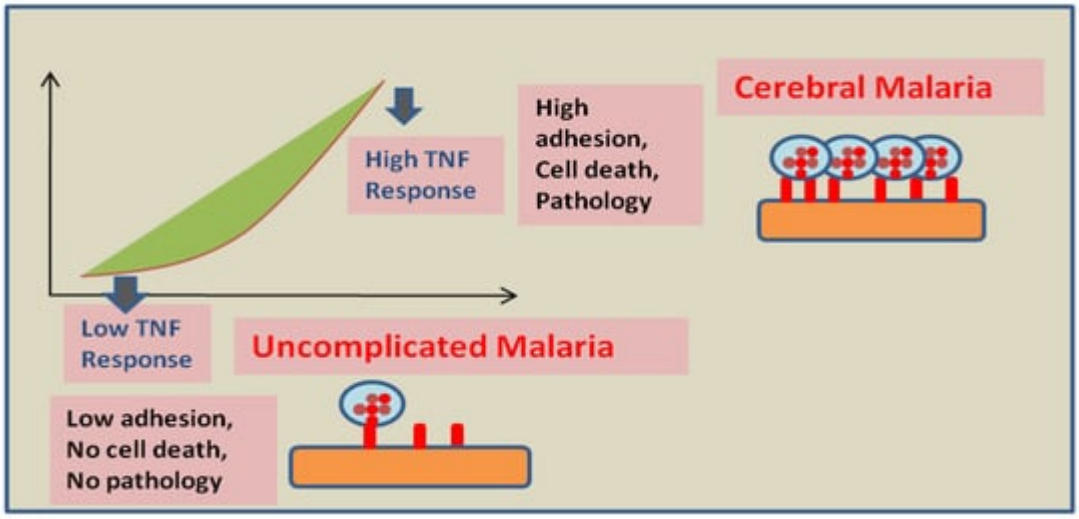

Figure 2. Proposed effect of the host endothelial response to TNF on disease severity: Low endothelium response to TNF leads to a minimal adhesion of infected RBCs and host cells and might be responsible for the absence of pathology. However, high endothelium response to TNF leads to elevated adhesion of infected RBCs. Moreover, a strong pro-apoptotic signal is generated in high responders that possibly results in BBB breakage and vasogenic edema (this figure is adopted and modified from Wassmer SC et al. [17]).

Malaria parasite during infection suppresses erythropoiesis in the bone marrow, and hemozoin deposition in the tissues are mostly responsible for malarial anemia. Dyserythropoiesis results in the removal of infected and uninfected erythrocytes from the system and suppressed the production of mature erythrocytes in the bone marrow, which are primary contributors to the severity of malaria infection [18][19], followed by the development of severe malarial anemia leading to increased mortality and morbidity. The proliferation of erythroid progenitor cells is challenged during malaria infection due to decreased expression of erythroid-specific transcription factors such as GATA-1 and GATA-2 [20][21]. It has been reported that erythroblasts, BFU-E, and CFU-E numbers decrease after infection with *P. berghei* [22]. GATA-1 facilitates the survival and late-stage differentiation of BFU-E to CFU-E, which is facilitated by the mediator subunit MED1/TRAP220 [20][21], whereas GATA-2 is critical for the maintenance and proliferation of hematopoietic progenitors.

2. Therapeutic Approaches

The sequestration of infected RBCs can also be prevented by using drugs targeting cytoadherence on the endothelium. In experimental cerebral malaria reports, the use of rapamycin restricts the cytoadherence of infected RBCs on endothelial cells by VCAM-1 and ICAM-1 reduction [23]. Moreover, the fungal ethanolic extract

of *Trichoderma stromaticum* helps in reducing inflammation in experimental cerebral malaria by reducing the expression of VCAM-1 and ICAM-1, thus protecting the blood-brain barrier's integrity [24].

The activation of endothelial cells by cytoadherence of infected RBCs and the pro-inflammatory effects of released cytokines play important roles in cerebral malaria pathophysiology [25]. Anti-TNF treatments can be helpful in managing the severity of disease and mortality rate. Endothelium activation triggers a TNF dependent pro-apoptotic pathway [26]. The difference in response of endothelial cells to TNF can affect the severity of the disease [27]. Anti-TNF therapy showed a positive result in vitro. However, it showed no reduction in mortality in the patients. Moreover, the use of antibodies against endothelial protein C receptor inhibited PfEMP1′s ability to bind to human endothelial cells [28]. Further studies on endothelial cells confirm the activation of Rac1 signaling via cross binding of VCAM-1 that results in Rho-dependent inductions of stress fibers, leading to the weakening of tight junctions [29].

Moreover, the interaction of EPCR with activated protein C is prevented by binding PfEMP1 to EPCR. This binding shows two impacts: (i) promoting the activation of tissue factors Va and VIIIa, which results in the disablement of the aPC-mediated anti-coagulative pathway. These factors are responsible for thrombin generation on activation, which eventually results in fibrin deposition. (ii) The initiation of NF-kB and Rho A pathways through thrombin-mediated scissions of PAR1 is also observed. The activation of these pathways generates a pro-inflammatory response, which leads to impairment and breakage of the blood-brain barrier [11][30][31]. The administration of aPC may prevent blood-brain barrier dysfunction by inhibiting thrombin activity [32]. Other approaches embrace the utilization of endothelial cells isolated from patients suffering with cerebral malaria. Moreover, measuring the consequences of Ang-1 on endothelial cells pre-treated with TNF will be crucial for the development of new additional therapies and improving disease outcomes in CM. Since Ang-1 acts as a serenity agent for endothelial cells in the exquisite model of sepsis [33], maintaining a higher Ang-1concetration in Ang-2/Ang-1 ratio during infection can potentially block and reverse ongoing inflammatory processes in CM patients.

3. Mesenchymal Stem Cells as A Regenerative Therapy

3.1. Mesenchymal Stem Cells Mediated Cellular Mechanism of Protection

Mesenchymal stem cells (MSCs) first observed in the bone marrow are multipotent cells [34], which can differentiate into various cell lines lineages such as adipocytes, chondrocytes, and osteoblasts and display strong tissue protective and restorative properties. During malaria infection, MSCs become accumulated in primary and secondary lymphoid organs. Different surface antigens are expressed by MSCs in different tissues, which facilitate tissue repair and regeneration through the secretion of various soluble factors. Souza MC et al. demonstrated that, in *P. berghei*-infected mice, neurons were damaged, with an increased number of astrocytes and oligodendrocytes. However, in *P. berghei*-infected mice treated with BM-MSCs, brain damage was repaired, which leads to a reduction in parasitemia and mortality [35]. There was a substantial increase in phagocytic neutrophils in the brain [36]; hepatocytes and Kupffer cell regeneration in MSC-infused mice indicate regenerative ability of MSCs. MSCs promote hematopoiesis by releasing different critical molecules involved in the self-renewal, proliferation, and differentiation of hematopoietic stem and progenitor cells (HSPCs). Other than that, MSCs encourage the formation

of colony-forming units-erythroid (CFU-E) cells in the bone marrow [37], which ultimately helps in preventing malarial anemia. These observations suggest that cell-based therapeutics for intervention in malaria may be useful in achieving sterile clearance and preventing disease reactivation.

3.2. Mesenchymal Stem Cells Mediated Molecular Aspects of Protection

Studies on anti -$CD3^-$, -$CD19^-$, -$Sca\text{-}1^+$, and -$CD34^+$ antibodies revealed a significant elevation in Sca- 1^+ and $CD34^+$ cells in the lymph node and spleen of MSC-treated mice as compared to untreated mice [36]. A drop in GATA-1 and GATA-2 expression was observed in plasmodium-infected mice. Since GATA-1 is a key erythroid cell differentiation factor, the low level of GATA-1 expression may be responsible for reduced RBC formation in malaria-infected animals. However, after MSCs treatment, an increase in the expression of GATA-1 and GATA-2 has been reported in animals infused with MSCs. Increased CFU-E formation and reduced hemozoin content in MSC-infused mice support the conclusion that MSCs elicits signals to support erythropoiesis [38].

3.3. Application of Mesenchymal Stem Cells in Animal Model of Cerebral Malaria

Malaria infection causes a reduction in $CD4^+$ T cells in mice models of cerebral malaria, which are crucial for immunity development against malaria, leading to impaired T cell-mediated immunity [39]. However, an increase in the number of $CD4^+$ and $CD8^+$ T cells was reported in MSC-infused mice, and MSCs are able to rescue the proliferation of $CD4^+$ T cells [37]. Ongoing research studies are focused on signaling molecules engaged in restoring immune responses by MSCs. MSCs also inhibited the induction of the negative co-stimulatory receptor programmed death-1 by T cells in recipient animals. Taken together, MSCs help in the protection against malaria infection by reprogramming hematopoiesis, by enhancing the differentiation of $CD34^+$ cells, restoring $CD4^+$ and $CD8^+$ T cell proliferation, and by suppressing the expression of negative co-stimulators on T cells. The increased production of interleukin IL-12, which is crucial for self-renewal and differentiation of multipotent progenitor cells and suppressed production of IL-10, was reported in MSC infused animals. The transfer of MSCs isolated from secondary lymphoid organs of *P. berghei*-infected mice conferred host resistance against malaria through the enhanced production of pro-inflammatory cytokines IL-6, IL-12, and TNF-α, and the suppression of IL-10 [38][40].

Preclinical studies on several metabolic disorders, tissue regeneration, cancer, heart disorders, and other disorders suggest the robust use of MSC-based therapy [41]. Despite multiple preclinical studies on MSC-based therapy, no promising clinical studies are available for cerebral malaria. The multipotent nature and the potential of MSCs of modifying the tissue microenvironment makes them appropriate candidates for the development of stem cell-based regenerative medicinal therapy.

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