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MECHANISMS OF PATHOGENESIS IN SEVERE COMPLICATED HUMAN MALARIA

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ABSTRACT

Invasion of human red blood cells by *Plasmodium* parasites, particularly *P. falciparum*, in some cases lead to progressive and dramatic structural, biochemical, and morphological modifications of the infected red cells. These changes are linked to development of severe complications associated with human malaria. Many aspects of severe and complicated course of the disease are not clearly understood. It is known that a combination of parasite-specific factors and mechanisms, such as adhesion and sequestration in the vasculature and the release of bioactive molecules, together with human inflammatory responses mediated by cytokine and chemokine production and cellular infiltrates are involved.

However, the contribution of each of the factors is not adequately addressed in biomedical literature. The multiple processes involved together lead to various forms of organ dysfunction which need a better understanding. Severe and complicated malaria is fatal in the absence of prompt diagnosis and identification of its clinical features and resultant complications, and urgent appropriate patient management should be considered as priority. This review provides a better understanding of the disease process and should lead to improvements in prevention and treatment of severe and complicated forms of human malaria. Areas reviewed and remain unclear and therefore require further inquiry in terms of research are highlighted as basis for further research.

Keywords: Severe Complicated Malaria, Cytoadherence, Nitric Oxide, Inflammatory Reactions, Cellular Infiltration, Sequestration, Rosetting

INTRODUCTION

Human malaria is caused by protozoan parasite species in the genus *Plasmodium.* Four species have been associated with disease, *P.falciparum*, *P.ovale*, *P.malariae* and *P.vivax*. Recently, *P.knowlesi* has been reported to cause human malaria in Asia. The most prevalent malaria parasite is *P.falciparum* and is most consistently associated with severe human malaria worldwide. The World Health Organization estimates that about 300-500 million global malaria cases are recorded annually, with 90% of this burden being in Africa while Southeast Asia contribute only 2.5 million **[1]**.

The disease in humans is categorized into uncomplicated and severe complicated malaria. The presentation of uncomplicated *P. falciparum* malaria is very variable and mimics that of many other diseases. Symptoms may include fever, headache, and aches and general body pain, occasional abdominal pain and diarrhea. This clinical presentation may be misdiagnosed as influenza, typhoid, brucellosis and, unless diagnosed and treated promptly the clinical picture may deteriorate and often with catastrophic consequences **[2]**.

Most cases of severe and complicated human malaria are caused by *Plasmodium falciparum* which manifests clinically as a spectrum of diseases ranging from asymptomatic or mild infection through severe to fatal disease. People who develop severe and complicated malaria may die from the disease. Groups at greater risk include young children and pregnant women in malaria endemic regions; non-immune migrants, laborers and visitors to endemic regions; and residents of regions where malaria has been recently reintroduced and or re-emerged **[2]**. For reasons largely unknown, not all individuals within the risk groups appear to be at equal risk for severe disease. It is hypothesized that determinants of severe and complicated disease include factors associated with a population, the individual (biologic, immunologic), and the parasite. Human-*Plasmodium* parasite interaction is thought to play major part in the development of severe disease but the contribution of each is largely unknown.

The development of severe malaria probably results from a combination of parasite-specific factors, such as cytoadherence, rosetting and sequestration in the vasculature and the release of bioactive molecules. These in combination with host inflammatory responses, including cytokine and chemokine production and cellular infiltrates are widely cited in many cases of severe malaria without elaboration. Cerebral malaria is the most common

clinical presentation and accounts for the majority of deaths in severe malaria **[3]**.

Many cases of severe and complicated malaria are being reported globally than before. These cases are associated with debilitating, demoralizing, and impoverishing consequences to the society in many developing countries. This warrants a better understanding of the disease process that lead to improvements in preventing and treating severe and complicated forms of human malaria.

***Plasmodium* Developmental Stages Associated with Malaria**

In the human host, the malaria parasite undergoes two phases of growth and multiplication (schizogony). These are, exo-erythrocytic phase (exo-erythrocytic schizogony) that takes place exclusively in the liver cells and erythtrocytic phase (erythrocytic schizogony) in which the parasites invade and multiply in the red blood cells. It is the invasion by merozoite stage (asexual forms), feeding and growth of trophozoite stage (feeding stage) and multiplication of merozoite stage of the parasite in the red blood cells that is associated with the disease, malaria.

All the manifestations of malarial illness are caused by the infection of the red blood cells by the asexual forms of the malaria parasite, and makes malaria a potentially dangerous multi-systemic disease, as every organ of the body is reached by the blood. All types of malaria manifest with common symptoms such as fever, while some patients may progress into severe malaria, which is more often seen in cases of *P. falciparum* infection, though complications and even deaths have been reported in malaria cases caused by other species **[4, 5]**.

Erythrocytic Schizogony and Pathogenesis of Severe Complicated Malaria

Erythrocytic schizogony within the red cells, releases newly developed merozoites by the lyses of infected erythrocytes and along with them, numerous known and unknown waste substances, such as red cell membrane products, hemozoin pigment, and other toxic factors such as glycosylphosphatidylinositol (GPI). These products, particularly the GPI, activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such as tumor necrosis factor (TNF), gamma interferon (IFN-γ), interleukin 1 (IL-1), IL-6, IL-8, macrophage colony-stimulating factor, and lymphotoxin, as well as superoxide and nitric oxide (NO). Many studies have implicated the GPI tail, common to several merozoite surface proteins (MSP) such as MSP-1, MSP-2, and MSP-4, as a key parasite toxin **[6, 7]**.

The systemic manifestations of malaria such as headache, fever and rigors, nausea and

vomiting, diarrhea, anorexia, tiredness, aching joints and muscles, thrombocytopenia, immune-suppression, coagulopathy, and central nervous system manifestations have been largely attributed to the various cytokines released in response to the parasite and red cell membrane products **[8]**. In addition to these factors, the plasmodial DNA is also highly pro-inflammatory and can induce cytokinemia and fever. The plasmodial DNA is presented by hemozoin (produced during the parasite development within the red cell) to interact intra-cellularly with the Toll-like receptor-9, leading to the release of pro-inflammatory cytokines that in turn induce COX-2-upregulating prostaglandins induction of fever **[9, 10]**. Hemozoin has also been linked to the induction of apoptosis in developing erythroid cells in the bone marrow, thereby causing anemia **[11, 12]**.

Accumulating evidence suggests that hemozoin (HZ) is immunologically active, but the molecular mechanism through which it modulates the human innate immune system has not been elucidated. However it has been demonstrated that HZ purified from *P. falciparum* is a novel non- DNA ligand for Toll-like receptor (TLR) 9. Hemozoin activates innate immune responses *in vivo* and *in vitro,* resulting in the production of cytokines, chemokines, and up-regulation of costimulatory molecules. Such responses were severely impaired in the TLR9 $^{-/-}$ and myeloid differentiation factor 88 (MyD88) $^{-/-}$ but not in the TLR2, TLR4, TLR7, or toll/interleukin 1 receptor domain-containing adaptor-inducing interferon $\beta^{-/-}$ mice. Synthetic HZ, which is free of the other contaminants, also activates innate immune responses *in vivo* in a TLR9-dependent manner **[11]**. It is therefore evident that immune-pathogenic processes play a central role in severe complicated malaria, with pro-inflammatory cytokine cascades leading to complex downstream metabolic changes in patients **[13]**.

Infected RBC Membrane-Surface Proteins and Endothelial Receptors Involvement in the Pathogenesis of Severe Complicated Malaria

The entry of the *Plasmodium* merozoites into the RBC induces changes in cell surface proteins and chemical changes that have been implicated in the pathogenesis of severe complicated malaria. This may cause cytoadherance in which infected RBC adhere to the endothelial cell lining of capillaries and or resetting that is sticking together of infected RBC. Both cytoadherence and resetting are known to cause sequestration, a situation in which the malaria parasite resides in RBC causes obstruction of the capillaries of the brain and may lead to fatal cerebral malaria.

Plasmodium falciparum is the only species among all malaria parasites that induces cytoadherence to vascular endothelium of erythrocytes containing the mature forms of the parasite whose proteins are transported and inserted into the erythrocyte membrane. This high molecular trans-membrane protein of approximately 240 kDa known as *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) is an antigenically diverse protein family that is expressed on the thousands of knob-like excrescences on the surface of red cells infected with *P. falciparum* trophozoites and schizonts and is the most important ligand for cytoadherence **[14]** Febrile conditions enhance expression of P*f*EMP1 mediated cytoadhesion and it presence in significant numbers, is a poor prognostic sign representing a large sequestered parasite load **[15]**.

Plasmodium falciparum related *Pf*EMP1 is encoded by the highly variable *VAR* gene family, comprising around 60 genes. The high switch rate between these genes gives rise to a new variant *Pf* EMP1 in 2% of the parasite's every new cycle, and this clonal antigenic variation helps the parasite escape the immune system **[16]**. P*f* EMP1 is expressed on the surface of 'knobs', which can be identified electron-microscopically as protrusions from the erythrocyte membrane acting as points of attachment to the vascular endothelium **[15]**. Other surface proteins that might play a role in cytoadherence are rifin **[17]** and sequestrin **[18]**.

The *PfEMP1* is known to bind to several adhesion receptors expressed on the endothelial cells. Of these only CD36, which is constitutionally expressed on most vascular beds but remarkably absent in brain vessels, and chondroitin sulphate A (CSA), the main receptor in the placenta, are able to support firm adhesion under flow conditions **[10]**. The intercellular adhesion molecule 1 (ICAM-1) is the most important receptor on brain endothelium **[19]**, and its expression is up-regulated by the pro-inflammatory cytokine TNF-α. Electrostatic forces are indeed important in addition to steric factors in the binding of *Pf*EMP1 to its receptors since surface potential spectroscopy of knobs has revealed that knobs are positively charged (+20 mV), whereas the endothelial plasma membranes and receptors have a negative surface charge **[19]**. This could contribute to the affinity of *Pf*EMP1 for a great variety of receptors. Recently it has been suggested that platelets, which express CD36, might serve as a sticky bridge between infected erythrocytes and the endothelium, which could be particularly important in the brain microvasculature lacking CD36 **[20]**.

The infected red cells may also adhere to uninfected red cells, resulting in the

formation of red cell rosettes, process known as rosetting. Rosetting is mediated by binding of *Pf*EMP1-DBLα on the surface of infected red cells to complement receptor 1, CD31, and heparan sulfate-like glycosaminoglycans of uninfected RBCs **[21-23]**. Rosetting is found to be lesser in blood group O erythrocytes compared with groups A, B, and AB suggesting that patients with blood group O may be protected from severe malaria **[24, 25]**.

Cytoadherence is also known to lead to sequestration of parasitized erythrocytes in the microcirculation involving mainly capillaries and post-capillary venules. Autopsy studies have shown that sequestration is not distributed equally throughout the body and is greatest in the brain (cerebrum, cerebellum, medulla oblongata), but also prominent in the heart, eyes, liver, kidneys, intestines, subcutaneous tissue, adipose tissue and placenta **[26]**. Sequestration of the growing *P. falciparum* parasites in these deeper tissues provides them the microaerophilic venous environment that is better suited for their maturation and the adhesion to endothelium allows them to escape clearance by the spleen and to hide from the immune system. These factors help the falciparum parasites to undergo uncontrolled multiplication, thereby increasing the parasite load to very high numbers **[8, 27]**.

It is widely believed that sequestration plays a key role in cerebral malaria and other fatal complications of the disease. However, there are conflicting theories about how this occurs. The traditional explanation for cerebral malaria is that sequestration, perhaps combined with the reduction in the deformability of red blood cells that occurs when the cells are infected with *Plasmodium*, leads to the blockage of capillaries in the brain, depriving the tissue of oxygen **[15]**. However, measurements made using Near Infrared Spectroscopy and Doppler sonography show that levels of blood flow in the brains of cerebral malaria patients are not abnormally low **[6]**, and individuals who recover from cerebral malaria do not generally exhibit permanent brain damage that is typically associated with acute oxygen deprivation. Sequestered cells infected with *P. falciparum*, however, harm the brain by causing an excessive but localized immune reaction, rather than by physically blocking the capillaries. Nitric oxide (NO) generated by activated macrophages to kill parasites is known also to be toxic to human cells at high concentrations. Nitric oxide has been implicated and plays a role in the damaging localized immune reactions already described. It has been reported that a toxin produced by *P. falciparum* can induce the NO-synthesizing enzyme iNOS (nitric oxide

synthase) in human endothelial cells, and iNOS has been found in samples of brain tissue taken during autopsies of cerebral malaria victims **[27]**.

During pregnancy, *Plasmodium* infected RBCs (pRBCs) typically sequester in the placenta and maternal malaria is associated with intra-uterine growth retardation and premature delivery, resulting in neonatal mortality. Maternal health also suffers through the development of maternal anemia and the resultant increased likelihood of maternal death. The consequences of sequestration to the host are thus variable and can be severe. Although the link between sequestration and clinical disease in humans remains indirect, a degree of obstruction to blood flow will occur in vasculature. This could lead to hypoxia, reduction of metabolite exchange and the release of inflammatory mediators. In the brain, it could contribute directly to cerebral edema and raised intracranial pressure **[8]**.

Due to the sequestration, *P. falciparum* asexual forms often circulate in the peripheral blood, while trophozoites and schizonts are bound in the deep microvasculature, hence seldom seen on peripheral blood examination. If massive cytoadherence-rosetting-sequestration of infected and uninfected erythrocytes in the vital organs continues uninhibited, it ultimately blocks blood flow, limits the local oxygen supply, hampers mitochondrial ATP synthesis, and stimulates cytokine production - all these factors contribute to the development of severe and complicated disease **[3, 28, 29]** Microscopic studies indicate that patients dying from cerebral malaria have more prominent sequestration in the brain microvasculature compared to severe but non-comatose fatal cases **[28, 30]**. Recent surveys from Malawi and Southeast Asia have found a significant association between the amount of sequestration and ante-mortem diagnosis of cerebral malaria **[30]**. Furthermore, there is growing evidence that parasite-induced sequestration of infected and uninfected erythrocytes changes blood–brain barrier function **[31-34]**.

Infected Red Blood Cell Membrane Rigidity, Deformability and Involvement in Pathogenesis of Severe Complicated Malaria

Plasmodium invasion of RBC induces major chemical, morphological and structural cellular changes. Altered red cell membrane rigidity and deformability contribute to the pathogenesis of severe malaria. In patients with severe *falciparum* malaria, the entire red cell mass, comprising mostly of un-parasitized and parasitized, becomes rigid **[35]**. Several mechanisms have been suggested to explain how the changes come

about and eventual role in pathogenesis of severe malaria. The suggestions include mechanisms like hemin-induced oxidative damage of the red cell membrane, alterations in the phospholipid bilayer of RBC membrane and attached spectrin network by the proteins transported to the red cell membrane. Others are thermally driven membrane fluctuations due to fever, and inhibition of the Na+/K+ pump on the red cell membrane, possibly by nitric oxide (NO) may be responsible for the increase in rigidity and reduction in deformability of the red cells in *falciparum* malaria **[36, 37]**.

Reduced red cell deformability of infected RBCs leads to increased splenic clearance and loss of red cells, causing anemia, a common clinical feature in severe malaria. In addition hemolysis, suppression of erythropoeisis by cytokines, and hemozoin-induced apoptosis in developing erythroid cells also contribute to the development of anemia in severe malaria **[8, 36]**. Compared to infection with *P. falciparum*, in which red cell deformability is reduced, the red cell deformability is increased in *P. vivax* infection. While this may enable *P. vivax* infected red cells to survive the passage through the splenic sinusoids, the accompanying increase in fragility of both infected and non-infected red cells may contribute to severe anemia in *P. vivax* malaria. Increased deformability of *P. vivax* infected red cells also makes sequestration and obstruction to blood flow unlikely **[27, 38]**. This is the likely explanation of why *P.vivax* is not associated with human cerebral malaria.

Comparative studies in adults and children indicated a strong association in outcome between low red-cell deformability and severe disease in adults **[39, 40]** without tissue necrosis. However, there are reports of critical reduction in the supply of metabolic substrates to the brain and this may be exacerbated by increased demand during seizures and fever, and may be worse in patients with severe anemia or hypoglycaemia **[41]**. Cerebral blood flow may also be reduced by high intracranial pressure while inflammatory cytokines can result in inefficient use of substrates in malaria patients **[34]**.

Pro-Inflammatory Cytokines Involvement in the Pathogenesis of Severe Complicated Malaria

Pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), IL-6 and IL-18 which become raised in malaria infection, have been implicated in the pathogenesis of severe malaria, while anti-inflammatory T – helper 2 (Th2) cytokines (IL-4, IL-10), have been proposed to have a protective or counter-regulatory role. TNF is also raised in placental malaria

and is associated with low birth weight **[42, 43]**.

Malaria infection can cause generalized vital organ dysfunction as a result of the release of systemic cytokines such as TNF ά, or local release of mediators such as nitric oxide (NO). Initially TNF, which plays a pivotal role, IL-1, and IFN γ are produced and these in turn induce the release of cascade of other pro-inflammatory cytokines including IL-8, IL-12 and IL-18. These are balanced by production of the anti-inflammatory cytokines IL-6 and IL-10. IFN- γ has been associated with both pathogenesis and protection against human disease **[44, 45]**. Cytokines are responsible for many of the symptoms and signs of the infection, particularly fever and malaise. Whereas high concentrations of cytokines may appear to be harmful, lower levels may probably benefit man **[46]**.

Potent stimulators that induces pro-inflammatory cytokine production by leucocytes are the glycosylphosphatidylinositol (GPI) anchors of *P. falciparum* GPI which stimulates the production of TNF-α and possibly also the lymphokine 'lymphotoxin'. Both cytokines can up-regulate the expression of ICAM-1 and VCAM-1 on endothelium cells, and could thus promote sequestration of parasitized erythrocytes in the brain, contributing to coma and unconsciousness. High plasma concentrations of TNF-α in patients with f*alciparum*-malaria correlate with disease severity, including coma, hypoglycemia, hyperparasitemia and death **[47]**.

The severity of infection with malaria depends heavily on the levels of IFN- and TNF- . As long as the immune system is capable of properly regulating the production of pro-inflammatory cytokines at a moderate level while avoiding severe pathology, then clinical immunity is achieved. Studies indicate that certain antibodies to malarial GPI block TNF-generation from macrophages in order to regulate inflammatory responses. On the other hand, it is hypothesized that pro-inflammatory (Th1/ IFN-) response would switch to an anti-inflammatory response via cytokines TGF-or IL-10 **[47]**.

Therefore, the role of TGF-, and IL-10, response is critical and their timing and levels determine the disease outcome in terms severity. High levels achieved too early in malaria infection compromises cell-mediated effector mechanisms while low levels that appear late during infection would risk failure in regulating inflammatory cytokine response **[46]**.

There is strong evidence emphasizing the importance of cytokine stability and balance in order to fight malaria infections without causing severe damage to the human host.

Post-mortem performed on the brain tissue of patients who die from cerebral malaria report localized build up of TNF- , IFN-, and IL-1 possibly related to parasite-infected erythrocytes, lymphocytes, and monocytes. Adults patients diagnosed with severe malaria suggest a link between organ failure and increased plasma concentration of inflammatory cytokines while those who died showed lower plasma concentration levels. In a study involving the correlation of pro-inflammatory and anti-inflammatory cytokine responses, it is reported that elevated ratio's of IFN-, IL-12 or TNF- , to TGF- reduce the risk of severe malaria infection **[42]**. Overall, it is accepted that anti-inflammatory cytokines are essential in controlling the concentration of pro-inflammatory cytokines in order to avoid the detrimental effects of high concentrations in malaria infection. Equilibrium must therefore be achieved between the pro-inflammatory cytokines working to contain the parasite and inhibit its growth and development, and the anti-inflammatory cytokines working to control disease manifestation **[42]**.

Furthermore, studies indicate that concentrations of IL 6 (pro-inflammatory) were higher in patients with cerebral malaria than in those with non-cerebral malaria—but there was no increase in IL 1, IL 8, IL 12, or TNF **[48]**, while in a related study, the concentrations of TNF and its receptor were higher in those with cerebral malaria than in those with mild or uncomplicated malaria **[49]**. Similarly, in Vietnamese adults, concentrations of IL 6, IL 10, and TNF were high in patients with severe multi-organ disease but low in patients with cerebral malaria alone, suggesting their involvement in the process leading to severe malaria **[44]**. In addition, post-mortem analysis of Malawian children with cerebral malaria suggests increased local production of TNF and IL 1. However, there is no evidence of association between production or staining for these cytokines and sites of parasite sequestration.

Nitric oxide (NO) Involvement in the Pathogenesis of Severe Complicated Malaria

Several studies support an association between protection from severe malaria and nitric oxide (NO) production, measured indirectly by examining NO metabolites, NO synthase 2 (NOS2) expression in peripheral blood monocytes and NOS2 promoter polymorphisms. Nitric oxide has been suggested as possible cause of coma in patients with severe malaria because of its ability to interfere with neurotransmission. NO synthesis requires extracellular arginine, and recent studies report an association between hypo-argininemia and severe malaria and death in children **[27]**. This

might be consistent with the protective role of NO against severe malaria, or hypo-argeninemia might have resulted from activation of inducible nitric oxide synthetase (iNOS) in severe malaria **[27]**. Furthermore, cerebral tissue postmortem analysis report increased iNOS expression and markers of NO production in severe malaria **[6]**. This suggests that an acute induction of iNOS expression occurs in the brain during cerebral malaria and this is known to occur in a number of different cell types. As NO may activate a number of secondary neuro-pathological mechanisms in the brain including modulators of synaptic function, induction of iNOS expression in cerebral malaria may contribute to coma, seizures and death **[6]**.

Nitric oxide has been implicated in the pathogenesis of severe sepsis, and could alternatively play a role in the pathogenesis of severe malaria disease **[50]**. However, when low arginine limits NO production, it also favors the generation of oxidants that might contribute to pathogenesis in severe malaria infection **[51-54]**. The administration of systemic arginine might therefore be a form of beneficial adjunctive therapy in the management of severe complicated malaria, or have a role in preventing severe complicated malaria **[6]**. Post-mortem staining of brain specimens in African children and Southeast Asian adults indicate increased iNOS in blood vessel walls associated with sequestered parasites in cerebral malaria **[54]** whereas in other studies, nitric oxide is associated with protection **[27]**.

The beneficial and harmful effects of NO and TNF in malaria cases depends on the up-regulation and down-regulation of their plasma levels. The up-regulation of iNOS by TNF may set off a negative feedback mechanism through NO to control the stimulatory action on iNOS. However, in some individuals, production of NO happens too slowly to down-regulate the primary wave of TNF induction, such that a slow build up of iNOS-induced NO allows iNOS and NO to reach the harmful concentrations seen in cerebral malaria patients **[55]**.

CONCLUSION

Malaria has been and continues to be a major public health problem in many parts of the world. The disease clinically recognized in two main forms, uncomplicated and deadly severe malaria, predominantly caused by *Plasmodium falciparum*. Mechanisms involved in the evolution of severe complicated human malaria include but not limited to a combination of *Plasmodium*-parasite-specific factors and human-host inflammatory responses. Severe complicated malaria is a complex multi-

systemic disorder presenting with a range of clinical features that vary from one individual to another. If not adequately understood, diagnosed and promptly and appropriately treated, severe malaria rapidly leads to death. We have reviewed possible processes involved and provided basis for further research to elucidate any other mechanisms that may be involved in this deadly form of malaria.

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