CEREBRAL MALARIA. I. THE ROLE OF ADHESION MOLECULES IN ITS PATHOGENESIS

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ABSTRACT

Cerebral malaria (CM), a complication in Plasmodium falciparum infection, causes high mortality among children in malaria endemic areas, with coma as major clinical symptom. Conventional explanation for the pathogenesis of the coma in CM is obstruction of small blood vessels in the brain by parasitized red blood cells which are sequestered there, that cause inadequate blood supply causing hypoxia and ischemia. Sequestration of parasitized rbc is started with the adherence of p-rbc to the endothelial cells lining blood vessels called cytoadherence, a specific ligand-receptor process involving adhesion molecules expressed on the surface of vascular endothels (i.e. CD 36, thrombospondin, ICAM-1, VCAM-1, E-selectin, Chondroitin-4-sulfate) and molecules on the surface of p-rbc which are related to the infecting parasites (e.g., Pf-EMP-1, Pf-HRP-1), followed or enhanced by rosette formation of normal rbc around p-rbc. Rosetting could involve similar p-rbc molecules involved in cytoadherence and facilitated by several serum proteins. The expression of adhesion molecules on the surface of endothelial cells is stimulated by parasite antigens and up-regulated by cytokines especially TNF-α (and IFN-γ). Efforts have been done to search antibody and medicine that could prevent cytoadherence and/or disrupt rosetting, and to control the production and effects of TNF-α.

***Keywords**: cerebral malaria,* Plasmodium falciparum*, adhesion molecules, sequestration.*

INTRODUCTION

Cerebral malaria (CM) is the most severe complication of *Plasmodium falciparum* infection and one of the major causes of mortality among children in malaria endemic areas. Major clinical symptom of CM is disturbance of consciousness, from mild form to deep, unrousable coma. Conventional explanation for the pathogenesis of human cerebral malaria is deprivation of adequate blood supply (that could end in ischemia or hypoxia) due to obstruction of blood vessels by parasitized red blood cells which are sequestered there (Nauck, 1967; Warrell, 1990). But Clark (1991 and 1999) doubted it considering the minimal sequels found when the patients recovered. From histopathological view, most researchers reported that sequestration of infected erythrocytes in the brain blood vessels is a constant feature in CM (Berendt, 1990; Hommel, 1993; Oh, *et al.,* 1997), but in Vietnam, hemorrhages were found more prominent than sequestration (Eling and Kremsner, 1994).

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The different findings and opinions raise questions concerning the real pathogenesis of CM. While no animal model fits properly to conditions in human cases because, for instance in mice, the most frequent animal model studied, sequestration involves leucocytes and platelets instead of infected red blood cells (Warrell, *et al.,* 1990).

This study reviews literature to explore factors playing role in the pathogenesis of human CM that will be written in two consecutive papers.

Sequestration of parasitized red blood cells (p-rbc) in microvasculatures of the brain.

It has been seen and reported more than a century ago by Bastianeli and Bignami, that in *falciparum* malaria only erythrocytes bearing young trophozoites (ring forms) and gametocytes can be found in blood smears, while erythrocytes infected with late stages (older trophozoites and schizonts) disappear from peripheral blood. Later it was found that the late stages are sequestered within the blood vessels in the deep organs. After schizogony, merozoites enter new rbc and spread again into systemic circulation (cited in Howard, 1988). Only *Plasmodium falciparum* are sequestered, and it is correlated with symptoms of severe malaria (Manson-Bahr and Bell, 1987; Clark, 1999).

It was presumed before that sequestration of p-rbc (parasitized red blood cells) is caused by mechanical trapping of p-rbc which are distended and lose their elasticity (so called deformability) and cannot slip

through small capillaries. But later on, other mechanisms have been reported to play role in CM as will be reviewed on the next paragraph.

According to the latter opinion, two major processes contribute to sequestration of p-rbc, i.e. cytoadherence, that means adherence of malaria infected erythrocytes and other cells to the endothelium lining blood vessels, and rosette formation or rosetting, that means the attachment of several normal erythrocytes to/around one or more infected erythrocytes, which could have been adhered to endothelial cells (Berendt, *et al.,*1992; Ockenhouse, *et al*.,1991; Wahlgren, *et al.,* 1994). Together with recruitment and adherence of other cells like monocytes, neutrophils, NK cells and platelets, they may form a large cell-complex that could occlude small vessels. Complete occlusion can cause petechien and ring hemorrhages.

The reduced local blood flow can lead to functional impairment of the organs. Accumulation of cells which brings them in close contact, together with the release of parasite's antigen, will activate and stimulate the cells to release all kinds of substances like cytokines, eicosanoids, reactive oxygen intermediates and reactive nitrogen intermediates (Eling and Kremsner, 1994). These substances can act locally and cause local tissue damage and more functional impairments, or spread systemically in the blood circulation.

Sequestration involves the role of adhesion molecules

Biopsy on patients died from CM shows that the vessels where the parasitized-erythrocytes (p-rbc) are sequestered, are mostly postcapillary venules, not the vessels with the smallest diameter.

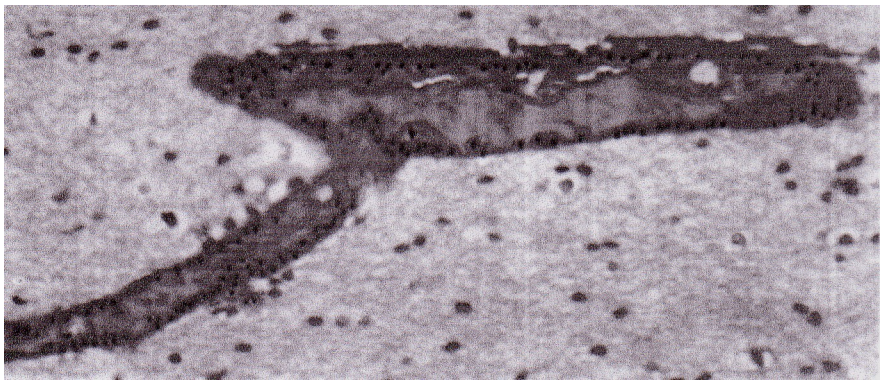


Figure 1. A photograph of a brain slice from a *Plasmodium falciparum* infected case showing attachment of parasitized red blood cells to the wall of blood vessels. The figure demonstrates that sequestration is not merely caused by trapping of distended p-rbc. Photograph is copied from Zaman, 1984.

It looks alike that mechanical trapping and sludging (Warrell, *et al*., 1990) are not the only causes of the phenomenon. This was also confirmed by the fact that the percentage of p-rbc in the sequester is higher than in the systemic circulation (Gilles and Warrell, 1993). Photograph made by Viqar Zaman (1984) cited in Figure 1 shows clearly that p-rbc attach to the endothelial surface not only in the small vessels but also in broader ones.

Those evidence brings the ideas that sequestration is started by an active process that later was recognized as cytoadherence. Cytoadherence represents a specific ligand-receptor binding that involves adhesion molecules on the surface of endothelial cells lining the vasculature and their counter-receptor on p-rbc. The idea concerning the role of adhesion molecules in CM began when MacPherson in 1985 reported the presence of "endothelial pseudopodia" from the brain of severe falciparum malaria deaths (cited in Clark, 1987). Clark proposed that the presence of malaria antigen makes the adjacent cells more "sensitive" including endothelial cells, which express adhesion molecules on their surface and be ready for the next stimulus, i.e. upregulating actions of cytokines.

Adhesion molecules involved in cytoadherence in malaria are ((Chulay and Ockenhouse, 1990; Berendt, *et al*., 1992; Ockenhouse, *et al*., 1991 and 1992; Esslinger, *et al.,* 1994; Graninger, *et al*., 1994; Maubert, *et al.,* 1997):

1. CD36 = platelet glycoprotein IIIb or IV, expressed on the surface of endothelial cells, monocyte-macrophage or platelets.
2. Thrombospondin (TSP), the secreted glycoprotein from platelets, endothelial cells and melanoma cells; it mediates cell-cell and cell-matrix interaction.
3. ICAM-1 (intercellular adhesion molecule-1) or CD54, expressed on the surface of vascular endothels, lymphocytes, monocyte-macrophage, and some tumor cell lines.
4. VCAM-1 (vascular adhesion molecule-1),
5. E-selectin or ELAM-1 (endothelial leukocyte adhesion molecule-1)
6. (Chondroitin-4-sulfate, special for syncytiotrophoblast in human placenta.

Most of those molecules showed good adhesion to the erythrocytes infected with mature trophozoites and schizonts but not to uninfected and ring-infected ones (Chulay and Ockenhouse, 1990). There were evidence of systemic endothelial activation in acute and severe malaria, and increased plasma levels of soluble ICAM-1, sVCAM-1 and sE-selectin was confirmed that could be correlated with disease severity (Turner, 1998 and 2004). Soluble adhesion molecules also increase in Plasmodium vivax patients (Jacobsen, 1994). Sections of brain tissue from patients died from CM showed expression of multiple adhesion molecules (Ockenhouse, *et al.,* 1992). Those adhesion molecules also have important role in the recruitment of leucocytes to the tissue, which involves CD18/CD11a and CD18/CD11b as counter-receptor on the leucocytes (Ockenhouse, 1992).

It was noted that there was morphological alteration on the surface of sequestered p-rbc at the focal junction of the attachment to the endothelial surface This was seen as protuberances consist of electron-dense materials measuring around 100 nm, namely "knobs" (Howard, 1988; Aikawa, *et al.,* 1988) and supposed to be parasite products exported to the host cell membrane, or part of host cell membrane that was altered by the parasite.. The molecules were then identified as PfEMP1 (Plasmodium falciparum-infected erythrocytes membrane protein 1), PfHRP2 (Plasmodium falciparum histidine-rich protein 2), KAHRP (knob-associated HRP), RESA/Pf155, glutamate-rich protein, or band 3 protein (pfalhesin) (Howard, 1988; Magowan, *et al.,* 1988; Berendt, 1992; Schlichtherle, *et al.,* 1996; Oh, *et al.,* 1997; Saul, 1999*).* These molecules were also suspected to be implicated in rosetting (Wahlgren, *et al.,* 1998).

Non-sequestering malaria parasites or parasites having no knobs (K-) do not have association with severe malaria (Berendt, 1992). Parasites isolated from CM patients have the highest binding capacity to ICAM-1 (Newbold, *et al.,* 1997). Organ predilection, i.e. in which organ sequestration will proceed, is affected by both different expression of adhesion molecules and specific tissue/organ tropism of parasite sub-populations (Berendt 1992, Grau, *et al.,* 1990).

From the parasite point of view, sequestration is beneficial in the following way:
1. By sequestration in other organs, they can evade from the clearance effect of the spleen.
2. By sequestration in the postcapillary venules, they obtain low oxygen tension they need as microaerophylic organism.
3. It is easier to meet new rbc host in the crowd (for the next generation).

Rosetting is commonly seen in malaria, and rosetting ability of isolates was associated with severity of malaria disease (Ringwald, *et al.,* 1993), and giant rosettes consisting of 10-20 i-rbc and 30-50 N-rbc, were found especially in CM. Binding to endothelial cells which expressed adhesion molecules, up-regulate rosette formation. Only erythrocytes infected with older parasite stages are able to form rosettes. Some erythrocyte surface molecules were suspected as rosetting ligands or/and receptors, i.e., Pf-EMP-1 and some other molecules like rosettin, Pf-HRP-1, heparan sulphate, CD35/CR1, CD36 (Wahlgren,1998).

Some proteins in the serum are suspected to support rosette formation, i.e., blood group A, AB and B, and the presence of medium such as (non specific-) IgM, IgG, fibrinogen, albumin and may be vW Factor and thrombospondin. Abnormal erythrocytes like thalassemia and sickle cells, form smaller rosettes than the normal ones (Wahlgren, 1994 and 1998). Rosette formation is suspected to give similar benefits to the parasites that the parasites in the middle of big rosettes have no direct contact with host immune response while they are easier to get new host cells (Wahlgren, 1998).

The role of cytokines in sequestration

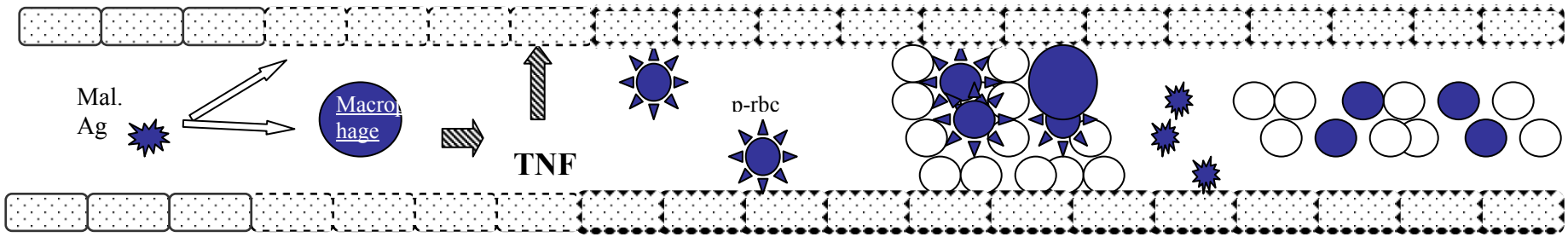
Schizont burst at the end of erythrocytic cycles stimulate phagocytic cells to produce TNF, IL-1, IL-6 and other mediators. The response evokes symptoms typical for malaria, i.e. periodic fever attacks preceded by chills and rigors (Kwiatkowski, et al, 1989). It was realized that TNF and other mediators also play role in sequestration of p-rbc. Ringwald, *et al*. (1993) noted that cytoadherence capacity was increased on stimulation with TNF-a but not increased by IL-6, IL-3 and GM-CSF. IL-3 enhances the effects of TNF-a. On the other hand rosetting was not stimulated by the

presence of cytokines. TNF-a and IL-1 induce and upregulate the expression of vascular adhesion molecules like ICAM-1, VCAM-1 and ELAM-1 (Berendt, 1992; Ockenhouse, *et al*., 1992; Ringwald, *et al*., 1993). TNF and IL-1 change the anticoagulant activity on the endothelial surface to a pro-coagulant activity and increase adhesiveness for PMN, monocytes and lymphocytes (Grau, *et al*., 1989b). IFN-γ also has the ability to upregulate ICAM-1 expression (Grau, *et al.,* 1989a). Lou, *et al*., (1997) found platelet sequestration and fusion to endothelial cells lining microvessels of the brain and lung of malaria-infected animals, which was inducable by TNF and IFN-γ. The presence of TNF and IFN-γ and its local production in the brain tissue has been reported (Grau, *et al*., 1990; Medana, 1997; Udomsangpetch, 1997). More about the role of TNF and other cytokines in CM will be reviewed in the next paper.

Efforts in prevention and treatment of CM in relation with adhesion molecules

There were efforts to analyze and to use antibody against molecules involved in cytoadherence and rosetting (Wahlgren, *et al*., 1994; 1998). Some successes have been achieved but many problems have to be overcome. Analysis over Pf-EMP-1, the molecule that has been extensively studied and recognized as parasite virulence factor, revealed that the parasite creates new antigen variants from time to time to evade the immune response and to keep their cytoadherence capacity (Howard, 1988) and there are immense number of variants exist (Sutherland, 1998; Saul, 1999).

Early time use of Heparin to disrupt rosetting in CM patients was discontinued because of lethal hemorrhage outcomes (cited in Wahlgren, *et al.*, 1994 and 1998). As TNF is needed for expression of sufficient number of adhesion molecules, there have been trials in taming TNF by controlling its production and using antibodies against TNF-inducing molecules. Hoffman launched his opinion that Chloroquine could be used for controlling TNF excessive production in severe malaria. But there was still controversy over clinical use of Chloroquine and some other anti-malaria drugs for that purpose (Hoffman, 1984; Picot; 1991; Kwiatkowski and Bate, 1995; Sri-Hidajati, 2002). While Playfair, Bate and Taverne pioneered the research towards production of vaccine against TNF-inducing parasite molecules (Bate, *et al*., 1990; Taverne, *et al.,* 1990).



1. Early infection : Schizont burst Release of merozoites and malaria antigens	2. Endothels are sensitized : expression of low number of adhesion molecules. Stimulation of macro-phage to produce & release of TNF-α, (amplified by IFN-γ)	3. TNF-α up-regulate expression of adhesion molecules to sufficient number	4. Cytoadherence of parasitized red blood cells to endothelial cells through special molecule on the membrane → sequestration.	5.Sequestration is enhanced by rosette formation around the adhered p-rbc → occlusion of blood vessels	6.Schizont burst → invasion of new rbc → ring-infected rbc circulate in systemic circulation (Summary and figure by Sri-Hidajati, 2003)

Figure 2 Schematic diagram to illustrate the role of adhesion molecules in sequestration of parasitized red blood cells and occlusion of blood vessel in cerebral malaria.

CONCLUSION

From the literature study above a conclusion can be formulated that sequestration of p-rbc causing obstruction of blood vessels is consistently evident in CM. Sequestration starts with cytoadherence, the adherence of p-rbc to the endothelial cells lining blood vessels which is a specific ligand-receptor process involving adhesion molecules expressed on the surface of vascular endothelial cells (i.e. CD 36, thrombospondin, ICAM-1, VCAM-1, E-selectin, Chondroitin-4-sulfate) and molecules on the surface of p-rbc which are parasite products or a part of host cell altered by the parasites (e.g., Pf-EMP-1, Pf-HRP-1). Sequestration could be enhanced by rosette formation, i.e. the attachment of several normal rbc to one or more p-rbc which could have been adhered to the endothelia. Rosetting could involve similar p-rbc molecules involved in cytoadherence and facilitated by several serum proteins.

The expression of adhesion molecules on the surface of endothelial cells is stimulated by parasite antigens and upregulated by cytokines especially TNF-α (and IFN-γ), mediator produced by macrophages (and T cells) upon stimulation with parasite antigen. By studying parasite life cycle correlated with TNF production stimulation and the mechanism of cytoadherence and rosetting, it is easier to understand different pathological findings.Efforts are being done to search antibody and medicine that could prevent cytoadherence and/or disrupt rosetting, and to control production and the effects of TNF-α. From the factors studied above, a pictorial scheme can be designed to summarize the sequence of events leading to sequestration of p-rbc and occlusion of blood vessels in CM as shown in figure 1.

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