

Platelet-endothelial cell interactions in cerebral malaria: The end of a cordial understanding

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Summary

Cerebral malaria is an acute encephalopathy evolving from an infection with *Plasmodium falciparum* which kills more than one million people each year. Brain tissues from patients who died with cerebral malaria revealed multifocal capillary obstruction by parasitised red blood cells, platelets, and leukocytes. Many studies are unified in their proposal of two major hypotheses consisting of cell adhesion to the brain endothelium and excessive immune stimulation resulting in further vascular inflammation, prothrombotic cell activation, mechanical obstruction of cerebral capillaries and, consequently, blood-brain barrier disruption. Platelets and endothelial cells communicate on multiple

levels. Infection-induced changes in platelets and endothelial cells occur in cerebral malaria, resulting in their concomitant activation, increased interactions between these two cell types, and a secondary procoagulant or hypercoagulable state. Here we review evidence for these mechanisms and highlight the possible role of platelets as effectors of endothelial damage in cerebral malaria. A better understanding of the complex regulation of these various interactions between brain endothelial cells and platelets in the context of cerebral malaria may prove useful in the development of new approaches to the treatment of this disease.

Keywords

Endothelial cells, platelet immunology, infectious diseases, microparticles

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Introduction

Malaria is a leading cause of illness worldwide causing approximately 250 million clinical attacks annually and almost 1 million deaths in sub-Saharan Africa, most commonly in children under 5 years of age (1). Human malaria infections are caused by four *Plasmodium* parasites, of which *P. falciparum* induces the most severe complications including anaemia, respiratory distress, renal failure, acidosis and cerebral malaria (CM) (2). Cerebral malaria has a high mortality rate of about 20% despite active chemotherapy and support (3). Clinically, CM is defined as a diffuse encephalopathy causing unrousable coma often associated with seizures in the presence of asexual forms of *P. falciparum* in peripheral blood, in the absence of other causes of unconsciousness such as coexistent infections of the central nervous system (2). In many patients, this coma reverses rapidly but neurological sequelae persist in more than 10% of survivors (4).

Although extensively studied, CM pathogenesis remains incompletely understood (see Fig. 1). CM results from a combination of vascular and immune system dysfunctions. During the intra-erythrocytic phase of the *P. falciparum* cycle, mature stages of parasitised red blood cells (PRBC) adhere to the deep microvascular beds of the brain and other organs and disappear from the peripheral circulation (5). This phenomenon, called sequestration, is quantitatively linked to the incidence of coma during CM (6) and supports the mechanical hypothesis in which obstruction of brain microvessels leads to decreased blood flow and hypoxia (7, 8). Nevertheless, the presence of sequestered parasites in the brain microvasculature of asymptomatic infected people (9), the poor correlation between parasitaemia and mortality, the lack of evidence for irreversible hypoxic damage in neurones in fatal CM (10) and the relatively low prevalence of neurological sequelae in survivors suggest that, although sequestration is necessary for the development of CM, other factors

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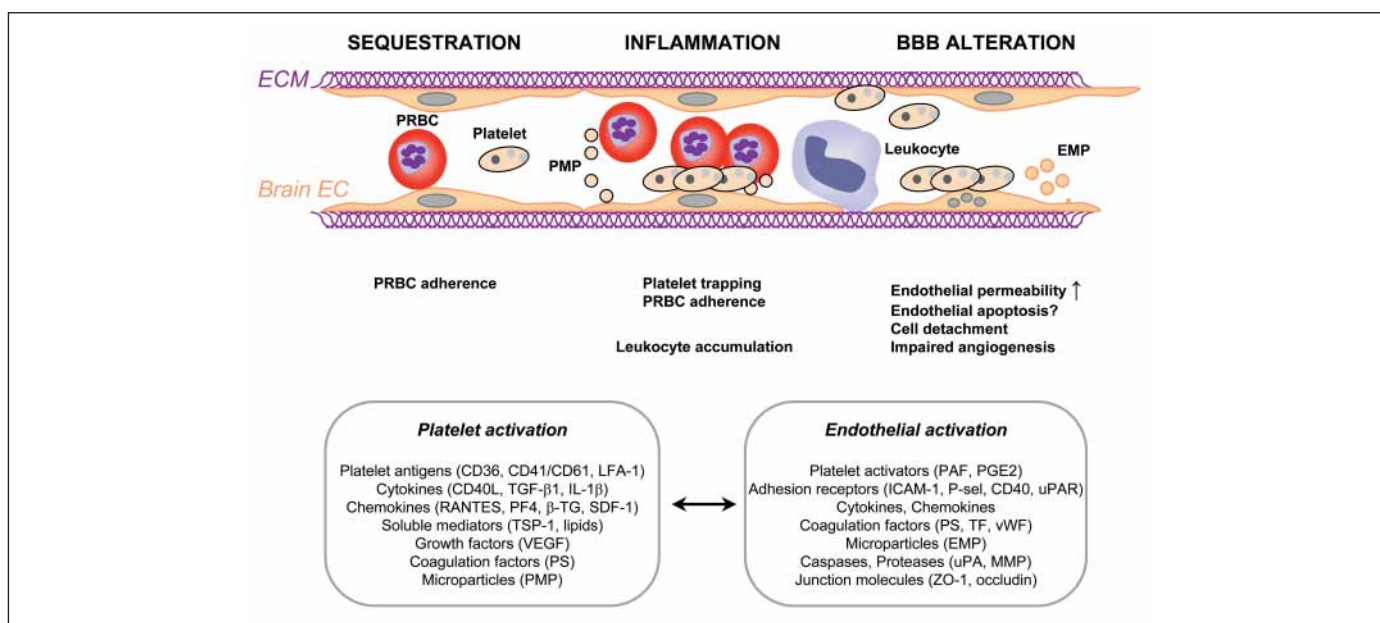


Figure 1: Schematic representation of the involvement of platelets and brain endothelium in the pathogenesis of CM. During the initial phase of CM, elevated levels of cytokines promote widespread endothelial activation leading to an increased binding of circulating cells including platelets and PRBC. Adherent platelets then enhance PRBC binding to EC bringing new adhesion molecules between the two cell types and inducing further endothelial activation. Activated endothelium, in turn, releases potential platelet activating mediators. Also, platelets are capable of potentiating PRBC-induced alterations of the endothelial monolayer integrity, notably by promoting matrix degradation and apoptosis and impairing vascular repair. The two text boxes summarise the mediators produced by either EC or platelets upon activation that are able to potentiate the sequence of events observed during CM, namely

sequestration, inflammation and BBB alteration. Abbreviations: β -TG, β -thromboglobulin; CD40L, CD40-ligand; CM, cerebral malaria; EC, endothelial cells; ECM, extra-cellular matrix; EMP, endothelial-derived microparticles; BBB, blood-brain barrier; ICAM-1, intercellular cell adhesion molecule 1; IL-1 β , interleukin-1 β ; LFA-1, lymphocyte function-associated antigen-1; MMP, matrix metalloproteinases; PF4, platelet factor 4; PMP, platelet-derived microparticles; PRBC, parasitised red blood cells; PS, phosphatidylserine; P-sel, P-selectin; RANTES, regulated on activation T-cell expressed and secreted; SDF-1, stromal cell-derived factor-1; TF, tissue factor; TGF- β 1, transforming growth factor- β 1; TSP-1, thrombospondin; uPAR, urokinase plasminogen activator receptor; VEGF, vascular endothelial growth factor; VWF, Von Willebrand factor; ZO-1, zona occludens-1.

must be involved at the onset of the disease as well as during its advanced stages.

Besides the mechanical hypothesis, the “cytokine” or “immune” theory suggests that, in response to the presence of PRBC and malaria toxins, an overproduction of inflammatory cytokines and of soluble mediators by host cells, notably tumor necrosis factor (TNF) and interferon-gamma, and lymphotoxin, is responsible for the development of the neurological syndrome, alongside sequestration (11). Most likely, a combination of the two theories is operational (12). More recently, a role for haemostatic dysfunction in CM pathogenesis has been proposed (13–15). Indeed a profound thrombocytopenia and the activation of both coagulation cascade and the fibrinolytic system are common findings in malaria and correlate with parasitaemia and disease severity (16–18). Histopathologically, petechial and ring haemorrhages are often observed in the brain parenchyma of patients who died from CM (19, 20). Pathogenesis of malaria-induced thrombocytopenia is complex and might implicate antibody-mediated platelet destruction, coagulopathy and adhesion to the activated vascular endothelium (21–23). Besides being a target during the disease, platelets are thought to play a major role in the immune response to *Plasmodium* (24). Thus, a unifying hypothesis suggests the combination of sequestration, inflammation and haemostasis for the genesis of CM (13).

The blood-brain barrier (BBB), especially the endothelium, is the key interface between the brain parenchyma and the parasite. Not only does it mediate parasite cytoadhesion through the expression of surface adhesion receptors (25) but it also regulates solute and cellular transport into the brain. Brain endothelium phenotype and function are thus critical to understand how the parasite, which remains within the cerebral microvasculature, can cause neurological disturbances. Intact, non-activated endothelium normally prevents platelet aggregation and adhesion to the vascular wall. The presence of platelets trapped in the microvasculature of mice (26) and patients with CM (27) raised the hypothesis of a role for platelets in the initiation of endothelial activation, parasite sequestration and neurovascular lesions. Platelets have well-defined roles in haemostasis but are also important in promoting inflammation and regulating immune responses. Indeed, platelets express surface markers and secrete molecules that contribute to vascular inflammation and are in turn activated by substances released from cells of the vascular wall, notably endothelial cells (EC) (28).

While both endothelial and platelet activations occur during malaria, the question remains which one of these two processes is critical for the genesis CM. Here we review the latest data from clinical, animal and in-vitro studies to highlight the dual roles played by both EC and platelets in the development of CM.

Brain endothelium and platelets in the triggering of CM: roles in parasite sequestration

The virulence of *P. falciparum* is thought to be due to its ability to adhere to EC lining small blood vessels. PRBC adherence to EC is mediated by electron-dense structures called knobs on the PRBC surface (29), which contain the main parasite ligand *P. falciparum* erythrocyte membrane protein 1 (*PfEMP-1*). *PfEMP-1* belongs to a large family of clonally variable antigens encoded by *var* genes. Its variants expressed by different *P. falciparum* clones have different binding affinities for various receptors that can be expressed by the endothelium, including glycoprotein IV (GPIV, CD36), E-selectin (CD62-E), intercellular cell adhesion molecule-1 (ICAM-1, CD54), vascular cell adhesion molecule-1 (VCAM-1, CD106) (30), thrombospondin-1 (TSP-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31) (31) or chondroitin sulfate A (32). CD36 and TSP-1 are used by all parasite isolates, whereas ICAM-1 or VCAM-1 are used by a subset of field and laboratory isolates. Interestingly, ICAM-1 binding is more likely to be associated with the development of CM (25). Both parasite binding phenotype and the expression of receptors in the critical vascular beds are thus crucial in influencing the site of sequestration and the pattern of disease caused by *P. falciparum*.

Basal endothelial phenotype

Each vascular bed has unique structural and functional properties (33). Postcapillary venules are the predilection site for PRBC sequestration (34), and also for leukocyte trafficking and platelet rolling (35). This is promoted by a low flow rate and the expression of adhesion receptors such as P-selectin on the endothelial surface (36). However, the expression of these receptors is not restricted to EC, nor to the brain, which makes it difficult to determine the specific role of the endothelial compartment and to determine whether they play a preferential role in brain circulation. For example, ICAM-1 is widely expressed in vascular beds of brain, liver, spleen, kidney and lung. In contrast, CD36 does not appear to be prominent in cerebral microvessels, but is expressed in other organs such as kidney, liver, spleen, lung and muscle. Basal levels of expression of TSP-1 and VCAM-1 are very weak on brain endothelium and E-selectin is absent in virtually all vascular beds (37). Because most parasite isolates adhere to CD36 and ICAM-1, ICAM-1 has been implicated as the primary adhesion molecule in mediating sequestration in the brain microvessels where CD36 is absent. In this context, the murine model of CM due to *Plasmodium berghei* ANKA (*PbA*) infection with mice deficient for those receptors has been helpful to identify critical receptors for the development of CM. For example, in mice, ICAM-1 deficiency confers full protection against CM which correlates with a decrease of PRBC trapping and a less severe thrombocytopenia (38). However, little is known on the specific role of endothelial-derived ICAM-1 in this protection. Extrapolating these results to humans is even more delicate than no association between the variation in the ICAM-1 gene in humans and CM phenotype could be observed (39). Chimeric mice deficient only in endothelial P-selectin do not show any sign of CM compared to mice lacking only platelet P-selectin or wild-type mice (40). Interestingly, whilst leukocyte

adhesion is not markedly altered in the absence of ICAM-1 (41) or P-selectin (40, 42), platelet rolling and adhesion in brain venules are reduced in *PbA*-infected mice lacking one of these receptors (43). Indeed, platelet rolling is independent of platelet activation but requires endothelial P-selectin and constitutive expression of P-selectin ligands on platelets (35).

In the same way, mice deficient for urokinase plasminogen activator (uPA) and its receptor (uPAR), a critical receptor for platelet trapping and kinetics (44), are partially but significantly protected against CM (45). In addition, in-vivo treatment with a monoclonal antibody to the $\beta 2$ integrin lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18), which is present on platelets, selectively abrogates the cerebral sequestration of platelets and protects mice from CM (26). Blockade of platelet glycoprotein (GP)IIb (CD41) or GPIb (CD42) also protects mice from CM and markedly alters cytokine production during infection with *PbA* (46, 47). All these findings converge to elucidate the pivotal role of platelet functions and interactions with inflamed endothelium in CM development.

Modulation of the endothelial adhesion phenotype

Acquisition of new adherence molecules via platelet binding

Governed by disturbed flow, platelets adhere to the vessel wall *in vivo*, even in the absence of EC denudation, thereby initiating lesion formation (48). Once they have adhered to the endothelium, platelets can either act as a bridge or sometimes fuse with the endothelial membrane, leading to the transfer of platelet antigens onto the endothelial surface, rendering it more adhesive for other circulating cells such as PRBC or leukocytes (49). Platelet fusion occurs at a low rate under physiological conditions but exhibits some obvious different features in pathological conditions. Remarkably, platelet adhesion and fusion are found in diseased organs or tissues but not in others (26). One consequence of this interaction is the acquisition by EC of platelet adherence molecules. While the vast majority of field isolates of *P. falciparum* adhere to CD36 (50), CD36 is not expressed by the capillary endothelium of the brain (37, 51). *In vitro*, PRBC can bind to CD36 or gC1qR/HABP1/p32 on platelets to form clumps (52, 53). Hence, platelets can act as a bridge between CD36-binding PRBC and CD36-deficient EC but, conversely, can hide constitutively *P. falciparum* receptors such as chondroitin sulfate A expressed on EC. Thus, platelets may provide an adhesion receptor to microvascular beds originally devoid of it and reorient the sequestration of different parasite phenotypes (54). However, platelet sequestration alone is not sufficient for CM to occur and platelet activation might be required for subsequent sequelae (40). CM is associated with an increased activation and apoptosis of platelets and EC. During activation or apoptosis, cells release small membrane fragments known as microparticles (MP). MP are characterised by the increased exposure on the external membrane leaflet of anionic phospholipids, such as phosphatidylserine, and by the presence of proteins, including functional adhesive receptors, and also bioactive lipids, derived from the membrane or cytoplasm of the cell of origin (55). During CM, thrombocytopenia is associated with an increased caspase-mediated death of thrombocytes and increased plasma MP numbers, mainly of platelet origin (56). Platelet-derived MP

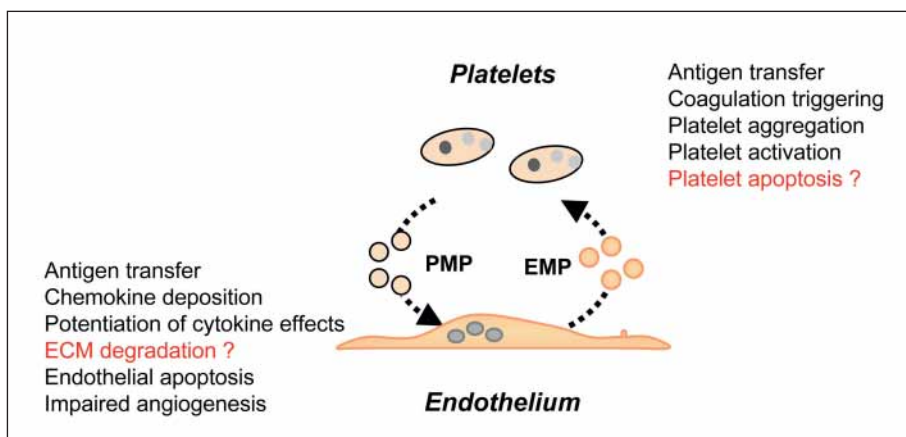


Figure 2: Microparticles as new effectors in platelet–endothelium interactions. During CM, MP from both platelets and endothelium are released, triggering additional cellular interactions with potential consequences on both inflammation and haemostasis. MP are able to transfer antigens, chemokines or bioactive lipids and to induce intracellular signalling in their target cells. Thus, PMP modulate EC adhesive and chemoattractive phenotype, but also EC apoptosis and angiogenesis. EMP modulate platelet activation and procoagulant phenotype. Abbreviations: CM, cerebral malaria; EC, endothelial cells; ECM, extra-cellular matrix; EMP, endothelial-derived microparticles; MP, microparticles; PMP, platelet-derived microparticles.

(PMP), which are more mobile than platelets and more susceptible to interaction with other cell types (57), are thought to modulate important aspects of endothelial functions (58). *In vitro*, PMP are able to modulate human EC phenotype by increasing the expression of adhesion receptors such as ICAM-1 and the resulting adhesion of other cells such as monocytes (59). PMP also induce cyclooxygenase-2 (COX-2) expression and prostacyclin (PGI₂) production by delivery of arachidonic acid (60). In addition, PMP transfer numerous platelet antigens (CD41, CD61, CD62, CXCR4, protease-activated receptor-1 [PAR-1]) to the surfaces of other cell types such as human or murine haematopoietic stem-progenitor cells or human neutrophils and increase their adhesion to endothelium or soluble fibrinogen (61, 62). It seems plausible, then, that PMP have the capacity to transfer platelet antigens to the surface of brain EC and modulate their adhesiveness to PRBC and leukocytes. Using an in-vitro model of CM, we found that PMP are actively internalised by human brain EC transferring platelet antigens such as PECAM-1 and CD36 to their surface and, as a result, enhance PRBC adherence (63). These findings indicate a new role for PMP in the modulation of endothelial phenotype and in PRBC sequestration, and raise the hypothesis that the pathogenic role of platelets in CM might, at least in part, involve the release of MP (see Fig. 2).

Endothelial activation and induction of adherence molecule expression

Several studies have shown the occurrence of widespread endothelial activation during the course of CM. Elevated plasma levels of von Willebrand factor (VWF) and its propeptide have been reported in field studies of patients with severe malaria (17, 23). Plasma levels of shed adherence receptors such as sICAM-1, sVCAM-1, and sE-selectin also correlate positively with the severity of malaria but might not reflect accurately the EC expression of adherence molecules in a particular vascular bed (64–67). More remarkably, at the tissue level, surface expression of ICAM-1, VCAM-1 and E-selectin is upregulated during CM and co-localise with sequestered PRBC (5, 37). PRBC or platelet adherence itself subsequently activates vascular endothelium (68, 69), but preliminary endothelial activation by systemic mediators might be required for their adherence during the initial stages of the disease.

Endothelial response to a systemic production of inflammatory cytokines

Plasmodium spp. are able to induce the production of several cytokines by mononuclear cells, regulating the cellular immune response and possibly influencing the mechanisms of clinical disease (70). A role for inflammatory cytokines in the pathogenesis of CM has been suggested, mainly through their capacity to up-regulate the expression of adhesion molecules on the cerebral vascular endothelium (12, 71–73). Thus, the role of the host response is a critical determinant of CM pathophysiology. Indeed, plasma levels of TNF are frequently increased in patients with severe *P. falciparum* malaria, particularly in those with CM, but decline during convalescence (74). While the production of cytokines varies among individuals according to their immune status, the distinctive sensitivity of brain endothelium might also explain the variations of clinical symptoms and signs. This is adduced from mouse experiments and cells purified from mice with distinct susceptibilities to CM. Upon infection with *PbA*, various strains of mice exhibit different susceptibility to the development of CM. Brain EC purified from CM-susceptible mice displayed a higher capacity to produce IL-6 and to up-regulate ICAM-1 and VCAM-1 in response to TNF. This difference may be partly due to a differential regulation of TNF receptor transcription via distinct protein kinase pathways (75, 76).

Elevated plasma levels of inflammatory cytokines are a common feature in severe malaria patients and are more likely to be associated with systemic changes rather than with a specific effect on cerebral endothelium. The local production of cytokines and chemokines at the site of the lesions by recruited cells, leukocytes but also platelets, appears of greater interest.

Inflamed endothelium promotes platelet adherence

While the healthy, non-activated endothelium normally provides a non-adhesive and anti-thrombotic environment for the circulating blood cells and plasma, proinflammatory cytokines, such as TNF and interferon-gamma, induce platelet adherence to cerebrovascular endothelium, notably via the overexpression of ICAM-1 on EC surface (49, 77). Also, increased circulating amounts of active and ultra-large VWF enabling spontaneous platelet binding, together with reduced VWF inactivation by ADAMTS13 (a VWF cleaving protease), may promote platelet

aggregation and adhesion to the endothelium, thrombocytopenia, and microvascular disease (78–81). This is supported by a strong relationship between platelet numbers and VWF levels (78, 82).

Platelet adherence and secretory mediators promote endothelial activation

Platelets, because of their rich content of mitogenic, proinflammatory and procoagulant mediators, can modulate the function, state of activation and survival of a wide variety of cells implicated in CM, notably EC, leukocytes, and neurones. In the adjoining EC, platelets alter the chemotactic, adhesive and proteolytic properties and promote the switch to an inflammatory endothelial phenotype. An important role in this context has been attributed to the rapid release of high amounts of CD40 ligand (CD40L, CD154) from activated platelets. Both membrane-bound and soluble forms of this ligand may interact with CD40, which is constitutively expressed on EC. *In vitro*, ligation of CD40 present on human EC by platelet-derived CD40L induces the release of chemoattractants, such as interleukin-8 (IL-8; CXCL8) and monocyte chemoattractant protein 1 (MCP-1; CCL2), and enhances endothelial expression of E-selectin, ICAM-1, VCAM-1 and tissue factor (83, 84). The role of CD40-CD40L in CM has been explored in the mouse model. Deficiency in CD40 or in CD40L or in-vivo treatment with an anti-CD40L monoclonal antibody prevents BBB breakdown and mortality although parasitaemia and TNF levels are similar. Thrombocytopenia and sequestration of macrophages in brain venules also are reduced in CD40- or CD40L-deficient mice. Thus, the mortality associated with CM requires CD40-CD40L interaction in mice that contributes to platelet consumption, macrophage sequestration and blood-brain barrier breakdown (85).

In addition, platelets can release multiple chemotactic factors that actively contribute to inflammation from their α -granules, including RANTES (CCL5), platelet factor 4 (PF4, CXCL4), as well as β -thromboglobulin (β -TG), thus triggering the recruitment of vascular cells or modulating crucial processes of EC such as angiogenesis (86, 87). Several studies suggest a potential role for platelet-derived chemokines in CM pathogenesis. During acute *P. falciparum* infection in humans, plasma concentrations of β -TG and PF4 are significantly elevated (88). Mice deficient for PF4 or its receptor CXCR3 have decreased T cell recruitment to the brain and less severe experimental CM (47, 89). Surprisingly, low levels of RANTES correlate with platelet counts and are independently associated with mortality in humans (90–92). RANTES-deficient mice present less leukocyte and platelet adhesion, BBB permeability and tissue infarction after middle cerebral artery occlusion and reperfusion (93). Mice deficient for RANTES receptor CCR5 are less susceptible to experimental CM (94). All these data suggest a deleterious but local role for this chemokine released by sequestered platelets during CM. PMP contain substantial amounts of RANTES and may also serve as a transcellular delivery system for RANTES, triggering monocyte arrest to inflamed endothelium under flow (95).

Platelets also secrete cytokines such as interleukin-1 β (IL-1 β) and transforming growth factor- β 1 (TGF- β 1). Levels of

both cytokines are elevated in CM (96) and their mRNA's are induced in the brains of CM cases but are not found to correlate with PRBC sequestration (97). *In vitro*, IL-1 β modulates the adhesive properties of endothelium, enhancing the surface expression of $\alpha_v\beta_3$ and promoting platelet-endothelium interactions (98). Moreover, even if produced on the vascular side of the brain endothelium, IL-1 β has profound actions in the brain, causing neuronal cell death and exacerbating brain damage (99). In malaria infected mice, IL-1 β synergies with TNF, especially by increasing plasma levels of nitric oxide (NO), a mediator argued to induce cerebral malaria (100). TGF- β 1 is thought to be an important immunoregulatory molecule that determines the balance of immune and immunopathological reactions in murine malaria (101), but less is known about its effect on the expression of the adhesion molecules in humans, especially during CM. Some data suggest that TGF- β 1 may down-regulate the expression of cell adhesion molecules such as VCAM-1 (102). It seems that TGF- β 1 could thereby have a protective role through decreasing PRBC cytoadherence. However, IL-1 β or TGF- β 1 can be produced by other cell types than platelets and neither the contribution of platelet-derived IL-1 β or TGF- β 1, nor their precise roles in modulating brain endothelial phenotype, have been studied in the context of CM.

Another major protein released from activated platelets is TSP-1 (103). Its expression is observed in cerebral microvessels with sequestered PRBC during CM (104) but TSP-1 plasma levels are not significantly elevated in CM (80). TSP-1 is able to bind PRBC, to stimulate ICAM-1, VCAM-1, and E-selectin expression and monocyte adhesion to EC (105) and to trigger endothelial apoptosis via CD36 activation (106).

Brain endothelium and platelets in the late stages of CM: roles in BBB disruption

The BBB is a highly specialised interface between the intravascular space and the central nervous system. It is composed of EC that line cerebral microvessels and form with astrocytes, pericytes and neurons functional "neurovascular units". This complex structure protects the brain from toxic substances in the blood, supplies brain tissues with nutrients, and filters harmful compounds from the brain back to the bloodstream. Transport across the BBB is strictly limited through both physical (tight junctions) and metabolic barriers (enzymes, diverse transport systems) (107). BBB breakdown or alterations in transport systems play an important role in the pathogenesis of many diseases of the central nervous system, including CM.

Platelets contribute to BBB permeability changes and brain endothelium apoptosis

Immunohistochemistry on post-mortem tissues from adults (108) and children (109) shows a loss of the EC junctional proteins zona occludens-1, occludin and vinculin in vessels with sequestered PRBC, associated with subtle changes in partition of albumin between circulating plasma and the cerebrospinal fluid compatible with impaired BBB function. A direct cytotoxic effect of PRBCs on brain EC can lead to the decrease in the integrity of the BBB monolayer through caspase activation and en-

endothelial apoptosis (110–112). Interestingly, the capacity of *P. falciparum* field isolates to induce endothelial apoptosis is more associated with neurological manifestations than their capacity to cytoadhere (113) and endothelial apoptosis can occur indirectly in the absence of physical interaction with PRBC (114, 115). BBB changes also are influenced by systemic metabolic changes observed during acute malaria, such as acidosis, hypoglycaemia, hypoxia and severe renal or hepatic insufficiency (8, 116).

Another potential mechanism by which BBB function might be altered is the consequence of platelet interactions with endothelial and circulating cells. *In vitro*, platelet binding enhances PRBC direct cytotoxic effect on EC activated by TNF or lymphotoxin, but not on resting, brain EC (49, 117). Both permeability and trans-endothelial electric resistance are strongly affected, and the apoptosis rate of brain EC is dramatically increased (117). Endothelial apoptosis can be triggered by platelet-derived secretory mediators such as TGF- β 1 (118) and requires both caspase-8 and caspase-9 activation, as well as EC production of reactive oxygen species (119). *In vivo*, mice with elevated levels of sP-selectin from EC show an increased vessel permeability in the brain but not in the skin or spleen, suggesting a vessel damage specific to the barrier function and specialised junctions of the brain endothelium (120). Interestingly, both endothelial-derived MP (EMP) and PMP contain caspase 3. The release of caspase 3-containing EMP might contribute to endothelial cell survival (121), whereas PMP induce apoptosis possibly due to the transfer of this caspase 3 into target cells (122, 123). In an *in vitro* model of sepsis, exosomes from platelets induce EC caspase-3 activation and apoptosis by generating reactive oxygen species (124). Both soluble mediators and membranous material delivered from activated platelets or the endothelium itself can thus mediate endothelial apoptosis and BBB breakdown. However, their precise role in the pathogenesis of CM has not yet been defined.

Platelets can induce extracellular matrix degradation by endothelial proteases

Enhanced turnover of the extracellular matrix is thought to be involved in the increased BBB permeability. In a complex cascade, uPA and tissue plasminogen activator (tPA) cleave plasminogen to plasmin, a serine protease of broad specificity, capable of degrading specific components of the extracellular matrix (125). In addition, plasmin activates matrix metalloproteinases (MMP), such as interstitial collagenase (MMP-1) and gelatinase A (MMP-2) (126). EC secrete uPA, tPA, MMP-1, MMP-2, and gelatinase B (MMP-9) in an activation-dependent manner (127). Platelets, via β 3-integrin-mediated adhesion and subsequent CD40L-induced signals, can enhance the proteolytic activity of EC, inducing the expression of uPAR and the secretion of uPA, tPA and MMP-1, MMP-2 and MMP-9 (128). During CM, focal accumulation of uPAR was observed in EC adjacent to petechial haemorrhages in the brain of patients who died from CM (129) and metalloproteinases are found active in the BBB environment in CM (130). uPAR has been shown to promote platelet trapping in cerebral venules and thrombocytopenia during experimental CM. Lesion-associated uPAR expression by EC could thus contribute to BBB alteration in CM patients directly, by allowing

proteolysis, but also indirectly by enhancing intravascular platelet trapping and cytotoxicity.

Platelets modulate brain angiogenesis

Increasing evidence indicates that platelets mediate potential regenerative mechanisms of the vascular endothelium. Platelets provide the critical signal that recruits circulating progenitor cells to sites of vascular injury by secreting the chemokine, stromal cell-derived factor (SDF-1), and influence progenitor cell biological activity and maturation (131). Failure to mobilise sufficient circulating endothelial progenitor cells to the cerebral microvasculature is a pathophysiologic feature of CM. Children with CM have low levels of circulating endothelial progenitor cells, but high levels of SDF-1, compared with those with uncomplicated malaria, asymptomatic parasitaemia, or healthy controls (132). The latter finding of the increased expression of SDF-1 in platelets from patients with decreased circulating progenitor cells is consistent with recent reports in patients with cardiovascular disease (133, 134). However, it is unclear why increased SDF-1 levels are not able to induce endothelial progenitor migration. It has been proposed that the cleavage of SDF-1 by proteases such as MMP may contribute to these findings (135, 136). Indeed, MMP-2-dependent cleavage of SDF-1 yields a neurotoxic remnant, lacking part of the biological activities (137).

Besides platelets, PMP modulate biological functions of progenitor cells and angiogenic processes. In addition to platelet-endothelium adherence receptors (61, 62), PMP express G-protein-coupled seven transmembrane-span receptors such as CXCR4 and PAR-1, cytokine receptors, including TNF-RI, TNF-RII and CD95, and ligands such as CD40L and PF4. PMP are thus able to chemoattract haematopoietic cells, to increase their adhesion, proliferation, and survival, and to activate in these cells various intracellular signalling cascades including MAPK p42/44, PI-3K-AKT, and STAT proteins (138).

Other platelet-derived lipid mediators such as lysophosphatidic acid, sphingosine 1-phosphate and phosphatidic acid are known to play a key role in many aspects of the angiogenic response and provide a novel link between haemostasis and angiogenesis (139). sphingosine 1-phosphate displays a tissue-specific, anti-angiogenic role in the brain, which may help to stabilise the cerebral vasculature and thus have a crucial impact on the regulation of normal brain vascularisation (140). However, the role of such mediators in brain neo-vascularisation has never been studied in CM.

Damaged brain endothelium activates platelets in CM: disrupting the homeostasis

Vascular endothelium is strategically located at the interface between tissue and blood. It is pivotal for protecting against vascular injury and maintaining blood fluidity. Normal endothelial surfaces express thrombomodulin and heparin-like molecules, and secrete tPA, which activates fibrinolysis. Normal endothelium also releases PGI₂ and NO, potent vasodilators and inhibitors of platelet activation (81). Conversely, activated endothelium secretes VWF from its Weibel-Palade bodies, which

mediates platelet adhesion and shear-stress-induced aggregation (81, 141) and rapidly generates platelet activating factor (PAF), a potent activator of platelets (142).

von Willebrand factor

In malaria, EC activation with increased circulating amounts of active and ultra-large VWF, together with reduced VWF inactivation by ADAMTS13, may result in intravascular platelet aggregation, thrombocytopenia, and microvascular disease (78, 79).

Prostaglandins

Altered prostaglandin concentrations were also revealed by clinical trials in peripheral blood of CM patients. Prostaglandin synthesis is controlled by cyclooxygenases (COX) and COX expression has been attributed a key role in immunomodulation, haemostasis and inflammation in a wide variety of pathologically altered brain tissues. In healthy vessels, the endothelium expresses constitutive forms of COX-1 and produces PGI₂ that inhibits platelet activation. In contrast, in diseased vessels, inducible forms of COX-2 are expressed, resulting in the release of large amounts of PGI₂ but also prostaglandin (PGE₂). Accumulation of COX-2 expressing EC was detected in the brain parenchyma of patients with CM (144) and expression of COX-2 mRNA in the brain is highly induced in experimental CM (145), suggesting that prostaglandins may play a role in human CM. However, an impaired systemic production of PGE₂ is associated with the adverse outcomes of CM (146) and salicylates in the blood of children correlates with poor outcomes in severe malaria (147). The administration of COX-2 inhibitors in murine CM (145) does not have any beneficial effect, suggesting a beneficial role for prostaglandins in the outcome of malaria. One potential beneficial effect of prostaglandins in CM is the induction of the expression of the immunosuppressive cytokine IL-10 by dendritic cells (148).

Nitric oxide

Nitric oxide (NO) is widely known to inhibit platelet and leukocyte adhesion to endothelium through its regulatory effect on adhesion molecule expression. In the context of CM, NO production by the inducible form of NO synthase can be induced by engagement of CD23 antigen and reduces the number of adherent PRBC on resting and TNF-stimulated EC by down-regulating ICAM-1 and VCAM-1 expression (149, 150). Low NO bioavailability in the vasculature during malaria might contribute to pathologic activation of endothelium and platelets during CM pathogenesis. Agents that improve endothelial NO production and endothelial function, such as L-arginine, may serve as potential adjunctive therapy, effective in the acute phase of CM (151). However, NO can also have a deleterious role, enhancing PRBC-induced apoptosis in EC through an oxidative stress pathway (111).

Endothelial-derived MP

Elevated plasma levels of circulating MP of endothelial origin have been identified in children with severe *P. falciparum* malaria

complicated with coma (152). These levels positively correlated with corresponding plasma TNF levels (Valéry Combes, unpublished data). *In vitro*, endothelial MP released upon TNF stimulation display pro-coagulant properties via a tissue factor-dependent pathway (153) and promote platelet ristocetin-induced aggregation via ultra-large VWF multimers (154). Endothelial MP also expose endothelial protein C receptor, which is able to bind activated protein C and to cleave endothelial protease-activated receptor 1 to modulate inflammation and increase EC survival (155, 156). *In vivo*, the phosphatidylserine exposed on the surface of EMP is an endogenous ligand for CD36 that transmits an activating signal to platelets leading to GPIIb/IIIa activation, P-selectin expression, and aggregation in response to low doses of ADP (157). In CM, endothelial MP could thus have a pathogenic role in the development of the cerebral syndrome and in the worsening of the lesion by locally disturbing the haemostatic balance and potentiating leukocyte adhesion.

Conclusion

Endothelium and platelets maintain a constant dialogue that regulates vascular homeostasis. Platelets release or transfer substances that impact on EC function, and vice versa. Miscommunication between these two cell types, usually in the way of excessive crosstalk, underlies several disease states, either as a causative factor and/or as a consequence of the disease process. Platelet-endothelium interactions have been particularly well described in macrovascular diseases such as atherosclerosis and it is now widely accepted that platelets play a significant role in the initiation, propagation and total extent of the atherosclerotic lesion (158). Less well-known are these interactions in microvascular diseases such as sepsis, sickle cell disease, stroke or CM. Although of distinct pathogeneses, these disorders share several common clinical features, allowing physiopathological hypotheses to be put forward and tested. Acquired abnormalities in endothelium and platelets, and their interactions occur in CM, and some data suggest a role for platelets in parasite sequestration and BBB disruption, two key processes involved in CM pathogenesis. Unfortunately, only a small part of these processes have been well characterised in the context of CM. Further *in vitro* or *in vivo* studies are needed to offer new therapeutic approaches, as current efforts using drugs affecting platelet-endothelium interactions (heparin and acetylsalicylic acid) did not show any improvement in the clinical course of human *P. falciparum* malaria (159).

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