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MULTI-AUTHOR REVIEW

The role of platelets in the pathogenesis of cerebral malaria

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Abstract Malaria is a major cause of morbidity and mortality in the developing world and cerebral malaria is responsible for the majority of malaria-associated deaths. There is a strong association between thrombocytopenia and outcome in malaria, suggesting a role for platelets in the pathogenesis of malaria. This thrombocytopenia is likely due to platelet activation possibly through an interaction between PfEMP1 on plasmodium and CD36 on platelets. Platelet activation by plasmodium has two potential consequences. It can lead to the formation of micro-aggregates of infected red blood cells and platelets which can occlude blood vessels and it also leads to binding to and activation of the endothelium.

Keywords Platelet · Plasmodium · Malaria · Thrombocytopenia · CD36 · GPIV · PfEMP1

Introduction

Malaria is a mosquito-borne parasite infection and is a major cause of mortality and morbidity in much of the developing world. Recent WHO statistics show that each year over 1 million people die from malaria, mostly children under five and pregnant women who live in sub-Saharan Africa. In addition, over 500 million people per year suffer illness due to malaria [1]. Most cases of malaria present as uncomplicated malaria with characteristic symptoms of fever, nausea and aches; however, some can present with severe malaria that involves impaired function of various organs. The most serious form of severe malaria is cerebral malaria, which is estimated to occur in 10% of hospitalised cases and is associated with 80% of deaths. Cerebral malaria occurs when infected red blood cells (RBCs) occlude cerebral blood vessels. However, the exact cause is not known. Of the four organisms (*Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*) causing clinical malaria, the vast majority of mortality and morbidity is due to *P. falciparum*.

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Malaria

The first clinical description of fevers consistent with malaria infection is recorded by the Greek, Hippocrates, about 400 BC, in his book *Epidemics*. In Europe, these were thought for centuries to be caused by bad air emanating from the swamps and marshes, around which these fevers were more common. Thus, the name malaria literally means "bad air" from the Italian name for the disease. The popularity of germ theory and bacteriology in the 1870s may have contributed to some investigators, erroneously as it turned out later, ascribing the cause of malaria to a bacterium.

Alphonse Laveran and Protozoa

In his Nobel Lecture of December 11th 1907, Charles Alphonse Laveran the Paris-born military doctor working in Algeria, recounted that "in 1880 in a military hospital at Constantine, I discovered on the edges of the pigmented spherical bodies in the blood of a patient suffering

from malaria, filiform elements resembling flagellae which were moving very rapidly displacing the neighbouring red cells" [2]. He realised at that stage that these were parasitic elements likely to be the aetiological agent of malaria. He also realised that these were not conventional bacteria and were a new form of pathogen. At that time, chemical dyes for staining blood cells did not exist but over the following 10 years the staining methods developed by Romanowsky allowed any of the various blood stage forms to be visualised in detail. This confirmed the presence of intraerythrocytic protozoan parasites in other malaria patients from a variety of countries. What Laveran discovered was small round elements between 1 and 2 microns in diameter inside erythrocytes. The erythrocytes, or red cells in the blood, are each 7–10 μm in diameter. Again, to quote Laveran's Nobel Lecture of 1907 "in stained preparations a nucleus can be detected in each of these small elements" [2]. Secondly, he described amoeboid elements inside the red cells and recognised that these elements destroy the parasitised red cells. These may be equivalent to what we now know as mature trophozoites. He recognised that multiplication of the two forms mentioned above is by halving or by multiple divisions. The third form of malaria parasite described in the blood by Laveran was "crescentic-shaped bodies measuring 8–9 μm long which he correctly identified as the gametocytes of sexual phase of reproduction. Finally, Laveran noticed flagellae which he described as 20–25 μm long, and as these are visible without staining they were the first components to come to his notice. These he correctly identified as being associated with motile gametocytes. Thus, through diligent observation of many patient's blood using very rudimentary stains, Laveran was able to identify several of the polymorphic forms of the pathogen causing malaria and to make some deductions about their likely significance. With no success, he spent several years searching in soil, air, marshes and the environment for the extracorporeal forms of the infection. This led him to speculate that a vector, for example mosquitoes, might be important in the transmission of the protozoa from one patient to another.

Malaria and mosquitoes

As noted in the previous paragraph, the idea that malaria might be transmitted by mosquitoes was suggested by Laveran. In addition, Patrick Manson, based in Liverpool, showed that the helminth disease elephantiasis was transmitted by mosquitoes and strongly suspected this was the case for malaria. Ronald Ross was born in India of English parents and worked for the Indian Medical Service. He collected mosquito larvae, allowed these to pupate and develop into adult forms in experimental conditions and then invited patients with active malaria and gametocytes in the blood to allow the adult mosquitoes to feed on their blood. He followed the gametocytes into the mosquitoes stomach and noted the mixed flagellation there of gametocytes. In 1897, after 8 years of researching by dissection and microscopy of the stomach, mouth parts and other tissues of mosquitoes he found 12 cells in the "thickness of the stomach wall" and these contained pigmented granules familiar to that found in the blood stream of people with malaria [3]. He discovered these parasitic cells only in the stomach of an anopheles mosquito and realised then that he had spent most of the previous years looking at the insides of the wrong species of mosquitoes, and there were many other species of mosquito that were present in India. In the following year, 1898, by re-feeding infected mosquitoes and keeping them alive for several weeks with serial harvesting, he was able to describe in detail the microscopic features of the life cycle of *P. falciparum* in anopheles mosquitoes. He noted that the pigmented cells in the wall of the stomach of the mosquito enlarged, matured and subsequently ruptured with "discharge of their contents into the body cavity of the grey mosquitoes". The contents of the mature pigmented cells consisted of "a multitude of delicate threadlike bodies, which on the rupture of the parent cell, were poured into the body cavity of the insect". He showed that these threadlike bodies (sporozoites) migrated anteriorly in the body of the mosquito and invaded the cells in the salivary glands. Thus, by artificially breeding anopheles mosquitoes and deliberately feeding them on an infected patient with gametocytes, keeping them alive for several weeks with serial dissections, Ronald Ross was able to demonstrate the full life cycle of the plasmodium. He showed that the plasmodium gametocytes combine in the stomach of the mosquito to form an oocyte which burrows into the stomach wall, matures, ruptures with the release of very many sporozoites into the abdominal cavity which migrate via the thoracic cavity into the acinar cells of the salivary glands of the mosquito. From there, they proceed into the salivary fluid and are injected through the mosquito's proboscis into a human host when a subsequent blood-feeding event occurs. Using experimental conditions of feeding mosquitoes on birds with malaria, and after appropriate incubation re-feeding the same mosquitoes on uninfected susceptible birds, Ross was able to confirm very high rates of infection in a repeatable reliable fashion. He also studied the natural history of malaria infection in the birds showing the gradual appearance of parasites in the blood stream, then increase to extremely large numbers. Some of the birds died off from severe disease and involvement of liver and other organs. In this way, the basic life cycle of a pathogen causing malaria was identified.

From herbal treatments to chemistry: artemisinin, quinine and chloroquine

The wormwood plant *Artemisia annua* known in China as *qinghaosu* has been used for more than 2,000 years as a Chinese medical remedy. Its use for fever was specifically described in AD 340. Subsequently, an active extract from the plant (artemisinin) was shown to be effective against malaria infection in animals and humans in the 1970s, 1980s and 1990s. Since 2002, with the advent of very significant international funding for control of malaria from the Global Fund for AIDS, Tuberculosis and Malaria based in Geneva, its use, with partner drug lumefantrine, has escalated and it is now first-line treatment for malaria in many African countries. It is produced commercially by Novartis in Switzerland, and a treatment course for a child is approximately US $1. The story of the history of quinine has been recounted many times and describes how a local remedy using the bark of a South American tree was used to treat fever there. The bark was introduced to Europe by the members of the Jesuit society around 1630 and was used to treat several of the royalty in Europe. Unfortunately, as quinine does not eliminate the relapsing form of malaria, some of the cures proved short lived. In the 1920s and 1930s, several of the chemical companies tried to manufacture synthetic anti-malarial drugs including chloroquine and several similar compounds and proguanil. While these were extremely widely used after the Second World War, recently the parasites of *P. falciparum* have become resistant to chloroquine on a very widespread basis precluding its effective use. It is, however, still valuable in the treatment of other forms of malaria, although some resistance has also been described in other species [4].

Immunoglobulin-mediated immunity

Surprisingly as it may seem, some of the less pathogenic species of plasmodium were used as therapy for syphilis infection in the 1920s onwards when few if any effective remedies for syphilis were available. Starting in 1917, neurosyphilis patients were treated by inoculating them with some blood from a patient with *P. vivax* or *P. ovale* infection to produce periodic fever every 2 days, which was known as tertian malaria. This remedy led to a 30% recovery rate in patients with neurosyphilis while a smaller proportion had a partial improvement due to this treatment. This continued as a treatment for neurosyphilis until the late 1940s when penicillin became widely available. Through the widespread practice of these natural experiments, the natural history and host parasite interaction between plasmodium and the host were described in detail. This showed that some patients who were inoculated with one particular strain of plasmodium were partially immune to infection with that strain in the future. However, inoculation with a different strain of *P. vivax* or *P. ovale* led to repeated infection and fever, indicating that the host acquires strain-specific immunity to infection. The mechanism of immunity has been investigated in detail. It is partial and develops slowly, and it appears that plasmodium infection can, in some way, diminish the immune response to its own antigens. However, particularly after repeated infections an effective immunity develops, initially against severe disease, and subsequently against infection and parasitaemia. This was demonstrated in the Gambia by Ian McGregor working at British MRC Unit with his wife Joan. He showed that taking antibody-rich hyper-immune serum from people who had been extensively exposed to malaria and infusing this into infected children led to less parasitaemia and partial cure of the young children with acute severe malaria [5]. He has also shown that serum from hyper immune patients in one part of the world is also partially effective at treating malaria in patients from other parts of the world, indicating that there is some cross-strain immunity which develops in highly exposed patients who have hyperimmunity. This finding stimulated vaccine research and showed that acquired immunity could effectively control parasitaemia. That use of serum from hyper immune Gambian donors could be used to control malaria infection in Tanzania also suggests that a single vaccine might be effective at strains from across the continent of Africa [6].

Continuous culture of *Plasmodium falciparum*

While working at Rockefeller University in New York on animal forms of malaria and on other protozoa, William Trager developed the skills of tissue culture and in 1976 described continuous culture of the blood form of *P. falciparum* [7]. At the time, WHO announced great optimism that this would soon lead to a vaccine against the disease. However, more than 30 years later a marketable licensed vaccine does not exist. The continuous culture of plasmodia is currently relatively straight forward. A method has been described using conventional tissue culture medium and preparation of human erythrocytes which are regularly manually changed and replenished. Several automated apparatus have been designed. The most exciting perhaps called the “Tipper” developed in Niemegen in The Netherlands by Ponnudurai and co-workers [8]. The ability to culture plasmodia continuously and to cryopreserve, thaw and re-establish growth of the erythrocytic forms as required has greatly facilitated ease of study of malaria biology.

Use of irradiated sporozoites for vaccination

Is malaria vaccine even possible? Is it possible to artificially mimic the naturally acquired immunity to malaria disease and to plasmodium infection? These questions were answered definitively in the affirmative in 1975 when David Clyde and others working in Baltimore for the US Army Medical Research & Development Command showed that acquired immunity could be reliably induced in humans [9]. They used repeated biting and feeding of human volunteers by multiple infected X-irradiated mosquitoes. The irradiation attenuates the plasmodial sporozoites so that they are live but cannot replicate after inoculation. This led to temporary protection of a volunteer from malaria for less than 3 months duration. Thus, acquired immunity, presumably due to antibody and/or cell-mediated immunity directed against antigens of the sporozoites, could lead to sterilising immunity. Irradiated mosquitoes and the irradiated sporozoites are unable to productively infect the parasites or to produce merozoites or the erythrocytic forms of malaria. These results indicate that the sporozoite antigens alone are adequate in this system to confer protective immunity. These sporozoites are still alive and entering the host's body and appear to traverse through tissues and cells perhaps as far as the parasites but are just unable to replicate because of DNA damage. However, these experiments were only a proof-of-principle, because manufacturing a commercial preparation of irradiated sporozoites on the scale needed to protect 500 million people who get it each year is not possible at present.

Culture of gametocytes

One of the technical obstacles to testing of interventions against malaria, particularly testing vaccines directed against sporozoites or liver stages of the pathogen which precede the intraerythrocytic stages, was the difficulty of artificially infecting mosquitoes with plasmodia grown in vitro. The transition from conventional blood stage trophozoites to gametocytes was found to be unpredictable and erratic until 1982 when, in Niemegen in The Netherlands, Ponnudurai discovered the need for particular culture conditions to effect this change. In particular, hypoxanthine was required in the culture medium to encourage maturation of trophozoites into gametocytes [10]. This can be combined with feeding of mosquitoes on a latex membrane from blood containing infected red cells and gametocytes to allow the laboratory-bred anopheline mosquitoes to become artificially infected with laboratory strains of *P. falciparum*. These strains, for example 3D7, known to be chloroquine sensitive, have been used in human challenge experiments in Niemegen, Maryland and London. This has proven an effective way to assess rapidly the efficacy of new malaria vaccines in adults; in particular, vaccines directed against the sporozoite and liver stage antigens of plasmodium. Without this development of a safe predictable challenge model and of the safe in vitro culture and infection of laboratory strains of chloroquine-sensitive plasmodia, the rapid screening of vaccine candidates which use sporozoites and liver stage antigens would have been delayed very significantly. The only alternative would have been to plan and fund large field trials for each new candidate vaccine.

The complete *Plasmodium falciparum* genome

Within a year of the first draft of the sequence of the human genome, an international consortium sequenced the 3D7 Clone of *P. falciparum* in 2002. They found 23 million base pairs in a genome of 14 chromosomes which encodes information for about 5,300 proteins. Several of these relate to immune invasion and 59 *var* genes were found coding for erythrocyte membrane protein one [11, 12]. These proteins are found on the surface of red cells and mediate adherence to host endothelium receptors. These proteins, which are exported from the intra-erythrocytic trophozoite stage through the cytoplasm of the erythrocyte into its lipid membrane, mediate adherence to receptors on the endothelium of the host capillaries and other vascular elements. This produces sequestration, accumulation of infected erythrocytes close to the wall of blood vessels, in particular, in capillaries. As described below, this may be an important feature in the pathogenesis of severe malaria, particularly cerebral malaria. In the placenta, chondroitin sulphate is a major receptor and a particular *var* gene encodes for the plasmodial protein which adheres to this placental receptor [13]. The sequestration in the placenta of pregnant women particularly in the primigravida is associated with low birth weight and other serious complications for the infant. The complete genetic sequence of the main vector of malaria, *Anopheles gambiae,* was also published in the same year [14]. This has provided the basic tools for a genomic and proteomic systemic approach to malaria research in the fields of pathogenesis, therapeutics and vaccine development.

Cerebral malaria

P. falciparum produces the clinical syndrome of cerebral malaria, characterised by various kinds of severe but often reversible brain dysfunction, including seizures, diminished level of consciousness and focal neurological deficits. While this has been accepted as a clinical entity for some years, only in 2004 was the pathology of this condition described systematically [15]. The research showed that, in

about a quarter of children who died with the clinical syndrome of severe cerebral malaria, other illness caused their death, probably unrelated to malaria. Thus, the clinical syndrome was not as clear-cut as was expected. However, in those who had malaria, there were dramatic changes in the brain including sequestration of parasites in the vessels and peri-vascular pathological changes, for example, haemorrhages and accumulation of malarial pigment [16]. This group have gone onto show that platelets are involved in clumping of parasitized red cells [17]. They suggested that the thrombocytopenia, so commonly seen in severe malaria, may be a protective adaptive response of the host to avoid additional clumping of red cells [18]. Thus, vascular endothelium, red cells and platelets and the interactions of these three components probably play a key role in the pathogenesis of severe malaria.

Platelets in malaria

Thrombocytopenia in malaria

A drop in the platelet count (thrombocytopenia) is a common feature in malaria. In fact, it is considered to be diagnostic in suspect febrile patients [19, 20]. The extent of the thrombocytopenia is also a predictor of outcome [21, 22] as well as a predictor of both outcome and severity in children [23]. However, some studies have suggested that it only predicts the level of parasitemia and not disease severity [24, 25]. Thrombocytopenia has also been shown to be associated with asymptomatic malaria infection [26]. Thrombocytopenia also occurs with *P. vivax* infection [27] and *P. berghei* in mice [28]. While thrombocytopenia has been associated with outcome in cerebral malaria, the role of platelets is somewhat controversial [22, 29–32]. Previous electron microscopy studies using post-mortem pathology sections from patients with cerebral malaria identified no platelets at sites of RBC adherence [31]. However, a more recent immunohistopathological study, where human cerebral blood vessels were stained for platelet-specific markers, showed that there is significantly higher platelet accumulation in the brains of patients with cerebral malaria than those without [17]. Together these data suggest that thrombocytopenia is very common in all forms of malaria, and since thrombocytopenia appears to be very specific for malaria, it strongly suggests that it is probably part of the pathogenesis of malaria and clearly indicates a role for platelets in the progression of the disease.

While the cause in malaria is not clear, thrombocytopenia is usually due to decreased production or increased consumption of platelets. As the effects of decreased production takes time to affect the platelet count, this is more likely to be seen in chronic thrombocytopenia rather than during an acute infection. The two principal mechanisms that drive platelet consumption are activation of the platelet and immune-mediated clearance. The two are often related as anti-platelet antibodies often trigger platelet activation. An example of this is heparin-induced thrombocytopenia where antibodies are formed to heparin–platelet factor 4 complex on the surface of platelets [33]. In some cases, these antibodies lead to platelet activation [34]. Platelet turnover has been shown to be increased in plasmodium-infected patients with thrombocytopenia and platelet life-span reduced [35]. In these patients with uncomplicated malaria, sequestration was diffuse.

Platelet activation

There is a paucity of data on the cause of platelet consumption in patients with malaria. There are a few studies suggesting that malarial thrombocytopenia is driven by platelet activation. P-selectin levels have been shown to be increased in patients with malaria [36]. Platelet microaggregates [37], enhanced platelet activation [38] and increased levels of platelet factor 3 [39] were identified in patients with malaria.

There have been a number of studies on thrombocytopenia in animal malaria models using *P. berghei* rather than *P. falciparum*. Increased thromboxane levels have been found in hamsters [40], P-selectin levels were increased in infected mice [41, 42] and platelet caspase activation was seen in infected mice [43], as well as increased levels of platelet microparticles [43], all of which are markers of platelet activation. Using MRI in a mouse model of cerebral malaria, adhesion of activated platelets to the cerebrovascular endothelium was detected [44]. One study suggested a role for an interaction between urokinase plasminogen activator and its receptor in platelet sequestration [45]. Thus, it is likely that at least in animal models platelet activation occurs and probably drives the platelet consumption.

Immune mechanisms

One study identified an increased level of platelet-associated antibodies in thrombocytopenic malaria (falciparum) patients [46] as well as in vivax patients [47]. This was supported by a study that showed an association between polymorphisms in FcγRIIa, the platelet IgG receptor and disease severity [48]. IgE levels have also been associated with severity of malaria [49] and an increase in IgM levels has also been reported [50]. IgE response to parasites and allergens has been shown to involve an IgE-mediated activation of platelets [51–53].

Whatever the cause of the thrombocytopenia, platelets appear to play a key role in cerebral malaria as platelet-mediated infected RBC agglutination has been shown to be associated with severe malaria [22]. In an animal model of malaria, blocking platelet adhesion was found to be beneficial [54] especially in the early stages of the disease [55], and in vitro studies showed that platelet binding to activated endothelial cells increased cell permeability [56]. However, mice rendered thrombocytopenic prior to infection had a much higher mortality rate compared with normal mice, and thrombocytopenia only occurred in normal rats and not in splenectomised although mortality was much higher in splenectomised rats. These data suggest a protective role for platelets in malaria [57].

There is also evidence of an immune-mediated thrombocytopenia in mice. Thrombocytopenia was found to be $CD4^+$ T-cell-dependent and antibody-dependent [58] but was independent of Toll-like receptors [59]. Thrombocytopenia was attenuated in ICAM-1-deficient mice [60] and involves CD40-CD40L interaction [61]. In mice, antibody-dependent mechanisms only play a minor role in the destruction of platelets with cell-mediated immunity being the most important factor [28]. Rats infected with *P. chabaudi* also developed thrombocytopenia while nude rats did not [62], and thrombocytopenia was antibody-dependent [63].

Complement and thrombocytopenia

Complement formation is known to lead to platelet activation. The formation of C3d, indicating complement activation, was associated with thrombocytopenia in malaria-infected patients [64]. The formation of immune complexes is also associated with thrombocytopenia [63, 64]. Blockade of the interaction of C5a with C5 receptor protected mice from cerebral malaria [65]. Complement generation has been shown to play a role in thrombocytopenia in haemolytic uremic syndrome [66], and bacteria have been shown to induce platelet activation in a complement-dependent manner [67–69].

Bacterial-induced platelet activation

While we do not know for sure the mechanism for the thrombocytopenia, we can look for similarities with other pathogens. A number of bacteria are known to cause thrombocytopenia [70] usually by activating platelets. Some bacteria such as the oral pathogen *Porphyromonas gingivalis* secrete a platelet-activating factor, gingipain. Some bacteria interact directly with platelets, such as the interaction between *Streptococcus sanguinis* SrpA and platelet GPIb [71], while others interact via a bridging ligand such as occurs with *Staphylococcus aureus* where fibrinogen binds to ClfA on the bacteria and GPIIb/IIIa on the platelet [68]. These interactions all cause platelet activation and aggregation but only in the presence of a specific antibody which engages with the platelet FcγRIIa receptor [68, 72]. A more generalised mechanism was also identified where, in the absence of specific bacterial–platelet interactions, antibodies to the bacteria could trigger complement formation. The interaction of bound antibody and complement with receptors on the platelet surface can also lead to platelet activation although it occurs at a much slower rate [68].

Plasmodium receptors on platelets

CD36

GPIV (CD36) was first identified on platelets as a glycosylated 88-kDa protein [73] and has subsequently been shown to be widely expressed on cells and to have an equally broad range of ligands and functions. As well as platelets, it is found on macrophages, dendritic cells, adipocytes, muscle and some types of endothelial cells. Originally identified as a thrombospondin receptor, it also binds to modified lipids particularly oxidized lipids such as LDL and phosphatidylserine. Functionally, it acts as a scavenger receptor on phagocytic cells where it is involved in clearing modified lipids from the circulation as well as removing some pathogens by acting as a co-receptor for TLR 2. On adipocytes, it acts as a fatty acid transporter [74].

Despite being originally identified on platelets [73], its role in platelet function is unclear. It mediates platelet binding to thrombospondin [75, 76] and there is evidence that it may play a role in platelet adhesion to collagen [77, 78]. CD36 was also identified as an endothelial cell receptor for *P. falciparum*-infected erythrocytes [79] and subsequently as a platelet receptor for infected erythrocytes [80, 81].

There is evidence that CD36 plays a role in platelet activation. Antibodies to CD36 induce platelet aggregation [80, 82–84] although this might be a due to a more general response of platelets to antibodies [85]. However, there are significant data to support a role for CD36 in platelet activation. Misfolded proteins have been shown to induce platelet aggregation in a CD36-dependent manner [86]. VLDL enhances collagen-induced platelet aggregation [87] and oxidized HDL induces platelet aggregation [88] in a CD36-dependent manner. Thrombospondin, a CD36 ligand, has also been shown to stimulate platelet activation by blocking cGMP accumulation in platelets through a process that partially involves CD36 [89].

Other platelet receptors

While CD36 is a key platelet receptor in the interaction with infected RBC's there is evidence for other interactions as antibodies to CD36 did not inhibit interactions with all isolates [22, 90]. The complement receptor gC1qR/HABP1/p32 on both endothelial cells and platelets has been shown to support an interaction with infected RBC's and supports platelet-mediated clumping of infected RBC's although the parasite ligand is not known [90]. PECAM-1 was also shown to be an endothelial receptor for infected RBC's [91], and as this is also expressed on the platelet surface, it is likely to mediate an interaction with platelets as well.

Plasmodium adhesion molecules

Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1)

Some strains of falciparum malaria parasites induce the formation of small membrane protrusions known as knobs on erythrocytes. These knobs have been identified as the site of contact with endothelial cells, and high molecular weight malarial proteins expressed on these knobs mediate this interaction [92–94]. PfEMP1 was subsequently identified as the knob protein that binds to CD36 [95]. PfEMP1 is a high molecular weight protein (200–400 kDa) [93, 96] encoded by the *var* multigene family [97, 98] and a member of a highly variant antigenic family that is responsible for antigen variation in *P. falciparum* [99]. Thus, as well as mediating adhesion to endothelial cells, it also plays a key role in immune evasion.

As PfEMP1 can bind to numerous proteins including CD36, thrombospondin, ICAM-1 and VCAM-1 it contains a number of different binding domains. PfEMP1 contains two different modules, the Duffy binding-like domain (DBL) of which there are six and the cysteine-rich interdomain regions (CIDR) of which there are three [100]. The CD36 binding domain has been localised to one of the CIDRs, CIDRα. A C-terminal 166 amino acid sequence appears to be responsible for the interaction with CD36 and that amino acids 106–166 appear to be especially important [101]. The region of CD36 that binds to PfEMP1 has been located in the region aa139–184 [102]. While PfEMP1 can directly bind to CD36 and a number of CD36 ligands can induce platelet aggregation, there is no evidence that PfEMP1 triggers platelet activation. However, *P. falciparum* does trigger clumping of infected erythrocytes that is mediated by platelets in a CD36-dependent manner [22], and thus it is possible that this may be due to PfEMP1-CD36-mediated platelet activation.

Role of the endothelium

The endothelium plays an important role in the pathogenesis of malaria. The clumped RBCs bind to the endothelium and can ultimately occlude smaller blood vessels, especially in the brain. Thus, it is necessary to understand the relationships between platelets, infected RBCs and endothelium. This is complicated by the presence of shear stress in the vasculature.

Platelet-RBC-endothelium interactions

While infected RBCs can bind to both endothelial cells and to platelets via CD36 and other receptors, these are unlikely to be either/or interactions. Using in vitro co-cultures, it was shown that platelet binding to activated endothelial cells potentiated the cytotoxic effect of infected RBCs [56]. In a mouse model of cerebral malaria, early platelet depletion was found to be protective. The adverse effects of platelets was not due to adherence to the endothelium but was thought to be due to modulation of cytokine production [55].

Activated endothelium is a key component of cerebral malaria and activation of the endothelium has been shown to occur in children [103]. Overproduction of cytokines plays a major role in the activation of the endothelium [55, 104]. One of the key cytokines involved is TNF which is produced by macrophages in response to malaria antigens [105], possibly acting on TNFR2 [106]. Platelets play a significant role in the destruction of TNF-activated endothelial cells [30, 107, 108] while TGFβ_1 released from activated platelets can kill TNF-activated endothelial cells [109]. CD40–CD40L interaction was also shown to be important in thrombocytopenia in infected mice [61]. Endothelial cell P-selectin but not platelet P-selectin was found to play a significant role in cerebral malaria in a mouse model [42].

Blood is continually flowing and as a result the cells are exposed to a range of shear conditions in vivo. Although several studies have investigated the interaction of plasmodium-infected RBCs with the endothelium [56, 109–112], there is a paucity of data examining this phenomenon under physiologically relevant shear conditions. In vivo endothelial cells are constantly exposed to haemodynamic forces, namely shear stress and cyclic strain. These forces profoundly affect endothelial gene expression, morphology and cell fate [113–115] and lead to the expression of a blood–brain barrier (BBB) phenotype in cerebral endothelial cells [116–120]. Moreover, regions of the macrovasculature exposed to low levels of shear stress, such as arterial branch points and bifurcations, exhibit a greater occurrence of atherosclerotic plaque formation,

where platelets play a pivotal role in both the development and progression of the disease [121–123].

When infected RBCs were sheared over a range of substrates, it was found that they rolled over immobilised endothelial cells in an ICAM-1-dependent manner. However, they formed static interactions with platelets in a CD36-dependent manner [124]. The interaction with platelets could be inhibited by peptides from PfEMP1 [125] or by CD36 antibodies[126]. Using a mutant *P. falciparum* strain that had the knob-associated histidine-rich protein (KAHRP) deleted, it was shown that infected RBCs were capable of adhesion to CD36 under static conditions but not under shear conditions [127]. This is analogous to results with *Staphylococcus aureus* where both Clf A and Fnbp A could support adhesion to fibrinogen under static conditions but only Clf A could support adhesion under shear conditions [128].

Targeting the platelet for treatment of cerebral malaria

There is still a lot to learn about the interactions of platelets with plasmodium; however, there is extensive evidence to suggest that platelets are implicated in the pathogenesis of malaria as they are also involved in the pathogenesis of other infectious diseases [70]. The precise role of the platelet is not clear as the animal studies suggest that blocking platelet function in mice is beneficial [54–56]; however, other studies suggest that platelets play a beneficial role in malaria [18, 57]. This contradiction can be explained by the different roles platelets play. The evidence would suggest that platelets play an important role in the clumping of infected RBCs and possibly in their interaction with the endothelium. Activation of the platelet will have two effects. Firstly, it will release many cytokines (there are up to 300 biologically active proteins in platelet releasate) [129] and these play an important role in activation of the endothelium. Secondly, however, the platelet activation will also lead to thrombocytopenia which will reduce the platelet-mediated RBC clumping.

Thus, targeting the plasmodium–platelet interaction should be an ideal drug target. The strategy should be to prevent platelet activation and to inhibit platelet-mediated RBC clumping. The most obvious target is the PfEMP1–CD36 interaction although the role of other interactions needs to be determined.

There is some evidence to suggest that the platelet may have a role to play as a drug target in cerebral malaria. The pro-vitamin pantethine is a low molecular weight thiol with anti-platelet activity. In a mouse model of cerebral malaria, it was shown to reduce platelet reactivity, endothelial cell activation and prevented the cerebral syndrome [130]; however, neither the use of heparin nor aspirin influenced the course of falciparum malaria [131]. In a mouse model of cerebral malaria, antibodies to GPIIb/IIIa significantly reduced morbidity [54].

A recent study showed that platelet binding to infected red blood cells aids in the killing of the parasites [132] which has raised the issue of the wisdom of using aspirin to treat fever in patients with malaria as this may compromise the ability of platelets to kill the parasites [133]. However, in a patient with cerebral malaria this system has been overwhelmed and it is unlikely that inhibition of platelets would have any impact and anti-malarial agents will be used anyway. Thus, while the platelets play a complex role in malaria it is still unclear whether they are a suitable drug target to aid in the treatment of cerebral malaria.

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