

Title:

Immune role of platelets in malaria.

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Short title:

Platelets in malaria

No disclosures

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Abstract

Plasmodium infections causing malaria threaten over 3 billion people from more than ninety countries. Despite control efforts spanning several decades there are still more than 400,000 fatalities annually. Survival to malaria is largely determined by the host response mounted against the parasite, and the ensuing balance of inflammatory, tissue-damaging reactions and immune-mediated mechanisms directed towards controlling pathogen growth. Platelets appear to play important roles in each of these processes. On one hand, platelets have been implicated adversely in cerebral malaria, a severe disease manifestation where microvascular occlusions develop in the brain, leading to inflammatory foci and brain swelling, and causing coma and often death. Platelets adhere to the cerebral endothelium and mediate accumulation of infected erythrocytes in the brain, with the postulated outcome of increasing severity or of even mediating this disease. On the other hand, platelets can directly kill parasites in the periphery by binding to infected red cells and thereby contribute to the host's ability to control the infection. Platelet binding to infected red cells releases an antimicrobial protein, platelet factor 4 (PF4), which accumulates inside the cell and kills the parasite by lysing the food vacuole. The Duffy-antigen, a red cell-expressed receptor of PF4, is required for the PF4 accumulation and parasite killing. Further understanding of the roles of platelets in malaria, especially in clinical disease is required. This knowledge may provide novel interventions and therapeutic tools, which are so desperately needed.

Main text (Word count: 3822)

Introduction

Malaria is responsible for over 400,000 deaths each year [1]. While most deaths are caused by *Plasmodium falciparum*, four *Plasmodium* species known to infect humans, namely *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, can also cause severe and fatal infection [2-5]. Malaria is a haematological disease and all its clinical consequences are due to the erythrocytic life stage of *Plasmodium*. Living in the bloodstream within the erythrocyte, interactions between parasitized cells and platelets are inevitable and frequent. These interactions are known to have a range of consequences that are important for both host and parasite survival.

Malaria

Plasmodium is a single cellular eukaryotic organism. It undergoes a complex life cycle, requiring mosquito and vertebrate hosts. Human malarial infections are initiated from the bite of an infected Anopheles mosquito, which delivers the sporozoite form of the parasite into the bloodstream. Sporozoites travel to the liver, where they infect hepatocytes and replicate many times into merozoites, which are then released into the bloodstream. The lifecycle in the human host is thereafter centred on the erythrocyte. Merozoites, the red cell infectious form of the parasite, invade and establish within erythrocytes, and then develop through morphologically distinct stages, including ring-stage (early development) and trophozoite-stage (late development), before finally resolving into new daughter merozoites. Lysis of the infected cell releases these merozoites, which then invade new erythrocytes. Rounds of cell invasion, replication and lysis may continue to increase parasite burden. Disease pathogenesis is driven largely by parasite biomass in the circulation [6-8]. Host innate and adaptive immune responses directed at controlling parasite development and propagation in the bloodstream are therefore most critical for modulating disease severity and determining host survival [9, 10]. An effective adaptive immune response against malarial parasites takes years to develop as the parasite population houses a large suite of antigenic variation. The lifecycle is continued by the development of dimorphic sexual stages (gametocytes) in the bloodstream, which are ingested by mosquitoes feeding on infected individuals. Gametocytes undergo meiosis in the mosquito. This produces recombinant, segregated parental chromosomes in the progeny. If the mosquito ingests more than one type of parasite, sporozoites will be produced with different combinations of variant antigens resulting in an immunologically unrecognisable parasite to hosts previously infected with either parental parasites.

Platelets and malaria

Platelets are the second most abundant cell in the bloodstream after erythrocytes. They are small (2 – 3 μ M diameter) and discoid in appearance, anucleate, and derive from the megakaryocytes of the bone marrow. Their primary physiological role is to regulate hemostasis, where they accumulate at sites of vascular injury and initiate coagulation to prevent bleeding; they also have well-known pathological roles in atherosclerosis and thrombosis.

In malaria platelets are known to be involved in all but the hepatic stage of infection. Their activation and adhesion are inhibited during mosquito feeding by the presence of the anopheline antiplatelet protein (AAPP) in the insect saliva. The mechanism involves direct binding of AAPP to collagen, which blocks platelet adhesion to collagen and its subsequent activation [11]; AAPP may also have value as an alternative anti-platelet drug [12]. Soon after the onset of erythrocytic stage, an acute and profound reduction in platelet count, or thrombocytopenia is commonly observed. The phenomenon

in humans is observed in infections caused by all *Plasmodium* species, as well as in most animal models of the disease. It has also been regarded a risk factor for mortality in African children with falciparum malaria [13], Southeast Asian adults and children with falciparum and vivax malaria [14] and adults with knowlesi malaria [5]. However, the mechanisms leading to thrombocytopenia are not fully understood, nor its subsequent effects on disease progression. This is important to understand because multiple studies indicate that platelets appear to both ameliorate infection by killing parasites and compromise the host by mediating adhesion of infected erythrocytes to the vascular wall. Herein, the possible causes of malaria-induced thrombocytopenia as well as the roles of the platelets in the erythrocytic stage of the disease will be discussed.

Thrombocytopenia in malaria – occurrence, clinical corollaries and causes.

A constant companion to a malarial infection is a decrease in circulating numbers of platelets. The phenomenon was first observed in rodent infection experiments in the 1950s [15] and has been regularly (and frequently) reported over the decades since. Thrombocytopenia is consistently seen in animal models of the disease, including in hamsters infected with *Plasmodium berghei*, a rodent malaria [16], in *P. vinckei*-infected mice [17] and *P. chabaudi*-infected mice [18] (Fig. 1), and is particularly severe in *P. knowlesi*-infected macaque monkeys [19].

In humans, normal platelet counts range between 150,000 to 450,000 platelets per microliter of blood; thrombocytopenia is normally defined as a decrease in platelet count to below 50,000. Thrombocytopenia is a common feature of all human malarias [20-36]. The phenomenon is most extensively analyzed in *P. falciparum* and *P. vivax* infections, mainly because of the relatively higher occurrence of malaria caused by these species. The proportion of malaria-infected patients with thrombocytopenia varies from study to study [13, 21, 28]. However, there is little doubt that the majority of patients with malaria show some level of thrombocytopenia. Percentages range from close to 100% of all malaria-infected individuals being thrombocytopenic, in an early study in Singapore [37], to 85% of infected children in India [38] and to 56 % of children in Kenya [28]. Moreover, platelet numbers are decreased in asymptomatic as well as symptomatic malaria [26, 27].

Malaria severity has been associated with thrombocytopenia and considered an independent predictor of death, including in falciparum malaria [13] and vivax malaria [14]. However, there are other reports showing that there is no direct link between the level of thrombocytopenia and the severity of malaria [21, 28]. Strong inverse relationships between thrombocytopenia with the level of parasitemia have also been reported, for example, in a study of 240 Nigerian children with falciparum malaria [26] and in vivax and falciparum-infected adults [39].

Malaria-associated thrombocytopenia may increase the propensity to bleed. However, hemorrhages are not common in malarial infections and studies of clotting parameters have reported conflicting findings. Abnormalities in prothrombin time, activated thromboplastin time and thrombin time were noted in one study of children with severe falciparum malaria [38], but not observed in another [40]. Prasad et al [38] also demonstrated that several platelet function indices, such as ADP-aggregation response, were depressed in greater than 90 % of malaria cases, although bleeding was rare. Bleeding has been reported some severe cases of malaria, with frequencies just above 5 % in one study [41], and 55 % of a cohort of autopsy cases [42]. The most common site of hemorrhage was in the retinas of children with malaria, part of the malaria retinopathy, which is a quartet of changes common in

cases of malaria [37, 43-45]. However, the relationship between retinal hemorrhage and clotting deficiencies and/or platelet changes has not been studied.

Regarding the mechanism for the loss of platelets during malarial infection, decreases in their production, or increases in the consumption and destruction of platelets, or a combination of both can be considered. Malarial parasites are known to profoundly suppress erythropoiesis [46-48], but no decreases in the number of megakaryocytes have been observed in the bone marrow of patients [37], and no compromise to thrombopoiesis was observed in experimental studies [49]. Some cytokines produced during malaria infection such as interleukin 10 (IL10) can suppress megakaryocytosis when administered to humans [50]. Based on this knowledge, Casals-Pascual and colleagues [51] have proposed that the elevated plasma levels of interleukin 10 (IL10) observed in a large cohort of children with malaria as a contributing factor to the concomitant thrombocytopenia present in these patients. However, no additional studies directly testing the potential suppressive effects on thrombopoiesis during malaria infection have been reported.

Multiple lines of evidence support a mechanism of accelerated clearance or consumption of platelets during malarial infection. Clearance has been suggested to occur by disseminated intravascular coagulation (DIC), immune-mediated destruction, pooling within the reticuloendothelial system, sequestration in the microcirculation and platelet apoptosis. Although early studies suggested DIC, variously characterized by increased prothrombin time and fibrin degradation, as a cause of thrombocytopenia [33, 52], more recent studies indicate the phenomenon is restricted to mainly cases of end-stage severe malaria [53-56]. As platelet number begins to decrease early in infection prior to the onset of severe symptoms, it is unlikely that this is due to DIC.

Immune-mediated clearance of platelets during malarial infection has been proposed as another important mechanism underpinning thrombocytopenia. In one small patient cohort of mainly *P. vivax*-infected patients with thrombocytopenia, high platelet-associated IgG and platelet-IgG complexes were detected during infection in the presence of malarial antigens; IgG levels reduced when platelet numbers recovered [57]. Platelet-associated IgG and IgM, and antibodies binding to the platelet antigens CD41 and CD49b [58] were detected in a single case study, however there was no evidence given that these were responsible for the thrombocytopenia. In another two-sample study from Japan, platelet-associated antibodies were found in two patients with vivax malaria [59]. The common thread linking these different studies is that upon treatment with antimalarial drugs, the binding of the antibodies disappeared. This is inconsistent with a hypothesis of autoimmune antiplatelet antibodies, because presumably, these would persist for some time after the malarial infection was cleared. This phenomenon appears instead to resemble heparin-induced thrombocytopenia, where antibodies directed against PF4-heparin complexes are captured by platelet Fc receptors, causing the activation and removal of platelets [60]. This disease is stopped when heparin administration is ceased. In a similar fashion, the binding of IgG to platelets in malarial infection requires the presence of malarial antigens and stops as soon as antigen is withdrawn upon successful treatment of disease. Further work is required to explore this hypothesis.

Malaria is often accompanied by splenomegaly. The increase in spleen size is due to an accumulation of macrophages, which phagocytose both infected and noninfected red cells [61]. There have been suggestions that platelets pool in these large spleens and are phagocytosed by the reticuloendothelial system [62]. However, there is no correlation between splenic size and platelet density [37]. Scintigraphy studies, which transfused radio-labelled platelets into normal and malaria-infected patients found no evidence for platelet pooling in the spleen, even in individuals with very large

spleens; the distribution of platelets was indistinguishable from control individuals. However, a greatly reduced platelet half-life was noted in the malaria patients [63]. Consistent with these studies, there were no differences in platelet numbers between splenectomized and spleen intact rhesus monkeys infected with *P. cynomolgi* [64]. These data would tend to rule out the reticuloendothelial system in playing a large role in the clearance of platelets in malarial infection.

Platelets may also directly bind to the endothelium during malaria, and thus be sequestered from the circulation. Under *in vitro* laminar flow conditions, cultured human endothelium exposed inflammatory cytokines induced expression of cytoadherent molecules such ICAM1 and von Willebrand factor multimers (VWF) that could bind to the introduced platelets [65]. Production of VWF has been observed in patients infected by *P. falciparum* [66] and *P. vivax* [67], and production of VWF was concomitant with the onset of thrombocytopenia in a controlled blood stage human infection study [68].

Malaria-induced thrombocytopenia may also occur as a result of platelet death, which can be mediated through a process analogous to apoptosis in nucleated cells involving the activation of platelet caspases [69]. In a mouse model of malaria, caspases have been shown to be activated causing platelet apoptosis [70]. This was associated with an increase in the number of circulating platelet microparticles, a possible corollary of platelet apoptosis. CD40 is a cell receptor belonging to the TNF receptor superfamily that can modulate cell proliferation, differentiation, and apoptosis. The thrombocytopenia in these mice could be decreased with pretreatment with anti-CD40L antibodies or with caspase inhibitors, indicating that apoptosis was initiated by CD40L and that caspases were crucial.

Roles of platelets in cerebral malaria

Based on the platelet's well-known abilities to bind both infected and noninfected red cells and the endothelium, they are thought to be a major mediator of cerebral malaria. Platelet binding to infected red cells involves the platelet receptors, CD36, and gC1qR [71, 72], although on the erythrocyte only one molecule, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) has been identified as a ligand for platelet CD36 [73]. Platelet-erythrocyte adhesion in human malaria, involving both infected and uninfected cells, has been variously observed as cell rosetting and clumping phenotypes [74, 75]. Infected red cells also bind to the endothelium, principally to avoid the reticuloendothelial system and enable parasites to reproduce more effectively. Some of the endothelial receptors involved in parasite sequestration are also involved in platelet-erythrocyte binding. It is therefore possible that adhesion to platelets is a side effect of the development of endothelial binding by infected red cells to avoid splenic clearance.

Platelets are implicated in the development of cerebral malaria (CM), which is a complex collection of syndromes specific to *P. falciparum* infections and a major cause of death. The pathology of CM appears to involve the physical binding of infected red cells to the endothelial cells of small vessels in the brain [76-78]. Erythrocyte-endothelial binding occurs in many organs, but in the brain, this produces cerebral malaria. Binding is believed to result in the obstruction of blood flow, as well as stimulating leukocyte accumulation, which leads to localized intravascular inflammation, and activation and damage of the endothelium; ultimately this could lead to disruption of the blood- brain barrier. Evidence for platelet involvement in CM has come from early observations reporting that platelets are seen in plugs in cerebral blood vessels. Microscopy studies on the brains of patients who

have died from CM have revealed the presence of infected red cells bound to the cerebral endothelium and with platelets [76, 79]. These and subsequent observations using experimental approaches in cell cultures [71] have led to the popular hypothesis that the binding of platelets to infected red cells mediates their adhesion to the endothelial surface. Other experimental model studies have also shown that platelets can activate the endothelium and release molecules with immunostimulatory functions [65]. However, a quantitative analysis of pathological manifestations in post-mortem cerebral tissues from CM patients found that only 6 % of all cerebral lesions contained platelets [80]. This would indicate that perhaps the role for platelets in CM is not as important as previously thought.

There is a large literature implicating a role for platelets in CM using a murine model of the syndrome: infection of mice with a rodent parasite strain, *P. berghei* ANKA. Infected mice develop a cerebral malaria-type syndrome early in the infection, called experimental cerebral malaria (ECM). However, the pathophysiology of ECM is quite different from human CM [81], and only certain mouse strains develop the condition, so-called ECM-susceptible strains. The basis for the differences between mouse and human CM is not known, but likely include both host and parasite factors. The cerebral lesions that develop in the mice involve mostly uninfected red cells and large mononuclear phagocytic cells but contain very few infected cells. This is very different from human cerebral malaria where the large mononuclear cells are absent, and the lesions contain mainly infected cells. It is, however, possible that there are some shared effector pathways between the human and mouse cerebral malaria [82]. Evidence that platelets are involved in ECM development include observations that platelet binding to the endothelium of cerebral capillaries is increased in *P. berghei*-infected mice, and that antibody administration to deplete platelets early in the infection prevents ECM [83, 84]. That mice deficient in either ICAM-1 or P-selectin are resistant to ECM is also taken to indicate a role for platelet binding to the endothelium [83]. However, if platelets were depleted later in the infection, one or two days prior to the onset of ECM symptoms, the syndrome still developed [84]. This would counter the argument that adherent platelets play a role in the development of CM as there were no platelets present in these mice, and yet the development of cerebral malaria continued unabated. More recent studies suggest that requirement of platelets in ECM involves activation very early after the parasite infection is established, and that this activation releases molecules that contribute to a systemic inflammatory response, which is a major feature of ECM. Inhibition of platelet activation early but not late after *P. berghei* infection prevents an acute phase response and concomitant ECM [85], and release of a platelet chemokine, platelet factor 4 (PF4) early after infection is required for ECM development [86]. In this later study, PF4 was also shown to stimulate monocyte cytokine production and depletion of monocytes prevented ECM. PF4-deficient mice also showed less trafficking of monocytes to the brain following *P. berghei*, an effect mediated through expression of CXCL3, which is a chemokine receptor that binds PF4. In support of these findings are the observations that mice either lacking the platelet-activating factor (PAF) receptor or treated with a PAF receptor antagonist, have an ameliorated ECM response [87]. Therefore, platelets appear more important for the inflammatory responses that go on to produce the ECM pathology.

Protective roles of platelets in malaria

Alternatively, and not exclusively, platelets have been shown to bind to infected red cells and to kill the parasites within. Early studies by Peyron and colleagues have shown that purified platelets added to cultures of *P. falciparum* inhibit the growth of the parasites [88]. This inhibition of growth required

direct physical contact between the platelets and parasitized cells and appeared to be mediated by a soluble platelet factor. The importance of these observations in malaria infection was recognized nearly a decade later where Polack and colleagues showed that antibody depletion of platelets in animals that were normally resistant to *P. berghei* infection (i.e. ECM-resistant) resulted in an increased susceptibility, manifested as greater a propagation of parasites in the circulation and reduced survival to the infection [89]. My laboratory reproduced these mouse findings in an *Mpl* knockout mouse (*Mpl*^{-/-}) that, due to a targeted deletion of the murine thrombopoietin receptor gene, has only 10 % of the normal number of circulating platelets [90]. The *Mpl* deletion also affects the generation of other hematopoietic progenitor cells, but their complement of mature leukocyte populations is relatively normal [90]. These mice were highly susceptible to infection with the murine malaria, *P. chabaudi* [18]. An important feature of this study was to measure the proportions of dead and dying parasites in the circulation of infected mice by applying an adapted version of the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). TUNEL labels fragmented DNA and reacts against nuclei of parasites that are undergoing necrosis or apoptosis. Remarkably, even though the *Mpl*^{-/-} mice developed similar parasitemia to wild-type animals during the initial stages of infection, only 20% of the parasites were labelled by TUNEL in the platelet-deficient mice compared to more than 40% in wild type mice. In other words, the platelet-deficient mice contained substantially greater numbers of viable circulating parasites, suggesting an ability of platelets to control the growth of circulating intraerythrocytic parasites. Further support of this hypothesis came from observations that these dead parasitized cells were frequently bound by platelets. Aggrey and colleagues have also shown in mice that platelet depletion just prior to *P. berghei* infection resulted in greater parasite burdens [85]. In contrast, another study reported that depletion platelets in mice just prior to *P. chabaudi* infection did not cause any changes to initial parasite growth [91]. However, this study did not distinguish the absolute parasite growth rates from the proportions of viable and dead parasites during the infection.

We have also confirmed Peyron and colleagues' observations of the *in vitro* parasite killing activity of purified human platelets against *P. falciparum* [18, 92], and observed a similar action against cultured *P. knowlesi* [93]. Further, we have demonstrated that platelets bind and kill intraerythrocytic parasites in malaria patients infected with *P. falciparum*, *P. vivax*, *P. malariae* or *P. knowlesi* [93]. In parasite cultures, infected mice and in human patients, platelets were observed preferentially bound to infected cells, although non-infected red cells also bound. Parasites in cells bound to platelets were dead. Killing of cultured *P. falciparum* by platelets also required the activation of the platelet, since killing was blocked by treating the platelets with molecules that block platelet activation, including nitric oxide, a P2Y₁ purinergic receptor antagonist (MRS2179), or aspirin [18]. Aspirin (acetylsalicylic acid) prevents platelet activation through the irreversible inhibition of cyclooxygenase-1 (COX1) and prostaglandin synthesis. This COX1 and platelet inhibitory effect is shared with other commonly used antipyretic drugs such as ibuprofen, collectively known as nonsteroidal anti-inflammatory drugs (NSAIDs). These observations may bring doubt to the wisdom of the continued use of aspirin and other NSAIDs in malarial infections.

The molecular basis of the parasite killing activity of platelets involves PF4. As well as its aforementioned monocyte-activating chemokine functions, PF4 is cytotoxic against several microorganisms, especially bacteria [94], and has been classified as a kinocidin based on these dual chemotactic and antimicrobial functions [95]. The antimicrobial activity is located in a domain near the carboxyl-terminus of PF4 that is similar in structure to the amphipathic alpha-helical configuration of antimicrobial peptides (AMP). AMPs interact with, penetrate and disrupt cell membranes

containing exposed negatively-charged phospholipids, which are absent in healthy mammalian cells but feature prominently in microbes and *Plasmodium*-infected cells [95]. Indeed, peptides containing the AMP domain of PF4 show modest anti-plasmodial activity [96], while a circularized dimer of the domain displays relatively potent activity, and kills parasites without the requirement for red cell DARC expression [97] (see below). PF4 and these PF4 AMP domain peptides were also shown to accumulate within the parasitized cell. Interestingly the accumulation resulted in the selective dissolution of the digestive vacuole, presumably due to the disruption of the membrane encompassing this organelle [96]. We have also observed that mice with a genetic disruption of murine *Pf4* experience higher burdens of viable parasites when infected with *P. chabaudi*, although their survival is not affected, perhaps indicating that in mice additional platelet molecules with anti-parasite functions molecules may be required for platelet protection against Plasmodium infection [98].

The Duffy antigen is important for the killing action of PF4 (and platelets). Also known as the Duffy antigen receptor for chemokines (DARC), the molecule is expressed on the surface of red cells and binds chemokines, including PF4; its proposed function is to modulate concentrations of serum chemokines by acting as a decoy or scavenger receptor [99]. Platelet and PF4-directed killing were shown to absolutely require DARC, both through the use of DARC ligands and antibodies that outcompeted PF4 for binding and prevented parasite killing, and in DARC-negative red cells, where the platelet and PF4 killing activities were abrogated. In addition, PF4 accumulation was significantly impeded if parasites were grown in DARC-negative red cells [92]. It is not known how DARC enables PF4 accumulation and parasite killing. Receptor-ligand binding may trigger cell uptake of PF4, or DARC may increase the surface concentration of PF4 such that it can diffuse inside the cell by virtue of its membrane penetrating AMP domain. There is a null allele of the Duffy antigen which is fixed as a homozygous allele in most people living in sub-Saharan Africa [100]. If the role of platelets in parasite killing and protection against infection is as important as is predicted from the *in vitro* culture and animal studies, the absence of PF4-dependent killing of parasites by platelets in these individuals could represent a novel malaria susceptibility factor.

Protective roles for platelets in other infectious diseases?

Looking beyond malarial infection, there is substantial evidence for platelets as a general mechanism of protection against blood borne pathogens. By both number and cellular mass, platelets outnumber leukocytes, and are well-placed to sense and respond to microbial infections of the circulation. They have been shown to be integral for many immune responses, directed through both the production and secretion immunomodulatory molecules, and by cell-to-cell interactions with various leukocytes. These properties and functions have been extensively reviewed by others [101, 102]. Platelets are also an abundant source of antimicrobial molecules [94, 103] and have been shown to directly kill a range of microbial pathogens, including *Staphylococcus aureus*, *Streptococcus*, Schistosomones and *Aspergillus* [104-110]. As a corollary, many studies show they are required for the efficient control of pathogen growth and/or host survival. For example, platelet-depleted mice are more susceptible to *Staphylococcus*, *Streptococcus* or *Pseudomonas* infection [111-114]. In other animal infection studies, platelets were shown to enhance the antimicrobial functions and pathogen killing actions of other immune cells, including promotion of leukocyte infiltration and neutrophil extracellular trap formation [115-117]. In humans, thrombocytopenia is a frequent correlate of many clinically significant infections. Interestingly, low platelet counts have been associated with poor prognosis in tuberculosis patients [118] and reduced survival in sepsis patients [119], as well as increased risk of fungal infections in liver transplant patients [120]. One study has also suggested the natural history of

HIV infection may be influenced by platelet count [121]. Further studies on the relationships and roles of platelets in human infectious disease are required to determine the importance of all of these observations, especially their functions early in infection before thrombocytopenia develops. Furthermore, to underscore the fact that there is still much to learn about platelets and infection, Chapman and colleagues reported data showing that platelets have the ability to present malaria parasite antigens on a class II MHC molecule. Platelets contain proteasomes, β 2-microglobulin and HLA, and can present parasite antigen and stimulate CD8+ T cells [122]. This raises the important question as to whether platelets also play a role in the adaptive immune response. Clearly, we have only just begun to understand how the seemingly innocuous platelet lies at the interface of many important infections, including malaria.

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