

The Role of Platelet Physiology in the Pathogenesis of Severe (Complicated) Malaria

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Abstract

This review represents an attempt to shed light on the pathogenic role of platelets in severe cases of malaria infection. It was found that platelets contribute to an increase in severity by way of inflammatory-mediated processes, including but not limited to: receptor functions, secretion of platelet-derived cytokines and chemokines, and adhesion of parasitized RBCs to the endothelium of critical microvasculature. The additive effect of these pro-inflammatory and pro-coagulant properties makes platelets pathogenic during the course of malaria infection, adding to the severity of the infectious disease.

Malaria is one of the leading causes of death in tropical, underdeveloped countries throughout the world. Upon infection, the life cycle of the *Plasmodium sp.* begins and ends in the bloodstream where erythrocytes are infected and consequently destroyed, causing symptoms such as anemia, respiratory sequelae, cerebral malaria, metabolic acidosis, and eventually organ failure, leading to death (Achidi et al. 2012; Barbier et al. 2011). However, before the total destruction of erythrocytes occurs, there seems to be a mode by which the parasitized red blood cells (pRBCs) become sequestered in particular sites throughout the vascular system (CDC 2010). In many severe cases of malaria, occlusion of vessels seems to further complicate the overall severity of the disease. Cerebral malaria (CM), possibly the most severe type, is synonymous with its specific location of impairment, evidenced by the sequestration of pRBCs mainly in the central nervous system (CNS). The consequent occlusion of brain microvasculature can lead to convulsions and coma, and death is reported often (Beare et al. 2009; Hirayasu et al. 2012; von Seidlein et al. 2012).

The pathway by which pRBCs are sequestered is an important topic of discussion among the scientific community. During CM, severe complications are primarily the result of occluded vessels, and severe retinopathy has been questioned as a possible indicator of CM (Essuman et al. 2010). In these retinal and brain vessels, a common finding in virtually all CM patients is an increased platelet profile. Platelets become activated as early as *Anopheles* mosquito feeding, when the mosquito creates the blood vessel, and the continuance of activation at sites deep to the injection site is thought to be one cause of an increase in the severity of symptoms (Yoshida et al. 2008). Upon activation, platelets adhere to the walls of vessels, and may play an important role in sequestration of pRBCs (Faille et al. 2009). Even more interestingly is the cooperation between platelet activation and a cascade of immune responses that seem to follow. All

infectious agents promote the generation of immune responses, and many cytokines like TNF, which has been measured in large amounts during CM infection, contribute to cell signaling cascades that lead to the expression of adhesion molecules on the surface of endothelial cells. ICAM and P-selectin have been associated with the cytoadherence of platelets and pRBCs to endothelial cells, and interestingly enough, platelets themselves have the ability to present these same molecules on their surfaces and are also able to secrete immune factors that help to initiate the systemic immune response (Francischetti et al. 2010). In this paper, the pathogenic role of platelets is discussed as a means of increasing complication severity in those with malaria.

Platelets not only engage in pRBC sequestration, but they also are involved in the pathogenesis of endothelial cell (EC) damage during *Plasmodium sp.* infections (Combes et al. 2004; Barbier et al. 2011; Sarfo et al. 2005; van der Heyde et al. 2011; Wassmer and de Souza et al. 2006). Although platelets have gained a reputation as effector cells in clot formation and subsequent cellular repair, it has been shown that the excessive contribution of platelets during infectious disease can lead to damage (Sarfo et al. 2005). Platelets seem to be used as a type of initiator of the immune response, which can induce apoptosis of ECs (van der Heyde et al. 2011; Wassmer and Combes et al. 2006; Wassmer and de Souza et al. 2006). The contribution of the production of cytokines during the systemic response to infection and inflammation gives rise to EC gene changes (Barbier et al. 2011). Human studies have shown that ECs infected with the malarial agent *Plasmodium falciparum* and exposed to TNF exhibit an increase in platelet cytoadherence and in return, platelets potentiate the toxic effects of the cytokine, increasing the likelihood of apoptotic damage (Barbier et al. 2011; Wassmer and Combes et al. 2006). Many pro-apoptotic genes are upregulated during this type of immune response, and platelets propound the biosignaling that causes transcriptional changes (Barbier et al. 2011). Platelets also

upregulate the expression of a monocyte transcription factor that is needed for differentiation and resulting innate inflammatory properties (Srivastava et al. 2010). The secretion of a chemokine called PF4 (platelet factor 4) helps to induce the transcriptional change that promotes inflammatory activation of monocytes. However, competition for parasite binding between the two cell types has been reported to negate this possible positive effect exhibited by platelets (Dzik et al. 2010).

Monocytes, phagocytic cells that contribute to the killing of parasites and foreign bodies, have CD36 receptors on their surfaces (Dzik et al. 2010). CD36 is a cell marker and scavenger receptor on the surface of many different cell types, including ECs, monocytes, and platelets (Dzik et al. 2010). Platelet CD36 has been implicated in the pathogenesis of malaria by binding to pRBCs and apoptotic ECs. Dzik et al. studied the influence of increasing platelet counts on the decreased median fluorescence intensity (MFI) of monocytes. Using flow cytometry to indicate the immunophenotype of monocytes and platelets, it was indicated that increasing the amount of platelets in the sample mixture influences the amount of antibody that binds to the CD36 receptors of monocytes (Dzik et al. 2010). This shows competition between the two cell types for antibody that interacts with the CD36 receptor (Dzik et al. 2010). This means that platelets directly affect the phagocytic actions of monocytes by competing for the foreign body. As a means of contributing to the pathogenesis of malaria, one can visualize the lasting effects of such competition: increased parasitemia due to decreased phagocytic activity, just another way that platelets contribute to disease severity.

By providing receptors on their surfaces for cytoadherence and circulating immune cell capture, platelets increase their pathogenic effects during infection (Wassmer and Combes et al. 2006; Wassmer et al. 2004). Although evidence of the non-association between platelet-mediated

clumping and severity has been reported, there are several studies that show the negative influence of platelet-mediated phenomena, especially that of surface receptor instigation (Arman et al. 2009; Biswas et al. 2007; Chotivanich et al. 2004; Cserti-Gazdewich et al. 2008; Srivastava et al. 2008; Pain et al. 2001; Wassmer and Combes et al. 2006; Wassmer et al. 2004; Wassmer et al. 2008). Platelets become activated as a result of pRBC binding to CD36 receptors, and this activation is said to cause sequestration, keeping in mind that this is not the only path to platelet activation (Srivastava et al. 2008; Wassmer et al. 2008). The process of autoagglutination in malaria patients shows the involvement of this platelet receptor. Autoagglutination, the clumping of RBCs due to the presence of antibodies on their surfaces, occurs with the help of the CD36 receptor on platelets (Chotivanich et al. 2004; Cserti-Gazdewich et al. 2008; Pain et al. 2001). The receptor acts as a lodging place for the parasitized portion of the pRBC. A protein on the parasite surface binds with high affinity to the CD36 platelet receptor, which is categorized as a platelet-mediated ligand-receptor interaction, and is associated with severity (Chotivanich et al. 2004; Cserti-Gazdewich et al. 2008; Pain et al. 2001). It has also been shown that this receptor acts in conjunction with another receptor on the surface of platelets to bind pRBCs, consequently inducing sequestration. The gC1qR/HABP1 receptor was identified in a study by Biswas and colleagues in an attempt to find the cause of remaining severity when antibodies were used against the CD36 receptor, proving that this was not the only receptor involved in the sequestration of pRBCs (Biswas et al. 2007). Using *E. coli* and recombinant human DNA, the investigators found that the presence of the gC1qR/HABP1 receptor increases upon platelet activation and that this receptor is used directly in the clumping and sequestration of pRBCs. There has also been strong evidence that supports the theory of an EC protein multimer that has been previously shown to mediate platelet adhesion at sites of vascular damage (Bridges et al.

2010). The protein complex VWF (von Willebrand factor) is said to reach out in string-like projections upon activation of the endothelium and provide an adhesive area for platelets to bind. These platelet-decorated strings support the clumping of platelets, and in turn, support the severe consequences of pRBC sequestration by binding to platelet receptors. Here, yet again, is an example of EC activation coupled with platelet activation that ultimately leads to an increase in severity of malarial infection (Bridges et al. 2010). These VWF proteins give rise to an increased exposure and susceptibility of platelet receptors by way of stabilization and capture of platelets.

Studies using murine models to associate the role of platelet activation to increased cytokine production show a magnification in the apoptotic pathway by using P-selectin, an integrin/receptor found on both the surface of ECs and platelets and used in leukocyte rolling and adherence, as a predictor of infection severity (Combes et al. 2004; Sun et al. 2003). When platelets bind EC P-selectin, they become activated and begin to express their own P-selectin (Sun et al. 2003). The increased expression of platelet P-selectin via activation by EC P-selectin increases the number of receptors that can be used in the cytoadherence of pRBCs. Using EC P-selectin knock-out (KO) mice and control groups, it was found that these KO mice were protected against CM (Combes et al. 2004). EC P-selectin has been shown to be expressed before platelet P-selectin, proving that EC activation may be needed to activate subsequent effector cells, such as platelets. Working as a receptor for the parasite protein on the pRBC just like that of platelet CD36 and gC1qR/HABP1, platelet P-selectin increases the magnitude of sequestration (Wassmer et al. 2008). In a synergistic manner, both CD36 and P-selectin on the platelet surface increase this phenomenon (Wassmer et al. 2008). All of the afore-mentioned platelet receptors increase the likelihood of pRBC sequestration, adding to the severity of the

disease. With the help of VWF proteins on ECs, even more susceptibility to platelet receptors is made possible.

When platelets adhere to ECs, they not only express their own adhesion molecules, but they also activate TNF production. This proves the coexistence and synergistic nature of platelets and TNF in the pro-apoptotic pathway. Using P-selectin as an indicator of consequent cytoadherence of platelets, it was shown that platelets continue the cascade of signaling events that lead to cellular damage and possible cytoadherence to the microvasculature (Combes et al. 2004; Sun et al. 2003). However, Wassmer, et al. proposed that TNF-stimulated ECs cause platelet binding and the addition of yet another cytokine that has apoptotic capabilities (Wassmer and de Souza et al. 2006). Upon binding to the activated ECs, platelets secrete TGF- β , a cytokine that is usually indicative of anti-apoptotic events. In this study, using human ECs co-cultured with prepared platelets and exposed to immune factors, the investigators found that platelet-derived TGF- β during infection with malaria has the opposite effect, inducing apoptosis, permeability changes, cerebral edema, and hemorrhaging (Wassmer and de Souza et al. 2006).

There are also implications for the blood-brain-barrier (BBB) and platelet activation. In an experiment using a murine model, the presence of a signaling molecule was shown to be associated with cellular damage inflicted upon cells of the BBB. The chemokine RANTES is secreted by platelets in large quantities upon adherence to ECs (Sarfo et al. 2005). Chemokines are immunoregulators that help to guide immune cells to the appropriate site to carry out the response. The presence of RANTES yields a destructive response in malaria patients due to its receptors being located on numerous cells that form the BBB. Upregulation of the gene that encodes for this chemokine causes an overshoot response that induces apoptosis and vascular leakage in and around the BBB. Damage to this invaluable site leads to death preeminently by

way of hemorrhage and pRBC leakage into the brain space (Sarfo et al. 2005). The ECs that are affected contain numerous receptors on their surfaces that facilitate this destructive force.

Working in conjunction with platelet receptors, ECs provide a lattice upon which immune cells and pRBCs can be bound. Platelets are also precursors of cellular debris called microparticles that like RANTES, have been shown to increase the inflammatory response. Evidence of an increased pro-coagulant state in response to an increase in microparticle release is another secreted pathogenic factor that contributes to the disease severity in malaria (Combes et al. 2005; Faille et al. 2009).

As malaria continues to affect millions of people across the globe, it becomes evident that continued research is crucial to help prevent the epidemic that causes so much death each year. It is evident that platelets, the inflammatory molecules that take part in clot formation and cellular repair, also work against the body in an overshoot process during malarial infections. This autoimmune-like process can also be seen in non-pathogenic inflammatory diseases like atherosclerosis and other coronary-related diseases, and in both the autoimmune pathway and that of the pathogenic, the major contribution implicative of platelets seems to be the increase of the immune response (Combes et al. 2005, Sun et al. 2003). The negative effects of TNF are potentiated by platelets, and the activation of the latter causes increased sequestration of pRBCs. Adhesion molecules are not only expressed on the surface of ECs, but also on the surfaces of platelets, providing a binding area for ECs and pRBCs. These pathogenic effects of this well-known biological molecule contribute tremendously to the severity of malarial infections, and this is made apparent by their removal early in the course of disease. The decreased signs of severity seen in conjunction with their removal proves that platelets play a major role in the processes that further the destructive nature of *Plasmodium sp.* infections. Early detection

methods like visualizing the retina and using specific contrast agents during scans could provide evidence of severity and consequently give reason for the early removal of platelets at sites where damage has been associated with severe complications (von zur Muhlen et al. 2008). Even the possibility of using the mosquito's own saliva proteins may help to deplete platelets in severe cases (Yoshida et al. 2008). Although platelets have been shown to aid in the killing of pathogens early in the course of disease, in severe cases of malaria infection, platelets eventually begin to contribute to the virulence of the disease (McMorran et al. 2009). Overall, this review attempts to shed light on the pathogenic nature of platelets and how their destructive presence could possibly be controlled to decrease the severity of symptoms and the number of deaths that result from the infectious disease malaria.

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