Review Article

Malaria and Thrombopoiesis: A Possible Mechanism for the Malarial Thrombocytopenia

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Introduction

Platelets, traditionally considered as thrombogenic cells in mammals are now being considered as an important immunological entity. In recent years, the antiparasitic role of platelets have also been recognized [1,2]. Platelets and their specific chemokines (viz. CXCL4) act on infected erythrocytes (iRBC) to reduce the host parasite load [3]. It is also suggested that parasite too influences the platelet count in their host for its survival [4]. In fact, elevation of parasite load is directly proportional to the progression of thrombocytopenia (the loss of platelets) [5,6]. This suggests that maintenance of appropriate platelet count is indispensable for combating the infection and controlling the severity of the infection [1]. Previous studies have shown that the infection results in an increase in thrombopoietin possibly due to the feedback process initiated by the liver to counter the decrease in platelet number [7]. Nevertheless, this is not sufficient to prevent the loss of platelets. Suggesting that destruction of platelets in malarial thrombocytopenia is accompanied by simultaneous suppression of thrombopoiesis. We believe that one of the possible mechanism resulting to this phenomenon involves parasite factor(s) such as metabolic products and parasite specific protein. Therefore, it will be valuable to explore whether the parasite factor(s), which also have access to the bone marrow and megakaryocytes, suppresses the thrombopoiesis. These studies would be able to explain the mechanism and thereby open the possibility of extending the standard Immune Thrombocytopenia (ITP) therapy to the malaria patient, specially for severe malaria.

Overview

It has long been accepted that platelets play an important role in the pathogenesis of malaria, however their mechanistic role remains unclear [3]. Thrombocytopenia in malaria is a hallmark of severity and is often proposed as a mark of malaria infection when combined with fever [8,9]. Platelets may act as an agent of innate immunity that reduces parasitemia [10,11], while they may also participate in thrombo-inflammatory response of the body. Despite a little controversy [12], the innate immunological role of platelets is now being widely accepted [13]. Thrombocytopenia may provide a survival advantage to the parasite upon the onset of the infection. Since, the concomitant increase in thrombopoietin level does not result into the increase in platelet count in malaria, a parasite factor(s) yet to be identified might contribute to thrombocytopenia. We hypothesize that parasite factor(s) released in circulation may interact with the megakaryocytes in the bone marrow and can suppress its function resulting in to thrombocytopenia (Figure 1).

Malaria

Malaria is a parasitic protozoan disease caused by plasmodium species and transmitted by the female Anopheles (mosquito) vector. Malaria continues to be a worldwide malady, with one third of the global population being at risk of infection. Approximately 250 million people develop clinical infections annually and at least half a million die each year, where children and pregnant woman form a majority. There are six human species malaria parasites namely; *P falciparum, P vivax, P. malariae, P. Knowlesi and two subspecies of P ovale* [14]. Among these, *P falciparum* causes the most severe form of disease, morbidity and mortality. Uncontrolled malaria can result to conditions such as severe anemia, organ failure, cerebral damage and even death.

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Figure 1: Formation of platelet.
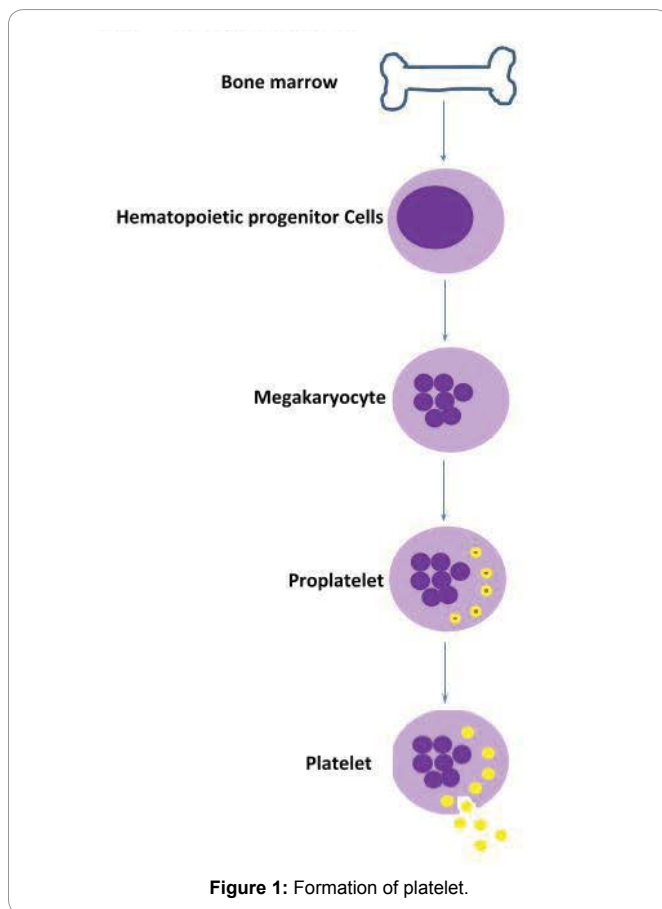
Each type of malarial infection is associated with anemia and thrombocytopenia. Severe anemia has been correlated with poor prognosis but the role of thrombocytopenia is less clear. Malaria parasite causes erythrocyte (RBC) and liver stage infection inside its human host [15]. In mammals, it undergoes asexual life cycle where it first invades the RBC, divides exponentially, erythro-lytically released and later invades the other RBC. This results into the anemia in the affected the host, and uninfected RBC also lost due to bystander effects. Apart from the destruction of RBC there are other damages caused by the parasite e.g organ failure. These are basically the aftermath of the parasite's attempt of survival inside the affected host. Parasites release numerous proteins/ molecules that can potentially switch on or off the host's normal physiological and immunological functions. Interestingly, there are strong evidences suggesting the parasite antigen can affect the bone marrow of the human host [16–18]. These antigens are released either directly by the parasite via RBC's cellular transportation mechanism or synthesized and released by the infected red blood cells (iRBC) [19].

Platelets

Platelets are terminally differentiated cells of myeloid origin derived from megakaryocytes that are primarily responsible for human hemostasis [20-22]. They are also associated with the progression of thrombosis and atherosclerosis. In recent decades platelets have also been identified in inflammatory processes and immune responses [23,24]. Platelets hosts numerous chemokines and inflammatory cytokines that are stored in its granular structures known as alpha, dense and lysosomal granules. These chemokines are released from activated platelets upon the onset of infectious disease. Among these an important chemokine is CXCL4 also known as platelet factor 4 (PF4). In malaria,CXCL4 has been correlated to the progression of cerebral malaria [25]. Also, the CXCL4 promotes the neurovascular damage in case of malaria by acting as a chemoattractant for immunological cells such as T Cells, monocytes and macrophages [26]. This results into the blood brain barrier breakdown resulting into the cerebral malaria. Simultaneously, both platelet and its chemokine CXCL4 also participate in parasiticidal activity [27]. CXCL4 interacts with the Duffy antigen of erythrocytes to carry out parasiticidal activity [26]. Therefore, platelets and their chemokines may play a dual role in malaria infection. However, the fact that there is increase in thrombocytopenia with increased parasitemia and there is platelets involvement in parasite clearance, supports the idea that the benefit of platelets activation in infection might outweighs its deleterious effects of platelets. In fact, a basal level of CXCL4 in serum is supposed to provide a survival advantage to the host against the parasite infections. It has been shown that cellular immunity against the malaria could largely contribute to the thrombocytopenia in case of malaria [28,29]. In general the thrombocytopenia is an aftermath of exaggerated immune response e.g. autoimmune diseases [30]. The role of parasite produced hemozoin in thrombocytopenia cannot be underestimated, which can proceed with or without the involvement of immune response [31,32]. We assume that maintaining normal count of platelets could result into efficient clearance of platelets and therefore, understanding the parasiticidal role of platelets may help developing new generation of therapeutics for malaria. Most importantly, understanding the parasite component that participates in platelet destruction and suppression of thrombopoiesis would also give the target for development of therapeutics for thrombocytopenia in malaria (Figure 2).

Thrombopoiesis

Thrombopoiesis is the process of platelet production from megakaryocytes, the platelet precursor cells found mostly in bone marrow. The steps from megakaryocyte formation to platelet production essentially involves the six stages, (i) megakaryocyte development in bone marrow from hematopoietic progenitor cells (ii) polyploidization involving the process known as endomitosis (iii) cytoplasmic maturation (iv) proplatelet/proplatelet formation and release to the adjoining microvessel endothelium (v) proplatelet - pre-platelet interconversion (vi) a true platelet formation [33]. These steps, from hematopoietic stem cell differentiation to platelet release, are critically dependent upon thrombopoietin [34]. Among all the hematopoietic progenitor cells megakaryocytes are the only ones with the tendency to undergo the polyploidy before differentiation. Megakaryocytes achieve this through a process of nuclear replication without an effective cytoplasmic disintegration, known as endomitosis. This increased nuclear content and polyploidization is fundamentally important for the platelet production. The large size and abundant cytoplasm allows the megakaryocytes to produce 2000 to 5000 platelets per megakaryocytes. The polyploidization and progression into the proplatelet formation stage is termed as maturation process. The maturation process constitutes establishment of demarcation membrane system
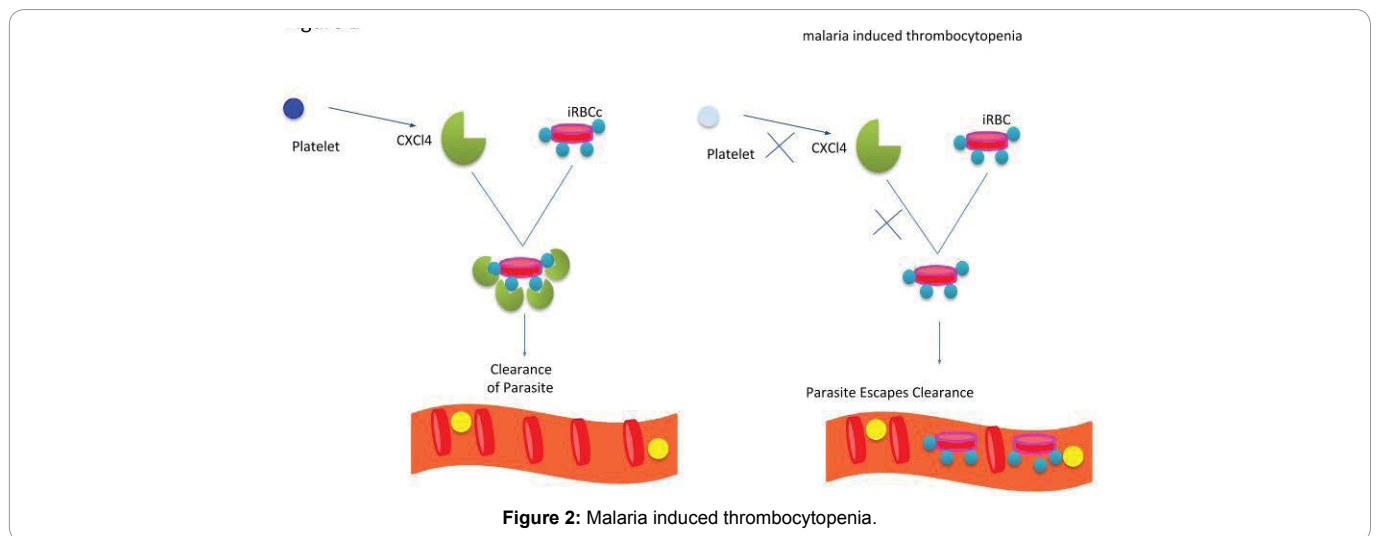
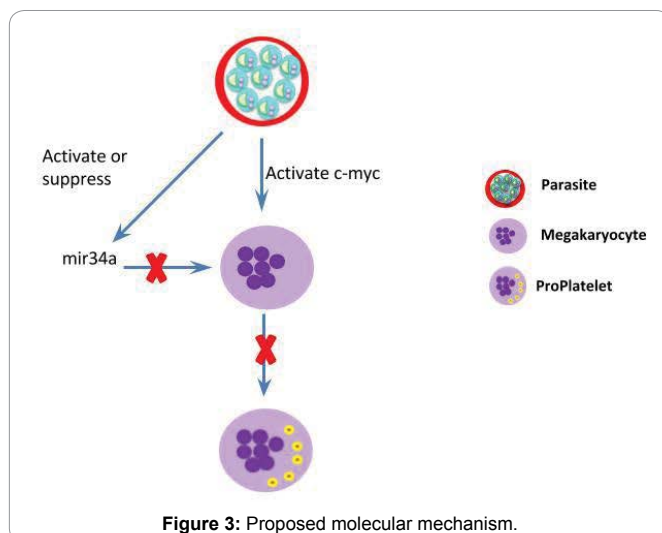**Figure 2:** Malaria induced thrombocytopenia.

Figure 3: Proposed molecular mechanism.

for effective assembly and release of platelets [35]. A significant progress has been made in understanding the mechanism by which the megakaryocytes undergo the process of maturation, polyploidization, proplatelet formation and resulting into the platelet release. In recent years, it is being highly appreciated that actin polymerization and apoptosis play an important role in differentiation of megakaryocytes into the platelets [36]. This has accelerated our efforts to understand the pathogenesis of thrombocytopenia (Figure 3).

Malaria and Stem Cells

N early each infectious disease has some degree of influence on stem cell production, count, phenotype and differentiation of stem cells [37-39]. Using systematic studies mesenchymal stem cells were reviewed for their protective ability in neurodegeneration [37,40]. However, hematopoietic stem cell (HSCs) have also been studied in infectious diseases [41]. Malaria has strong influence on HSCs and the number of the HSCs are generally reduced in the host [42]. This effect has been found to occur at the level of the bone marrow [43,44]. The intracellular SDF-1/CXCL12–CXCR4 axis commonly considered as responsible for the hematopoiesis [45]. Indirect studies have indicated that this malaria can affect this axis resulting into the impaired hematopoiesis [46-48]. It has also been indicated the hematopoietic growth factors such as erythropoietin and GM-CSF can play a possible protective role is malaria [49,50]. These evidences suggest that production of damage myeloid cells induces pathogenesis in case of malaria.

Thrombocytopenia in Malaria

Malaria is known to cause hematological changes such as anemia and thrombocytopenia. The anemia is a manifestation of the infection, RBC hemolysis, splenic sequestration, an inflammatory response and erythropoietic suppression. Anemia in general is well understood and is a predictor of morbidity and mortality. Thrombocytopenia however is yet to be understood for their correlation to the morbidity. Thrombocytopenia is a major complication encountered in conditions such as idiopathic thrombocytopenic purpura (ITP), myelodysplastic syndromes, chemotherapy, aplastic anemia, complications during pregnancy, surgery and infectious diseases such as malaria, HIV and dengue. Onset of malaria affects all types of blood cells in patients including platelets. Thrombocytopenia is a common symptom, caused by falciparum, vivax and knowlesi parasite infection [5,51]. Although, its correlation with the severity is yet to be established it may still present conditions such as bleeding diathesis in patient. The studies show that increase in parasitemia in patient is proportional to the degree of thrombocytopenia [6,52]. This suggests that the parasite multiplication in host might result into the destruction of platelets. Vice versa, the presence of platelet might contribute to the destruction of parasite. Therefore, thrombocytopenia somehow contributes to the survival of the parasite in the host. However, the level of thrombopoietin (TPO) in malaria patients is either unaffected or even goes up with the progression of the disease [7]. This is probably due to the feedback process of liver and kidney in response to the thrombocytopenia. However, the TPO released may not contribute to the thrombopoiesis, since no effective increase is observed in platelets count. Therefore, the thrombocytopenia in malaria might occur at two levels (i) destruction of platelets by the spleen under the influence of parasite antigen bound to the surface (ii) suppression of thrombopoiesis by parasite antigen that access to the bone marrow. Since, use of TPO may not be a viable answer,

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studying the antigen that suppresses the megakaryocyte function can be valuable.

Thrombocytopenia in other Diseases

Similar to malaria, viral infections are supposed also have adverse effect that results in thrombocytopenia. The most common example is dengue hemorrhagic fever [53-55]. Platelet count is severely reduced in dengue infection causing severe vascular disturbances [56]. Platelet count is severely affected in many viral infections in similar fashion [57-59]. The major mechanisms for a reduced platelet count are decreased production from the bone marrow megakaryocytes or the increased destruction of platelets in circulation [60]. The onset of disease can accelerate either or both of the processes. In case of defect in thrombopoiesis this can occur at any of stages involved [61]. This implies that the error at any of the steps viz. differentiation of megakaryocyte from CD34+ lineage cells, maturation or the biogenesis of the platelets from the cytoplasm can result into the reduction of platelet production. The onset of various diseases can also result into the impaired production of TPO, resulting into the impaired platelet production [34].

Future Direction

Malaria continues to kill hundreds of thousands of patients around endemic zone, including the travellers visiting these area. It remains a major deleterious factor for social and economic well being of these area. With only limited success of ongoing vaccine project, the drug discovery and drug repurposing remains one of the dependable ways of malaria therapy development. However, complexity has been added by the emergence of drug resistance. We therefore suggest development of novel yet cost effective cellular and immunological therapies. This includes the recruitment of host's own immunological machinery to combat the parasite growth. Our hypothesis is that increase in platelet count in malaria might have improved outcomes to the patients. Platelets produced from rescued thrombopoiesis can contribute to the improved therapeutic outcome. Reversing thrombocytopenia can also simultaneously bolster the effect of drug such as artemisinin and chloroquine.

It is imperative to understand the mechanism by which the parasite suppresses the thrombopoiesis. However, the investigation of mechanism does not come without potential roadblocks. These include failure to obtain material such as cord blood as per schedule and to obtain appropriate stage specific parasite culture. Several investigators believe that megakaryocytes obtained by *in vitro* differentiating progenitor cells from cord blood may not resemble megakaryocytes present in bone marrow. Also, the possibility that parasite may suppress the megakaryocyte activity indirectly cannot be ruled out. This means that parasite antigen may interact with other tissues, such as hepatic and renal cells, to produce yet unknown thrombopoietic suppressor. A cell culture study may not be able to identify such an indirect process. However, studies using ITP model have usually engaged the cell culture model by adding the cultured megakaryocytes with the patient serum. Therefore, one can utilize several cell culture strategy to perform these studies even for preclinical evaluation.

There is little information about the mechanism by which parasite and its antigen suppresses the platelet production from megakaryocytes. This mechanism may have prohibitory effect over events such as proplatelet formation. It is believed that the platelet biosynthesis is accompanied by the initiation of apoptotic pathway in the cell. We have discovered that c-myc is correlated with the inhibition of apoptosis in megakaryocytes (unpublished data). We anticipate that the parasite antigen may activate this gene in megakaryocytes and inhibit the proplatelets formation. We have also observed that miR34a is microRNA responsible for the suppression of the c-myc activity (unpublished data). Therefore, its expression is supposed to be elevated under thrombocytopenic conditions. We also employed RNASeq studies to identify other potential miRNA that participate in proplatelet formation. We expect that these miRNA are either over-expressed or underexpressed under the influence of parasite antigen. Standard thrombocytopenia therapy can also be implemented to revert the thrombocytopenia in malaria. An advantage of utilizing these therapy is that they would not require additional clinical trial steps, as they have already been tested for efficacy, efficiency and toxicity in human. We postulate that Nplate and Promacta might successfully block the megakaryocyte suppression in malaria.

Conclusion

In conclusion we hypothesize that malaria parasite produces factors that reduce the platelet production from the megakaryocytes. This saves the parasite from the platelet mediated clearance as a survival mechanism. We hypothesize that reversing this phenomenon could increase the number of platelets in the host and would result into the parasite clearance by the platelets.

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