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Review Article

Hemozoin Inhibition and Control of Clinical Malaria

Chibueze Peter Ihekwereme,[1] Charles Okechukwu Esimone,[2] and Edward Chieke Nwanegbo[1]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka 420281, Nigeria*
[2] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka 420281, Nigeria*

Correspondence should be addressed to Chibueze Peter Ihekwereme; chibuezep@yahoo.com

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Malaria has a negative impact on health and social and economic life of residents of endemic countries. The ultimate goals of designing new treatment for malaria are to prevent clinical infection, reduce morbidity, and decrease mortality. There are great advances in the understanding of the parasite-host interaction through studies by various scientists. In some of these studies, attempts were made to evaluate the roles of malaria pigment or toxins in the pathogenesis of malaria. Hemozoin is a key metabolite associated with severe malaria anemia (SMA), immunosuppression, and cytokine dysfunction. Targeting of this pigment may be necessary in the design of new therapeutic products against malaria. In this review, the roles of hemozoin in the morbidity and mortality of malaria are highlighted as an essential target in the quest for effective control of clinical malaria.

1. Introduction

Malaria has plagued humankind since ancient times and is still a significant threat to half of the world's population [1]. Malaria is the fifth most common cause of death from infectious diseases worldwide (after respiratory infections, HIV/AIDS, diarrheal diseases, and tuberculosis) and the second in Africa, after HIV/AIDS [2]. Recent estimates show that as many as 3.3 billion people live in areas at risk of malaria in 109 countries or territories [3]. In addition to its health toll, malaria puts a heavy economic burden on endemic countries and contributes to the cycle of poverty people face in many countries. For example, it is estimated to have in Africa alone contemporaneous costs of at least US$12 billion per year in direct losses (e.g., illness, treatment, and premature death), but many times more than that in lost economic growth [4]. Malaria continues to be a major global health concern, with an estimated 243 million cases of malaria worldwide [5]. The vast majority of cases (85%) were in the African region, followed by Southeast Asia (10%) and Eastern Mediterranean regions (4%) [5]. Malaria accounted for an estimated 863 000 deaths in 2008 [5]. Malaria is a leading cause of child mortality in Africa, claiming a life nearly every 30 seconds [6]. Children are at highest risk for severe malarial illness and death during the first five years of life while their immune systems are developing [5]. Malaria causes anemia in pregnancy and is associated with a higher HIV-1 viral load in pregnant women [7, 8]. In Sub-Saharan Africa, *Plasmodium falciparum* is responsible for most cases of malaria.

The parasite's life cycle includes a cycle of asexual division in the human liver, another cycle of pigment-producing asexual division in red blood cells (RBCs), and a sporogenic development in the female anopheles mosquito. During the erythrocytic stage of development, the malaria parasite develops into a trophozoite form in a vacuole formed by the internal membrane of the host red cells. The trophozoite feeds on hemoglobin (Hb) by ingesting small amounts of red cell cytoplasm. The globin component is further digested into amino acids for the parasite's metabolic needs. However, heme is toxic to the parasite and is thus aggregated into the insoluble dark-brown crystal called hemozoin (Hz) which can accumulate in the parasitized red blood cell (pRBC). Both circulating and resident phagocytes acquire Hz through

phagocytosis of pRBCs or free Hz crystals released after schizont rupture [9]. The presence of Hz disturbs normal cellular function and physiology of host whereas the parasite is less affected by it. The mechanism of inhibition of cellular function by Hz is thought to be the production of lipoperoxides from arachidonic acid [10, 11]. These compounds have been identified from Hz-fed macrophages and shown to inhibit macrophage function *in vitro* [12]. Furthermore, several studies have demonstrated that acquisition of malarial pigment by circulating monocytes and neutrophils is significantly associated with disease severity [13–16]. The Hz-monocyte complex has been associated with severe malaria anemia (SMA) [17], immunosuppression [9, 16], and cytokine dysregulation [18–20]. These findings demonstrate the pathological roles of Hz during malaria parasitaemia. However, recently researchers demonstrated that Hz may be an effective adjuvant for malaria vaccine [21–25]. This is because Hz was found to be a good ligand for Toll-like receptor 9 (TLR9) and induced both humoral and cellular immune responses in animals immunized with crude extract of Hz [21, 22].

Taken together, these findings suggest that Hz may be involved in both induction of immunity against malaria and the pathogenesis of the disease. In this brief review, we will evaluate reported roles of this pigment in the pathogenesis of malaria and provide the rationale for its targeting in antimalaria therapeutics development.

2. Hemozoin and Anemia

Structural abnormalities and extensive deposition of Hz were observed in the livers and bone marrows of children with SMA [26–28]. The abnormalities in these tissues indicate the pathological role of Hz in the development of SMA. Although there are many unexplained complex factors involved in the development of anemia during malaria infection, three mechanisms appear to be responsible for the problem, namely, direct destruction of infected red blood cells, increased destruction of unparasitized blood cells, and marrow suppression. Hz plays crucial roles in these three mechanisms.

2.1. Removal of Parasitized RBC. Anemia from *P. falciparum* malaria infection is usually responsible for severe morbidity and mortality in children and pregnant women in Sub-Saharan Africa. *P. falciparum* invades red cells of all ages [29] and digests Hb using parasite proteases [30] into small fragments consisting of about 20 different amino acids and free ferrous protoporphyrin IX, which is rapidly oxidized to heme [31]. Free toxic heme is rapidly oxidized to hematin and sequestered into inert, nontoxic crystalline Hz, which is present in the pRBC [32]. The presence of Hz in RBC results in appearance of antigenic molecules on membranes of these cells [33]. Recognition of Hz-containing RBC results in phagocytosis by circulating macrophages and removal from circulation. A study of the antigens exposed on the surface of pRBCs with different isolates of *P. falciparum* showed that the surface antigens could induce isolate-specific immunity [34]. Parasite-derived erythrocyte membrane protein (PfEMP1) facilitates rosetting and mediates the binding of pRBC to endothelial cells [35]. In another study which described clinically relevant cytoadhesive phenotypes of *P. vivax* isolates, it was observed that the intensity of rosetting was higher among anaemic individuals compared to non-anaemic and decreased with increasing haematocrit and haemoglobin levels [36]. Consequently, the study concluded that rosetting may contribute to development of anaemia. Previous studies involving *P. falciparum* suggest that rosetting and cytoadhesion contribute to anaemia [37, 38]. In a study in Kenya, the presence of Hz-containing monocytes in children infected with *P. falciparum* was shown to be associated with SMA [17].

2.2. Hemolysis of Unparasitized RBC. Trophozoites in the RBC grow and develop into schizonts which rupture the cell releasing merozoites and free Hz into blood circulation [39]. Circulating free Hz released after rupture of the RBC may be deposited on unparasitized RBC and cause lipid peroxidation of RBC membranes resulting in loss of deformability of the cell [40–42]. This change in membrane predisposes unparasitized RBC to increased sequestration and lysis in the spleen and other reticuloendothelial organs. A study investigating the mechanisms behind the structural and functional effects of haem products on infected and uninfected red cells showed that hemolysis induced by haematin was dose and time dependent [41]. Since red cells preincubated with haematin were more sensitive to haemolysis induced by hypotonic shock, low pH, H_2O_2, or haematin itself, the study concludes that the destabilising effect of haem products (haematin and beta-haematin) on red cell membrane may not result from oxidative damage of membrane lipids but from direct binding or incorporation to membrane. Furthermore, direct binding or incorporation of haem products to npRBC membrane is expected to initiate immunological responses and phagocytosis of npRBC.

Another mechanism involves 4-hydroxynonenal (4-HNE). Rosetting is a specialized form of cytoadherence of late hemozoin-containing pRBCs to npRBCs [43]. The aldehyde 4-HNE is synthesized in the parasite when iron, present in Hz, peroxidizes polyunsaturated fatty acids [42]. Uyoga et al. (2012) having studied the role of transfer of 4-HNE from parasitized to nonparasitized erythrocytes in rosettes concluded that the transfer plays a role in the phagocytic removal of large numbers of npRBCs and may be key to the SMA found in malaria patients [43].

2.3. Marrow Suppression. Hz has also been associated with direct inhibition of reticulocyte formation [15, 44]. Previous autopsy investigation in children that died from severe malaria demonstrated large deposition of Hz in bone marrow suggesting direct inhibition of erythropoiesis [28]. In addition, dyserythropoietic changes, including multinuclear erythroblasts, karyorrhexis, incomplete and unequal amitotic nuclear divisions, and cytoplasmic bridging, were also noted by Abdalla and colleagues in children with SMA in 1980 [45]. Similarly, proinflammatory cytokines such as TNF-α have been shown to inhibit all stages of erythropoiesis [46, 47].

Hz increases the secretion of TNF-α in both human and animal malaria infection [48–50].

In all, Hz directly promotes SMA through enhancing increased hemolysis of RBC and inhibition of erythropoiesis. It also indirectly promotes SMA by triggering production of inflammatory cytokines such as TNF-α and nitric oxide (see below).

3. Hemozoin, Cytokine, and Chemokine Dysfunction

Previous investigations had shown that ingestion of Hz by monocytes may enhance malarial pathogenesis by causing dysregulation in the production of cytokine, chemokines, and effector molecules, including TNF-α, interleukin IL-12, IL-10, macrophage inflammatory protein (MIP)-1α, MIP-1β, nitric oxide (NO), and prostaglandin (PG)-E_2 [18–20, 33].

3.1. Tumor Necrosis Factor-Alpha. Systemic symptoms of malaria like fever occur after the rupture of malaria schizont [51, 52] and are caused by the release of proinflammatory cytokine TNF-α [35]. In both human and animal studies, Hz ingestion by mononuclear cells enhances production of TNF-α [48–50, 53] and nitric oxide [54, 55]. High systemic level of TNF-α is seen in acute *P. falciparum* malaria [56] and is believed to be protective by restricting parasitaemia [57]. However it also inhibits all stages of erythropoiesis and is associated with increased malaria pathogenesis [46, 58]. Similarly, inhibition of bone marrow and RBC destruction as a result of high TNF-α was reported in murine malaria [59, 60]. Higher TNF-α was also reported in fatal compared to nonfatal cerebral malaria in African children [61]. Similarly, higher TNF-α was seen in cerebral malaria compared to uncomplicated malaria [62]. In addition, elevated serum TNF-α level was associated with abortions [58, 63, 64]. Taken together, transient elevation of TNF may be beneficial since it enhances parasite clearance while sustained release of TNF in severe malaria is associated with increased malaria morbidity and perhaps mortality.

3.2. Nitric Oxide. Hz and pro-inflammatory cytokines including TNF-α increases the expression of nitric oxide synthase 2 (NOS_2) gene and generation of nitric oxide (NO). Initial high levels of NO appear to be protective against severe malaria [65, 66]. However sustained high levels are associated with *P. falciparum* malaria anemia in children [55] by inhibiting erythropoiesis [67, 68].

3.3. Prostaglandins. In children with acute malaria, plasma prostaglandin E_2 and COX-2 gene expression by PBMC are significantly reduced [69]. This is partly due to Hz ingested by PBMCs [70]. PGE_2 inhibits TNF-α [71] and appears to decrease malaria severity. It also enhances erythropoiesis by inducing burst forming unit erythroid formation [72]. Inhibitors of PGE_2 synthesis such as Hz, acetaminophen, and salicylates are associated with high levels of TNF-α and increased malaria severity and mortality [73–75]. In addition low circulating bicyclo-PG_2/TNF-α is associated with decreased Hb concentration [55]. Hz has also been known to affect serum levels of IL-10 [33, 76] and IL-12 [77]. IL-10 may be increased in severe malaria while low IL-12 was also reported to be associated with increased malaria disease severity in children [13, 78].

In summary, Hz appears to trigger pathological levels of pro-inflammatory cytokines and chemokines like TNF-α, NO and at the same time reducing the level of more beneficial IL-12 and prostaglandin E_2. The role of anti-inflammatory cytokine IL-10 in malaria appears to enhance parasitemia since the Th1 immunological responses against the parasite are inhibited and IL-12 secretion is also suppressed by this cytokine. Taken together, Hz plays important role in deregulation of pro- and anti-inflammatory cytokines during malaria resulting in altered immunological responses to the disease, anemia, and host tissue damage.

4. Immunosuppression

Suppression of innate immune response during malaria infection has been reported in previous studies [9, 79]. One of the reported mechanisms is the suppression of dendritic cell (DC) function [33]. Dendritic cells play important roles in innate and adaptive immune responses. In contact with pathogen, these cells phagocytize the antigen, undergo a process of maturation, upregulate the requisite molecules and present to NK cells and naive and memory T and B lymphocytes. Ingestion of pRBC by DCs has been reported to impair the natural ability of DCs to stimulate both allogenic and antigen-specific T-cell immunity [80, 81]. This inhibition of DCs function is probably partially due to phagocytized Hz in pRBC. Moreover, Hz direct inhibition of DC maturation [33, 82] and suppression of general leucocytes proliferative responses [76] were also reported in previous studies. Similarly, ingestion of Hz by monocytes, macrophages, and neutrophils had been known to affect the functions of these cells resulting in defective phagocytosis and expression of MHC Class II, CD54, and CD11c [9, 79]. Specifically, decrease in IL-12 during malaria may be partly responsible for decreased immunity to the parasite. As mentioned above, low IL-12 has been associated with severe malaria infection in children [13, 78]. The derangement in the innate immune responses ultimately results in defective adaptive immune responses. As mentioned earlier, suppression of DC function is associated with failure of adequate priming and presentation of malaria antigens to CD4+ and CD8+ cells for appropriate Th1 and Th2 immune responses.

The suppression of immunity during malaria infection may be responsible for associated clinical problems. For instance, studies have demonstrated increased viral load in HIV patients [83] as a result of suppression of CD8+ function. Otieno et al. demonstrated increased SMA in HIV-1 positive infants and children in Kenya [84]. Also ineffective CD8+ function during malaria was linked to the association of Burkitt's lymphoma and endemic malaria [85, 86]. In addition, frequent association of bacteremia in children with clinical malaria was reported previously [87–89]. In all, presence of circulating malaria pigment in macrophages

and neutrophils was associated with poor prognosis [90] or increased morbidity [91, 92].

On account of the global inhibition of innate and adaptive immune responses, Hz was described in previous studies as a potent immunosuppressant [93, 94]. However, these findings have been challenged by recent reports that demonstrated the potent immunogenicity of crude extract of Hz. These studies reported that Hz is an effective TLR9 ligand and can enhance immunity against malaria [50, 95].

5. Other Harmful Effects of Hemozoin

A number of harmful effects have been documented or associated with Hz. Hz compromises the functions of human monocytes, as previously noted, and this dysfunction has been related to its lipoperoxidation products, namely, 15-hydroxyeicosatetraenoic acid (15-HETE) and 4-HNE [96]. Hz has also been associated with malaria-associated acute respiratory distress syndrome (MA-ARDS). By quantifying Hz in the lungs and measuring the disease parameters of MA-ARDS, a highly significant correlation between pulmonary Hz concentrations, lung weights, and alveolar edema was demonstrated [97]. Another study has shown that Hz is implicated in cerebral malaria since it modulates matrix metalloproteinases and induces morphological changes in human microvascular endothelium [98]. Histological examination in the study revealed that human microvascular endothelial cell line (HMEC-1) treated with natural Hz appeared elongated instead of polygonal and formed microtubule-like vessels on synthetic basement membrane.

6. Summary

One of the factors responsible for malaria pathogenesis is the suppression of host immune responses. This suppression enhances parasitemia and diminishes the host immune response to malaria-expressed proteins and other pathogens. Hz plays both direct and indirect roles in orchestrating this immunosuppression which may be important for the survival of the parasite and completion of its life cycle in the human host [99]. Blocking of Hz formation will ultimately increase immune mediated parasite clearance and can prevent formation and transmission of the gametocytes. The host can also maintain immunological surveillance to other pathogens. Development of new chemotherapeutic agents that will prevent the formation of the pigment in pRBC may greatly reduce morbidity associated with malaria. Association of Hz and SMA has been demonstrated in several previous studies (see above).

Use of agents that will prevent formation of the pigment will prevent Hz-induced inhibition of erythroid precursors and also reduce the production of other mediators of SMA. Chloroquine, quinine, and artemisinin block the formation of Hz from heme [21, 22, 95]. Perhaps, reduction of morbidity and mortality with these drugs may be attributable to decreased circulating levels of Hz. In developing countries where malaria is endemic, effective targeting of Hz may greatly reduce high morbidity and mortality presently associated with the disease. In this regard, development of new therapeutics that will block formation of Hz by the parasite will be necessary. Modification of quinine, chloroquine, or artemether to overcome parasite resistance may also achieve the same goal. Furthermore, it may be important to investigate the prevalence of anti-Hz immunity in healthy, mild, and severe malaria in people living in malaria endemic regions. Similarly, evaluation of crude extract of Hz as an adjuvant for human application requires further studies. This may provide the rationale for the inclusion of Hz as a component of candidate malaria vaccines. The applications of these potential Hz targeting measures warrant further studies.

Conflict of Interests

The authors have neither financial issues nor conflict of interests to disclose.

References

[1] Roll Back Malaria, "Global malaria action plan," Roll Back Malaria, 2008, http://rbm.who.int/gmap/gmap.pdf.

[2] World Health Organisation, *Global Burden of Disease Estimates*, World Health Organisation, Geneva, Switzerland, 2002.

[3] World Health Organization, *World Malaria Report*, World Health Organization, Geneva, Switzerland, 2008.

[4] J. L. Gallup and J. D. Sachs, "The economic burden of malaria," *American Journal of Tropical Medicine and Hygiene*, vol. 64, no. 1-2, pp. 85–96, 2001.

[5] World Health Organization, *World Malaria Report*, World Health Organization, Geneva, Switzerland, 2009.

[6] R. W. Snow, M. Craig, U. Deichmann, and K. Marsh, "Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population," *Bulletin of the World Health Organization*, vol. 77, no. 8, pp. 624–640, 1999.

[7] L. Molineaux, "Malaria and mortality: some epidemiological considerations," *Annals of Tropical Medicine and Parasitology*, vol. 91, no. 7, pp. 811–825, 1997.

[8] J. A. G. Whitworth and K. A. Hewitt, "Effect of malaria on HIV-1 progression and transmission," *The Lancet*, vol. 365, no. 9455, pp. 196–197, 2005.

[9] E. Schwarzer, F. Turrini, D. Ulliers, G. Giribaldi, H. Ginsburg, and P. Arese, "Impairment of macrophage functions after ingestion of Plasmodium falciparum-infected erythrocytes or isolated malarial pigment," *The Journal of Experimental Medicine*, vol. 176, no. 4, pp. 1033–1041, 1992.

[10] E. Schwarzer, P. Ludwig, E. Valente, and P. Arese, "15(S)-hydroxyeicosatetraenoic acid (15-HETE), a product of arachidonic acid peroxidation, is an active component of hemozoin toxicity to monocytes," *Parassitologia*, vol. 41, no. 1–3, pp. 199–202, 1999.

[11] E. Schwarzer, O. Müller, P. Arese, W. G. Siems, and T. Grune, "Increased levels of 4-hydroxynonenal in human monocytes fed with malarial pigment hemozoin. A possible clue for hemozoin toxicity," *FEBS Letters*, vol. 388, no. 2-3, pp. 119–122, 1996.

[12] B. C. Urban and D. J. Roberts, "Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells," *Current Opinion in Immunology*, vol. 14, no. 4, pp. 458–465, 2002.

[13] A. J. F. Luty, D. J. Perkins, B. Lell et al., "Low interleukin-12 activity in severe *Plasmodium falciparum* malaria," *Infection and Immunity*, vol. 68, no. 7, pp. 3909–3915, 2000.

[14] K. E. Lyke, D. A. Diallo, A. Dicko et al., "Association of intraleukocytic *Plasmodium falciparum* malaria pigment with disease severity, clinical manifestations, and prognosis in severe malaria," *The American Journal of Tropical Medicine and Hygiene*, vol. 69, no. 3, pp. 253–259, 2003.

[15] C. Casals-Pascual, O. Kai, J. O. P. Cheung et al., "Suppression of erythropoiesis in malarial anemia is associated with hemozoin in vitro and in vivo," *Blood*, vol. 108, no. 8, pp. 2569–2577, 2006.

[16] P. Arese and E. Schwarzer, "Malarial pigment (haemozoin): a very active "inert" substance," *Annals of Tropical Medicine and Parasitology*, vol. 91, no. 5, pp. 501–516, 1997.

[17] E. M. Novelli, J. B. Hittner, G. C. Davenport et al., "Clinical predictors of severe malarial anaemia in a holoendemic *Plasmodium falciparum* transmission area: research paper," *British Journal of Haematology*, vol. 149, no. 5, pp. 711–721, 2010.

[18] D. O. Ochiel, G. A. Awandare, C. C. Keller et al., "Differential regulation of β-chemokines in children with *Plasmodium falciparum* malaria," *Infection and Immunity*, vol. 73, no. 7, pp. 4190–4197, 2005.

[19] C. C. Keller, G. C. Davenport, K. R. Dickman et al., "Suppression of prostaglandin E2 by malaria parasite products and antipyretics promotes overproduction of tumor necrosis factor-α: association with the pathogenesis of childhood malarial anemia," *Journal of Infectious Diseases*, vol. 193, no. 10, pp. 1384–1393, 2006.

[20] C. C. Keller, O. Yamo, C. Ouma et al., "Acquisition of hemozoin by monocytes down-regulates interleukin-12 p40 (IL-12p40) transcripts and circulating IL-12p70 through an IL-10-dependent mechanism: in vivo and in vitro findings in severe malarial anemia," *Infection and Immunity*, vol. 74, no. 9, pp. 5249–5260, 2006.

[21] C. Coban, M. Yagi, K. Ohata et al., "The malarial metabolite hemozoin and its potential use as a vaccine adjuvant," *Allergology International*, vol. 59, no. 2, pp. 115–124, 2010.

[22] H. Wagner, "Hemozoin: malaria's "built-In" adjuvant and TLR9 agonist," *Cell Host and Microbe*, vol. 7, no. 1, pp. 5–6, 2010.

[23] M. Jaramillo, M.-J. Bellemare, C. Martel et al., "Synthetic Plasmodium-like hemozoin activates the immune response: a morphology—function study," *PLoS ONE*, vol. 4, no. 9, Article ID e6957, 2009.

[24] C.-C. Hou, M. J. Day, T. J. Nuttall, and P. B. Hill, "Evaluation of IgG subclass responses against Dermatophagoides farinae allergens in healthy and atopic dogs," *Veterinary Dermatology*, vol. 17, no. 2, pp. 103–110, 2006.

[25] C. Coban, K. J. Ishii, D. J. Sullivan, and N. Kumar, "Purified malaria pigment (hemozoin) enhances dendritic cell maturation and modulates the isotype of antibodies induced by a DNA vaccine," *Infection and Immunity*, vol. 70, no. 7, pp. 3939–3943, 2002.

[26] S. H. Abdalla, "Hematopoiesis in human malaria," *Blood Cells*, vol. 16, no. 2-3, pp. 401–416, 1990.

[27] S. N. Wickramasinghe and S. H. Abdalla, "Blood and bone marrow changes in malaria," *Bailliere's Best Practice and Research in Clinical Haematology*, vol. 13, no. 2, pp. 277–299, 2000.

[28] G. Giribaldi, D. Ulliers, E. Schwarzer, I. Roberts, W. Piacibello, and P. Arese, "Hemozoin- and 4-hydroxynonenal-mediated inhibition of erythropoiesis. Possible role in malarial dyserythropoiesis and anemia," *Haematologica*, vol. 89, no. 4, pp. 492–493, 2004.

[29] P. A. Tamez, H. Liu, S. Fernandez-Pol, K. Haldar, and A. Wickrema, "Stage-specific susceptibility of human erythroblasts to Plasmodium falciparum malaria infection," *Blood*, vol. 114, no. 17, pp. 3652–3655, 2009.

[30] R. Banerjee, J. Liu, W. Beatty, L. Pelosof, M. Klemba, and D. E. Goldberg, "Four plasmepsins are active in the Plasmodium falciparum food vacuole, including a protease with an active-site histidine," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 2, pp. 990–995, 2002.

[31] N. T. Huy, Y. Shima, A. Maeda et al., "Phospholipid membrane-mediated hemozoin formation: the effects of physical properties and evidence of membrane surrounding hemozoin," *PLoS One*, vol. 8, no. 7, 2013.

[32] A. K. Tripathi, S. K. Garg, and B. L. Tekwani, "A physiochemical mechanism of hemozoin (β-hematin) synthesis by malaria parasite," *Biochemical and Biophysical Research Communications*, vol. 290, no. 1, pp. 595–601, 2002.

[33] P. Giusti, B. C. Urban, G. Frascaroli et al., "Plasmodium falciparum-Infected erythrocytes and β-hematin induce partial maturation of human dendritic cells and increase their migratory ability in response to lymphoid chemokines," *Infection and Immunity*, vol. 79, no. 7, pp. 2727–2736, 2011.

[34] N. Kalantari and S. Ghaffari, "Identification and characterization of the antigens expressed on the surface of human erythrocytes infected with *Plasmodium falciparum*," *Iranian Journal of Parasitology*, vol. 8, no. 2, pp. 197–206, 2013.

[35] J. Alexandra Rowe, J. M. Moulds, C. I. Newbold, and L. H. Miller, "*P. falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1," *Nature*, vol. 388, no. 6639, pp. 292–295, 1997.

[36] A. Marín-Menéndez, A. Bardají, F. E. Martínez-Espinosa et al., "Rosetting in *Plasmodium vivax*: a cytoadhesion phenotype associated with anaemia," *PLOS Neglected Tropical Diseases* , vol. 7, no. 4, 2013.

[37] A. Mayor, A. Hafiz, Q. Bassat et al., "Association of severe malaria outcomes with platelet-mediated clumping and adhesion to a novel host receptor," *PLoS ONE*, vol. 6, no. 4, Article ID e19422, 2011.

[38] J. A. Rowe, J. Shafi, O. K. Kai, K. Marsh, and A. Raza, "Non-immune IgM but not IgG binds to the surface of *Plasmodium falciparum*-infected erythrocytes and correlates with rosetting and severe malaria," *American Journal of Tropical Medicine and Hygiene*, vol. 66, no. 6, pp. 692–699, 2002.

[39] R. Stiebler, J. B. R. C. Soares, B. L. Timm et al., "On the mechanisms involved in biological heme crystallization," *Journal of Bioenergetics and Biomembranes*, vol. 43, no. 1, pp. 93–99, 2011.

[40] K. Mohan, M. L. Dubey, N. K. Ganguly, and R. C. Mahajan, "*Plasmodium falciparum*: role of activated blood monocytes in erythrocyte membrane damage and red cell loss during malaria," *Experimental Parasitology*, vol. 80, no. 1, pp. 54–63, 1995.

[41] F. Omodeo-Salè, A. Motti, A. Dondorp, N. J. White, and D. Taramelli, "Destabilisation and subsequent lysis of human erythrocytes induced by *Plasmodium falciparum* haem products," *European Journal of Haematology*, vol. 74, no. 4, pp. 324–332, 2005.

[42] O. A. Skorokhod, L. Caione, T. Marrocco et al., "Inhibition of erythropoiesis in malaria anemia: role of hemozoin and hemozoin-generated 4-hydroxynonenal," *Blood*, vol. 116, no. 20, pp. 4328–4337, 2010.

[43] S. Uyoga, O. A. Skorokhod, M. Opiyo et al., "Transfer of 4-hydroxynonenal from parasitized to non-parasitized erythrocytes in rosettes. Proposed role in severe malaria anemia," *British Journal of Haematology*, vol. 157, no. 1, pp. 116–124, 2012.

[44] A. A. Lamikanra, M. Theron, T. W. Kooij, and D. J. Roberts, "Hemozoin (malarial pigment) directly promotes apoptosis of erythroid precursors," *PLoS One*, vol. 4, no. 12, Article ID e8446, 2009.

[45] S. Abdalla, D. J. Weatherall, S. N. Wickramasinghe, and M. Hughes, "The anaemia of *P. falciparum* malaria," *British Journal of Haematology*, vol. 46, no. 2, pp. 171–183, 1980.

[46] C. Dufour, A. Corcione, J. Svahn et al., "TNF-alpha and IFN-gamma are overexpressed in the bone marrow of Fanconi anemia patients and TNF-alpha suppresses erythropoiesis in vitro," *Blood*, vol. 102, no. 6, pp. 2053–2059, 2003.

[47] C. Grigorakaki, F. Morceau, S. Chateauvieux, M. Dicato, and M. Diederich, "Tumor necrosis factor alpha-mediated inhibition of erythropoiesis involves GATA-1/GATA-2 balance impairment and PU. 1 over-expression," *Biochemical Pharmacology*, vol. 82, no. 2, pp. 156–166, 2011.

[48] B. Mordmüller, F. Turrini, H. Long, P. G. Kremsner, and P. Arese, "Neutrophils and monocytes from subjects with the Mediterranean G6PD variant: effect of Plasmodium falciparum hemozoin on G6PD activity, oxidative burst and cytokine production," *European Cytokine Network*, vol. 9, no. 3, pp. 239–245, 1998.

[49] S. Biswas, M. Karmarkar, and Y. Sharma, "Antibodies detected against Plasmodium falciparum haemozoin with inhibitory properties to cytokine production," *FEMS Microbiology Letters*, vol. 194, no. 2, pp. 175–179, 2001.

[50] C. Coban, K. Ishii, T. Kawai et al., "Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin," *The Journal of Experimental Medicine*, vol. 201, no. 1, pp. 19–25, 2005.

[51] D. Kwiatkowski, J. G. Cannon, K. R. Manogue, A. Cerami, C. A. Dinarello, and B. M. Greenwood, "Tumour necrosis factor production in Falciparum malaria and its association with schizont rupture," *Clinical and Experimental Immunology*, vol. 77, no. 3, pp. 361–366, 1989.

[52] D. Kwiatkowski and P. Perlman, *Inflammatory Processes in the Pathogenesis of Malaria*, Harwood Academic Publishers, 1999.

[53] B. A. Sherry, G. Alava, K. J. Tracey, J. Martiney, A. Cerami, and A. F. G. Slater, "Malaria-specific metabolite hemozoin mediates the release of several potent endogenous pyrogens (TNF, MIP-1α, and MIP-1β) in vitro, and altered thermoregulation in vivo," *Journal of Inflammation*, vol. 45, no. 2, pp. 85–96, 1995.

[54] M. Jaramillo, D. C. Gowda, D. Radzioch, and M. Olivier, "Hemozoin increases IFN-γ-inducible macrophage nitric oxide generation through extracellular signal-regulated kinase- and NF-κB-dependent pathways," *Journal of Immunology*, vol. 171, no. 8, pp. 4243–4253, 2003.

[55] C. C. Keller, P. G. Kremsner, J. B. Hittner, M. A. Misukonis, J. B. Weinberg, and D. J. Perkins, "Elevated nitric oxide production in children with malarial anemia: hemozoin-induced nitric oxide synthase type 2 transcripts and nitric oxide in blood mononuclear cells," *Infection and Immunity*, vol. 72, no. 8, pp. 4868–4873, 2004.

[56] M. Odeh, "The role of tumour necrosis factor-α in the pathogenesis of complicated Falciparum malaria," *Cytokine*, vol. 14, no. 1, pp. 11–18, 2001.

[57] P. G. Kremsner, S. Winkler, C. Brandts et al., "Prediction of accelerated cure in *Plasmodium falciparum* malaria by the elevated capacity of tumor necrosis factor production," *American Journal of Tropical Medicine and Hygiene*, vol. 53, no. 5, pp. 532–538, 1995.

[58] I. A. Clark and G. Chaudhri, "The balance of useful and harmful effects of TNF, with special reference to malaria," *Annales de l'Institut Pasteur. Immunologie*, vol. 139, no. 3, pp. 305–306, 1988.

[59] J. Taverne, N. Sheikh, J. B. de Souza, J. H. L. Playfair, L. Probert, and G. Kollias, "Anaemia and resistance to malaria in transgenic mice expressing human tumour necrosis factor," *Immunology*, vol. 82, no. 3, pp. 397–403, 1994.

[60] I. A. Clark and G. Chaudhri, "Tumour necrosis factor may contribute to the anaemia of malaria by causing dyserythropoiesis and erythrophagocytosis," *British Journal of Haematology*, vol. 70, no. 1, pp. 99–103, 1988.

[61] G. E. Grau, P.-F. Piquet, P. Vassalli, and P.-H. Lambert, "Tumor-necrosis factor and other cytokines in cerebral malaria: experimental and clinical data," *Immunological Reviews*, no. 112, pp. 49–70, 1989.

[62] D. Kwiatkowski, A. V. S. Hill, I. Sambou et al., "TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria," *The Lancet*, vol. 336, no. 8725, pp. 1201–1204, 1990.

[63] G. Entrican, "Immune regulation during pregnancy and host-pathogen interactions in infectious abortion," *Journal of Comparative Pathology*, vol. 126, no. 2-3, pp. 79–94, 2002.

[64] N. Y. Kim, H. J. Cho, H. Y. Kim et al., "Thyroid autoimmunity and its association with cellular and humoral immunity in women with reproductive failures," *American Journal of Reproductive Immunology*, vol. 65, no. 1, pp. 78–87, 2011.

[65] D. J. Perkins, P. G. Kremsner, D. Schmid, M. A. Misukonis, M. A. Kelly, and J. B. Weinberg, "Blood mononuclear cell nitric oxide production and plasma cytokine levels in healthy gabonese children with prior mild or severe malaria," *Infection and Immunity*, vol. 67, no. 9, pp. 4977–4981, 1999.

[66] J. F. Kun, B. Mordmüller, D. J. Perkins et al., "Nitric oxide synthase 2Lambaréné (G-954C), increased nitric oxide production, and protection against malaria," *Journal of Infectious Diseases*, vol. 184, no. 3, pp. 330–336, 2001.

[67] P. J. Shami and J. B. Weinberg, "Differential effects of nitric oxide on erythroid and myeloid colony growth from CD34+ human bone marrow cells," *Blood*, vol. 87, no. 3, pp. 977–982, 1996.

[68] S. Reykdal, C. Abboud, and J. Liesveld, "Effect of nitric oxide production and oxygen tension on progenitor preservation in ex vivo culture," *Experimental Hematology*, vol. 27, no. 3, pp. 441–450, 1999.

[69] D. J. Perkins, P. G. Kremsner, and J. Brice Weinberg, "Inverse relationship of plasma prostaglandin E2 and blood mononuclear cell cyclooxygenase-2 with disease severity in children with Plasmodium falciparum malaria," *Journal of Infectious Diseases*, vol. 183, no. 1, pp. 113–118, 2001.

[70] C. C. Keller, J. B. Hittner, B. K. Nti, J. B. Weinberg, P. G. Kremsner, and D. J. Perkins, "Reduced peripheral PGE2 biosynthesis in *Plasmodium falciparum malaria* occurs through hemozoin-induced suppression of blood mononuclear cell cyclooxygenase-2 gene expression via an interleukin-10-independent mechanism," *Molecular Medicine*, vol. 10, no. 1–6, pp. 45–54, 2004.

[71] S. L. Kunkel, M. Spengler, M. A. May, R. Spengler, J. Larrick, and D. Remick, "Prostaglandin E2 regulates macrophage-derived

tumor necrosis factor gene expression," *The Journal of Biological Chemistry*, vol. 263, no. 11, pp. 5380–5384, 1988.

[72] F. Dupuis, N. Gachard, A. Allegraud, C. Dulery, V. Praloran, and Y. Denizot, "Effect of platelet-activating factor on the growth of human erythroid and myeloid CD34+ progenitors," *Mediators of Inflammation*, vol. 7, no. 2, pp. 99–103, 1998.

[73] M. English, V. Marsh, E. Amukoye, B. Lowe, S. Murphy, and K. Marsh, "Chronic salicylate poisoning and severe malaria," *The Lancet*, vol. 347, no. 9017, pp. 1736–1737, 1996.

[74] H. J. Ball, H. G. MacDougall, I. S. McGregor, and N. H. Hunt, "Cyclooxygenase-2 in the pathogenesis of murine cerebral malaria," *Journal of Infectious Diseases*, vol. 189, no. 4, pp. 751–758, 2004.

[75] L. Xiao, P. S. Patterson, C. Yang, and A. A. Lal, "Role of eicosanoids in the pathogenesis of murine cerebral malaria," *American Journal of Tropical Medicine and Hygiene*, vol. 60, no. 4, pp. 668–673, 1999.

[76] P. Deshpande and P. Shastry, "Modulation of cytokine profiles by malaria pigment—Hemozoin: role of IL-10 in suppression of proliferative responses of mitogen stimulated human PBMC," *Cytokine*, vol. 28, no. 6, pp. 205–213, 2004.

[77] M. Cambos, S. Bazinet, E. Abed et al., "The IL-12p70/IL-10 interplay is differentially regulated by free heme and hemozoin in murine bone-marrow-derived macrophages," *International Journal for Parasitology*, vol. 40, no. 9, pp. 1003–1012, 2010.

[78] D. J. Perkins, J. B. Weinberg, and P. G. Kremsner, "Reduced interleukin-12 and transforming growth factor-β1 in severe childhood malaria: relationship of cytokine balance with disease severity," *Journal of Infectious Diseases*, vol. 182, no. 3, pp. 988–992, 2000.

[79] E. Schwarzer, H. Kühn, E. Valente, and P. Arese, "Malaria-parasitized erythrocytes and hemozoin nonenzymatically generate large amounts of hydroxy fatty acids that inhibit monocyte functions," *Blood*, vol. 101, no. 2, pp. 722–728, 2003.

[80] B. C. Urban, D. J. P. Ferguson, A. Pain et al., "Plasmodium falciparuminfected erythrocytes modulate the maturation of dendritic cells," *Nature*, vol. 400, no. 6739, pp. 73–77, 1999.

[81] M. F. Good and D. L. Doolan, "Immune effector mechanisms in malaria," *Current Opinion in Immunology*, vol. 11, no. 4, pp. 412–419, 1999.

[82] O. A. Skorokhod, M. Alessio, B. Mordmüller, P. Arese, and E. Schwarzer, "Hemozoin (malarial pigment) inhibits differentiation and maturation of human monocyte-derived dendritic cells: a peroxisome proliferator-activated receptor-γ-mediated effect," *Journal of Immunology*, vol. 173, no. 6, pp. 4066–4074, 2004.

[83] I. F. Hoffman, C. S. Jere, T. E. Taylor et al., "The effect of *Plasmodium falciparum* malaria on HIV-1 RNA blood plasma concentration," *AIDS*, vol. 13, no. 4, pp. 487–494, 1999.

[84] R. O. Otieno, C. Ouma, J. M. Ong'echa et al., "Increased severe anemia in HIV-1-exposed and HIV-1-positive infants and children during acute malaria," *AIDS*, vol. 20, no. 2, pp. 275–280, 2006.

[85] D. Burkitt, "A sarcoma of the jaw in African children," *British Journal of Surgery*, vol. 46, pp. 218–223, 1958.

[86] A. Chene, D. Donati, J. Orem et al., "Endemic Burkitt's lymphoma as a polymicrobial disease. New insights on the interaction between Plasmodium falciparum and Epstein-Barr virus," *Seminars in Cancer Biology*, vol. 19, no. 6, pp. 411–420, 2009.

[87] J. Berkley, S. Mwarumba, K. Bramham, B. Lowe, and K. Marsh, "Bacteraemia complicating severe malaria in children," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 93, no. 3, pp. 283–286, 1999.

[88] Q. Bassat, C. Guinovart, B. Sigaúque et al., "Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital," *Tropical Medicine and International Health*, vol. 14, no. 9, pp. 1011–1019, 2009.

[89] T. Were, G. C. Davenport, J. B. Hittner et al., "Bacteremia in Kenyan children presenting with malaria," *Journal of Clinical Microbiology*, vol. 49, no. 2, pp. 671–676, 2011.

[90] N. H. Phu, N. Day, P. T. Diep, D. J. P. Ferguson, and N. J. White, "Intraleucocytic malaria pigment and prognosis in severe malaria," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 89, no. 2, pp. 200–204, 1995.

[91] W. G. Metzger, B. G. Mordmuller, and P. G. Kremsner, "Malaria pigment in leucocytes," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 89, no. 6, pp. 637–638, 1995.

[92] O. K. Amodu, A. A. Adeyemo, P. E. Olumese, and R. A. Gbadegesin, "Intraleucocytic malaria pigment and clinical severity of malaria in children," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 92, no. 1, pp. 54–56, 1998.

[93] N. Morakote and D. E. Justus, "Immunosuppression in malaria: effect of hemozoin produced by *Plasmodium berghei* and *Plasmodium falciparum*," *International Archives of Allergy and Applied Immunology*, vol. 86, no. 1, pp. 28–34, 1988.

[94] T. Scorza, S. Magez, L. Brys, and P. de Baetselier, "Hemozoin is a key factor in the induction of malaria-associated immunosuppression," *Parasite Immunology*, vol. 21, no. 11, pp. 545–554, 1999.

[95] C. Coban, Y. Igari, M. Yagi et al., "Immunogenicity of whole-parasite vaccines against *Plasmodium falciparum* involves Malarial Hemozoin and host TLR9," *Cell Host and Microbe*, vol. 7, no. 1, pp. 50–61, 2010.

[96] M. Polimeni, E. Valente, E. Aldieri, A. Khadjavi, G. Giribaldi, and M. Prato, "Role of 15-hydroxyeicosatetraenoic acid in hemozoin-induced lysozyme release from human adherent monocytes," *Biofactors*, vol. 39, no. 3, pp. 304–314, 2013.

[97] K. Deroost, A. Tyberghein, N. Lays et al., "Hemozoin induces lung inflammation and correlates with malaria-associated acute respiratory distress syndrome," *American Journal of Respiratory Cell and Molecular Biology*, vol. 48, no. 5, pp. 589–600, 2013.

[98] M. Prato, S. D'Alessandro, P. E. van den Steen et al., "Natural haemozoin modulates matrix metalloproteinases and induces morphological changes in human microvascular endothelium," *Cellular Microbiology*, vol. 13, no. 8, pp. 1275–1285, 2011.

[99] B. C. Urban and D. J. Roberts, "Inhibition of T cell function during malaria: implications for immunology and vaccinology," *The Journal of Experimental Medicine*, vol. 197, no. 2, pp. 137–141, 2003.