Chapter

Malarial Inflammation-Driven Pathophysiology and Its Attenuation by Triterpene Phytotherapeutics

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Abstract

Malaria driven pathophysiology inimically conjoined to systemic inflammation response cascade in a vicious feed-forward cycle destined to a terrible debilitation or demise of the host. The *Plasmodium* parasite initiates physiological changes when it is transmitted into the human host by intermediate host and vector. Sporozoites injection elicits immunological and inflammatory response suppression facilitating movement into the blood stream undetected, destined to hepatocyte. Subsequently, hepatocyte invasion culminates in intracellular growth and conversion of the parasites rapturing hepatocytes releasing merozoites into the extrahepatic circulation. Inflammatory and immunological response initiation results in overt malarial disease symptoms. Initially, inflammatory response alleviates and curtails infection. Activation of leukocytes, lymphocytes, monocytes, and phagocytes secretes inflammatory mediators, chemokines, cytokines cytoadhering molecules which accelerate infection patency. Hormonal processes influence disease tolerance without necessarily interfering with parasitemia. Current treatment is anti-parasitic. Phytotherapeutic intervention in malaria is anti-parasitic and anti-disease effects that terminate the vicious cycle and alleviating disease. The phytochemicals, in malarial experimental and clinical work, include asiatic acid, maslinic acid, oleanolic acid, and inflammatory and immunological aberrations evolving in malaria and the effects of phytochemical therapeutics in the alleviation of the disease to enable leverage of future treatment regimens through harnessing existing plants materials is explored.

Keywords: malaria inflammation, phytochemicals, Asiatic acid, Maslinic acid, Oleanolic acid, anti-parasitic, anti-disease, *Plasmodium falciparum*, *Plasmodium berghei*, pathophysiology

1. Introduction

Malaria and inflammation seem to be intricately connected with the *Plasmodium* parasite having evolved mechanisms to evade its initiation before the parasite has set a strong and indelible foot print in either the intermediate (mosquito) or definitive (animal or human) hosts. Pathogen-driven tissue damage complements inflammatory response building a debilitating state against host fitness and survival [1, 2].

The disease is premised upon an immunological disease and a systematic inflammatory syndrome with a strong cachectic constituent driven by an endogenous cytokine milieu in the form of the pyrogens tumor necrosis factor alfa (TNF-α) and interleukin-1 (IL-1) [3, 4]. Eliciting and chaperoning this immunological-inflammatory response to malarial infection are the glycolipid glycosylphosphatidylinositol (GPI) moieties which are covalently connected to the antigens on the exterior topological aspect of malaria parasites or free in circulation. Whether linked to the parasite related proteins or free, GPI induce elevated levels of TNF-α and IL-1 from macrophages resulting in pyrexia, cachexia and, through their insulin memetic effect, regulate glucose homeostasis in adipocytes with profound hypoglycemia initiation [5]. In attends to these GPI-induced cytokine inflammatory effects and other malaria parasites by products like hemozoin, are the common malarial syndromes of severe malaria anemia (SMA), hypertriacylglycerolemia, hypotension, pulmonary vasculature hyperneutrophilia and acute respiratory distress syndrome (ARDS), diffuse intravascular coagulation, upregulated expression of endothelial cell and vascular cell intercell adhesion molecule 1 (ICAM1 and VCAM 1) [6, 7]. Mature parasites tend to recognize these molecules leading to their sequestration in cerebral malaria (CM) [8].

In a bid to neutralize the disease sequelae and induce disease tolerance (anti-disease effects), albeit without reduction in parasitemia (anti-infection effects) are the adrenal glands hormones which mediate immunological-inflammatory responses but do not affect the pathogen load [9]. Other hormones play a critical role in the amelioration of malarial inflammation.

Investigations and diagnosis of malarial-related inflammation and immunological aberrations have been vast owing to the importance of the tropical and subtropical disease. However, a lull in research has been experienced in the past making malaria one of the forgotten diseases. Interest has resurged in both disease investigations and management.

Malarial inflammatory response yields itself into the pathophysiology of malaria intimating that management needs to be directed at both the parasite and the disease, antiparasitic and anti-disease, respectively. The animal host develops disease tolerance through immunological and inflammatory response to a limited extent depending on the rate and duration of exposure to potent infection transmitting mosquito bites. Management of the disease with antiparasitic regimens alone or anti-disease management alone does not result in a radical cure but provides opportunity for future parasite recrudesces and drug resistance.

Phytotherapeutics and their derivatives have been administered in both experimental research and clinical practices with astonishing results. The drug artemisinin (extract from Chinese artemisinin *annua* herb) and its derivatives provide current mainstay of malaria treatment such that any meaningful parasite resistance to the drug will thrust the world into an era without effective malaria treatment. Anti-inflammatory activities have been reported for the drug. However, the proposed mode of action of the drug has been through a pro-oxidant capacity driven the endoperoxidase activity of the molecule which eventually is a compelling proinflammatory oriented outcome that rapidly kills the parasites. This process

leaves behind an oxidative environment with possible fatal hemolytic episodes occurring days after successful treatment of the disease in what has been termed post artemisinin administration hemolytic diseases. Alternative and safer treatment regimens are urgently required.

Experimental phytotherapeutics administration in murine malaria, the likes of triterpenes Asiatic acid (AA), Maslinic acid (MA) [10] and Oleanolic acid (OA) have shown potential antiparasitic and anti-disease facultative propensities. Salvaging of inflammation-driven glucose homeostasis, acute renal injury (ARI), hyperlipidemias, hyperparasitemia, hyperinsulinemia, cerebral malaria, weight loss and reduced feeding disease patterns have been reported. Triterpenes have pleiotropic characteristics with both antioxidant and pro-oxidant properties dependent on the environmental homeostasis. A plethora of challenge bedevil malaria management. There is antimalaria multidrug resistance, high cost of antimalarials, poverty amongst those most affected by malaria, lack of 100% effectiveness of current antimalaria drugs, no efficacious vaccine against malaria in place yet and adverse drug related post treatment side effects are common constraints in malaria management. Therefore, it is imperative that alternative drugs, as suggested here, be explored or leads compounds with antimalarial disease alleviation properties be reported for either more work to be done or be implemented in human trials.

2. Malarial cycle and potential interventional areas

2.1 Parasitemia drives malarial inflammatory response

Malaria is a highly inflammatory condition characterized by acute periods of fever, headache and nausea which correspond to the release of merozoites into the blood stream as the erythrocytes rapture. Excessive erythrocyte rupture leads to anemia and high parasitemia. Molecules from the parasite and ruptured RBCs trigger host inflammatory responses [11]. The erythrocyte stage is characterized by up-regulation of inflammation-driving cytokines such as IL-1b, IL-6, IFN-g, TNF-a and IL-12 [12, 13], which may lead to excessive inflammation if not curtailed [14]. Some parasite molecules that have been associated with host inflammatory responses include glycosylphosphatidylinositol (GPI) anchors, hemozoin, uric acid and parasite DNA [5, 15–17].

In vitro studies showed induction of nitric oxide, TNF and IL-1β by parasite GPI-anchors while synthetic and purified [19] *Plasmodium* GPI had immunogenic properties in vivo. *Plasmodium* species produce hemozoin as they detoxify heme in pRBCs. The hemozoin induce IL-1β production by immune cells such as monocytes and macrophages once released into circulation during pRBCs lysis [20]. Hemozoin has been demonstrated to activate the inflammasome protein complex [21, 22] and injection of parasite-derived hemozoin in disease-free mice induce transcription of inflammatory genes [23]. Parasite DNA has also been shown to induce cytokine and chemokine responses by human plasmacytoid dendritic cells by the activating the TLR9-MyD88 signaling pathway [24].

Parasite DNA is also detected by several cytosolic DNA sensors in the cytoplasma upon release of phagolysosomal contents [25]. Uric acid derived from the parasite and from the rupture of infected pRBCs has also been reported to induce strong inflammatory responses in patients [11]. In vitro studies show that uric acid derived from the parasite promotes secretion of pro-inflammatory cytokines that include TNF, IL-1β and IL-6 by [26]. The uric acid levels are specifically elevated in periods correlating with parasitemia [27, 28]. Overall, the role of parasitemia and components of the parasite in pathological inflammation response is an on-going concern poised for novel target development for diagnosis and anti-inflammatory antimalarials.

2.2 Inflammatory cytokines in severe malaria

P. falciparum infection is associated with the release of proinflammatory cytokines which are crucial in mediating the control of parasite growth and sickness behaviors in the host e.g., lethargy, fever, anorexia, pain [7, 29, 30]. The excessive production of these mediators is implicated in host-harming effects associated with infection. However, the inflammatory response is a highly regulated response regulated, in part, by the production of cytokines. Apparently, malaria disease outcome depends on the delicate balance between proinflammatory and anti-inflammatory cytokines. Development of severe forms of the disease and death depends on the rapture of this balance [31–33].

During malarial infection, the recognition of different pathogen related molecules expressed by the parasite or released by the host stimulate the immune system and cytokine production. A glycolipid toxin of *P. falciparum*, glycosylphosphatidylinositol (GPI), is a potent pathogen associated molecular pattern (PAMP) expressed on the parasite whose interaction with the host immune system induces the expression of genes encoding pro- and anti-inflammatory cytokines including TNF-α, IL-1, IL-6, IL-12, IL4, IL10 and the enzyme inducible nitric oxide synthase (iNOS) [5, 34]. GPI interacts with toll like receptor (TLR)1/TLR2 and TLR2/TLR6 dimers, and possibly C-type lectins on dendritic cells and macrophages to produce proinflammatory cytokines. Hemozoin, a PAMP and detoxification crystal from hemoglobin binds to TLR9 and mediates the production of proinflammatory cytokines in dendritic cells and macrophages [35, 36].

Pure hemozoin is not a ligand for TLRs but acts a carrier molecule for malarial DNA which has the immunostimulatory effects [37–39]. The *P. falciparum* genome contains CpG motifs which act through the MYD-88-NFkβ to induce cytokine production. *P. falciparum* DNA also contains the highest AT content and the AT motifs induce the production of interferons [16]. Other proteins, sugars, RNA motifs and other phosphorylated non-peptidic antigens are known to be PAMPs with cytokine induction potential [7, 36, 40]. In addition to parasite expressed proinflammatory molecules, several host-derived molecules, commonly known as damage associated molecular patterns (DAMPs), are also involved in evoking the inflammatory response and cytokine release in severe malaria. These include nucleic acids and urate crystals, heme and microvesicles derived from platelets, endothelial cells and leukocytes [11, 41–43].

2.2.1 Tumor necrosis factor-α (TNF-α)

TNF-α is involved in the pathogenesis of malaria associated with disease severity and death. TNF-α is dramatically increased with up to two- and ten-fold concentrations in cerebral malaria (CM) and in fatal cases, respectively [44, 45]. TNF-α concentrations are associated with parasite clearance and blocking TNF-α function increases the risk of hyperparasitemia [46]. The administration of recombinant TNF also induce clinical manifestations characteristic of malarial pathology including fever, anemia and hypotension [47, 48].

The susceptibility of an individual to cerebral malaria is linked to the presence of a genetic variant of the gene that encodes the TNF promoter [49]. TNF-α exacerbates sequestration in cerebral blood vessels in the brain by increasing the expression adhesion molecules e.g., ICAM-1 in the endothelial cells [50]. However, there is contrary knowledge on the role of TNF-α in the pathogenesis of severe and cerebral malaria [51–53]. Blocking TNF-α using anti-TNF antibodies does not seem to reduce mortality in CM [54] although it successfully stops fever suggesting that the full biological effects of TNF may be a concerted effort with a complex network of other cytokines. TNF-α is produced quite early during infection and has a short half-life, hence the timing of TNF-α neutralization in inflammation has to be carefully

considered. Blocking TNF-α when patients are already severely ill may be too late as an intervention to show anti-inflammatory efficacy of this process.

2.2.2 Lymphotoxin-α

Other cytokines demonstrate similar proinflammatory activity as TNF-α. Lymphotoxin-α (previously known as TNF-β) is a proinflammatory cytokine produced by lymphocytes which shares the same receptors with TNF and exhibits similar biological effects as TNF-α. Like TNF-α, lymphotoxin acts in synergy with IL-1 to increase the production of IL-6 and induce hypoglycemia; both are which are prominent clinical features in severe malaria [55]. Lymphotoxin also mediates the cell-mediated killing of *P. falciparum* parasites in infected erythrocytes [56].

2.2.3 Interferon γ

Another prominent proinflammatory cytokine in malarial pathology is IFNγ. IFNγ is produced by both CD4+ and CD8+ T lymphocytes during malarial infection. IFNγ activates macrophages and monocytes to produce other proinflammatory molecules including TNF, IL-1, IL-6, TGF-β and NO intermediates which help to kill the malarial parasite. As a result of its ability to induce TNF production, IFNγ has been implicated in the pathogenesis of cerebral malaria and blockage of IFNγ action was reported to prevent against cerebral malaria in murine models [57]. Additionally, IFNγ knockout mice showed resistance against cerebral malaria but were still susceptible to severe malaria and death. The release of IFNγ is controlled by IL-12 [58] and its relevance in severe disease may depend on the levels, timing and balance with other cytokines.

2.2.4 Interlukin-1

Interleukin-1 is a proinflammatory cytokine produced by macrophages, natural killer cells, B cells, dendritic cells and other immune cells. Serum concentrations of IL-1 correlate strongly with severity of disease with higher IL-1 levels observed in patients with CM and greater than in those in severe malarial anemia [59]. IL-1 has synergistic interactions with TNF-α and increases the expression of the adhesion molecule ICAM-1 in cerebral vasculature thus exacerbating sequestration.

2.2.5 Interleukin-6

IL-6 is proinflammatory cytokine released by monocytes/macrophages and Th2 cells. Serum concentrations of IL-6 vary with severity of disease with higher levels in CM than in severe and in uncomplicated cases [59]. IL-6 mediates TNF-α functions in severe malaria.

2.2.6 Interleukin-10

While proinflammatory cytokines play a critical role in the host immune defense against the malaria parasite, poorly regulated release of these chemical molecules results in the immunopathological response characteristic of severe malaria. Proinflammatory cytokines release is closely regulated by anti-inflammatory cytokines. IL-10 inhibits the release of Th1 type of cytokines but not Th2 showing its negative correlation with TNF-α, IL-1β and IL-8 [60].

Progression of malaria from uncomplicated to more severe forms is the functional balance of anti-inflammatory to proinflammatory cytokines than it is about individual cytokine levels. This explains why severe malaria does not occur in all cases where proinflammatory cytokine concentrations are very high. A downregulation of IL-10 coupled to the upregulation of TNF is usually associated with severe and CM. Thus, it is important to consider the ratio of IL-10 to TNF-α [61, 62].

2.2.7 Transforming growth factor-β (TGF-β)

TGF-β is an anti-inflammatory cytokine that immune-regulates malaria. A TGF-β neutralizing antibody in malaria infected mice is associated with dramatic increases in TNF-α and IFN-γ concentrations. TGF-β also upregulates IL-10 production while downregulating the expression of adhesion molecules, decreasing sequestration of pRBCs in severe malaria. Interestingly, at low concentrations TGF-β exhibits proinflammatory effects but has anti-inflammatory effects at high concentrations [63]. In malaria, the multifunctionality of TGF-β serves two important functions; (1) enhancing Th1 mediated parasite control during early infection, and (2) regulating the inflammatory response in later phases to prevent immunopathology [63].

3. Inflammation-induced mitochondrial dysfunction and cellular energy depletion in malaria

Parasitized red blood cells (pRBC's) agglutination to the endothelium causing blood vessels occlusion solely has been the ascribe process by which tissue hypoxia in malaria occurred. However, other mechanisms have since been elucidated as contributing to this phenomenon [64, 65]. Sepsis shares the same systemic pathology and disease presentation of tissue underlying tissue hypoxia with malaria but does not experience RBC's sequestration. Which may indicate other causes of the complication. In sepsis tissue oxygen tension is usually normal or elevated in the rat, patients or pigs [66]. This bring to the fore the aspect that poor oxygen utilization, compared to supply, as the major cause of malaria-related hypoxia.

Excessive reactive oxygen species (ROS), nitric oxide (NO) and peroxynitrite ($ONOO^-$), as seen in malaria, tend to cause mitochondria dysfunction. Proinflammatory cytokines induce excessive inducible nitric oxide synthase (iNOS) expression by monocytes and macrophages tend to increase NO and subsequently oxidative stress (OS). Increase OS reversibly inhibits cytochrome oxidase and aconitase [67, 68] with concomitant energy reduction with respiration fatigue and tissue hypoxia. Together or as separate phenomena, reduction in energy utilization potential and oxygen transportation, play a crucial role in the generation of hypoxic conditions of malaria in an escalating feed-forward mechanism.

Another mechanism for hypoxia in malaria involves the nuclear enzyme poly (ADP-ribose) polymerase (PARP). PARP catalyzes transfer of ADP-ribose units from β-nicotinamide adenine dinucleotide (NAD^+) to produce linear and branched polymers from a number of different proteins. The normal and malarial inflammation-driven DNA damages repair mechanism is the same mechanism that activates PARP. Activation of PARP invariably consumes NAD^+ and conversely leads to the depletion of ATP. Intriguingly, breaks of DNA strands may be initiated by oxygen free radicals or their NO reaction products $ONOO^-$ as OS. ROS and DNA strands breaks reigns supreme in malaria inflammation with consequential depletion of NAD^+ and decreased ATP generation without the critical NAD^+ [69]. Aerobic respiration is thus invariably compromised with the possibility of bioenergetics failure through increased

glycolysis which generates insufficient energy and hyperlactemia. In malarial inflammation, there is increased generation of $ONOO^-$ through induction of iNOS and subsequently NO, which activates over expression of PARP through increased DNA damage. Concomitantly, a vicious cycle of mitochondrial dysfunction leading to ATP rundown predisposes to polymyopathy, hypoglycemia, hyperlactemia. The facets have been hitherto mostly attributed to poor oxygen delivery in malaria [70].

The solution to energy regeneration may be through prevention of PARP-induced energy rundown and may comprise of (i) increasing supply of NAD^+, (ii) PARP activation-inhibition, (iii) quenching the inflammatory drivers of PARP activation, i.e., NO and $ONOO^-$, or stopping DNA damages by other means [71]. However, supplementation with NAD^+ in sepsis or malaria is somewhat impractical; inhibition of PARP may have deadly effects if it was possible; elimination of DNA damage is impossible. Quenching the pro-oxidant drivers of activation of PARP through DNA damage seem the most plausible method to protect against both the inflammasome and energy rundown. Indeed, certain agents have been reported to protect against free radical oxidation inhibiting PARP activation salvaging brain ischemia, splanchnic ischemia and reperfusion [72–74], lipopolysaccharide-induced toxicity, local inflammation and brain pathology in mice, multi-organ failure in rats and sepsis of the pigs [75, 76].

The other fascinating feature in PARP is that transcription factor NF-kβ is intricately connected the activation of the nuclear enzyme. Also, NFkβ is involved in DNA repair, immunological response and apoptosis placing it pivotally in the expression of genes essential to systemic inflammatory disease mainly, TNF-α and interleukin-1 (IL-1), IL-1β, IL-6, IAM-1, E-selectin and iNOS [77–82].

Essentially, PARP activation through $ONOO^-$ as a result of inflammation, consumes NAD^+ causing poor energy utilization and energy depletion [69]. Subsequent to inflammation-driven PARP activation through $ONOO^-$, tissue hypoxia (from increased NAD^+ consumption) sets in causing more inflammation-induced tissue damage, increased inflammatory cytokines synthesis and more $ONOO^-$ production [69]. Coma and death are inevitable from the vicious cycle of severe inflammation breeding more of itself leading to multi-organ failure, ultimately [70]. As a result, anti-inflammatory agents like Asiatic acid may have indispensable roles, through inhibition of NF-kβ, in the assuagement of malarial disease which essentially is underscored by inflammatory processes.

4. Malarial oxidative stress-driven inflammatory response

Reactive oxygen species have destructive effects through their ability to increase oxidative stress. Malaria disease is able to orchestrate multi-organ injury and disintegration through the ROS's disparaging properties. Oxidative stress (OS) has a fundamental role in pRBC's influence to disease manifestation comprising of pRBC's vascular sequestration, AKI, CM, SMA and ARDS [83, 84]. The defense mechanism of ROS and reactive nitric oxide species (RNOS) against disease and their signal transduction capacities make them have a both beneficial and pathological role in malaria necessitating regulation.

Plasmodium parasites have contracted capacities to mobilize amino acids and depend on hemoglobin (Hb) breakdown, a process with a high potential to generate ROS. Hb breakdown yields heme and globin (protein), with the former being a highly toxic compound which generates high OS activity at very low concentrations. Hb-free heme contains ferri/ferroprotoporphyrin IX (FP-IX) a very reactive iron-containing compound which generates an OS environment. Detoxification of heme by the parasite to hemozoin (β-hematin) is necessary and critical [85, 86]. Failure to convert heme to its biocrystallization or biomineralization form will oxidize the

parasite food vacuole membranes destroying it in the process. Chloroquine and other 4-aminoquinolines use this principle to dislocate parasite proliferation by inhibiting heme biocrystallization in pRBC's and increasing OS [85–89].

There is a high overall oxidative load that the host cell immune response to parasitemia likewise yields to the pRBC. Nevertheless, the parasite has developed mechanisms for amplified antioxidant capacity, which may only be overcome by an extremely oxidative agent [83, 90–93]. Agents that inhibit hemozoin creation from heme have since been rendered impotent through multidrug resistance (MDR) processes that extrude the drug from the food vacuole protecting the parasite from possible oxidative stress.

On the other hand, ROS may be deliberately generated and targeted at certain parasite enzymes and membranes, as what is witnessed in the use of endoperoxidase antimalarials, with higher chances of faster parasitemia clearance although with higher tissue inflammation induction as well [94].

Antioxidants may have an anti-inflammatory effect in malaria where inflammation is generated from OS. However, OS is beneficial in malarial parasite eradication. Pleiotropic characteristics of triterpenes, antioxidant and pro-oxidant, seem to be very ideal properties for combating malaria. Indeed, it has since emerged that certain phytotherapeutics do eradicate parasitemia while ameliorating the pathophysiology of malaria like inflammation [95] and severe malaria anemia [96]. Buttressing the antioxidant capacity of triterpene phytotherapeutics are findings that oral administration of Asiatic acid (20 mg/kg) in streptozotocin-induced diabetic rats up-regulated both enzymatic and non-enzymatic antioxidants with subsequent lipid peroxidation abating [97] and salvaged diabetic rats [98] where increased OS is common. Of note is that superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST), ascorbic acid and reduced glutathione (GSH) tend to be elevated in the phytochemical is administered animal experimental DM [97–99]. Moreover, severe malaria is accompanied by marked lipid peroxidation, prevention of which by triterpenes testifies their efficacy against OS driven inflammation in malaria and acute renal injury [100].

5. Hormonal anti-inflammatory processes in malaria mediate glucose homeostasis

Complications of malaria including CM, SMA, placental malaria, hypoglycemia, ARDS are not efficaciously resolved despite the effective parasitemia inhibition by current antimalarial agents [9]. Parasite infection and/or exaggerated immune reaction [101] contribute to malarial complications necessitating treatment emphasis on more than just pathogen clearance but extending it to host defense mechanism that do not interfere with parasitemia load. The malarial disease tolerance or anti-disease process has been linked to heme oxygenase [102] and to Fe^{3+} sequestration protein ferritin [103] and some novel phytotherapeutics [96]. Glucocorticoids (GC), cortisol in the human and corticosterone in rats and mineral corticoids (MC) are adrenal gland cortex products and the adrenaline-noradrenaline combination are from the adrenal medulla. Adrenal cortex responds to the circadian rhythm, when the hypothalamus-pituitary–adrenal axis is activated, in stress/trauma situations, infection or systemic inflammation [104] by producing hormones to influence metabolism, immunity, bone remodeling, cardiovascular function, reproduction and cognitive processes [105].

GC's have anti-inflammatory properties and differential effects on numerous leukocytes phenotypes [106, 107]. In response to GC's, liver, muscle, adipose tissue increase gluconeogenesis, protein catabolism and lipolysis, respectively, to increase glucose directly or indirectly [108]. Upon human malarial (*P. falciparum* or *P. vivax*)

infection, GC's (cortisol) are the only hormones of the adrenal gland [109]. Malaria infection and pregnancy-associated corticosterone concentration increases cause the loss malarial immunity and recrudescence [110–112] with adrenalectomy reducing survival in mice infected with *P. berghei* K173 parasite [113]. Infection of adrenalectomized mice causes lethal hypoglycemia of insulin- and TNF-α-independent type with increased inflammation [9]. The phenotype is characterized by exhausted hepatic glycogen stores, no increase in glucogenesis and is rescued by dexamethasone administration. This shows that GC's are essential for malarial disease tolerance through modulation of inflammatory mediators. Notably, raised cytokine concentration tend to be observed in both the circulation and the brain; adrenal hormones differentially disturb inflammation in the brain and circulation compared to liver and lungs. Together with inflammation, marked hypoglycemia sufficient to cause functional brain failure and coma, has been observed with rapid brain death [114]. Hypoglycemia is a major life threat in malaria and glycemia correlates with clinical scores negatively regular consistence. Moreover, plasma glucose concentrations negatively correlate with brain concentrations of mRNA's encoding TNF-α, IL-1β, IL-6, CCL2 and iNOS, signifying the possible linkage between hypoglycemia and expression of these pro-inflammatory markers. Plasma concentrations of chemokines, cytokines and with the exception of IL-4, tend to be negatively interrelated to plasma glucose concentration. Notwithstanding hypoglycemia, hyperlactemia in acidosis, resulting from increased glycolytic flux, may complicate malarial infection.

After malaria infection, hepatic gluconeogenic transcriptional response is diminished regardless of increases in GC concentration showing the importance of adrenal glands in maintaining blood glucose concentration in malaria. The classical glycemia-regulating principles of adrenal corticosteroids includes transcriptional induction of gluconeogenic response in the liver and increase plasma free fat acids concentrations.

In the absence of adrenal hormones, like in adrenalectomy, malaria causes complete exhaustion of hepatic glycogen stores. This creates irreversible severe hypoglycemia that may not be rescued by glucose supplementation by oral or intraperitoneal (i.p.) injection route in mice. Parasitemia remains unaffected by glucose supplementation in such situations. Amazingly, even the neutralization of the most potent pro-inflammatory cytokine, TNF-α, does not seem to salvage from lethal hypoglycemia in the absence of adrenal glands. Also, adrenal hormone absence in malaria affect glycemia independent of insulin secretion. Overall, malaria infection is moderated by adrenal hormones to alleviate hypoglycemia which cannot be reversed by glucose administration, insulin blocking with clonidine, neutralizing of TNF-α but is rescued by dexamethasone, a GC analog [9]. A similar effect has been shown when triterpene phytotherapeutics, potent anti-inflammatory capacity, are administered in murine malaria [115] also showing inflammation involvement in malarial lethality.

6. Inflammation and the pathogenesis of cerebral malaria

Cerebral malaria is the most severe neurological complication of malaria. It is a clinical syndrome characterized by coma and other acute and or chronic neurological disturbances. In children, coma may develop with seizures often following weakness and prostration. Other neurological symptoms include encephalitis, intracranial hypertension, retinal changes and brainstem signs (impaired pupillary reflexes, posture problems and abnormal eye movements) [116, 117]. In adults, patients develop fever and headaches and progressive delirium and coma but seizures and retinal abnormalities are less common [116, 117]. Several neurological sequelae have been associated with cerebral malaria and these include; spasticity (hemiplegia, quadriparesis or quadriplegia), hypotonia, cranial nerve palsies, ataxia, visual disturbances, aphasias,

neurocognitive deficits, epilepsy and some behavioral and neuropsychiatric disturbances. These sequelae may occur in the short-term and resolve, or may persist long term [117].

The pathogenesis of cerebral malaria has been be explained according to two theories: (1) the occlusion theory, or (2) the inflammation theory. The occlusion theory, supported by vast scientific evidence, suggests that brain injury and the resultant neurological disturbances are the result of increased sequestration of blood cells to the brain microvasculature which reduces perfusion and may cause ischemia and tissue injury. Increased sequestration of infected red blood cells, leukocytes and platelets is well known to occur in cerebral malaria [118–120] but the occlusion theory does not adequately explain how some fatal cases of cerebral malaria occur with little to no sequestration. Additionally, although *P. vivax* is not likely to sequester in brain vasculature, there have been isolated cases of *P. vivax* infection-related cerebral malaria cases [121]. These gaps in our knowledge of the pathogenesis of cerebral malaria have led to an increased interest in other possible pathologic mechanisms which may work independently or together with occlusion to cause cerebral malaria and related neurological disturbances. Stimulation of a local inflammatory response in the brain has been coined as an alternative or accompanying mechanism in the pathogenesis of cerebral malaria and is summarized in **Figure 1**.

The blood brain barrier (BBB) endothelium responds to PAMPs, DAMPs and peripheral cytokines and is now regarded as an integral part of the neurovascular unit. In response to these immunostimulatory molecules, endothelial cells produce proinflammatory cytokines and chemokines that mediate leukocyte recruitment and thus trigger local inflammation [122, 123]. Leukocytes further release proinflammatory cytokines and thus set up a vicious inflammatory cycle which exacerbates local inflammation with the brain. As part of the BBB, the integrity of the endothelium is central to BBB function and brain protection. Inflammatory activation of the endothelium has been associated with increased BBB permeability through the induction of regulatory miRNAs that reorganize endothelial tight junctions [124, 125]. This renders the BBB leaky and allows passage of substances into neural tissue, leading to neurotoxicity. Clinically the progression of cerebral malaria has been closely associated with changes in BBB function as evidenced by hemorrhages in cerebral malaria and loss of endothelial intercellular junctions in pediatric fatal cerebral malaria (**Figure 2**) [132].

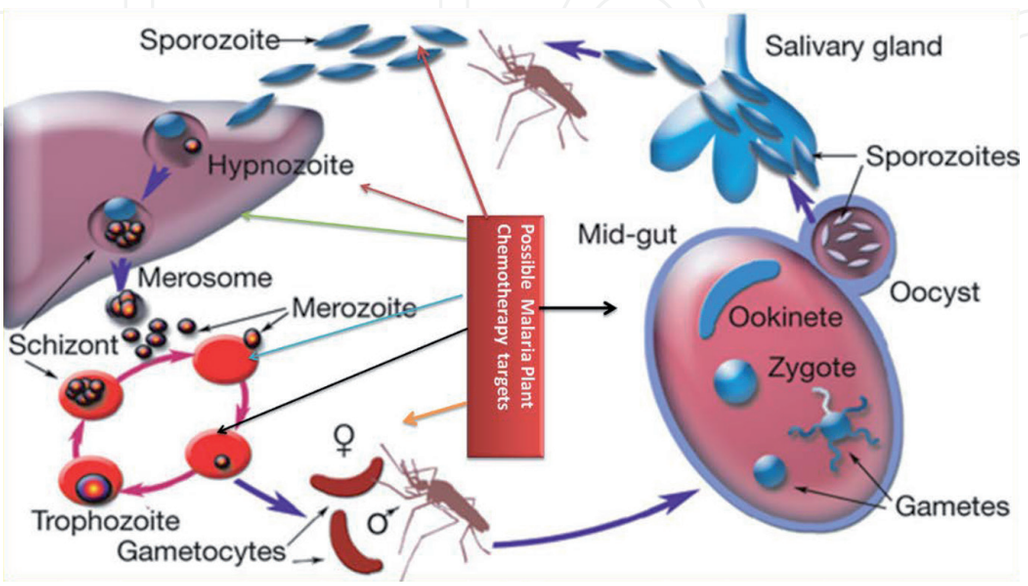

Figure 1.
Malaria Cycle and therapeutic possible cites [18].

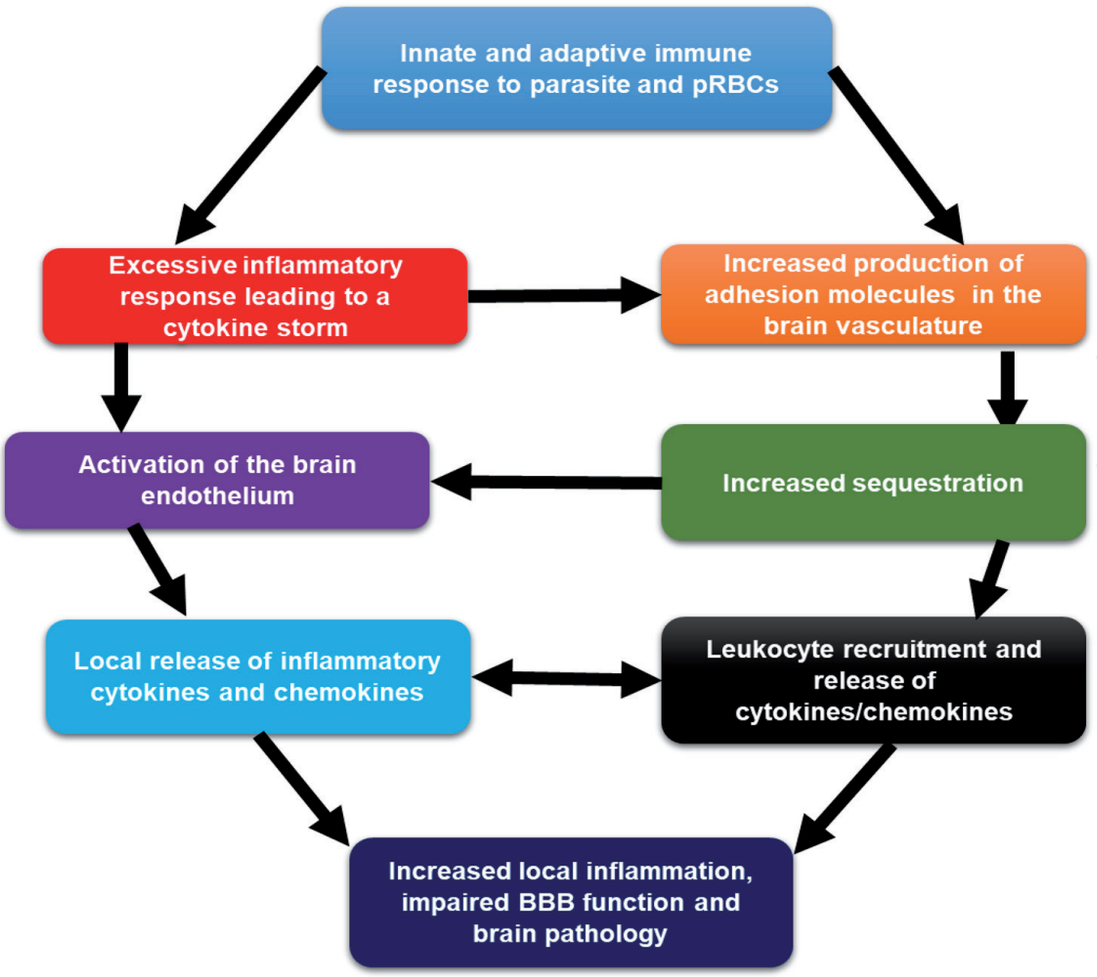

Figure 2.
Inflammatory events involved in the pathogenesis of cerebral malaria endothelial intercellular junctions in pediatric fatal cerebral malaria [126].

The production of proinflammatory cytokines in the brain could also be involved the development of encephalopathy. For example, TNF has been reported to regulate synaptic function and to cause glutamate neurotoxicity [127]; mechanisms which are closely linked to the development of seizures and neurocognitive deficits. IL-1 and TNF have also been shown to inhibit long term potentiation [128] and it is possible that high levels of these cytokines produced in severe malaria could be involved in the development of cognitive deficits associated with the disease.

7. Medicinal plants

Exposure to Plasmodium infection leads to elevation of pro-inflammatory markers such as TNF-α and interleukin-1 (IL-1) from macrophages and lymphocytes. Natural products have attracted interest due to their affordability to the general communities with low socio-economic status. Below is the description of the triterpenes that possess anti-inflammatory properties.

7.1 Synthetic oleanolic (SO) pentacyclic triterpenes derivatives

CDDO-EA (**Figure 3**) is a synthetic oleanolic derivative that has been shown to possess various biochemical activities which include efficacy against cerebral malaria (CM) [129]. The development of severe CM is associated with dysfunction of the

immune system as shown by plasma levels of TNF-α and IFN-γ [59, 130]. A single injection dose of CCDO-EA (200 μmol/kg) lowered circulating levels of TNF-α and IFN-γ which improved mice survival and lowered inflammation [129]. Indeed, studies have indicated that the host's response to malaria is excess production of pro-inflammatory molecules which are thought to be central causes of inflammation in malaria.

7.2 Ursolic acid

Ursolic acid (3-hydroxy-urs-12-ene-28-oic acid, **Figure 4**) is triterpene which is widely distributed on different medicinal plants. UA has been shown to exhibit a number of pharmacological activities which include, anti-microbial [132], anti-malarial [133] and potent anti-inflammatory properties [134]. Although

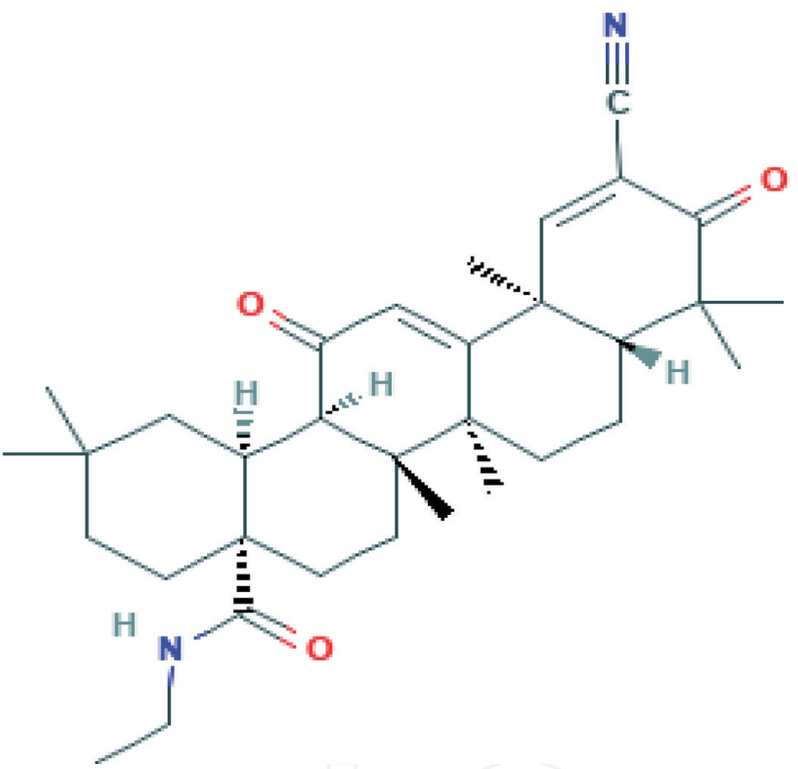

Figure 3.
Chemical structure of a synthetic oleanolae derivative [129].

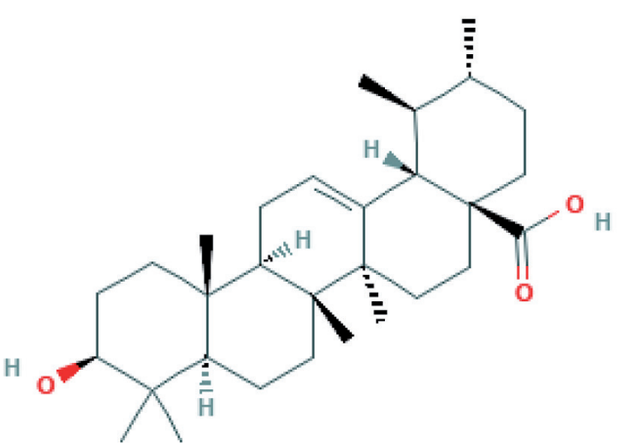

Figure 4.
2D chemical structure of ursolic acid [131].

no research has been done on anti-inflammatory properties of this triterpene in malaria rats, several studies have evaluated anti-inflammatory properties on other experimental models in vivo and in vitro [134]. However, several studies have evaluated the anti-inflammatory properties of the compounds in in-vitro and in vivo. Tsai and Yin's reports indicate that UA and oleanolic acid (OA) alleviate inflammation through reduction of IL-6 and TNF-α [135]. Additionally, studies also validate the anti-inflammatory potency of UA by reducing the production of IL-2 and through activation of T-helper cells [136]. Liu et al., have shown that UA suppressed T cell responses including NF-kB inhibition at 25 mM while Bharata et al. demonstrated the efficacy against a lowers dose of UA IS enough to lower immune cells such as T-cells, B-cell, and macrophage activation. Apart from this, Xu et al. have shown that anti-inflammatory effects of UA are mediated through.

7.3 Maslinic acid (MA) and oleanolic acid (OA)

Maslinic acid and oleanolic acid are two triterpenes widely abundant in olive trees and *Syzygium* spp. Among many pharmacological properties, these triterpenes have demonstrated efficacy against malaria [133]. The triterpenoids are generally highly hydrophobic, which reduces their bioavailability and efficacy. Sibiya et al. showed that once off application of an-OA patch reduces parasitemia and TNF-α plasma levels [137]. Exposure of the host to malaria activates macrophages which in turn induces production of TNF-α and then the release of other cytokines such as IL-6 which initiate inflammation. Reports also indicate the efficacy of another promising pentacyclic triterpene (maslinic acid) to alleviate malaria and inflammation in general. Extensive in vivo and in vitro studies indicate that MA reduces inflammation by reducing lipopolysaccharides (LPS)-induced production of nitric oxide (NO) and INOS gene expression [132] Márquez et al. also indicated that MA reduces the production of interleukin-6 (IL-6) on peritoneal macrophages [132] (**Figure 5**).

7.4 Anti-inflammatory effects of Asiatic acid (AA) in malaria

Many parasitic and metabolic diseases are built upon inflammatory processes. Ample indications exist that phytopharmaceuticals may moderate innumerable inflammatory mediators, govern the production and action of second messengers, direct the expression of transcription factors and key pro-inflammatory mechanisms [1, 2, 95, 115, 138–141]. The fundamental machinery of anti-inflammatory activity for AA in malarial may comprise: (i) anti-oxidative and radical scavenging;

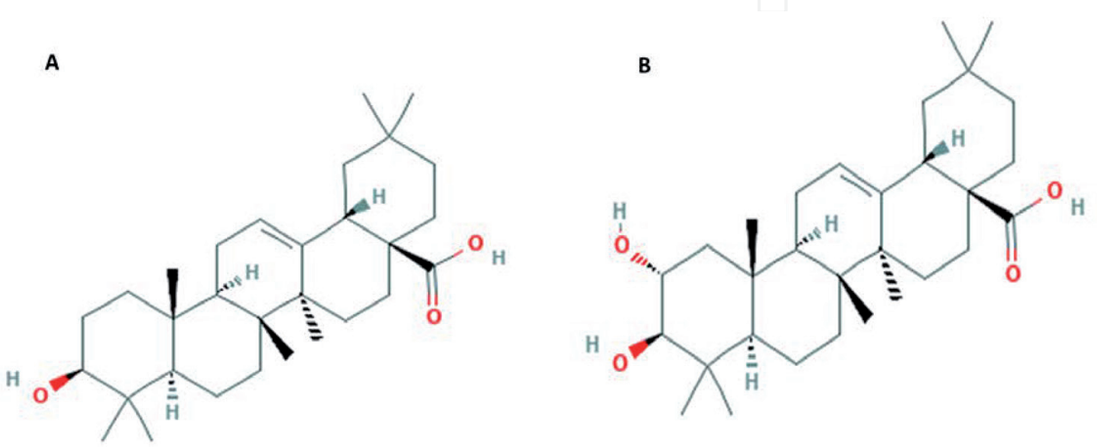

Figure 5.
2D chemical structure of oleanolic acid (A) and maslinic acid (B) adapted from the PubMed database [134].

(ii) inflammatory cellular components modulation (macrophages, lymphocytes neutrophils); (iii) modulation of expression and/or activity of pro-inflammatory enzymes such as phospholipase A2 (PLA2), cyclooxygenase (COX), lipooxygenase (LOX), iNOS and (iv) modulation of pro-inflammatory gene expression [138].

In malaria inflammation, the immune system-triggering-malaria-toxin is GPI which may be released pRBC rupture at erythrocytic schizogony [70]. GPI initiates TNF-α and lymphotoxin (formerly TNF-β) production [142], up-regulates ICAM-1 and IVCAM-1 [5, 143].

Hemopoietic mediators of inflammation comprise Th1/M1 cytokines largely TNF-α, IL-1, IL-6, IL-18 and Th2/M2 cytokines IL-4 and IL-10. When produced excessively as in severe malaria, Th1 cytokines may lead to the generation of fever, hypoglycemia, bone marrow suppression, coagulopathies, hypergammaglobulinemia, hypotension and elevated acute phase reactants [55, 144]. The works by Clark and Chaudhri [144], showing that TNF-α-induced dyserythropoiesis and erythrophagocytosis in malaria-infected animals, evidenced the association of SMA to inflammatory mediators and corroborated Peetre et al. who verified growth inhibition of culture hemopoietic cells [145].

Compounded, the anti-inflammatory outcome of AA may modify pro-inflammatory apparatuses in malaria in the same way it does in other inflammatory diseases. Indeed, AA displays a dose-dependent (10 and 20 μsg/kg AA) selective induction of selective mitochondria-dependent apoptosis in activated Th1 cells. This averted concanavalin (Con-A)-induced murine fulminant hepatitis in a fashion that disrupted mitochondrial transmembrane potential, released cytochrome c, activated caspases and cleaved poly(ADP-ribose) polymerase [PARP] [146].

In malaria, hematological differential counts display exaggerated leukocytosis. As inflammatory response is similar regardless of cause, AA may modify Th1 over expression in malaria by eradicating activated cells. Moreover, in a mouse model for pain and inflammation, AA blocked the activation of NF-kβ [147], a major transcription factor in the regulation of pro-inflammatory cells, cytokines and enzymes [148].

In unstimulated Th1 cells, NF-kβ subunit p65/p50, is sequestered in the cytoplasm bound to the inhibitory factor Ikβ-α. Proinflammatory signals in malaria comprising of GPI, cause the phosphorylation of Ikβ-α by Ikβ kinase (IKK) and its inactivation though the ubiquitin-mediated destruction. Liberated, NF-kβ translocate into the nucleus acting as pro-inflammatory mediator and transcription factor [70, 78, 148]. Eradication inflammatory responses is critical for overall health maintenance. AA may be able to inhibit GPI production or maintain inactivation of NF-kβ or both as this anti-inflammatory mechanism has been revealed in other diseases, and not malaria, when similar triterpenoid to AA, madecassoside (MA), was used [149–151].

By inhibiting activation of NF-kβ, AA may subsequently inhibit iNOS and COX-2 and reduce NO release. Moreover, AA (10 mg/kg) injected into Carrageenan-induced paw edema inhibited expression of iNOS, COX-2 and NF-kβ in mice [147]. This may mean, in malaria, reduction in unrestrained vasodilation related to vascular permeability, pulmonary edema or renal dysfunction. Toxic oxidative activities causing tissue injury may likewise be ablated by a NO reduction and possibly superoxide [$O_2^{\bullet -}$] [151]. Certainly, AA has been predicted by a computational model AutoDock v.3.05 to bind iNOS. This binding inhibits iNOS's strong affinity for arginine, exhibited as free energy binding (FEB) of −9.79 kcal.mol^{-1} [152–154].

Chemoattractant mediators hinging on NF-kβ activation may also be inhibited by AA resulting in abrogation of neutrophil-aggregation and inactivation of the linked oxidant and pro-inflammatory injury lytic enzymes [155]. Activation inhibition of peroxisome proliferator-activated gamma (PPAR-γ), which regulates inflammation through NF-kβ translocation, may be a route AA may confer anti-inflammatory activity. A similar process has been confirmed with curcumin, a multi-faceted phytopharmaceutical [156]. The consequent action of this activation

will be up-regulation of CD36 in monocytes/macrophages for non-opsonic pRBC's phagocytosis with parasite extrusion or destruction [157].

Credence of AA anti-inflammatory capacity in malarial pathophysiology has been shown. Indeed, the anti-inflammatory effect of AA has been reported in a murine malaria model where C-reactive protein was shown to be significantly reduced in infected transdermal AA administered animals as compared to infected chloroquine-treated animals and non-treated controls [95].

8. Conclusion

The strong connection between malaria pathophysiology and systemic inflammation mobilizes various mediators, metabolic processes consummating in toxic cachexia, hypoglycemia, neuronal damage, coma and death. Numerous immunological and inflammatory response mediators drive the disease. Initial inflammatory response directed at alleviating and curtailing the infection through parasite killing turns around and aberrantly militates against the host. Hormonal involvement is crucial in maintain malaria tolerance by the host. The phytotherapeutics AA, Ma and OA intervention in malaria promises to engage the parasitic as well the inflammation salvaging glucose homeostasis, neuronal death and other disease effects in malaria terminating the vicious cycle and alleviating the disease. Potential alternative treatment regimens for malaria are thus in the offing.

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References

[1] Mavondo GA, Kasvosve I. Antimalarial phytochemicals: Delineation of the triterpene asiatic acid malarial anti-disease and pathophysiological remedial activities—Part I. Journal of Infectious Disease and Pathology. 2017;**1**:104

[2] Mavondo GA, Kasvosve I. Antimalarial phytochemicals: Delineation of the triterpene asiatic acid malarial anti-disease and pathophysiological remedial activities—Part II. Journal of Infectious Disease and Pathology. 2017;**1**:103

[3] Dinarello CA. Interleukin-1 and the pathogenesis of the acute-phase response. The New England Journal of Medicine. 1984;**311**:1413

[4] Beutler B, Cerami A. Tumour necrosis factor, cachexia, shock, and inflammation: A common mediator. Annual Review of Biochemistry. 1988;**57**:505

[5] Schofield L, Hackett F. Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites. The Journal of Experimental Medicine. 1993;**177**:145-153

[6] Clark IA. Cell-mediated immunity in protection and pathology of malaria. Parasitology Today. 1987;**3**:300

[7] Schofield L, Grau GE. Immunological processes in malaria pathogenesis. Nature Reviews Immunology. 2005;**5**:722-735

[8] Hansen DS. Inflammatory responses associated with the induction of cerebral malaria: Lessons from experimental murine models. PLoS Pathogens. 2012;**8**(12):e1003045. DOI: 10.1371/journal.ppat.1003045

[9] Vandermosten L, Pham T-T, Knoops S, De Geest C, Lays N, Van der Molen K, et al. Adrenal hormones mediate disease tolerance in malaria. Nature Communications. 2018;**9**:4525. DOI: 10.1038/s41467-018-06986-5

[10] Moneriz C, Mestres J, Bautista JM, Diez A, Puye A. Multi-targeted activity of maslinic acid as an antimalarial natural compound. The FEBS Journal. 2011;**278**:2951-2961

[11] Gallego-Delgado J, Ty M, Orengo JM, van de Hoef D, Rodriguez A. A surprising role for uric acid: The inflammatory malaria response. Current Rheumatology Reports. 2014;**16**(2):401

[12] Dinko B, Pradel G. Immune evasion by *Plasmodium falciparum* parasites: Converting a host protection mechanism for the parasite's benefit. Advances in Infectious Disease. 2016;**6**(2):67759

[13] Artavanis-Tsakonas K, Tongren JE, Riley EM. The war between the malaria parasite and the immune system: Immunity, immunoregulation and immunopathology. Clinical and Experimental Immunology. 2003;**133**:145-152

[14] Dodoo D, Omer FM, Todd J, Akanmori BD, Koram K. Absolute levels and ratios of proinflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to *Plasmodium falciparum* malaria. The Journal of Infectious Diseases. 2002;**185**:971-979

[15] Griffith JW, Sun T, McIntosh MT, Bucala R. Pure hemozoin is inflammatory in vivo and activates the NALP3 inflammasome via release of uric acid. Journal of Immunology. 2009;**183**:5208-5220

[16] Sharma S, DeOliveira RB, Kalantari P. Innate immune recognition of an AT-rich stem-loop DNA motif in

the *Plasmodium falciparum* genome. Immunity. 2011;**35**:194-207

[17] Gowda NM, Wu X, Gowda DC. The nucleosome (histone-DNA complex) is the TLR9-specific immunostimulatory component of *Plasmodium falciparum* that activates DCs. PLoS One. 2011;**6**:e20398

[18] Winzeler EA. Malaria research in the post-genomic era. Nature. 2008;**455**:751-756. DOI: 10.1038/nature07361

[19] Naik RS, Branch OH, Woods AS. Glycosylphosphatidylinositol anchors of Plasmodium falciparum: Molecular characterization and naturally elicited antibody response that may provide immunity to malaria pathogenesis. The Journal of Experimental Medicine. 2000;**192**(11):1563-1576. DOI: 10.1084/jem.192.11.1563

[20] Olivier M, Van Den Ham K, Shio MT, Kassa FA, Fougeray S. Malarial pigment hemozoin and the innate inflammatory response. Frontiers in Immunology. 2014;**5**:25. DOI: 10.3389/fimmu.2014.00025

[21] Dostert C, Guarda G, Romero JF, Menu P, Gross O, Tardivel A. Malarial hemozoin is a Nalp3 inflammasome activating danger signal. PLoS One. 2009;**4**:e6510. DOI: 10.1371/journal.pone.0006510

[22] Shio MT, Eisenbarth SC, Savaria M, Vinet AF, Bellemare MJ, Harder KW. Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. PLoS Pathogens. 2009;**5**:e1000559. DOI: 10.1371/journal.ppat.1000559

[23] Deroost K, Lays N, Pham T-T, Baci D, Van den Eynde K, Komuta M. Hemozoin induces hepatic inflammation in mice and is differentially associated with liver pathology depending on the *Plasmodium* strain. PLoS One. 2014;**9**(11):e113519. DOI: 10.1371/journal.pone.0113519

[24] Pichyangkul S, Yongvanitchit K, Kum-arb U, Hemmi H, Akira S, Krieg AM. Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a toll-like receptor 9-dependent pathway. Journal of Immunology. 2004;**172**:4926-4933. DOI: 10.4049/jimmunol.172.8.4926

[25] Gowda DC, Wu X. Parasite recognition and signaling mechanisms in innate immune responses to malaria. Frontiers in Immunology. 2018;**9**:3006. DOI: 10.3389/fimmu.2018.03006

[26] Orengo JM, Leliwa-Sytek A, Evans JE. Uric acid is a mediator of the *Plasmodium falciparum*-induced inflammatory response. PLoS One. 2009;**4**:e5194

[27] Lopera-Mesa TM, Mita-Mendoza NK, van de Hoef DL. Plasma uric acid levels correlate with inflammation and disease severity in Malian children with *Plasmodium falciparum* malaria. PLoS One. 2012;**7**:e46424

[28] Mita-Mendoza NK, van de Hoef DL, Lopera-Mesa TM. A potential role for plasma uric acid in the endothelial pathology of *Plasmodium falciparum* malaria. PLoS One. 2013;**8**:e54481

[29] Mackintosh CL, Beeson JG, Marsh K. Clinical features and pathogenesis of severe malaria. Trends in Parasitology. 2004;**20**(12):597-603

[30] Lyke K, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, et al. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1β), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12 (p70) in Malian children with severe *Plasmodium falciparum* malaria and matched uncomplicated malaria

or healthy controls. Infection and Immunity. 2004;**72**(10):5630-5637

[31] Crompton PD, Moebius J, Portugal S, Waisberg M, Hart G, Garver LS, et al. Malaria immunity in man and mosquito: Insights into unsolved mysteries of a deadly infectious disease. Annual Review of Immunology. 2014;**32**:157-187

[32] Dieye Y, Mbengue B, Dagamajalu S, Fall MM, Loke MF, Nguer CM, et al. Cytokine response during non-cerebral and cerebral malaria: Evidence of a failure to control inflammation as a cause of death in African adults. PeerJ. 2016;**4**:e1965

[33] Clark IA, Budd AC, Alleva LM, Cowden WB. Human malarial disease: A consequence of inflammatory cytokine release. Malaria Journal. 2006;**5**(1):85

[34] Tachado SD, Gerold P, McConville MJ, Baldwin T, Quilici D, Schwarz RT, et al. Glycosylphosphatidylinositol toxin of *Plasmodium* induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signaling pathway. The Journal of Immunology. 1996;**156**(5):1897-1907

[35] Sherry BA, Alava G, Tracey KJ, Martiney J, Cerami A, Slater A. Malaria-specific metabolite hemozoin mediates the release of several potent endogenous pyrogens (TNF, MIP-1 alpha, and MIP-1 beta) in vitro, and altered thermoregulation in vivo. Journal of Inflammation. 1995;**45**(2):85-96

[36] Gazzinelli RT, Kalantari P, Fitzgerald KA, Golenbock DT. Innate sensing of malaria parasites. Nature Reviews Immunology. 2014;**14**(11):744

[37] Parroche P, Lauw FN, Goutagny N, Latz E, Monks BG, Visintin A, et al. Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to toll-like receptor 9. Proceedings of the National Academy of Sciences. 2007;**104**(6):1919-1924

[38] Schwarzer E, Turrini F, Ulliers D, Giribaldi G, Ginsburg H, Arese P. Impairment of macrophage functions after ingestion of *Plasmodium falciparum*-infected erythrocytes or isolated malarial pigment. The Journal of Experimental Medicine. 1992;**176**(4):1033-1041

[39] Scorza T, Magez S, Brys L, De Baetselier P. Hemozoin is a key factor in the induction of malaria-associated immunosuppression. Parasite Immunology. 1999;**21**(11):545-554

[40] Liehl P, Zuzarte-Luís V, Chan J, Zillinger T, Baptista F, Carapau D, et al. Host-cell sensors for *Plasmodium* activate innate immunity against liver-stage infection. Nature Medicine. 2014;**20**(1):47

[41] Butt AN, Swaminathan R. Overview of circulating nucleic acids in plasma/serum: Update on potential prognostic and diagnostic value in diseases excluding fetal medicine and oncology. The Annals of the New York Academy of Sciences. 2008;**1137**(1):236-242

[42] Figueiredo RT, Fernandez PL, Mourao-Sa DS, Porto BN, Dutra FF, Alves LS, et al. Characterization of heme as activator of Toll-like receptor 4. The Journal of Biological Chemistry. 2007;**282**(28):20221-20229

[43] Mantel PY, Marti M. The role of extracellular vesicles in *Plasmodium* and other protozoan parasites. Cellular Microbiology. 2014;**16**(3):344-354

[44] Kern P, Hemmer CJ, Gallati H, Neifer S, Kremsner P, Dietrich M, et al. Soluble tumor necrosis factor receptors correlate with parasitemia and disease severity in human malaria. The Journal of Infectious Diseases. 1992;**166**(4):930-934

[45] Molyneux M, Engelmann H, Taylor TE, Wirima JJ, Aderka D, Wallach D, et al. Circulating plasma receptors for tumour necrosis factor in Malawian children with severe falciparum malaria. Cytokine. 1993;**5**(6):604-609

[46] Geraghty EM, Ristow B, Gordon SM, Aronowitz P. Overwhelming parasitemia with *Plasmodium falciparum* infection in a patient receiving infliximab therapy for rheumatoid arthritis. Clinical Infectious Diseases. 2007;**44**(10):e82-e84

[47] Spriggs D, Imamura K, Rodriguez C, Horiguchi J, Kufe D. Induction of tumor necrosis factor expression and resistance in a human breast tumor cell line. Proceedings of the National Academy of Sciences. 1987;**84**(18):6563-6566

[48] Creagan ET, Kovach JS, Moertel CG, Frytak S, Kvols LK. A phase I clinical trial of recombinant human tumor necrosis factor. Cancer. 1988;**62**(12):2467-2471

[49] McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-α and interleukin-1 (IL-1) promoter region associated with susceptibility to cerebral malaria. Nature. 1994;**371**(6497):508

[50] Berendt A, Simmons D, Tansey J, Newbold CI, Marsh K. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. Nature. 1989;**341**(6237):57

[51] Esamai F, Ernerudh J, Janols H, Welin S, Ekerfelt C, Mining S, et al. Cerebral malaria in children: Serum and cerebrospinal fluid TNF-α and TGF-β levels and their relationship to clinical outcome. Journal of Tropical Pediatrics. 2003;**49**(4):216-223

[52] Armah HB, Wilson NO, Sarfo BY, Powell MD, Bond VC, Anderson W, et al. Cerebrospinal fluid and serum biomarkers of cerebral malaria mortality in Ghanaian children. Malaria Journal. 2007;**6**(1):147

[53] Thuma PE, Van Dijk J, Bucala R, Debebe Z, Nekhai S, Kuddo T, et al. Distinct clinical and immunologic profiles in severe malarial anemia and cerebral malaria in Zambia. The Journal of Infectious Diseases. 2011;**203**(2):211-219

[54] van Hensbroek MB, Palmer A, Onyiorah E, Schneider G, Jaffar S, Dolan G, et al. The effect of a monoclonal antibody to tumor necrosis factor on survival from childhood cerebral malaria. The Journal of Infectious Diseases. 1996;**174**(5):1091-1097

[55] Clark I, Gray K, Rockett E, Cowden W, Rockett K, Ferrante A, et al. Increased lymphotoxin in human malarial serum, and the ability of this cytokine to increase plasma interleukin-6 and cause hypoglycaemia in mice: Implications for malarial pathology. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1992;**86**(6):602-607

[56] Kumaratilake L, Ferrante A, Rzepczyk C. The role of T lymphocytes in immunity to *Plasmodium falciparum*. Enhancement of neutrophil-mediated parasite killing by lymphotoxin and IFN-gamma: Comparisons with tumor necrosis factor effects. The Journal of Immunology. 1991;**146**(2):762-767

[57] Grau GE, Heremans H, Piguet P-F, Pointaire P, Lambert P-H, Billiau A, et al. Monoclonal antibody against interferon gamma can prevent experimental cerebral malaria and its associated overproduction of tumor necrosis factor. Proceedings of the National Academy of Sciences. 1989;**86**(14):5572-5574

[58] Sedegah M, Finkelman F, Hoffman SL. Interleukin 12 induction of interferon gamma-dependent

protection against malaria. Proceedings of the National Academy of Sciences. 1994;**91**(22):10700-10702

[59] Mandala WL, Msefula CL, Gondwe EN, Drayson MT, Molyneux ME, MacLennan CA. Cytokine profiles in Malawian children presenting with uncomplicated malaria, severe malarial anemia, and cerebral malaria. Clinical and Vaccine Immunology. 2017;**24**(4):e00533-e00516

[60] Mahanta A, Kar SK, Kakati S, Baruah S. Heightened inflammation in severe malaria is associated with decreased IL-10 expression levels and neutrophils. Innate Immunity. 2015;**21**(5):546-552

[61] Othoro C, Lal AA, Nahlen B, Koech D, Orago AS, Udhayakumar V. A low interleukin-10 tumor necrosis factor-α ratio is associated with malaria anemia in children residing in a holoendemic malaria region in western Kenya. The Journal of Infectious Diseases. 1999;**179**(1):279-282

[62] Kurtzhals JA, Adabayeri V, Goka BQ, Akanmori BD, Oliver-Commey JO, Nkrumah FK, et al. Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. The Lancet. 1998;**351**(9118):1768-1772

[63] Omer F, Kurtzhals J, Riley E. Maintaining the immunological balance in parasitic infections: A role for TGF-β? Parasitology Today. 2000;**16**(1):18-23

[64] Bihari D, Smithies M, Gimson A, Tinker J. The effects of vasodilation with prostacyclin on oxygen delivery and uptake in critically ill patients. The New England Journal of Medicine. 1987;**317**:397-403

[65] Bersten A, Sibbald WJ. Circulatory disturbances in multiple systems organ failure. Critical Care Clinics. 1989;**5**:233-254

[66] Boekstegers P, Weidenhofer S, Kapsner T, Werdan K. Skeletal muscle partial pressure of oxygen in patients with sepsis. Critical Care Medicine. 1994;**22**:640-650

[67] Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Letters. 1994;**345**:50-54

[68] Castro L, Rodriguez M, Radi R. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. The Journal of Biological Chemistry. 1994;**269**:29409-29415

[69] Szabo C. The pathophysiological role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. Shock. 1996;**6**:79-88

[70] Clark IA, Cowden WB. The pathophysiology of *falciparum* malaria. Pharmacology and Therapeutics. 2003;**99**:221-260

[71] Khan AU, Delude RL, Han YY, Sappington PL, Han X, Carcillo JA, et al. Liposomal NAD(+) prevents diminished O_2 consumption by immunostimulated Caco-2 cells. The American Journal of Physiology. 2002;**282**:L1082-L1091

[72] Takahashi K, Pieper AA, Croul SE, Zhang J, Snyder SH, Greenberg JH. Post-treatment with an inhibitor of poly(ADP-ribose) polymerase attenuates cerebral damage in focal ischemia. Brain Research. 1999;**829**:46-54

[73] Jagtap P, Soriano FG, Virag L, Liaudet L, Mabley J, Szabo E, et al. Novel phenanthridinone inhibitors of poly(adenosine 5′-diphosphateribose) synthetase: Potent cytoprotective and antishock agents. Critical Care Medicine. 2002;**30**:1071-1082

[74] Mazzon E, Dugo L, De SA, Li JH, Caputi AP, Zhang J, et al. Beneficial effects of GPI 6150, an inhibitor of poly(ADP-ribose) polymerase in a rat model of splanchnic artery occlusion and reperfusion. Shock. 2002;**17**:222-227

[75] Koedel U, Winkler F, Angele B, Fontana T, Pfister HW. Meningitis-associated central nervous system complications are mediated by the activation of poly(ADP-ribose) polymerase. Journal of Cerebral Blood Flow and Metabolism. 2002;**22**:39-49

[76] Cuzzocrea S, Zingarelli B, Costantino G, Sottile A, Teti D, Caputi AP. Protective effect of poly(ADP-ribose) synthetase inhibition on multiple organ failure after zymosan-induced peritonitis in the rat. Critical Care Medicine. 1999;**27**:1517-1523

[77] Jimi E, Ghosh S. Role of nuclear factor-kappaB in the immune system and bone. Immunological Reviews. 2005;**208**:80-87

[78] Ogawa Y, Yoneda M, Tomeno W, Imajo K, Shinohara Y, Fujita K, et al. Peroxisome proliferator-activated receptor gamma exacerbates concanavalin A-induced liver injury via suppressing the translocation of NF-κ B into the nucleus. 2012;**2012**:940384. DOI: 10.1155/2012/940384

[79] Janssens S, Tschopp J. Signals from within: The DNA-damage-induced NF-kappaB response. Cell Death and Differentiation. 2006;**13**(5):773-784

[80] Niu J, Shi Y, Tan T, Yang CH, Fan M, Pfeffer LM, et al. DNA damage induces NF-kB-dependent micro RNA-21 up-regulation and promotes breast cancer cell invasion. The Journal of Biological Chemistry. 2012;**287**:21783-21795

[81] Lawrence T. The nuclear factor NF-κB pathway in inflammation. Cold Spring Harbor Perspectives in Biology. 2009;**1**(6):a001651. DOI: 10.1101/cshperspect.a001651

[82] Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. Oncogene. 2006;**25**(51):6758-6780

[83] Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogersone S, Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: Host-parasite interactions. International Journal for Parasitology. 2004;**34**:163-189

[84] Descamps-Latscha B, Lunel-Fabiani F, Kara-Binis A, Druilhe P. Generation of ROS in whole blood from patients with acute *falciparum* malaria. Parasite Immunology. 1987;**9**:275-279

[85] Hempelmann E. Hemozoin biocrystallization in *Plasmodium falciparum* and the antimalarial activity of crystallization inhibitors. Parasitology Research. 2007;**100**:671-676

[86] Egan TJ. Haemozoin formation. Molecular and Biochemical Parasitology. 2008;**157**:127-136

[87] Goldberg DE. Haemoglobin degradation. Current Topics in Microbiology and Immunology. 2005;**295**:275-291

[88] Foley M, Tilley L. Quinoline antimalarials: Mechanisms of action and resistance and prospects for new agents. Pharmacology & Therapeutics. 1998;**79**:55-87

[89] Bonilla-Ramírez L, Galiano S, Quiliano M, Aldana I, Pabón A. Primaquine-quinoxaline 1,4-di-N-oxide hybrids with action on the exo-erythrocytic forms of *Plasmodium* induce their effect by the production of reactive oxygen species. Malaria Journal. 2019;**18**:201

[90] Hiller NL, Bhattacharjee S, van Ooij C, Liolios K, e a HT. A

host-targeting signal in virulence proteins reveals a secretome in malarial infection. Science. 2004;**306**:1934-1937

[91] Etkin NL, Ross PJ. Malaria, medicine and meals: A biobehavioral perspective. In: Romanucci-Ross L, Moerman DE, Tancredi LR, editors. The Anthropology of Medicine. New York: Praeger Publishers; 1997. pp. 169-209

[92] Etkin NL. Co-evolution of people, plants, and parasited: Biological and cultural adaptations to malaria. Proceedings of the Nutrition Society. 2003;**62**:311-317. DOI: 10.1079/PN2003244

[93] Etkin NL. Plants as antimalarial drugs: Relation to G-6-PD deficiency and evolutionary implications. In: MED LSG, editor. Adaptation to Malaria: The Interaction of Biology and Culture. New York: Gordon and Breach Publishers; 1997. pp. 139-176

[94] Olliaro PL, Haynes RK, Meunier B, Yuthavong Y. Possible modes of action of the artemisinin-type compounds. Trends in Parasitology. 2001;**17**:122-126

[95] Mavondo GA, Musabauane CT. Asiatic acid-pectin hydrogel matrix patch transdermal delivery system influences parasitaemia suppression and inflammation reduction in *P. berghei* murine malaria infected Sprague-Dawley rats. APJTM. 2016;**9**:1172-1180

[96] Mavondo GA, Mkhwananzi BN, Mabadla MV, Musabayane CT. Asiatic acid influences parasitaemia reduction and ameliorates malaria anaemia in *P. berghei* infected Sprague-Dawley male rats. BMC Complementary and Alternative Medicine. 2016;**16**:357. DOI: 10.1186/s12906-016-1338-z

[97] Ramachandran V, Saravanan R. Asiatic acid prevents lipid peroxidation and improves antioxidant status in rats with streptozotocin-induced diabetes. Journal of Functional Foods. 2013;**5**:1077-1087

[98] Ramachandran V, Saravanan R. Efficacy of asiatic acid, a pentacyclic triterpene on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. Phytomedicine. 2013;**20**:230-236

[99] Ramachandran V, Saravanan R. Antidiabetic and antihyperlipidemic activity of asiatic acid in diabetic rats, role of HMG CoA: *In vivo* and *in silico* approaches. Phytomedicine. 2014;**21**:225-232

[100] Mavondo GA, Musabayane CT. Transdermal drug delivery of asiatic acid influences renal function and electrolyte handling in *Plasmodium berghei*-infected Sprague-Dawley male rats. Journal Diseases and Medicinal Plants. 2017;**4**(1):18-29. DOI: 10.11648/j.jdmp.20180401.13

[101] Deroost K, Pham T-T, Opdenakker G, Van den Steen PE. The immunological balance between host and parasite in malaria. FEMS Microbiology Reviews. 2016;**40**:208-257

[102] Ferreira A, Marguti I, Bechmann I, Jeney V, Chora A, Palha NR, et al. Sickle hemoglobin confers tolerance to *Plasmodium* infection. Cell. 2011;**145**(3):398-409. DOI: 10.1016/j.cell.2011.03.049

[103] Gozzelino R, Andrade BB, Larsen R, Luz NF, Vanoaica L, Seixas E, et al. Metabolic adaptation to tissue iron overload confers tolerance to malaria. Cell Host & Microbe. 2012;**15**(5):693-704. DOI: 10.1016/j.chom.2012.10.011

[104] Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: From HPA axis to glucocorticoid receptor dysfunction. Annals of the New York Academy of Sciences. 2012;**1261**:55-63

[105] Oakley RH, Cidlowski JA. The biology of the glucocorticoid receptor: New signaling mechanisms in health and disease. The Journal of Allergy and Clinical Immunology. 2013;**132**:1033-1044

[106] Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. Nature Reviews. Immunology. 2017;**17**:233-247

[107] Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Molecular and Cellular Endocrinology. 2011;**335**:2-13

[108] Patel R, Williams-Dautovich J, Cummins CL. Minireview: New molecular mediators of glucocorticoid receptor activity in metabolic tissues. Molecular Endocrinology. 2014;**28**:999-1011

[109] Muehlenbein MP, Alger J, Cogswell F, James M, Krogstad D. The reproductive endocrine response to *Plasmodium vivax* infection in Hondurans. The American Journal of Tropical Medicine and Hygiene. 2005;**73**:178-187

[110] van Zon AA, Eling WM, Hermsen CC, Koekkoek AA. Corticosterone regulation of the effecto function of malarial immunity during pregnancy. Infection and Immunity. 1982;**36**:484-491

[111] van Zon A A, Eling W M, Hermsen C C, Van de Wiel T J, and Duives M E. (1983) Malarial immunity in pregnant mice, in relation to total and unbound plasma corticosterone. Bulletin de la Société de pathologie exotique et de ses filiales 76:493-502

[112] van Zon AA, Eling WM, Schetters TP, Hermsen CC. ACTH-dependent modulation of malaria immunity in mice. Parasite Immunology. 1985;**7**:107-117

[113] Herms V, Baumgart E, Kretschmar W. Effects of adrenalectomy and cortisone treatment on the course of malaria (*Plasmodium berghei*) in NMRI mice. Zeitschrift für Tropenmedizin und Parasitologie. 1968;**19**:389-400

[114] Cryer PE. Hypoglycemia, functional brain failure, and brain death. The Journal of Clinical Investigation;**117**:868-870

[115] Mavondo GA, Mkhwananzi BN, Mabandla MV, Musabayane CT. Asiatic acid influences glucose homeostasis in *P. berghei* murine malaria infected Sprague-Dawley rats. African Journal of Traditional, Complementary, and Alternative Medicines. 2016;**13**(5):91-101

[116] Idro R, Jenkins NE, Newton CR. Pathogenesis, clinical features, and neurological outcome of cerebral malaria. The Lancet Neurology. 2005;**4**(12):827-840

[117] Idro R, Marsh K, John CC, Newton CR. Cerebral malaria: Mechanisms of brain injury and strategies for improved neurocognitive outcome. Pediatric Research. 2010;**68**(4):267

[118] Berendt A, Tumer G, Newbold C. Cerebral malaria: The sequestration hypothesis. Parasitology Today. 1994;**10**(10):412-414

[119] Patnaik JK, Das BS, Mishra SK, Mohanty S, Satpathy SK, Mohanty D. Vascular clogging, mononuclear cell margination, and enhanced vascular permeability in the pathogenesis of human cerebral malaria. The American Journal of Tropical Medicine and Hygiene. 1994;**51**(5):642-647

[120] Grau GE, Mackenzie CD, Carr RA, Redard M, Pizzolato G, Allasia C, et al. Platelet accumulation in brain microvessels in fatal pediatric cerebral malaria. The Journal of Infectious Diseases. 2003;**187**(3):461-466

[121] Anstey NM, Russell B, Yeo TW, Price RN. The pathophysiology of vivax malaria. Trends in Parasitology. 2009;**25**(5):220-227

[122] Dunst J, Kamena F, Matuschewski K. Cytokines and chemokines in cerebral malaria pathogenesis. Frontiers in Cellular and Infection Microbiology. 2017;**7**:324

[123] Medana IM, Turner GD. Human cerebral malaria and the blood–brain barrier. International Journal for Parasitology. 2006;**36**(5):555-568

[124] Barker KR, Lu Z, Kim H, Zheng Y, Chen J, Conroy AL, et al. miR-155 modifies inflammation, endothelial activation and blood-brain barrier dysfunction in cerebral malaria. Molecular Medicine. 2017;**23**(1):24-33

[125] Lopez-Ramirez MA, Wu D, Pryce G, Simpson JE, Reijerkerk A, King-Robson J, et al. Micro RNA-155 negatively affects blood–brain barrier function during neuroinflammation. The FASEB Journal. 2014;**28**(6):2551-2565

[126] Brown H, Rogerson S, Taylor T, Tembo M, Mwenechanya J, Molyneux M, et al. Blood-brain barrier function in cerebral malaria in Malawian children. The American Journal of Tropical Medicine and Hygiene. 2001;**64**(3):207-213

[127] Pickering M, Cumiskey D, O'connor JJ. Actions of TNF-α on glutamatergic synaptic transmission in the central nervous system. Experimental Physiology. 2005;**90**(5):663-670

[128] Cunningham A, Murray C, O'neill L, Lynch M, O'connor J. Interleukin-1β (IL-1β) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. Neuroscience Letters. 1996;**203**(1):17-20

[129] Crowley VM, Ayi K, Lu Z, Liby KT, Sporn M, Kain KC. Synthetic oleanane triterpenoids enhance blood brain barrier integrity and improve survival in experimental cerebral malaria. Malaria Journal. 2017;**16**(1):463. DOI: 10.1186/s12936-017-2109-0

[130] Ho M, Sexton MM, Tongtawe P, Looareesuwan S, Suntharasamai P, Webster HK. Interleukin-10 inhibits tumor necrosis factor production but not antigen-specific lymphoproliferation in acute *Plasmodium falciparum* malaria. The Journal of Infectious Diseases. 1995;**172**(3):838-844. DOI: 10.1093/infdis/172.3.838

[131] National Center for Biotechnology Information. Ursolic acid, CID=64945, PubChem Database. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Ursolic-acid

[132] Marquez Martin A, de la Puerta Vazquez R, Fernandez-Arche A, Ruiz-Gutierrez V. Supressive effect of maslinic acid from pomace olive oil on oxidative stress and cytokine production in stimulated murine macrophages. Free Radical Research. 2006;**40**(3):295-302. DOI: 10.1080/10715760500467935

[133] Moneriz C, Marin-Garcia P, Garcia-Granados A, Bautista JM, Diez A, Puyet A. Parasitostatic effect of maslinic acid. I. Growth arrest of *Plasmodium falciparum* intraerythrocytic stages. Malaria Journal. 2011;**10**:82. DOI: 10.1186/1475-2875-10-82

[134] National Center for Biotechnology Information. Maslinic acid, CID=73659, PubChem Database. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Maslinic-acid

[135] Tsai SJ, Yin MC. Antioxidative and anti-inflammatory protection of oleanolic acid and ursolic acid in PC12 cells. Journal of Food Science. 2008;**73**(7):H174-H178. DOI: 10.1111/j.1750-3841.2008.00864.x

[136] Zeng G, Chen J, Liang QH, You WH, Wu HJ, Xiong XG. Ursolic acid inhibits T-cell activation through modulating nuclear factor-kappa B signaling. Chinese Journal of Integrative Medicine. 2012;**18**(1):34-39. DOI: 10.1007/s11655-011-0858-0

[137] Sibiya H, Musabayane CT, Mabandla MV. Transdermal delivery of oleanolic acid attenuates pro-inflammatory cytokine release and ameliorates anaemia in *P. berghei* malaria. Acta Tropica. 2017;**171**:24-29. DOI: 10.1016/j.actatropica.2017.03.005

[138] Bellik Y, Boukraâ L, Alzahrani HA, Bakhotmah BA, Abdellah F, Hammoudi SM, et al. Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: An update. Molecules. 2013;**18**:322-353. DOI: 10.3390/molecules18010322

[139] Evans AG, Wellems T. Coevolutionary genetics of *Plasmodium* malaria parasites and their human hosts. Integrative and Comparative Biology. 2002;**42**:401-407

[140] Calixto JB, Otuki MF, Santos Adair RS. Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor κB (NF-κB). Planta Medica. 2003;**69**:973-983

[141] Gautam R, Jachak SM. Recent developments in anti-infammatory natural products. Medicinal Research Reviews. 2009;**29**:767-820

[142] Rockett KA, Awburn MM, Aggarwal BB, Cowden WB, Clark IA. In vivo induction of nitrite and nitrate by tumor necrosis factor, lymphotoxin, and interleukin-1-possible roles in malaria. Infection and Immunity. 1992;**60**:3725-3730

[143] Schofield L, Novakovic S, Gerold P, Schwarz RT, Mcconville MJ, Tachado SD. Glycosylphosphatidylinositol toxin of *Plasmodium* up-regulates intercellular adhesion molecule-1, vascular cell adhesion molecule-1, e-selectin expression in vascular endothelial cells, increases leukocyte and parasite cytoadherence via tyrosine kinase-dependent signal transduction. Journal of Immunology. 1996;**156**:1886-1896

[144] Clark IA, Chaudhri G. Tumour necrosis factor may contribute to the anaemia of malaria by causing dyserythropoiesis and erythrophagocytosis. British Journal of Haematology. 1988;**70**:99-103

[145] Peetre C, Gullberg U, Nilsson E, Olsson I. Effects of recombinant tumor necrosis factor on proliferation and differentiation of leukemic and normal hemopoietic cells in vitro. The Journal of Clinical Investigation. 1986;**78**:1694-1700

[146] Guo W, Liu W, Hong S, Liu H, Qian C, Shen Y, et al. Mitochondria-dependent apoptosis of Con A-activated T lymphocytes induced by asiatic acid for preventing murine fulminant hepatitis. PLoS One. 2012;**7**(9):e46018. DOI: 10.1371/journal.pone.0046018

[147] Huang SS, Chiu CS, Chen HJ, Hou WC, Sheu MJ, Lin YC, et al. Antinociceptive activities and the mechanisms of anti-inflammation of asiatic acid in mice. Evidence-based Complementary and Alternative Medicine. 2011;**2011**:895857

[148] Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: The control of NF-κB activity. Annual Review of Immunology. 2000;**18**:621-663

[149] Baker RG, Hayden MS, Ghosh S. NF-kB, inflammation, and metabolic disease. Cell Metabolism. 2011;**15**:11-22

[150] Cao W, Li X-Q, Zhang X-N, Hou Y, Zeng A-G, Xie Y-h, et al. Madecassoside suppresses LPS-induced TNF-α production in cardiomyocytes through inhibition of ERK, p38, and NF-κB activity. International Immunopharmacology. 2010;**10**:723-729

[151] Hogg N. Free radicals in disease. Seminars in Reproductive Endocrinology. 1998;**16**(4):241-248

[152] Musfiroh I, Muhtadi NA, KartasasmitaRE,DaryonoHT,IbrahimS.In silico study of asiatic acid interaction with inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2). International Journal of Pharmacy and Pharmaceutical Sciences. 2013;**5**(1):204-207

[153] Kartasasmitaa RE, Musfiroha I, Muhtadib A, Ibrahima S. Binding affinity of asiatic acid derivatives design against inducible nitric oxide synthase and ADMET prediction. Journal Applied Pharmaceutical Science. 2014;**4**(02):075-080. DOI: 10.7324/JAPS.2014.40213

[154] Lee SK, Lee IH, Kim HJ, Chang GS, Chung JE. The PreADME Approach: Web-based Program for Rapid Prediction of Physico-Chemical, Drug Absorption and Drug-Like Properties. Massachusetts: Blackwell Publishing; 2003. pp. 418-420

[155] Yoshikawa T, Naito Y. The role of neutrophils and inflammation in gastric mucosal injury. Free Radical Research. 2000;**33**(6):785-794

[156] Jain K, Sood S, Gowthamarajan K. Modulation of cerebral malaria by curcumin as an adjunctive therapy. The Brazilian Journal of Infectious Diseases. 2013;**17**(5):579-591. DOI: 10.1016/j.bjid.2013.03.004

[157] Serghides L, Kain KC. Peroxisome proliferator-activated receptor gamma-retinoid X receptor agonists increase CD36-dependent phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes and decrease malaria induced TNF-alpha secretion by monocytes/macrophages. Journal of Immunology. 2001;**166**:6742-6748