PATHOGENS AND GLOBAL HEALTH, 2018
VOL. 112, NO. 1, 1–12
https://doi.org/10.1080/20477724.2018.1429847

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REVIEW

A bite to fight: front-line innate immune defenses against malaria parasites

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ABSTRACT

Malaria infection caused by *Plasmodium* parasites remains a major health burden worldwide especially in the tropics and subtropics. *Plasmodium* exhibits a complex life cycle whereby it undergoes a series of developmental stages in the *Anopheles* mosquito vector and the vertebrate human host. Malaria severity is mainly attributed to the genetic complexity of the parasite which is reflected in the sophisticated mechanisms of invasion and evasion that allow it to overcome the immune responses of both its invertebrate and vertebrate hosts. In this review, we aim to provide an updated, clear and concise summary of the literature focusing on the interactions of the vertebrate innate immune system with *Plasmodium* parasites, namely sporozoites, merozoites, and trophozoites. The roles of innate immune factors, both humoral and cellular, in anti-*Plasmodium* defense are described with particular emphasis on the contribution of key innate players including neutrophils, macrophages, and natural killer cells to the clearance of liver and blood stage parasites. A comprehensive understanding of the innate immune responses to malaria parasites remains an important goal that would dramatically help improve the design of original treatment strategies and vaccines, both of which are urgently needed to relieve the burden of malaria especially in endemic countries.

KEYWORDS

Innate immunity; malaria; macrophages; natural killer cells; neutrophils; invasion; vaccines

Introduction

Malaria has been a significant threat to humans throughout history. According to the most recent World Malaria Report, 212 million clinical cases were reported in 2015 as well as 429,000 deaths [1]. Despite the fact that malaria cases have been steadily dropping during the last decade due to several World Health Organization (WHO) supported initiatives, the disease mortality and morbidity rates remain high exerting serious social and economic burdens on several populations, especially those living in poverty. Malaria transmission is ongoing in 91 countries worldwide with the heaviest burden occurring in Africa, particularly in the sub-Saharan region were poverty, inefficient access to adequate health services, poor infrastructure and presence of highly anthropophilic vectors, such as *Anopheles gambiae* and *Anopheles funestus*, aggravate the problem. WHO has come up with the Global Technology Strategy for Malaria 2016–2030 to reduce the number of malaria cases and deaths worldwide by at least 90% and to prevent the reestablishment of this parasite in malaria free countries [1].

The absence of an efficient vaccine has been a major hurdle in the elimination of malaria from endemic countries. In fact after a natural infection, the host's immune system does not elicit a long term protective immunity to re-infection [2]. Hence, the aim from a vaccine, at best, is to provide partial but significant protection mainly to children below the age of 5 where disease morbidity is often high. There are several reasons why a sterilizing immunity against natural *Plasmodium* infection fail to develop, most of which are not entirely understood. Some obvious reasons include the complex biology of malaria parasites with several stage-specific genes many of which undergo antigenic variation [3–8], the complex population structure of malaria parasites [9], evasion of innate immune responses and failure to develop long term memory responses [10]. In this review, we start with a synopsis of the parasite life cycle followed by a description of the major innate immune responses elicited by the mammalian host against the invasive stages, namely the sporozoites and merozoites, highlighting in particular the contribution of neutrophils, macrophages and Natural killer (NK) cells to the frontline control of these parasite stages in the skin, liver and blood.

Plasmodium life cycle

Malaria is a mosquito borne disease caused by parasites of the genus *Plasmodium*. It is transmitted through the bite of the female *Anopheles* mosquito [11,12]. There are four traditional species that infect humans namely *P. falciparum*, *P. vivax*, *Plasmodium ovale*, *P. malaria*

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[13,14] and recently the upsurge of a fifth human infective species in South-East Asia known as *P. knowlesi* [15]. *Plasmodium* species exhibit several variations in their life cycle, including severity and frequency of infection, fever paroxysm [16] number of merozoites produced per erythrocytic cycle [17], presence of hypnozoite stage [18] and gametocyte feeding density in relationship to subsequent infections [19], duration and stages of gametocytogenesis, gametocyte maturity [19], and other factors.

Despite the complicated variations in the parasites' life cycle, all species depend on two essential hosts, the *Anopheles* mosquito and the vertebrate host, for a successful completion and sustainability of the life cycle. During a blood meal, the female Anopheles injects sporozoites into the dermis from where they rapidly migrate to the liver initiating the hepatic schizogony. Infection of hepatocytes is initiated after the sporozoites traverse the sinusoidal wall by utilizing various microneme proteins including the sporozoite protein essential for cell-traversal (SPECT) [20], SPECT2 [21], and the cell-traversal protein for ookinetes and sporozoites [22]. Sporozoites traverse the sinusoidal cell wall by migrating through endothelial cells or Kupffer cells [23–25], however, cell traversal (CT)-independent routes have been also described [25]. CT traversal by sporozoites seems to be important for escaping clearance by Kupffer cells [25]. Following the CT phase, sporozoites switch to the invasive phase in order to infect hepatocytes. Sporozoite-specific molecules, such as the 6-cys domain proteins Pbs36p and Pbs36 have been shown to be required for hepatocyte invasion by *P. berghei* parasites [21]. Hepatic schizogony involves asexual parasite replication producing thousands of erythrocyte-infecting merozoites. In some species of the parasite, such as in *P. vivax* and *P. ovale*, there is a dormant stage referred to as hypnozoite [26,27] that resides in the liver cells and causes relapses by invading the bloodstream weeks or even years later [28–31].

Following their release into the blood stream, merozoites invade erythrocytes transforming initially into the ring form which eventually matures to form a trophozoite that grows substantially in size to prepare for the subsequent schizont stage characterized by multiple asexual cell divisions leading to the production of more merozoites. When the red blood cell ruptures, merozoites are released into the blood stream to infect new red blood cells [32]. Merozoites attach to the red blood cell at any point of its surface. After attachment, reorientation of the merozoites occurs in order to deploy the enzymatic content of a series of secretory organelles, such as rhoptries, micronemes, and dense granules [33–35]. Ligands interact with the erythrocyte surface receptors forming a junction. This junction is driven by an actin-myosin motor that is found in the inner membrane of the merozoites [36,37]. Further invasion occurs through the inward motion driven by the actin-myosin motor until the parasite is enclosed with a parasitophorous vacuole. After that, the junction is pinched off, the outer surface coat is shed, and the red blood cell and parasitophorous vacuole are resealed. After the completion of the process, the parasite matures to the ring form [38]. The pathogenesis of malaria is mainly linked to the erythrocytic stage, where known symptoms of the disease may occur. However, high prevalence of asymptomatic infections have been reported for certain *Plasmodium spp*, such as *P. falciparum in Haiti pool of individuals* [39]. Some parasites differentiate rather into gametocytes which are the sexual stage of the parasite. This commitment to sexual development occurs early on when merozoites are still inside the schizonts [40], and involves several epigenetic factors and transcription regulators [41]. When ingested by a mosquito, gametocytes differentiate into gametes initiating the sexual cycle of the parasite [42].

The fact that the early clearance of parasites by the innate immune system is almost always inefficient suggests that malaria parasites evolved several strategies to fend off host defenses in order to complete their development. Some of these evasion strategies include allelic diversity of parasite surface proteins such as the circumsporozoite protein [43,44], surfin [45], and the merozoite surface proteins (MSPs) [46] that form the merozoites outer coat surface [47,48]. In addition, certain parasites display variant surface antigens on infected red blood cells. For instance, *P. falciparum* possess three large multigene families, namely the *var* [49], the repetitive interspersed family (*rif*) [50], and the subtelomeric variant open reading frame (*stevor*) that encode variant antigens [51]. Interestingly, one of the products of *var* genes, PfEMP1, was shown to suppress IFN-γ secretion by naïve γδ-T cells and Natural Killer (NK) cells consequently further aiding in parasite immune evasion [48,52]. Other gene superfamilies have been recognized in *P. vivax* as variant interspersed repeats (*vir*) and in *P. knowlesi* as Schizont Infected Cell Agglutination (*sicavar*) that encode as well variant surface antigens related to parasite evasion. Functional analysis of *P. vivax* VIR proteins revealed different subcellular localizations and cytoadherence to the ICAM-1 endothelial receptor [7,53].

Despite these evasion strategies, several interactions do occur between cellular and humoral components of the innate immune system and the parasite. In the sections below, and as summarized in Table 1, we describe the significance and relative contribution of these interactions to parasite elimination.

The role of neutrophils in defense against Plasmodium parasites

Interaction of neutrophils with Plasmodium sporozoites

Neutrophils are the first to be recruited to the site of infection combating microorganisms via an array of strategies [54]. There is strong evidence as well pointing to their important function in tumor microenvironment. Their

infiltration and prognostic significance has been lately studied in esophageal squamous carcinoma [55] and in colorectal cancer [56]. Moreover, neutrophils are the first responders to the injection of *Plasmodium* sporozoites by the mosquito bite in the skin. Even a sterile insect bite can trigger neutrophil migration to the dermis and epidermis as has been previously shown for sand flies [57], suggesting that the damage inflicted to the skin by the probing behavior of the insect proboscis is sufficient to drive neutrophil migration. Using mice as model vertebrate host, it was shown that neutrophils increase steadily in the skin within 1–2 h after a mosquito bite [58]. Another study analyzed the influx of neutrophils for a longer duration after injecting the skin of mice with wild type sporozoites (WTS), radiation attenuated sporozoites (RAS), or salivary gland extracts (SGE) from uninfected mosquitoes. The neutrophil count decreased four hours after SGE infection reaching basal levels, but further increased in WTS and RAS injected mice indicating that sporozoites triggered a sustained neutrophil recruitment to the skin. In parallel, the levels of monocytes were relatively low in all cases during the first few hours of malarial infection; however, there was a sharp increase at 24 h in WTS and RAS injected mice [59,60]. This indicates that neutrophils are the first cells to be recruited to the site of infection. Recently, it was shown that a protein called Agaphelin is upregulated to several folds in the salivary glands of *P. falciparum*-infected *A. gambiae* mosquitoes and it exhibits inhibitory effects on neutrophil chemotaxis *in vitro* and *in vivo* in a mouse model of acute inflammation induced by carrageenan [61]. While this finding might seem contradictory to previous reports showing rapid neutrophil infiltration into the skin after sporozoite injection, it is not clear how much the carrageenan induced inflammation mimics that triggered by invading sporozoites. Also, it remains to be shown whether Agaphelin knockdown mosquitoes trigger differential neutrophil recruitment to the skin after sporozoite injection as compared to wild types. Despite the clear evidence for neutrophil influx as a result of sporozoite injection, it is not clear whether they contribute significantly to sporozoite killing [62]. Mac-Daniel et al. [59] showed that sporozoites can be phagocytosed by both neutrophils and monocytes leading to their imminent death, however, a small percentage of phagocytic cells harbored sporozoites that remained alive in parasitophorous vacuoles for at least 24 h before they die. These results suggest that sporozoites can actively invade phagocytic cells; however, it remains unclear whether a single parasite can traverse multiple phagocytes in the skin to facilitate the invasion mechanism.

Interaction of neutrophils with Plasmodium blood stages

Phagocytosis of *P. falciparum* merozoites have been observed *in vivo* [63,64] and *in vitro* [65,66]. Merozoite phagocytosis was examined morphologically by examining their adherence and ingestion by neutrophils. *In vitro*, phagocytosis of merozoites was shown to be enhanced in the presence of immune sera and furthermore if the inflammatory cytokines IFN-γ, TNF-α, IL-1β, and GM-CSF were added [67]. The fact that neutrophils from infected individuals were shown to be more effective in inhibiting parasite growth *in vitro* than those from naïve individuals [65] supports a potential role for inflammatory cytokines in boosting neutrophil activity. The mechanisms by which neutrophils kill blood stage parasites are not completely understood but several studies point to a role for the neutrophil respiratory burst in that process. This respiratory burst seems to be triggered by parasite specific antibodies, a process referred to as antibody-dependent respiratory burst (ADRB), and its intensity correlates with clinical protection from malaria [68]. In the ADRB, reactive oxygen species (ROS) production was shown to be produced intracellularly suggesting that the underlying driving mechanism is phagocytosis [69]. However, an earlier study revealed that neutrophils from chronic granulomatous disease (CGD) patients were still able to inhibit the growth of malaria parasites *in vitro* especially when stimulated with phorbol-myristate-acetate suggesting that, in addition to ROS, microbicidal components of neutrophil granules may also be involved in parasite killing [66]. TNF-α was also shown to boost the activity of neutrophils from CGD patients against *P. falciparum* blood stages. The inhibition of *Plasmodium* growth *in vitro* seems to depend on the ratio of polymorphonuclear leukocytes (PMNs) to parasitized-erythrocytes (PE). It was shown that at high ratios, even without stimulating neutrophils, parasite growth was almost completely blocked [70]. This observation was also supported by a study by Golenser et al. [28] whereby a PMN to PE ratio of 1/50 resulted in 82% inhibition of parasite growth after two days of culture. In this study, it was proposed that PMNs impede the growth of *P. falciparum* through an increase in oxidative stress, since glucose 6-phosphate dehydrogenase (G6PD)-deficient PE were more sensitive to PMNs than PE from normal individuals. This sensitivity in G6PD deficient PE is suggested to be due to the presence of an antagonistic environment for the *Plasmodium* thereby causing oxidative damage and destruction of the parasite [71].

In addition to phagocytosis and ROS release, neutrophil extracellular traps (NETs) contribute to host defense against malarial parasites. NETs are networks of extracellular fibers, mainly made up of web-like meshwork of DNA that were shown to be present in the peripheral blood circulation of children under the age of 6 years diagnosed with uncomplicated *P. falciparum* malaria. Circulating neutrophils exhibiting NETs had normal morphology, and were adherent to PE [72–74]. Interestingly, after a mosquito bite, NET formation at the level of the skin was inhibited by Agaphelin mainly by targeting elastase and reducing neutrophil infiltration [61].

Despite the presence of substantial evidence linking neutrophils to parasite killing as mentioned above, it remains unclear how significant is the contribution of neutrophils to the clearance of parasite blood stages. For instance, one study reported the presence of a dominant population of neutrophils in the blood of *P. falciparum* infected children with reduced oxidative burst as a result of the induced expression of the heme oxygenase-1 enzyme in neutrophil progenitors in the bone marrow [29].

On the other hand, neutrophils may also contribute to the pathophysiology of severe malaria cases. For instance, severe malaria cases were associated with significantly higher neutrophil counts compared to uncomplicated cases [75], and there was a positive correlation between ROS production in neutrophils and severe malaria and anemia in a cohort of Gabonese children [2]. A more recent study revealed that the secretion of IFN-γ- inducible protein 10, CXCL10, by inflammatory monocytes and neutrophils compromises the control of blood-stage malaria infection leading to severe diseases such as cerebral malaria (CM) [76]. CXCL10 seems to inhibit the accumulation of T-follicular helper cells in the spleen hence compromising the parasite-specific antibody response. In a mouse model of malaria-associated acute lung injury, depletion of neutrophils reduced lung pathology and increased mice survival [71]. On the same note, early depletion of neutrophils by injecting malaria infected mice with anti-GR1 IgG antibody prevented the appearance of acute lung injury/acute respiratory distress syndrome compared to untreated mice. Similarly inhibiting the CXCR4/CXCL12 signaling pathway that induces neutrophil migration to the lung tissue upon injuries protected almost 90% of the treated mice pool [77]. Hence, while neutrophils may play a protective role in the early phase of the disease, at later stages they might contribute to the pathophysiology that develops in severe cases.

The role of macrophages and monocytes in anti-Plasmodium immunity

Macrophages are known to be highly phagocytic cells with an array of surface receptors that allow them to interact with diverse classes of microorganisms. They possess different phenotypes and functions mainly based on the cytokine profile in their microenvironment [78]. They can either favor a pro-inflammatory or an anti-inflammatory immune response thereby playing a role in inflammation and tissue repair. Their function extends to several diseases and disorders, ranging from obesity, asthma, metabolic disorders, autoimmunity, anti-tumor activity [79,80] and fighting parasitic infections, such as Leishmaniasis [81]. In this section, we review the role of macrophages to defeat malaria infections, particularly against *Plasmodium* sporozoites and its blood stages.

Interaction of macrophages with Plasmodium sporozoites

Early studies utilizing peritoneal macrophages incubated with sporozoites of *P. berghei* and *P. knowlesi* in the presence of serum revealed that the fate of sporozoites and macrophages depends on the presence of *Plasmodium* antibodies in the serum. In normal serum, sporozoites invade macrophages actively leading to their destruction, while immune serum triggers the phagocytic uptake of sporozoites leading to their destruction [82]. Activated macrophages were also shown to phagocytose and kill *Plasmodium*-infected erythrocytes using oxidative burst and this response was enhanced in the presence of immune serum [83,84]. The protective role of parasite neutralizing antibodies has been documented in several studies and it is clear now that several mechanisms are involved [85]. However, the outcome of the interaction of sporozoites with macrophages seems to be context-dependent as other studies highlighted a certain level of immune dysfunction in Kupffer cells (KCs) upon encounter with sporozoites. For instance, upon contact with *Plasmodium yoelii* sporozoites, murine KCs in the liver directly undergo apoptosis as depicted by the blebbing of the cellular membrane, nuclear condensation and fragmentation. This process results from the down-regulation of the pro-inflammatory cytokine, TNF-α, and up-regulation of the anti-inflammatory cytokine, IL-10 [86]. Moreover, sporozoites and recombinant circumsporozoite protein suppressed the respiratory burst of KCs by increasing the levels of cAMP in these cells [87]. Additionally, Steers et al. [88] revealed that the uptake of sporozoites by KCs of naïve mice, not infected previously with malaria, lead to the impairment of antigen presentation by down-regulating the expression levels of MHC class I and co-stimulatory molecules. Sporozoites also exhibit a cell traversal (CT) activity that aids in KC evasion and liver invasion. Using intravital laser spinning disc microscopy, it was shown that CT activity prevents the clearance of sporozoites by KCs, as the majority of SPECT2$^-$ sporozoites established lasting interactions with KCs resulting in their clearance while a small percentage of wildtype sporozoites established such interactions [25]. In the same study, authors also examined the *in vitro* internalization of fluorescently labeled *Plasmodium* sporozoites by purified primary KCs and their association with the lysosomal marker, LAMP1. Interestingly, only half of the sporozoites were phagocytosed as marked by an overlapping stain with LAMP1. The remaining half was suggested to be packaged in parasitophorous vacuoles hence escaping phagocytosis through CT activity [25]. In addition to their role in parasite clearance, inflammatory macrophages may also contribute to the severity of malaria cases by priming type I IFN production by plasmacytoid dendritic cells [85]. On the other hand, M2 anti-inflammatory macrophages polarized by IL-33 administration contributed

to protection from cerebral malaria in a *P. berghei* model of infection [89].

Interaction of macrophages with Plasmodium blood stages

Monocytes have been also implicated in inhibiting the growth of blood stages of the parasite by antibody-dependent cellular inhibition (ADCI) in the presence of protective antibodies [90]. Recently, it was shown that human monocytes incubated with *P. falciparum*-infected erythrocytes in the presence of IgGs from malaria immune African human sera produced the antibacterial molecule trappin-2 that localized to the surface of merozoites and inhibited *P. falciparum* growth *in vitro* [91]. The systemic and lung overexpression of trappin-2 in mice reduced parasite sequestration in the spleen, liver, lung and brain leading to increased mouse survival after infection with a *P. berghei* strain that causes cerebral malaria. These results suggest that monocyte secretion of trappin-2 may contribute to the process of ADCI. On the other hand, there is evidence that ingestion of the parasite pigment hemozoin, the breakdown product of hemoglobin, or malaria-infected erythrocytes impairs the function of both monocytes and macrophages repressing their ability to produce inflammatory cytokines [92,93]. More recently, this functional impairment in response to the uptake of blood stage parasites was attributed to the rapid phagosomal acidification in monocytes and macrophages which impairs toll-like receptor (TLR) interactions with their parasite ligands, hence, preventing downstream cytokine responses [28]. This is particularly significant in malaria infections whereby *Plasmodium* parasites seem to be recognized exclusively by endosomal TLRs [2,29,94]. Moreover, CD47, a marker of self, expressed on the cell membranes of human cells including young red blood cells acts as a shield for blood stages parasites inhibiting their clearance by phagocytic cells [71]. CD47 exerts its effects by interacting with macrophage signal-regulatory protein alpha (SIRPα), which activates intracellular signaling pathways leading to reduced phagocytic uptake and decreased production of inflammatory cytokines [95].

Direct and indirect roles of natural killer cells in anti-Plasmodium immunity

Natural killer (NK) cells are key players in innate immunity [96]. They have been traditionally linked to fighting cancers and viral infections however recent studies point to a broader role in microbial infections. They form a heterogenous pool that is distinguished from other lymphocytes based on the expression of certain surface markers, such as CD56 and CD16 [97,98], along with the absence of surface marker CD3, which is specific to T cells [99,100]. NK cells normally express a wide variety of receptors, such as lectin-like and TLRs [101,102]; and inhibitory receptors, such as LT-2, CD94/NKG2A, CD161, and killer cell immunoglobulin-like receptors (KIRs) [103]. Their role in immunomodulating parasitic [104] and fungal infections [105] and in immunotherapy is of great concern [106,107]. Hereby, we provide the behavior of NK cells against malaria infections. A quick and robust pro-inflammatory response is needed to control malaria parasites in the early phases of infection and NK cells can contribute to that by the secretion of IFN-γ. Studies in mice revealed a crucial role for IFN-γ in controlling the levels of parasitemia and parasite clearance [85,92]. The depletion of NK cells in mice infected with *P. yoelii* and *P. chabaudi* triggered an early increase in parasitemia as a result of reduced IFN-γ production [85,108]. The protective role of IFN-γ is thought to occur through the activation of macrophages to phagocytose merozoites and parasitized erythrocytes in opsonization-dependent and independent manners [93].

In humans, the early production of IFN-γ also provides protection against malaria infection. IFN-γ production was shown to correlate with mild rather than severe diseases in children and to protect from reinfection within one year of initial infection [109]. Interestingly, IFN-γ production was not detected upon placing purified NK cells, instead of NK from whole blood donors, with infected RBCs. It was therefore deduced that peripheral blood mononuclear cells (PBMC) aid in NK cells activation, particularly by monocytes through the secretion of IL-12 and IL-18 [2,28,29,85] or through direct contact with macrophages [71,82]. In fact, the dependence of NK cells on other immune cells for activation during malaria infections have been observed long time ago. In a study by Ojo-Amaize et al. [110] *P. berghei* sporozoites were γ-irradiated to attenuate their virulence without affecting the erythrocyte stability prior to their culture with whole blood after their injection into mice. Such a modification still induced IFN-γ release *in vitro* and activation of splenic NK cells to lyse murine tumor cells. This piece of finding reflects the indirect activation of NK cells through immune players rather than by the parasite itself. Later studies supported this observation. For instance, NK cells from PBMC depleted of monocytes resulted in a relative decrease in IFN-γ production after incubation with P. falciparum-infected erythrocytes; however, when placed with purified macrophages only, NK cells re-established their IFN-γ production [111]. Hence, there seems to be a close cooperation between macrophages and NK cells that enhances IFN-γ by the latter in an IL-18 and MyD88 dependent manner. In fact NK cell activation in response to malaria infected RBCs (iRBCs) seems to depend on multiple factors, including the priming cytokine IL-2, the activating cytokines IL-12 and IL-18 [112,113], direct contact with macrophages through adhesion receptors [82,114] as well as direct contact with iRBCs [113]. The significance of such interaction and the receptors involved remain unclear. Supernatant from PBMC or NK

from blood donors cultured with Pf-infected red blood cells, showed high levels of IFN-γ production as assayed by ELISA [115]. It is worth noting that opposing viewpoints exist in the literature regarding the fast release of IFN- γ directly by NK cells [85,92]. Overall, the secretion of IFN- γ in humans whether via direct or indirect activation of NK cells showed a positive correlation with the protection against malaria infection.

In addition to the indirect role of NK cell in malaria defense through the release of cytokines, NK cells are also capable of directly killing parasitized cells, whether red blood cells or hepatocytes by cytotoxicity. When NK cells are activated, a contact-dependent elimination of the infected RBC occurs. Chen et al. were able to observe the process of conjugates formation between NK and infected RBC in real time where the infected red blood cells were flattened after NK cells interaction. Throughout this process, there was no sign of phagocytosis, therefore it was deduced that the killing of the infected cell was a result of the leakage of granzymes and loss of the cell volume and integrity [85]. Additionally, some NK cells showed actin relocalization at the site of contact with infected RBC, probably an indication of an immunological synapse where cytotoxic granules are released. The identity of the ligands or receptors mediating such synapse remains unclear [116].

An important role of NK cells during innate immunity is its natural killing capacity by surveilling MHC class I expression levels on nucleated cells and the binding affinity of MHC- I to certain KIRs. Such defense mechanism is most likely to take place in the pre-erythrocytic liver stage of infection since RBCs do not harbor MHC-I molecules on their cell surface. Moreover, it has been shown that individuals with certain haplotypes of KIR and MHC- I may be less prone to malarial infections [108]. Thus, the function of NK cells against malarial infections is quite heterogeneous based on the mode of NK activation, levels of pro-inflammatory cytokines, and its means of killing that culminate in variable protective and pathogenesis patterns among individuals.

Role of γδ-T cells in the pathogenesis of malaria

γδ T cells possess an immunoregulatory role by expressing pro-inflammatory molecules such as IFN-γ, TNF-α, TNF-β and releasing cytotoxic granules [117]. They occupy a small subset of T lymphocytes, and unlike normal T cells, they have restricted T cell repertoire and express abundant TLRs. γδ T cells do not recognize antigens presented by MHC molecules on antigen presenting cells and thus, are considered innate-like lymphocytes.

Analyzing the surface phenotype of T cells after a 6 day *in vitro* stimulation of *P.falciparum* schizonts on extracts from unprimed individuals showed a dominant proliferation of γδ T cells subset with T-cell receptors of Vγ9 + family type [118,119]. Such observations reflect the role of γδ-T cells in the pathogenesis of malaria as discussed in Langhorne's review, 1992 [120]. Recent studies demonstrate the release of phosphoantigens to the extracellular milieu during the egress of parasites at the end of the intraerythrocytic phase promoting the activation of γδ T cells [118] particularly a subset Vγ9 + Vδ2$^+$ T cells. However, the exact mechanism of action that Vγ9 + Vδ2$^+$ T possess in immunoregulating T cells is still not fully understood. Schofield et al. reported an upregulated expression of a potential inhibitory receptor, TIM3, in acutely infected mice or children with malaria. TIM3 may result in the impairment of γδ-T cells and consequently may have implications in the management of malarial diseases [121].

Other innate immune factors involved in defense against malaria

Certain studies have elucidated the role of the complement system against malarial infections [122]. Despite the well-established activation mechanism of the classical pathway through the increased binding of C1q to CRP and the alternative pathway through the activation of C3 by fragments from lysed infected erythrocytes, to date there is still no definitive mechanism on the mode of activation of the lectin pathway [14]. In addition to complement proteins, TLRs, such as TLR-2, TLR-4, and TLR-9, are considered key elements in initiating inflammatory response against malarial infections [123]. TLR-9 resides intracellularly, namely in the endoplasmic reticulum of resting non-specific immune cells, such as monocytes, NK cells, B cells and plasmacytoid dendritic cells [124]. Upon activation, TLR-9 are transported to a LAMP-1 positive compartment by trafficking through the Golgi complex [125] and interact with hemozoin aggregates found in engulfed parasitized erythrocytes [123]. In a study by Coban et al., TLR-9 deficient mice injected with *P. falciparum* hemozoin showed a drastic reduction in the serum levels of MCP-1 and IL-6 [126]. On the other hand, TLR-2 and TLR-4 reside on the surface of innate immune cells and are upregulated upon parasite infection especially in CD11c$^+$/CD14$^+$ monocytes [127]. These TLRs induce endosome/phagosome formation upon ligand binding and elicit an increase in TNF-α, IL-6, and IL-10 levels [123]. Furthermore, Toll/IL-1 receptor family induces NO synthase expression upon its binding to cytosolic factors released by ruptured host cells and impede the progress of parasite infection in the liver [128].

Another anti-malarial innate response is mediated through the secretion of cytokines and interferons, including both type I interferon (IFN-α/β) and IFN-γ (Figure 1). Type I interferon is released by hepatocytes [129] and by plasmacytoid cells (pDC) upon the recognition of sporozoites RNA by cytosolic sensors. Whereas, IFN-γ is released by several cellular sources including NK cells and CD8+ T cells upon activation by dendritic cells [130]. High serum levels of interferons have been shown

Table 1. Summary of the innate immunological responses against different stages of the malaria parasites as mounted in either human or rodent models.

Cell	Organism	Malaria species	Stage of infection	Immunological responses	References
Neutrophils	Rodent	*P. berghei*	Sporozoites	Increase in neutrophil recruitment to skin	[58] [59,60]
	Humans	*P. falciparum*	Merozoites	Phagocytosis of merozoites by neutrophils	[63,64] [65,66]
			Intra-erythrocytic	Blockage of parasite growth	[70]
				NETs formation	[72–74]
				Reduced oxidative burst	[29]
				Positive correlation between ROS production in neutrophils and severe malaria and anemia	[2]
				CXCL10 secretion leading to CM	[76]
	Rodent	*P. vinkei*	Intra-erythrocytic	Decrease in neutrophils thus decrease in lung pathology	[71]
Macrophages		*P. berghei* *P. knowlesi*	Sporozoites	Sporozoites invade macrophages and immune sera trigger phagocytic uptake of Sporozoites leading to their destruction	[82]
Kupffer cells	Rodent	*P. yoelii*		Phagocytosis of some sporozoites with others escaping through CT	[25]
Monocytes	Humans	*P. falciparum*	Intra-erythrocytic	Inhibiting growth by ADCI	[90]
				Production of trappin-2 on the surface of merozoites leading to growth inhibition *in vitro*	[91]
NK cells	Rodent	*P. yoelii* *P. chabaudi*	Blood stage	Increased parasitemia as a result of reduce IFNγ production	[85,92]
	Humans	*P. falciparum*	Intra-erythrocytic	NK cells activation aided by monocytes and macrophages	[2,28,29,85] [71,82]
				Increased IFNγ directly by NK cells	[85,92]
				Destruction of infected RBC after NKcell interaction through the release of granzymes and loss of cell volume and integrity	[85]
Splenic NK cells	Rodent	*P. berghei*	γ-irradiated sporozoites	Indirect activation of NK cells through immune players leading to the lysis of murine tumor cells	[110]
γδ T cells	Human	*P.falciparum*	schizonts	Activation of a subset of Vγ9+Vδ2^{+} T cells	[118]
	Human and rodent	*P.falciparum*	schizonts	Upregulation of TIM3 expression, impairment of γδ T cells	[121]

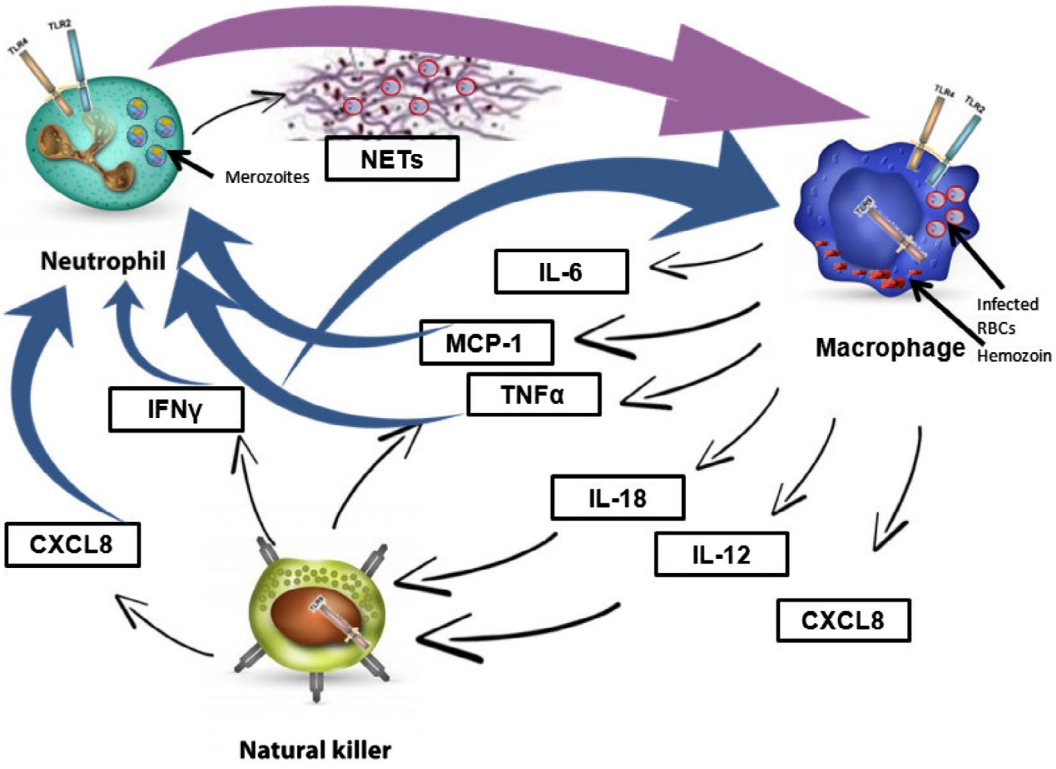

Figure 1. Major cytokines controlling the functions of key innate immune cells against malaria parasites.

to reduce the survival of the exo-erythrocytic forms of the parasite thus reducing the blood-stage population and impairing liver infection. It is worth noting that type I IFN activates the recruitment of neutrophils to the liver during *P.vivax* infection in both human and rodent malaria. Such an activation induces the expression of type I IFN stimulated genes (ISGs) by neutrophils. A positive correlation was detected between ISGs and increased serum levels of alanine and aspartate aminotransferases as an indicator of liver tissue damage. Thus, type I IFN modulates liver immunopathology by inducing the expression of certain chemokines and adhesion molecules involved in neutrophils recruitment [129,131].

During a malaria infection, a fine-tuned communication through different cytokines elicits the activation and recruitment of the three major players of the innate immune response, namely neutrophils, macrophages, and natural killer cells. Activated macrophages stimulate neutrophils via TNF-α and MCP1 and stimulate natural killer cells via IL-12 and IL-18. Natural killer cells also exhibit a feedback loop further activating macrophages and neutrophils via IFN-γ and CXCL8. Despite the feed-forward and feedback loops mediated through different cytokines to amplify the immune response, malaria parasites can still evade the immune players.

Abbreviations: TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; RBC, red blood cells; NETs, neutrophil extracellular traps.

Conclusion

Significant progress has been done in understanding the early innate immune responses triggered against malaria parasites and their relative contribution to parasite clearance and immune pathology. However, several questions remain to be addressed in order to gain better insight into these early events which are crucial in defining the profile through which the disease will evolve. An interesting question is to understand why several immune factors both humoral and cellular act in concert to cause protection while in other instances may contribute to severe malaria. What controls the levels of cytokines in the malaria infections and how and on what basis does this change from one individual to another? These questions are difficult to infer from most studies on malaria immunity due to the reductionist approach utilized. Understanding these early events in malaria immunity is important as it may inform the design of novel more efficient vaccines and possibly help designing novel original treatment strategies, both of which are urgently needed to relieve the burden of malaria especially in endemic countries.

Acknowledgment

We are grateful to Dr Mike Osta for his insightful comments and critical revision of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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