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REVIEW ARTICLE OPEN ACCESS

Toll-Like Receptors in Malaria: Gatekeepers of Innate Immunity and Pathogenesis

Khalid Abosalif[1]*, Muhammad Atif[1], Hasnain Farooq[2], Abualgasim Elgaili Abdalla[1], Albadawi Talha[1], Marwa Abdalla[3], Nada Amien[4], Bi Bi Zainab Mazhari[5] and Hasan Ejaz[1]*

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka 72388, Saudi Arabia.
[2]Department of Pathology Mayo Hospital, King Edward Medical University, Lahore 54000, Pakistan.
[3]Primary Health Care of Qara, Aljouf Health Cluster, Sakaka 72343, Saudi Arabia.
[4]Department of Microbiology, Faculty of Medical Laboratory Science, University of Khartoum, Khartoum, Sudan.
[5]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Qurayyat 75911, Saudi Arabia.

Abstract

***Plasmodium* parasites, transmitted to human blood via the bite of the *Anopheles* mosquito, cause malaria, an acute and severe disease that can potentially be fatal. These parasites and their mosquito vectors proliferate in warmer climates and, therefore, are more prevalent in certain regions. In 2021, fifty percent of the global population was at risk of malaria. Although this disease can affect any individual, specific demographic groups, including young children, pregnant women, neonates, and immunocompromised individuals, are more susceptible to infection and are at higher risk of mortality. Among *Plasmodium* species, only *P. falciparum* causes cerebral malaria and is behind the most severe symptoms and fatalities. The pathogenesis of *Plasmodium* malaria is associated with the downstream signaling pathways and Toll-like receptors (TLRs) of innate immunity. Owing to the potential role of TLRs in the pathophysiology of malaria, TLR gene polymorphisms may be subject to selection pressure in communities where the disease is endemic. This review paper summarizes the prevailing knowledge of the fundamental characteristics of TLRs and their role in malaria disease. In addition, it throws light on the potential role of the TLR signaling system in malaria pathogenesis.**

Keywords: Innate Immunity, Malaria, *Plasmodium* species, TLRs, Ligands, Nucleic Acid Motifs

***Correspondence:** : koabosalif@ju.edu.sa; hetariq@ju.edu.sa

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INTRODUCTION

Malaria poses a significant threat to approximately half of the global human population. An estimated 247 million individuals in 85 countries contracted malaria in 2021. In the same year, 619,000 people succumbed to the illness.[1] Flu-like symptoms, such as cyclic fever, chills, sweating, headache, muscular pains, exhaustion, and joint pain, are indicative of a malaria infection.[2] Additionally, there may be gastrointestinal problems such as diarrhea, vomiting, and nausea.[3] Complications include anemia, jaundice, and hepatosplenomegaly, which may arise as the infection worsens.[4] Severe malaria can result in respiratory distress, kidney and liver failure, cardiovascular collapse, and brain malaria (seizures, confusion, coma) if treatment is not received.[5] Immunocompromised people, children, and pregnant women are high-risk categories.[6] The most harmful species is *Plasmodium falciparum*, which frequently causes life-threatening complications. In order to avoid serious consequences, early identification and treatment are essential.[7] The *Anopheles* mosquito and humans are the two hosts in the complicated life cycle of *Plasmodium*, the parasite that causes malaria.[8] Sporozoites are injected into the circulation by an infected mosquito bite and go to the liver.[9] Sporozoites develop into schizonts within liver cells, which burst to release merozoites into the bloodstream.[10] These merozoites replicate asexually by invading red blood cells.[11] Malarial fever and cold cycles are caused by the bursting of infected red blood cells, which release more merozoites.[10] In order to continue the cycle of transmission, some merozoites undergo differentiation into sexual forms known as gametocytes, which may be ingested by another mosquito during a blood meal.[12] In human evolutionary history, malaria mortality has disproportionately affected children under the age of five, which aligns with the hypothesis that malaria has exerted a substantial influence on the selection of the human genome.[13]

A potential candidate of malaria vaccine, RTS, S/AS01, was authorized for a 2015 pilot deployment program in three African nations. This development was promising for addressing malignant malaria. But, RTS,S/AS01 has been associated with various limitations, of which, the most notable are ineffectiveness in specific age categories, insufficient immunity, and the requirement of nearly three boosters to achieve satisfactory performance. Therefore, a more thorough comprehension of naturally developed immune responses to the different phases of the parasite, including the accessible stages, may be essential for the development of a potent malaria vaccine.[14]

TLRs were first discovered in 1997 as proteins crucial for antifungal defense and dorsoventral fetal development of the fruit fly, *Drosophila melanogaster.*[15] The TLR relatives can be divided into two groups based on where they are found within the cell: TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are located externally on the cell surface, whereas TLR3, TLR7, TLR8, and TLR9 are found internally either in the endoplasmic reticulum membrane or in the endosomal/ lysosomal membranes. Exterior components of pathogens are recognized by cell-surface TLRs, but the nucleic acids of these infectious agents are mostly recognized by internal membrane-surface TLRs.[16] TLRs are the biggest family of pattern recognition receptors that can directly identify both extracellular and intracellular microbial antigens, including bacterial, fungal, viral, and protozoal antigens, with resulting activation of innate immune-signaling transduction-mediating inflammatory mediator production.[17]

Cell-surface TLRs consist of external, transmembrane, and cytoplasmic domains. They are classified as type I transmembrane glycoproteins. The basement domain (the first domain) is oriented toward either the cytoplasm or the outside of the cell, depending on the location of the binding site. It contains multiple (16-28) leucine-rich repeats (LRRs) comprising 24-9 amino acids and may exhibit either the typical (T) motif (LxxLxLxxNxLxxLxxxxF/LxxLxx) or the bacterial (S) motif (LxxLxLxxNxLxxLPx(x)LPxx).[18] The ectodomain structure accommodates the hydrophobic residues of LRRs, thus generating a hydrophobic area of attachment to the linker.[19] While the cytoplasmic membrane domain, commonly referred to as the Toll/interleukin-1 receptor (TIR) domain, interacts with signal transduction adaptors and initiates signaling, the LRR motif is responsible for pathogen recognition.[20] Although diverse

synthetic ligands bind to various TLRs, their sensitivity to microbial substances coincides.[21] The second domain of TLRs is the single-spanning transmembrane domain, which is analogous to the interleukin-1 receptor counterpart and secures the receptor in the appropriate orientation on the cell membrane.[18] The TIR domain is the third domain of the TLRs.[17,22] This domain is shared by almost all TLRs and typically consists of 150 amino acid residues.[23] TLRs exhibit a structure characteristic of type I membrane proteins: a cytoplasmic domain that protrudes from the inside of the cell and an extracellular domain projecting outside of the cell, which are connected by a transmembrane domain that traverses the membrane as shown in Figure 1.[24,25]

TLRs, which constitute the most extensively studied family of pattern recognition receptors that directly recognize protozoal antigens, function as the primary receptors of the innate immune response and are absolutely vital for the adaptive immune response, as observed in malaria.[26]

During a malaria infection, toll-like receptors (TLRs) are essential for bridging adaptive immunity.[27] When TLRs-specifically TLR2, TLR4, and TLR9-identify *Plasmodium*-derived compounds, including hemozoin and glycosylphosphatidylinositols (GPIs), they activate innate immunity cells, which results in the generation of type I interferons and pro-inflammatory cytokines.[28,29] This early signaling affects the adaptive immune response by influencing dendritic cell maturation, improving antigen presentation, and encouraging T-cell activation and differentiation.[30] Therefore, the establishment of efficient humoral and cellular defense against *Plasmodium* species depends on TLR-mediated pathways.[26]

With a focus on their signaling pathways and their role in malaria infection, this review explores the critical function of TLRs in the innate immune response. By elucidating the mechanisms through which TLRs recognize malarial components and modulate immune

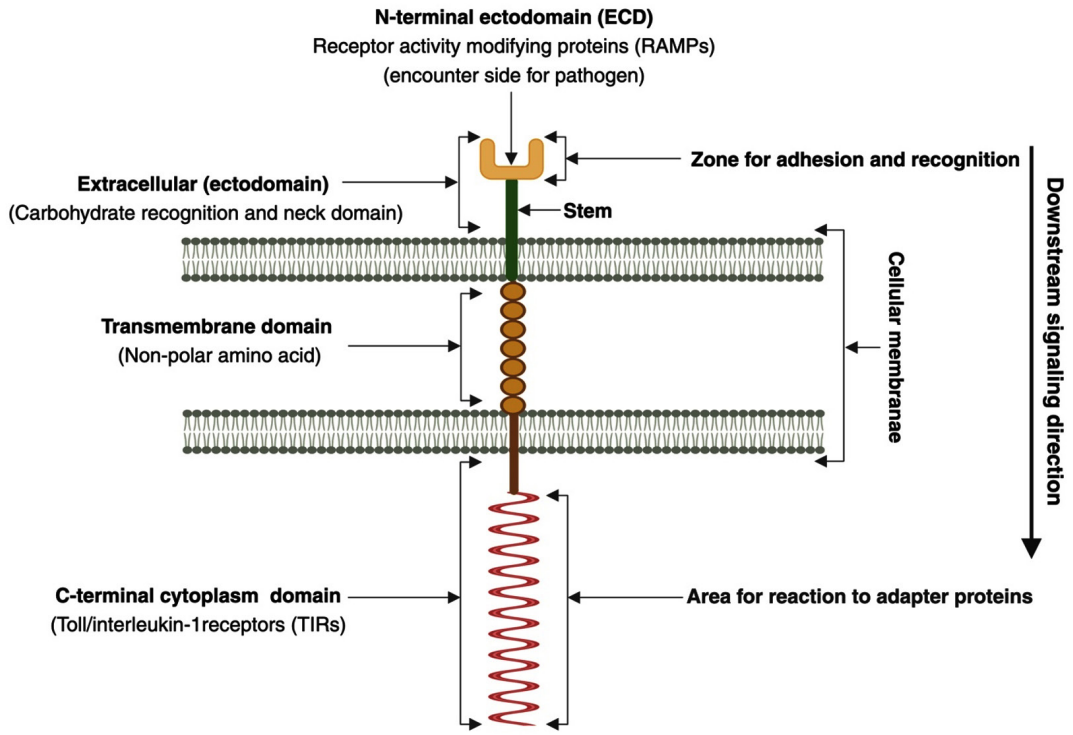

Figure 1. Schematic representation illustrating a transmembrane adhesion molecule comprising an extracellular domain, transmembrane segment, and cytoplasmic domain

responses, we aim to highlight their influence on the pathophysiology and progression of malaria. A deeper understanding of these pathways is essential for identifying potential therapeutic targets and improving disease management. Through a comprehensive analysis of recent advancements, this study underscores key aspects of TLR-mediated immunity in malaria and its broader implications for host-pathogen interactions.

Ligands (antigens) of TLR and malaria

In relation to the protozoan parasite *Plasmodium* and malaria, three primary TLR ligands have been identified: hemozoin, immunostimulatory nucleic acid motifs (schizonts and merozoites), and glycosylphosphatidylinositol (GPI) anchors as shown in Figure 2.[26] In addition, certain endogenous substances produced during malarial infection, including heme groups and microvesicles, function as damage-associated molecular patterns (DAMPs) and activate TLRs in conjunction with these pathogen-associated molecular pattern (PAMP) ligands.[31]

Hemozoin

During the heme detoxification process, all four species of *Plasmodium* that infect people synthesize an inorganic crystal termed hemozoin. This crystalline structure represents a significant byproduct of *Plasmodium*'s hemoglobin metabolism and performs a crucial function in the immune system's response to malaria infection.[32] During erythrocyte breakdown, hemozoin is discharged from the feeding vacuole into the bloodstream, and the liberated parasites invade additional uninfected red blood cells (RBCs). Hemozoin is released into the blood following merozoite egress when innate immune cells phagocytose it.[32] The hemozoin concentration in neutrophils and monocytes during phagocytosis serves as a reliable indicator of disease severity and parasite burden.[31] The precise mechanisms for its persistence remain unclear; however, one hypothesis suggests that hemozoin fails to induce lysosomal heme oxygenase, an enzyme necessary for catalyzing heme degradation.[33] To survive within erythrocytes, *P. falciparum* modifies the heme group and turns it into an insoluble crystal inside the digestive vacuole.[34]

Hemagglutinin itself possesses the capacity to activate natural immunity through direct interaction with TLR9 and the inflammasome.[35] Hemozoin, a parasite-derived pigment, has been conclusively demonstrated to facilitate immunosuppression by preventing dendritic cells from modulating the host's immune responses against the parasite and other antigens.[36] Upon erythrocyte rupture, merozoites, hemozoin, free heme, and other components of the parasite's cytoplasm and digestive vacuole are liberated, resulting in hemozoin accumulation. Numerous immune cells, including monocytes, neutrophils, dendritic cells, macrophages, and endothelial cells, engage with and internalize hemozoin, and malaria-infected RBCs (iRBCs) release microvesicles (Figure 2). Of these, monocytes and macrophages are the most extensively studied hemozoin-internalizing cells. Human monocytes have been observed to rapidly absorb hemozoin, which can occupy up to 30% of their whole cellular volume. Moreover, the ingested hemozoin can remain unaltered within monocytes for extended durations.[37]

Nucleic acid motifs (DNA)

Parasitic DNA activates natural immune system cells through detection by DNA sensors in the cytoplasmic matrix and TLR9 in endolysosomes.[38] One hypothesis suggests that TLR9 is activated by parasite DNA, thereby generating an initial signal necessary for hemozoin to activate the inflammasome and produce proinflammatory cytokines.[39] After being taken up by merozoites, entire diseased erythrocytes, hemozoin, or DNA-protein structures, parasite DNA infiltrates the endolysosomes of natural immune cells.[40] This leads to the generation of proinflammatory cytokines and the activation of the MyD88-NF-κB signaling pathway, as shown in Figure 2. Notably, TLR9 is mainly expressed in human B cells and plasmacytoid dendritic cells; consequently, monocytes are unlikely to contribute significantly in the detection of parasite DNA via this route.[31]

The cytoplasmic matrix DNA-sensing AIM2 and cGAS detect the presence of parasite DNA as it is released from endolysosomes into the cytoplasmic matrix.[41] This may result in the generation of recombinant IL-1β and stimulation of the AIM2 inflammatory process.[42] In addition,

the cGAS-stimulator of IFN genes (STING) process induces a type I interferon (IFN) response.[43] Recent research demonstrated that cGAS acts as a sensor that detects *P. falciparum* genomic DNA in the cytoplasm downstream of STING.[44] Type I IFNs are produced when activated cGAS synthesizes 2'3'-cGAMP, which functions as a secondary messenger to activate STING and phosphorylate TBK1 and IRF3-IRF7 (interferon regulatory factors).[45]

Nucleic acid motifs (RNA)

The natural immune response detects the RNA of parasites throughout hepatocytes and the bloodstream stages.[46] The melanoma differentiation-associated protein 5 (MDA5) exclusively detects RNA in the cytoplasmic matrix during the liver stage via MAVS and IRF3 and IRF7, two transcription factors, while TLR3, TLR7, and TLR8 may identify microbial RNA in endosomes.[47] Conversely, during the bloodstream stage of infection, TLR7 in dendritic cell phagolysosomes recognizes mouse parasite RNA, which results in the synthesis of type I IFN.[26] RNA sequencing in a liver-stage infection with rodent malaria elicits a robust innate immune response, involving type I IFN and IFNγ pathways.[48] *P. falciparum* RNA is recognized by endosomal TLR8 in human monocytes, which induces the production of IL-12p70 and IL-18, subsequently causing natural killer cells to produce IFNγ. Protozoan RNA-induced TLR8 activation demonstrates the distinct function of TLR8 in human immunity as well as its crucial involvement in human blood-stage malaria.[49] The notable differences between the innate immune systems of humans and mice in the detection of nucleic acids within endosomes may partially elucidate this phenomenon.[31]

Direct infusion of the plasma membrane produces microvesicles (Figure 2), which are small vesicles 0.1-1 µm in size. Microvesicles function as intercellular communicators and can contain RNA, proteins, and even organelles.[50] Individuals with malaria who are infected with *P. falciparum* or the related human pathogen *P. vivax* exhibit higher than normal concentrations of microvesicles derived from RBCs and platelets.[51] Peripheral blood parasitemia is associated with an increase in microvesicles in individuals with severe illness.[52] As the human malaria parasite, *P. falciparum*, develops, microvesicles derived from iRBCs (RMVs) are quantitatively released from the iRBCs. The majority of RMVs contain proteins originating from both *P. falciparum* and humans, and they are produced in the later stages of the asexual cycle. Mantel et al. demonstrated that RMVs function as messengers between iRBCs and are immunostimulatory. RMVs are transferred from one iRBC to another and influence the number of transmitting stages produced in infected individuals.[53]

Glycosylphosphatidylinositol

Glycosylphosphatidylinositol serves as a lipid anchor for numerous cell-surface proteins. The GPI anchor, which is extensively used in eukaryotes and potentially in certain Archaea but is absent in Eubacteria, is a posttranslational modification of proteins with a glycolipid.[8] GPI-anchored proteins constitute the predominant kind of cell-surface proteins in protozoa.[8] Many GPI-anchored proteins are incorporated into the cell wall of fungi.[54] At minimum of 150 GPI-anchored proteins are found in humans and perform a wide range of functions, for example as protease inhibitors, enzymes, adhesion molecules, receptors, and transcytotic receptors and transporters.[55] One of the first factors identified as a PAMP of the parasites associated with malaria was GPI of *P. falciparum*.[56] Parasite GPI consists of a varied array of molecules that are connected to the glucosamine moiety of glycan via triacylated phosphatidylinositol. These molecules have four mannose residues and one glucosamine residue.[57]

The compositional variability of malaria GPI is attributed to the acyl residues located at various locations on the phosphatidylinositol moiety, which vary in length and degree of unsaturation. This compositional variability does not affect GPI's capacity to elicit an immunological response.[58] Evidence for this is provided by the ability of sn-2 lyso GPI, generated by removing the acyl from the parasite GPI at the sn-2 position, to effectively elicit cytokine responses comparable to those of the original parasite GPI.[59] GPI is crucial for the survival of parasite as it binds many merozoite proteins that contribute to erythrocyte invasion of the plasma membrane.[60] Without GPI anchoring, the surface expression of these proteins does not take place, thus preventing merozoites from

invading RBCs.[61] Malaria parasites produce GPI in quantities significantly exceeding those required for attaching proteins to the exterior of merozoites, resulting in substantial amounts of unlinked GPI.[60] The unattached GPI particles, visible on the cell exterior, are presumably attacked by the innate immune system.[26]

The main proteins linked to the GPI particles throughout the *Plasmodium* circulation period of invasion are members of the merozoite surface protein family (MSP-1 and MSP-2) and the rhoptry-associated membrane antigen (RAMA).[62] The main GPI-attached protein in the pre-erythrocyte phase is circumsporozoite protein (CSP).[63] PF34, Cys6, and apical sushi protein are other *Plasmodium* proteins that include GPIs.[64] GPIs, which are PAMPs, have been demonstrated to be able to activate TLR4 homodimers or the TLR1-TLR2 and TLR2-TLR6 heterodimers to cause the generation of proinflammatory mediators such as tumor necrosis factor (TNF) and nitric oxide, through the MyD88 pathway.[65,66] Consequently, these compounds have been investigated as potential malaria vaccine components.[67]

Toll-like receptors

Humans have been demonstrated to possess 10 TLRs (TLR1-TLR10).[68] TLRs are located both on the cell surface (TLR1, 2, 4, 5, 6, and 10) or within the endosomal membrane (TLR3, TLR7, TLR8, and TLR9), enabling them to identify genetic material from pathogens, including nucleic acids found within intracellular pathogenic microorganisms.

In this context, TLRs recognize proteins, lipids, and carbohydrates present in the pathogen's exterior membrane.[69] TLR2 and TLR4 are surface receptor variants that are internally produced in epithelial, dendritic, and endothelial cells.[70] TLR1, 2, 4, 6, and 9 were identified in *P. falciparum* infections, whereas TLR1, 2, 4, 5, 6, and 9 were observed in *P. vivax* infections. In addition, TLR2 and 4 were detected in both species.[71] The immune response profile elucidated by TLR gene polymorphisms does not seem to be universally applicable to all malaria infection patterns globally. This variability may be influenced by both *Plasmodium* species and human genetic diversity.[72] Primary immunodeficiency diseases (PIDs), which are typified by an increased vulnerability to infections, can result from mutations in the genes encoding TLRs and associated signaling components.[73] For example, faulty innate immune responses caused by mutations in the MyD88 and IRAK-4 genes disrupt TLR signaling pathways, leading to repeated pyogenic bacterial infections.[74] Similarly, TLR3 signaling is disrupted by mutations in the UNC93B1 gene, which puts people at risk for herpes simplex virus encephalitis.[75] These genetic changes highlight how important TLRs and the pathways they are linked to are for preserving the integrity of the immune system (Table).

TLRs cell surface receptors

TLR1 (CD281)

TLR1 is a gene that encodes the Toll-like receptor 1 protein. It is an essential component of the innate immune system that facilitates the detection and response to pathogens like the parasite responsible for malaria.[79] Research on a Southeast Asian population affected by *P. falciparum* malaria demonstrated an association between elevated parasitemia and a common TLR1 variant. Parasitemia, the presence of parasites in the blood, is a critical indicator of malaria severity. The findings suggested that mutations in TLR1 influenced the host response to *P. falciparum* malaria in Asian people.[80]

The results for TLR1 and TLR6 are inconsistent. Two studies examining the impact of these TLRs on susceptibility reported statistical associations, albeit with differing outcomes.[71] However, another study suggested an increase in vulnerability.[81] The majority of studies found no effect on severity.[82] Only one investigation identified a significant association, indicating that the presence of this receptor exacerbated the severity of disease. This investigation included *P. falciparum*-infected adolescents and adults from Asia (Myanmar).[80] Research conducted in west-central Africa identified a link between TLR1 and higher levels of parasitemia in childhood.[83] Zhu et al. were able to detect TLR2-TLR6 with marginally higher discrimination than the dimeric pair TLR2-TLR1 due to their findings on sn-2 lyso GPIs, a malarial GPI variant with two fatty acid substituents. Nevertheless, the TLR2-TLR1 dimeric pair is more frequently engaged by malarial

Table. Malaria ligands of Toll-like receptors (TLRs)

TLRs	CD - Name	Other Name	TLR coreceptor	TLR-active version	Chromosome	Station	Binder	Signaling pathway	Ref.
TLR1	CD281	TIL; rsc786; KIAA001; DKFZp547I0610; DKFZp564I0682	TLR2	TLR1-TLR2	4p14	Cell membrane	Glycosylphosphati-dylinositol	MyD88/MAL, NF-κB, IRFs	17,26, 76-78
TLR2	CD282	TIL4	TLR1, 2, 6 and 10CD14, CD36, integrin, RP105, MBL, LBP	TLR1-TLR2TLR2-TLR2TLR2-TLR6	4q31.3	Cell membrane	Glycosylphosphati-dylinositol	MyD88/MAL, NF-κB, IRFs	17,26, 76-78
TLR4	CD284	TOLL; hToll	MD2, LY96, CD14, CD36, LBP, RP105	TLR4-TLR6	9q33.1	Cell membrane	Hemozoin, heme microvesicles	MyD88/MAL, NF-κB, IRFsOrTRIF, RIF3. IRFs	17,26, 76-78
TLR6	CD286	CD286 Antigen Q9Y2C9	TLR2, CD36, LBP	TLR2-TLR6 TLR4-TLR6	4p14	Cell membrane	Glycosylphosphati-dylinositol	MyD88/MAL, NF-κB, IRFs	17,26, 76-78
TLR7	CD287	IMD74; SLEB17; TLR7-like	CD14	TLR7-TLR7	Xp22.2	Endolysosome	RNA ligand	MyD88/MAL, NF-κB, IRFs	17,26, 76-78
TLR9	CD289	CD289 Antigen Q9NR96	CD14	TLR9-TLR9	3p21.2	Endolysosome	DNA ligand	MyD88/MAL, NF-κB, IRFs	17,26, 76-78

GPIs (Figure 2), which possess three fatty acid substituents, than TLR2-TLR6.[84]

TLR2 (CD282)

TLR2 is a protein-coding gene crucial to the innate immune system. It is expressed on multiple immune cells, such as dendritic cells, monocytes, neutrophils, macrophages, B lymphocytes, Th1, Th2, and Treg lymphocytes.[85] Host cellular responses are primarily elicited by GPI through TLR2/MyD88-mediated signaling.[65] As PAMPs, GPIs have been demonstrated to activate TLR4 homodimers or the TLR1-2 and TLR2-6 heterodimers, thereby inducing the synthesis of proinflammatory mediators, including TNF and nitric oxide, through the MyD88 pathway.[86]

According to the results of a meta-analysis on TLR2, the majority of studies assessing this TLR concluded that it does not affect susceptibility.[71] However, research conducted in Ghana, Africa, involving *P. falciparum*-infected individuals of various ages, found that this TLR played a role in protection with regard to malaria susceptibility.[87] Two studies have demonstrated that the CD282 receptor confers a protective effect against severe types of malaria.[87,88] These investigations, conducted in Ghana and Uganda, Africa, involved *P. falciparum*-infected individuals. In persons

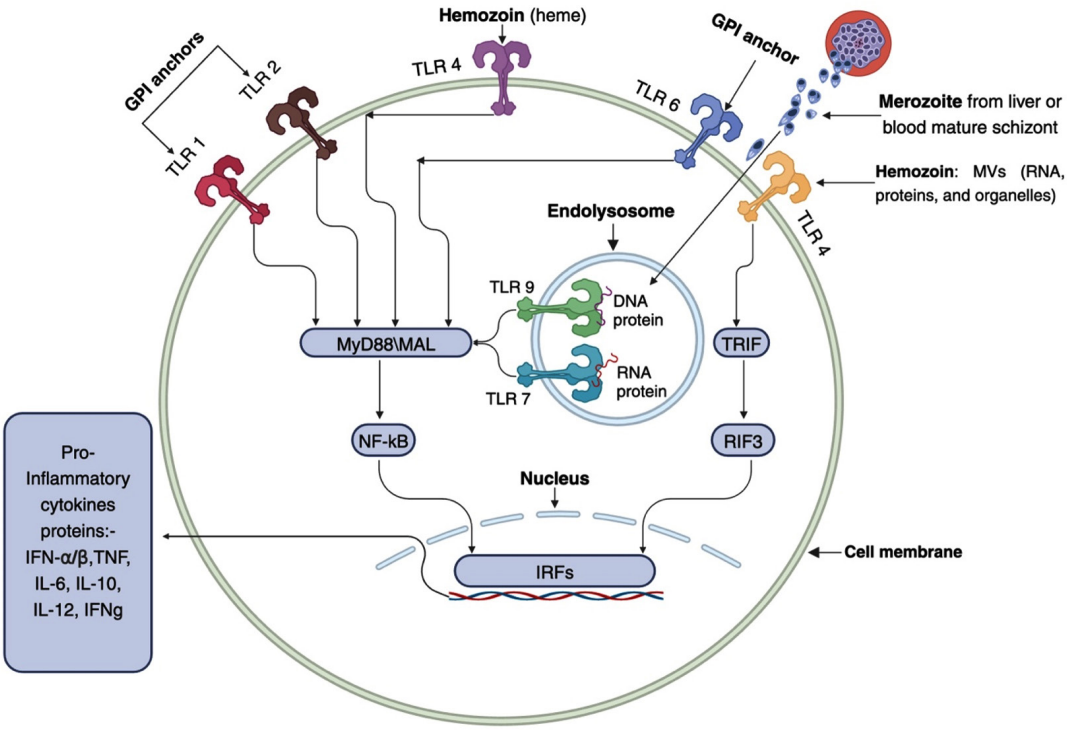

Figure 2. Three primary ligands can activate malarial Toll-like receptor (TLR) signaling pathways: glycosylphosphatidylinositol (GPI) anchors, immunostimulatory nucleic acid (DNA and RNA) motifs, and hemozoin (heme or microvesicles). TLR1, 2, and 6 are activated in the basement membrane (ectodomain) by parasite GPI, whereas heme or microvesicles containing RNA, proteins, and organelles can stimulate TLR4. Upon phagocytosis, infected erythrocytes release parasite DNA and RNA into phagolysosomes, where innate immunity is activated. TLR7 (an RNA ligand) and TLR9 (a DNA ligand) serve as receptors for these nucleic acid patterns. While TLR4 activation via TRIF (TIR domain-containing adaptor inducing interferon-β) induces the production of IFN-α/β, all other TLRs stimulated by malarial ligands promote the production of proinflammatory proteins, such as TNF, IL-6, IL-10, IL-12, and IFNγ, via the Myeloid Differentiation Primary Response Gene 88 (MyD88)/NF-κB pathway. The exception is the TLR4 pathway, which can use TRIF or MyD88 adapter proteins, also known as MyD88-adapter-like (MAL), with subsequent activation of interferon regulatory factors (IRFs)

heterozygous for the TLR2Δ22 mutation, reduced inducible production of TLR2 may result in diminished pro-inflammatory responses and potentially serve as an effective defense against cerebral malaria (Table).[88]

TLR4 (CD284)

The gene TLR4 is among the most extensively studied TLRs as it encodes the primary receptor for lipopolysaccharides, TLR4, a component of certain microorganisms such as malaria parasites.[89] TLR4 may be crucial to the clinical pathophysiology of malaria. This is supported by existing data and suggests that further investigation of TLR4 and associated genes in clinical malaria could lead to novel therapeutic approaches and pharmaceutical discoveries.[90] A previous study of patients from Ghana showed that this receptor exacerbated the condition.[87] A sequence of feedback events may be a potential explanation for the mechanism by which heme activates the TLR4 signaling pathway.[91] Elevated TLR2, 4, and 8 levels were noted in a case study of severe malaria.[92]

Heme, a byproduct of RBCs, is suggested as a DAMP that influences inflammatory responses in various pathophysiological contexts and has been associated with TLR4 signaling.[91,93] Moreover, heme can facilitate the recruitment of WBCs, RBCs, and leukocytes to the vascular endothelium. As initially demonstrated in macrophages, numerous pro-inflammatory actions of heme are linked to TLR4 signaling stimulation.[94] Heme-mediated TLR4 signaling seems to involve complex regulatory processes that vary depending on the experimental models and conditions.[91,95] The MyD88-dependent or MyD88-independent pathway is responsible for the production of pro-inflammatory cytokines after TLR4 activation by lipopolysaccharides or DAMPs.[96] In addition, DAMPs generated during malaria infection can activate TLR4. Macrophages have the capacity to internalize RMVs produced by *Plasmodium* species and trigger TLR4.[53,97] Couper et al.[97] showed that the macrophage activation pathway mediated by microvesicles is dependent on TLR-4 and MyD88. This represents a novel and significant mechanism of fundamental inflammation during malaria infection, and it associates the onset of severe malarial illness with microvesicles originating from parasitized RBCs (Table).[97]

TLR6 (CD286)

The human TLR6 gene encodes TLR6, also known as CD286.[98] TLR6 has been shown to increase the prevalence of acute malaria cases in African (Cameroonian) children with *P. falciparum* infections.[83] TLR6 and TLR2 form heterodimers, which enhance the ligand's potency against certain infections.[99] In conjunction with TLR1 and TLR6, TLR2 detects GPI in a heterodimeric form.[65] When host immune cells recognize GPIs on parasite surfaces, TLR2, in combination with TLR1 or TLR6 activates NF-κB, leading to the secretion of pro-inflammatory cytokines.[100] Murine TLR1 and TLR6 exhibit reduced selectivity compared to individual TLRs in identifying malarial GPIs; furthermore, TLR6 and TLR1 macrophages produce substantial quantities of nitric oxide and TNF-α (Table).[65]

TLR endosomal membrane receptors

TLR7 (CD287)

The endosomal natural immunity detector, TLR7, has the capacity to identify single-stranded ribonucleic acid.[101] Its mechanism of endosomal TLR placement is complex and tightly regulated and shares certain elements with other TLRs.[18] Research conducted on *P. chabaudi* clarified TLR7's function in IFN-1, IL-12, and IFN-γ production. TLR9 was previously identified as the primary indicator of infection. However, studies carried out without TLR7 and MyD88 demonstrated a significant decrease in pro-inflammatory cytokine-like IFN-1. On the other hand, animals lacking TLR2, 4, 9, interleukin-1 receptor, or IL18R had no effect on IFN production.[102]

This discrepancy is attributed to the difference in accessible ligands and the stimulation of TLR9 or TLR7 based on the duration or phase of infection.[103] Despite the lack of evidence for the parasite ligand that activates TLR7, it has been established that single-stranded RNA is necessary for TLR7-mediated IFN-I release in viral infection.[104] It has been postulated that the parasite's RNA might bind to the receptor and function as a ligand.[103] In addition, TLR7 responds to pathogens identified in the endosome by initiating an immune response to purine-rich single-stranded ribonucleic

acid.[101] Notwithstanding this hypothesis, the precise receptor-ligand interaction remains to be elucidated (Table).[100]

TLR9 (CD289)

A key component of the natural immunological response to malaria is the TLR9 protein. The TLR9 receptor facilitates an effective immunological response against malaria in a manner that is dependent on MyD88.[105] The innate immune system has been demonstrated to be activated by the murine malaria pathogens *P. berghei*, *P. chabaudi chabaudi* AS, and *P. yoelii*, as well as TLR2 and TLR9.[106,107] Dendritic cells are activated by parasite DNA through the TLR9 receptor.[108,109]

Parroche et al.[110] observed that TLR9 may be induced by natural hemozoin but not by purified heme. Experiments showed that stimulation was inhibited because nuclease prevented TLR9 from binding. Subsequent research confirmed that hemozoin's exterior contains DNA that interacts with TLR9.[110] Through direct interactions with TLR9 and the inflammatory response, in vitro experiments using synthetic versions have demonstrated that hemozoin per se may stimulate the innate immune response.[111] The initial signal necessary for hemozoin to activate inflammation and produce pro-inflammatory cytokines is likely caused by parasite DNA activating TLR9 as shown in Figure 2.[112]

CONCLUSION

The function of TLRs in malaria remains unclear and is potentially complex, although evidence suggests that these receptors may contribute to both immunological pathogenesis and prevention. Despite numerous research publications on the subject, there is a lack of consensus on whether TLR variations have a detrimental or beneficial effect on the clinical manifestations of malaria infections. Indeed, multiple variables may confound the findings and impede accurate comparison of studies. These variables include subject age, study population size, quality control measures employed during specimen processing, genetic diversity of *Plasmodium* species distributed across study locations, variation in malaria endemicities, and hereditary traits of the hosts. Nevertheless, the abundant published data strongly suggest that genetic variations of TLRs affect the incidence and progression of malaria. Future research on TLRs as mediators of the innate immune response may enhance comprehension of the immune system's balanced operation, which is crucial for developing novel treatments for a wide range of immune-related disorders, including malaria.

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The authors declare that there is no conflict of interest.

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