

Toll-Like Receptors and Malaria – Sensing and Susceptibility

Emily M Eriksson^{1,2*}, Natália G Sampaio^{1,2}, Louis Schofield^{1,2}

¹Walter and Eliza Hall Institute of Medical Research, Division of Infection and Immunity, Parkville, VIC 3052 Australia

²The University of Melbourne, Department of Medical Biology, Parkville, VIC 3052 Australia

Abstract

Toll-Like Receptors (TLRs) are important mediators of the innate immune response to pathogens, including malaria. Of the ten human and twelve mouse TLRs, TLR2, TLR4, TLR7 and TLR9 are known to detect malarial antigens and induce anti-malarial immune responses. Multiple immune cell populations express TLRs, and much has been done to elucidate the TLR-mediated immune response to malaria infections, in particular the involvement of TLRs in severe malaria pathogenesis. Here we review the role TLRs play in parasite detection, immune response, and severe malaria, with a focus on recent findings. Furthermore, the use of TLR ligands as malarial vaccine adjuvants is discussed, as this could have great potential in improving efficacy of vaccine candidates.

Keywords: Toll-like receptors; Interferon regulatory factors; X-ray crystallography

Introduction

The Toll-Like Receptors (TLRs) are a family of pathogen recognition receptors that recognize Pathogen-Associated Molecular Patterns (PAMPs). This initial detection of pathogens plays a central role in the activation of the innate immune system and the generation of an appropriate immune response to infection. To date, ten TLRs in humans (TLR1-10) and twelve TLRs in mice (TLR1-9, 11-13) have been identified, where each TLR binds specific ligands. TLR1-2, TLR4-6 and TLR10 are found on the cell surface, and interact with extracellular ligands, whereas TLR3 and TLR7-9 are found in endosomes and interact with intracellular ligands Table 1. Although TLR-induced responses to malaria have been described, the TLR-mediated contribution to malaria-associated pathogenesis has been difficult to determine and is a continued area of intense research. In addition, the use of TLR-based adjuvants for malaria vaccines is a quickly expanding field, which has thus far generated significant observations. This review focuses on recent advances in malaria-related TLR-dependent responses, pathogenesis, and vaccine developments.

TLR Structure, Adaptor Proteins and Signaling Pathways

TLRs are transmembrane glycoproteins that contain a ligand-binding domain in the extracellular N-terminus, and a signaling domain in the intracellular C-terminus. TLRs activate downstream signaling cascades that lead to activation of transcription factors, such as nuclear factor kappa light-chain enhancer of activated B cells (NF- κ B), activator protein-1 (AP-1) and Interferon Regulatory Factors (IRFs), which in turn activate a pro-inflammatory response. X-ray crystallography has confirmed that TLRs form homodimers, with the exception of TLR2, which forms heterodimers with TLR1 or TLR6 [1-5].

The initial signal transduction from TLRs to their adaptor proteins occurs via a unique signaling Toll-Interleukin-1 Receptor (TIR) domain. This domain is present in the C-terminus of all TLRs, as well as the IL-1 and IL-18 receptors, and on their intracellular signaling adaptors. There are six known TIR-domain containing adaptors for TLR signaling, and these are myeloid differentiation factor 88 (MyD88), MyD88 adaptor-like protein (MAL, TIRAP), TIR domain-containing adaptor inducing interferon- β (TRIF, TICAM-1), TRIF related adaptor protein (TRAM, TICAM-2), sterile- α and HEAT/Armadillo motifs-containing protein (SARM, MyD88-5), and the recently discovered B Cell Adaptor for PI3K (BCAP), further described in Table 1.

MyD88 was the first adaptor to be associated with TLR signaling [6] and has since been shown to be required for signal transduction of all TLRs with the exception of TLR3, and partially TLR4 [7,8]. Activation of MyD88 results in downstream NF- κ B activation via a signaling cascade involving members of the IL-1R-Associated Kinase family (IRAK), namely IRAK-1, 2 and 4 [9,10]. In addition to MyD88, MAL binds MyD88 via the TIR domain and is essential for TLR2 and TLR4 signaling [11,12].

Through studies using MyD88 KO mice, a MyD88-independent pathway for TLR3 and TLR4 signaling was demonstrated. TRIF was found to be the exclusive adaptor for TLR3, and also to mediate the MyD88-independent arm of TLR4 signaling [13]. The TRIF-mediated pathway of TLR4 signaling also requires an additional exclusive adaptor, TRAM [14,15] which functions to recruit TRIF to TLR4 after receptor-ligand interaction and endocytosis, resulting in the activation of IRF3 [16]. TRIF-mediated signaling controls production of Type I IFN through activation of IRF3 and possibly IRF5 [15,17]. In addition to NF- κ B, AP-1 and IRFs, TLR signaling also results in the activation of PI3K, which is thought to negatively regulate the pro-inflammatory TLR response. Most recently, it was discovered that BCAP contains a previously unidentified TIR domain that allows interaction with ligand-activated TLRs at the plasma membrane [18,19]. Additionally, BCAP was shown to directly bind and activate PI3K, and absence of BCAP resulted in an exacerbated TLR-induced cytokine response. This demonstrated that BCAP is the crucial link between TLR signaling and PI3K activation, and plays an important role in regulation of the immune response downstream of TLRs. Unlike the other adaptor proteins, the function of SARM is only now starting to emerge. SARM is predominantly expressed in neurons, with a suggested function of regulating neuronal death [20] and responding to viral infection in the

***Corresponding author:** Emily M Eriksson, The Walter and Eliza Hall Institute of Medical Research, Division of Infection and Immunity, The University of Melbourne, Department of Medical Biology Parkville, VIC 3052 Australia, Tel: 61393452644; Fax: 61393470852; E-mail: eriksson@wehi.edu.au

Received November 29, 2013; **Accepted** December 18, 2013; **Published** December 20, 2013

Citation: Eriksson EM, Sampaio NG, Schofield L (2013) Toll-Like Receptors and Malaria – Sensing and Susceptibility. J Trop Dis 2: 126. doi: [10.4172/2329-891X.1000126](https://doi.org/10.4172/2329-891X.1000126)

Copyright: © 2013 Eriksson EM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

TLR	Location	Ligand	Signaling adaptor
TLR1/2	Plasma membrane	Triacyl lipopeptide, intact malarial GPI	MAL, MyD88
TLR2	Plasma membrane	Peptidoglycan, LAM, hemagglutinin, phospholipomannan,	MAL, MyD88
TLR2/6	Plasma membrane	Diacyl lipopetide, malarial sn-2 lyso-GPI , LTA	MAL, MyD88
TLR3	Endosome	dsRNA, ssRNA (viral)	TRIF
TLR4	Plasma membrane and endosome	LPS, GPI (<i>Plasmodium</i> and <i>Trypanosoma</i>) , fibrinogen bound to malarial hemozoin , mannan, viral envelope proteins (RSV and MMTV), <i>Plasmodium</i> peroxiredoxin	MAL, MyD88, TRIF, TRAM
TLR5	Plasma membrane	Flagellin	MyD88
TLR7	Endosome	ssRNA from viruses (VSV, influenza), proposed <i>Plasmodium</i> RNA (?)	MyD88
TLR8	Endosome	ssRNA from viruses	MyD88
TLR9	Endosome	dsDNA viruses, DNA CpG motifs, malarial DNA-protein complex	MyD88
TLR10*	Plasma membrane	Undefined, possibly triacyl lipopeptide when dimerized with TLR2	MyD88
TLR11#	Endosome	Profilin-like protein from <i>Toxoplasma gondii</i> possibly as heterodimer with TLR12, uropathogenic bacteria	MyD88
TLR12#	Endosome	Undefined, likely bacterial products	MyD88
TLR13#	Endosome	Bacterial 23S ribosomal RNA, vesicular stomatitis virus	MyD88

Malarial ligands highlighted in bold. *Human only, #mouse only

Table 1: Description of TLRs, signaling adaptors and their ligands.

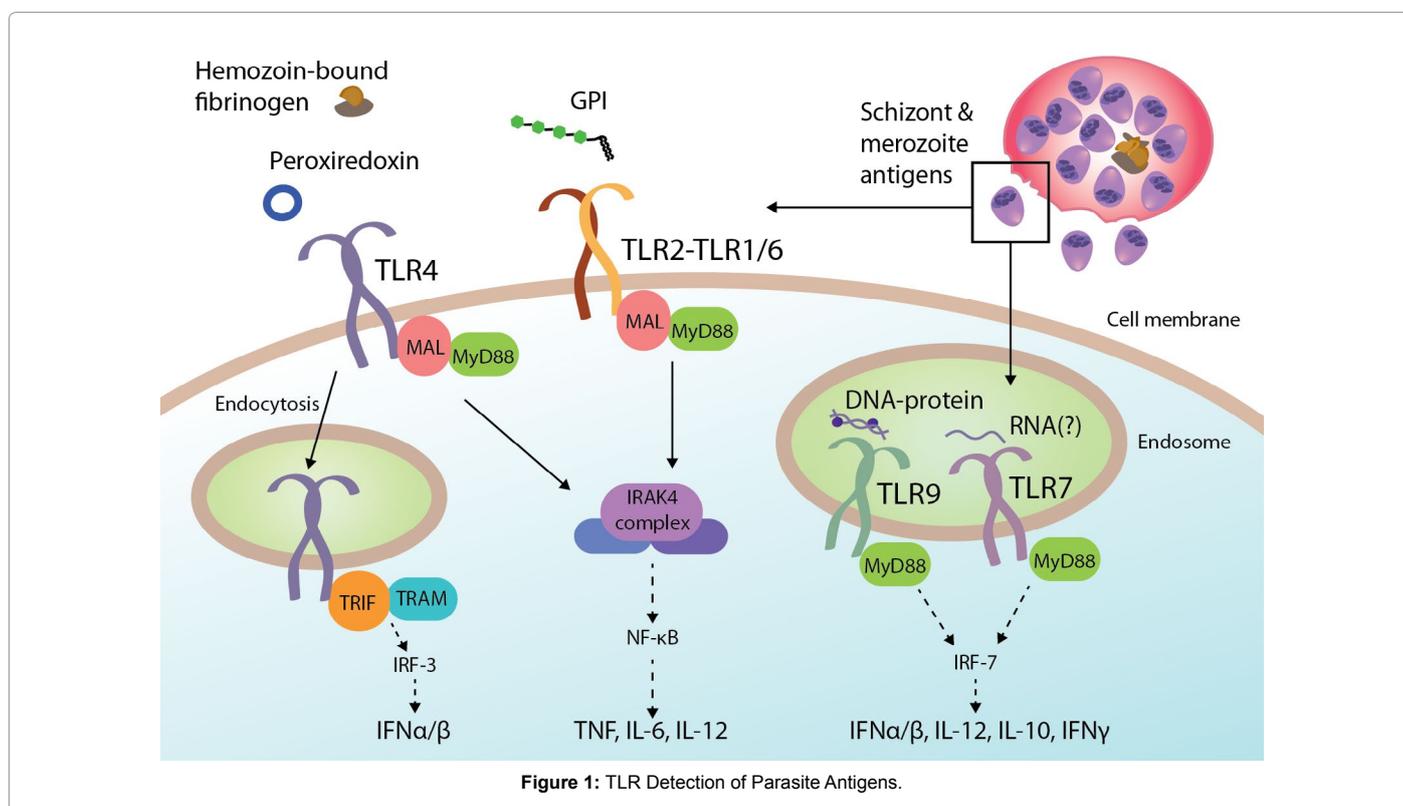


Figure 1: TLR Detection of Parasite Antigens.

brain [21]. More recently, studies have also suggested that SARM plays a role in apoptosis, although its cellular localization remains debated [22-24].

TLR Detection of Parasite Antigens

TLR2, TLR4 and TLR9, and most recently also TLR7, are activated by *Plasmodium* antigens (Figure 1). The first demonstration that parasite antigens could be detected by TLRs was by Campos et al. [25], who showed that Glycosylphosphatidylinositol (GPI) anchors from *Trypanosoma cruzi* activate TLR2. A later study demonstrated that TLR2 and, to a lesser extent, TLR4 are capable of detecting malarial GPI via a MyD88-dependent pathway, and that the TLR1/2 and TLR2/6

dimers preferentially bind to distinct structural forms of GPI [26]. Furthermore, the *Plasmodium* 2-Cys peroxiredoxin was identified as an additional TLR4 malaria antigen that induced cytokine production by both monocytes and mast cells [27]. However, it should be noted that to establish that a chemical agent is sufficient to activate a TLR requires proving the compositional purity of the putative ligand and the formal exclusion of any contribution by adventitious contaminants. Such compositional purity data were provided by Campos et al. [25] in the case of the *T. cruzi* GPI structure.

Early studies showed that malarial schizonts induced an immune response from human plasmacytoid Dendritic cells (DC) and mouse bone marrow-derived DC that was dependent on MyD88 and TLR9

[28]. There were several conflicting reports on the malarial antigen responsible for activation of TLR9. Initially, the parasite waste product hemozoin was suggested to be the activating antigen [29]. This finding was controversial as hemozoin is a by-product of parasite digestion of hemoglobin, composed of insoluble β -hematin crystals, whereas TLR9 was known to bind CpG DNA motifs from bacteria and viruses. It was later reported that the antigen binding to TLR9 was malarial DNA associated with hemozoin, but that purified hemozoin by itself was immunogenically inert [30]. Subsequent studies showed that a malarial DNA-protein complex activated TLR9, suggesting that previous results were likely due to DNA and protein contamination of hemozoin preparations [31]. Most recently it has also been reported that natural hemozoin from ruptured schizonts binds host fibrinogen and is recognized by human monocytes in a TLR4-dependent manner [32]. These highly discordant results reflect in part the issues relating to compositional purity of agents referred to above and previously [33,34], as hemozoin is not a single molecular species, but a morphological entity comprised of poorly characterized, variable aggregate of many different molecules.

A recent study has demonstrated that TLR7 might also be playing an important role in early detection of malarial infection [35]. Using the *P. chabaudi* acute malaria mouse model, the study showed that TLR7 and MyD88 KO mice, but not TLR2, TLR4, TLR9, IL-1R or IL-18R KO mice, had a significantly reduced Type 1 IFN response at 24 hr post-infection, which was dependent on IRF7 activation. They further demonstrated that absence of TLR7 and MyD88, but not TLR9, significantly reduced the IFN γ , IL-10, IL-12p40 and TNF response to infection at 24 hr, but not at 6 days post-infection. The authors propose that *Plasmodium* RNA might be the target for TLR7, although further work is required to confirm this.

TLR-induced Anti-malaria Responses

TLRs are expressed by a wide range of immune cells, though are predominantly present on innate cells. Consequently, malaria parasites have the potential to be targeted by multiple cell populations. However, a lot of focus has been on DC and other antigen presenting cells as these cells have been reported in several studies to play an important role during malaria infection, and are also crucial in shaping the subsequent adaptive immune response [36-39]. Evidence suggests that TLR-mediated signaling is required for DC maturation, cytokine production and upregulation of co-stimulatory molecules. Additionally, it has also been demonstrated that involvement of TLR4, TLR9, MyD88 and NF- κ B signaling, as well as cell-cell contact and internalization of infected RBC, are required for DC activation [40]. However, it appears that the DC cytokine profile progressively changes with infection. DC isolated early following infection produce pro-inflammatory cytokines, whereas DC isolated later during infection are anti-inflammatory IL-10-producing cells [41].

A distinct *Plasmodium*-mediated enhancement of TLR responsiveness in immune cells has also been described. Peripheral blood mononuclear cells of malaria naïve individuals that were experimentally infected with *P. falciparum* exhibited increased pro-inflammatory and anti-inflammatory cytokine production in response to TLR4 and TLR2/TLR1 ligation [42]. Similarly, acute infections in Ghanaian schoolchildren and a Brazilian cohort both presented with augmented reactivity to TLR stimulation [43,44], although these responses included not only stimulation with TLR2 and TLR4 ligands, but were attributed to all TLR ligands [43]. Further findings of this study indicated that IL-12, TLR9 and IFN- γ were contributing components to TLR hyperreactivity.

In contrast, accumulating evidence supports the implication that *Plasmodium* infection renders DC and other cells unresponsive to TLR stimulation. This has been reported using both murine systems and human cells. Although *Plasmodium yoelii* infection enhances TLR and parasite-specific responses of peritoneal macrophages [45], stimulation of DC with *P. yoelii*-infected erythrocytes inhibits response to a broad range of TLR stimulations [46]. A comparative study between West African ethnic groups Dogon and Fulani further emphasizes the potential for cell impairment during malaria infection. The different groups, which have equivalent malarial exposure, differ in their susceptibility to *P. falciparum* malaria where the Dogon are more susceptible compared to the Fulani. A significant difference in TLR-driven activation of DC subsets during *Plasmodium* infection was observed between the groups, and cytokine release following TLR stimulation was significantly inhibited in the infected Dogon individuals [47]. In addition, modulated immune responses caused by pregnancy-associated malaria have also been observed in neonates [48,49]. Cohort studies in Benin found that infant TLR-mediated cytokine profiles are affected by *in utero* exposure and maternal infection during delivery, and that IL-10 production in particular was associated with higher risk of *P. falciparum* in infancy [49]. These reports highlight the intricate relationship of TLR activation during malaria-induced immune responses, which conceivably are dependent on disease setting, exposure and duration of the infection.

TLR Polymorphism, Malaria Susceptibility and Pathogenesis

The function of TLR signaling in the development or protection from disease has been described by several genetic studies investigating polymorphisms within genes of the TLR pathways and links to disease outcome. Cohort studies of children in Ghana and in Kenya showed that polymorphisms in TLR4 [50] and TLR9 [51,52] were associated with severe malaria, symptomatic malaria, and severe malarial anemia whereas another common TLR9 polymorphism was correlated with high parasitemia [53]. Correspondingly, the same polymorphism in TLR9 has also been associated with susceptibility to malaria, but not with severity of disease [54]. Although in this study no differences in associations were detected with TLR4 and TIRAP polymorphisms between uncomplicated and severe malaria cases, a protective association with TIRAP S180L heterozygosity and malaria disease in a case study conducted in Gambia has been demonstrated [55]. Furthermore, examination of serum cytokine levels of IFN γ and TNF in children exhibiting cerebral malaria, where excess inflammation is an important basis for disease, revealed an association with polymorphisms in TLR9 and IFN γ production [56]. In addition, a common deletion in the 5' un-translated region of TLR2 conferred protection from cerebral malaria, but was not associated with serum cytokine levels. However, an insertion TLR2 polymorphism in the uncomplicated malaria group was linked to elevated inflammatory cytokines [57]. Collectively, this suggests that changes in expression of TLR2, TLR4 and TLR9 could potentially affect the inflammatory response and thus disease outcome.

A number of studies have investigated the potential selective pressure of malaria exposure on the presence of particular polymorphisms. In a study in India where polymorphisms between two genetically distinct populations with similar risk of malaria were investigated, it was found that TLR polymorphisms associated with protection were predominantly found in the population which had experienced longer exposure to malaria, suggesting that malaria may have exerted selective pressure [58]. However, studies in two Kenyan populations differing in malaria exposure showed that no malaria-related selective pressure was

observed in these populations, as similar frequencies of TLR2, TLR4, TLR9 and MAL polymorphisms were found [59]. Although there is some discrepancy in the results from these different genetic studies, and there is no robust evidence for selective pressure, it appears that genetic variations within the TLR signaling pathway have an effect on susceptibility and disease outcome.

TLR-Mediated Severe Malaria

Malaria-associated clinical manifestations are highly varied, but the more severe forms of disease typically involve life-threatening conditions including severe anemia, acute respiratory distress, metabolic acidosis and Cerebral Malaria (CM). Although the underlying mechanisms for severe malaria are not completely understood, inappropriate inflammatory responses are proposed to be a major contributing factor, supported by observations of high serum and plasma levels of cytokines at presentation of severe disease [60–63]. In addition, we have recently shown that an intrinsic ability to respond to parasite-infected red blood cells with high levels of pro-inflammatory cytokines is associated with severe malarial disease (manuscript submitted). Although TLR signaling undoubtedly is a significant aspect of pro-inflammatory responses and genetic studies suggest that TLRs are of importance to susceptibility of disease, the exact role of TLRs during severe malaria disease still remains inconclusive.

Rodent malaria models have commonly been utilized in an effort to improve understanding of severe malaria pathogenesis, including CM and TLR involvement. The development of CM has been demonstrated to depend on upregulation of adhesion molecules such as ICAM-1, production of Lymphotoxin- α and TNF [64–66], and IP-10-mediated leukocyte recruitment to the brain [67]. To address whether TLR signaling is a determinant of CM, various knockout mouse models have been used. MyD88, TLR2, and TLR9 KO mice, but not TRIF KO mice, were found to have increased resistance to *P. berghei*-induced CM. This was not due to control of parasitemia as parasite levels in KO mice were comparable to wild type mice, but a reduction in leukocyte brain infiltrate and cytokine production were observed [68]. Similar findings were obtained in a separate study, with the exception that TLR2 KO mice did not confer protection from CM [69]. In addition, specific blocking and inhibition of TLR9 increased survival rates of *P. berghei*-infected mice and reduced CM-related pathology [70,71], and TLR9 and MyD88 were found to be essential for protective immunity against *P. yoelii* infection [72]. However, in a contrasting study, a wide range of TLR and major adaptor protein KO mice did not change the course of *P. berghei* infection or susceptibility to CM, suggesting that TLR signaling is dispensable for development of CM [73]. This was further supported by a study using TLR2/4/9 triple KO mice where CM was concluded to be independent of TLR engagement [74].

Although the role of TLRs in severe malaria may still be contended, different studies have demonstrated that both IL-1 and IL-18 pathways were redundant for development of CM [69,73,75]. Another common observation in various studies was that parasitemia was not affected by absence of TLR or TLR-induced cytokine production, and was not a correlate of development of CM. This was further corroborated in a study using mice deficient in IRAK4 and challenged with *P. berghei*. However, when IRAK4 deficient mice were challenged with *P. chabaudi*, disruption of cytokine production and slight delay in control of parasitemia caused significant increase in mortality suggesting that the function of TLRs may be contingent on disease setting and model [76]. A recent study investigated the CM outcome in several KO mice following infection with *P. berghei* sporozoites and found that MyD88, TLR2 and TLR4 played an essential role in survival, but not in control

of parasitemia in the periphery or in the liver [75]. Considering these new results, TLR signaling in the liver stage may be an underlying component in relation to CM susceptibility and warrants further investigations.

Though human assessment of TLR protein expression in relation to disease outcomes is relatively limited, a study in Thailand examined TLR expression on Antigen-Presenting Cells (APC) from blood samples collected from individuals with either mild or severe malaria. It was observed that TLR2 expression was significantly increased on monocytes and myeloid DC from all malaria infected individuals. Conversely, TLR9 expression by plasmacytoid DC was decreased in malaria infected individuals. The lack of differences in TLR expression between the malaria groups may be due to the comparatively small number of individuals included in the study, but indicate that malaria infection affects the steady-state expression of TLRs on APC [77].

TLR Adjuvant in Malaria Vaccines

TLR agonists are increasingly being evaluated as potential vaccine adjuvants and have recently been assessed in various malaria vaccine formulations. The inclusion of TLR ligands as adjuvants was demonstrated to induce a more diverse antibody response by ‘massively parallel signature sequencing’, a proposed valuable characteristic in the ability to maintain protection against polymorphic pathogens such as malaria [78].

Even though TLR5 is not a receptor normally engaged during malaria infection, the adjuvanting effect of the *Salmonella enterica* serovar *typhimurium* flagellin (FliC), a TLR5 agonist, has been effective. The FliC agonist was demonstrated to increase immunogenicity of the 19kDa C-terminal fragment of *Plasmodium vivax* merozoite surface protein-1 (MSP1₁₉), inducing strong and long-lasting anti-MSP1₁₉ antibodies in mice. Furthermore, incorporation of an additional TLR9 ligand, CpG ODN 1826, resulted in IFN γ production [79]. Correspondingly, a chimeric protein containing repeat regions of *P. vivax* Circumsporozoite (CS) protein was fused to FliC and assessed for immunogenicity in mice. The fusion protein elicited robust antibody responses and was TLR5/MyD88 dependent. When Poly (I-C), a TLR3 agonist, was used as an additional adjuvant, antibody titers were significantly enhanced [80]. Another vaccine formulation utilizing either full-length flagellin or truncated flagellin of *Salmonella enterica typhimurium* linked to recombinant *P. falciparum* CS protein was administered either intranasally or subcutaneously to mice. The recombinant protein vaccine induced maturation of both human and murine DC, and immunogenicity was TLR5 dependent. This study produced promising results as the intranasal route of immunization elicited sporozoite-specific neutralizing antibodies and resulted in significant reduction in liver parasite burden. However, as concluded by the authors, further optimizations are required to improve protective efficacy [81].

The use of ligands that activate TLRs other than TLR5 as adjuvants has also been effective. Activation of TLR4 was demonstrated to further improve antibody and IFN γ levels in mice following immunization with a malaria fusion protein vaccine candidate consisting of the N terminal portion of Glutamate Rich Protein (GLURP) and merozoite surface protein 3 [82]. Furthermore, vaccines containing TLR9 ligand CpG enhanced the kinetics of acquiring malaria protein-specific memory B cell responses in malaria-naïve individuals, responses that were also prolonged [83]. In the mouse model, CpG inoculated in combination with *P. yoelii* CS protein promoted early CD8+ T cell expansion and IFN γ production [84]. A separate study showed that topical application

of the TLR7 agonist, imiquimod, at the injection site of *P. falciparum* CS peptides induced high levels of Th1 responses and antibodies, which conferred resistance to sporozoite challenge. Other vaccine approaches include investigation of self-assembling β -sheet fibrillar peptide fused to *P. falciparum* CS peptides. The adjuvanting effect was determined to be MyD88 dependent and the induced antibody responses were long-lived and protective against sporozoite infection [85].

Conclusions and Perspectives

The role of TLRs during malaria is decidedly complex and multifaceted, which is supported by the findings that TLR signaling may mediate both protection and immunopathogenesis. The various studies in TLR knockout mice have yet to generate a conclusive role for TLRs during malaria pathogenesis, indicating significant divergences in the use of rodent malaria model systems. Nonetheless, the collective findings from genetic analysis of single nucleotide polymorphisms in TLRs, which influence susceptibility or resistance to disease, and the immune responses induced by TLR signaling further implicate the importance of TLRs for disease outcome. Ongoing uncertainties in the field also reflect the need for quality-controlled reagents when sourced from native material. In the study of putative parasite-derived TLR agonists, contaminants can easily lead to erroneous conclusions in bioactivity. Progress thus requires a commitment to rigorous biochemistry. Further understanding of the function of TLRs during disease, in particular during life-threatening complications, could be beneficial in alleviating clinically severe malaria and in the development of vaccines.

Acknowledgement

Supported by National Health and Medical Research Council (NHMRC) Dora Lush Scholarship (NS) and NHMRC Project Grants # 516735, #513782, # 1058665, #1067222 and Program Grant #637406. This work was made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC Independent Research Institute Infrastructure Support Scheme.

References

- Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, et al. (2007) Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* 130: 1071-1082.
- Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, et al. (2009) Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. *Immunity* 31: 873-884.
- Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, et al. (2008) Structural basis of toll-like receptor 3 signaling with double-stranded RNA. *Science* 320: 379-381.
- Park BS, Song DH, Kim HM, Choi BS, Lee H, et al. (2009) The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 458: 1191-1195.
- Yoon SI, Kurmasov O, Natarajan V, Hong M, Gudkov AV, et al. (2012) Structural basis of TLR5-flagellin recognition and signaling. *Science* 335: 859-864.
- Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, et al. (1998) MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 2: 253-258.
- Kawai T, Adachi O, Ogawa T, Takeda K, Akira S (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 11: 115-122.
- Takeuchi O, Takeda K, Hoshino K, Adachi O, Ogawa T, et al. (2000) Cellular responses to bacterial cell wall components are mediated through MyD88-dependent signaling cascades. *Int Immunol* 12: 113-117.
- Burns K, Janssens S, Brissoni B, Olivos N, Beyaert R, et al. (2003) Inhibition of interleukin 1 receptor/Toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *J Exp Med* 197: 263-268.
- Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z (1997) MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 7: 837-847.
- Hornig T, Barton GM, Flavell RA, Medzhitov R (2002) The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* 420: 329-333.
- Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, et al. (2002) Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* 420: 324-329.
- Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, et al. (2002) Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. *J Immunol* 169: 6668-6672.
- Oshiumi H, Sasai M, Shida K, Fujita T, Matsumoto M, et al. (2003) TIR-containing adaptor molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-beta. *J Biol Chem* 278: 49751-49762.
- Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, et al. (2003) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol* 4: 1144-1150.
- Kagan JC, Su T, Hornig T, Chow A, Akira S, et al. (2008) TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. *Nat Immunol* 9: 361-368.
- Sasai M, Oshiumi H, Matsumoto M, Inoue N, Fujita F, et al. (2005) Cutting Edge: NF-kappaB-activating kinase-associated protein 1 participates in TLR3/Toll-IL-1 homology domain-containing adapter molecule-1-mediated IFN regulatory factor 3 activation. *J Immunol* 174: 27-30.
- Ni M, MacFarlane AW 4th, Toft M, Lowell CA, Campbell KS, et al. (2012) B-cell adaptor for PI3K (BCAP) negatively regulates Toll-like receptor signaling through activation of PI3K. *Proc Natl Acad Sci U S A* 109: 267-272.
- Troutman TD, Hu W, Fulenckek S, Yamazaki T, Kurosaki T, et al. (2012) Role for B-cell adapter for PI3K (BCAP) as a signaling adapter linking Toll-like receptors (TLRs) to serine/threonine kinases PI3K/Akt. *Proc Natl Acad Sci U S A* 109: 273-278.
- Kim Y, Zhou P, Qian L, Chuang JZ, Lee J, et al. (2007) MyD88-5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. *J Exp Med* 204: 2063-2074.
- Szretter KJ, Samuel MA, Gilfillan S, Fuchs A, Colonna M, et al. (2009) The immune adaptor molecule SARM modulates tumor necrosis factor alpha production and microglia activation in the brainstem and restricts West Nile Virus pathogenesis. *J Virol* 83: 9329-9338.
- Panneerselvam P, Singh LP, Ho B, Chen J, Ding JL (2012) Targeting of pro-apoptotic TLR adaptor SARM to mitochondria: definition of the critical region and residues in the signal sequence. *Biochem J* 442: 263-271.
- Panneerselvam P, Singh LP, Selvarajan V, Chng WJ, Ng SB, et al. (2013) T-cell death following immune activation is mediated by mitochondria-localized SARM. *Cell Death Differ* 20: 478-489.
- Sethman CR, Hawiger J (2013) The innate immunity adaptor SARM translocates to the nucleus to stabilize lamins and prevent DNA fragmentation in response to pro-apoptotic signaling. *PLoS One* 8: e70994.
- Campos MA, Almeida IC, Takeuchi O, Akira S, Valente EP, et al. (2001) Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. *J Immunol* 167: 416-423.
- Krishnegowda G, Hajjar AM, Zhu J, Douglass EJ, Uematsu S, et al. (2005) Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity. *J Biol Chem* 280: 8606-8616.
- Furuta T, Imajo-Ohmi S, Fukuda H, Kano S, Miyake K, et al. (2008) Mast cell-mediated immune responses through IgE antibody and Toll-like receptor 4 by malarial peroxidase. *Eur J Immunol* 38: 1341-1350.
- Pichyangkul S, Yongvanitchit K, Kum-arb U, Hemmi H, Akira S, et al. (2004) Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a Toll-like receptor 9-dependent pathway. *J Immunol* 172: 4926-4933.
- Coban C, Ishii KJ, Kawai T, Hemmi H, Sato S, et al. (2005) Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. *J Exp Med* 201: 19-25.
- Parroche P, Lauw FN, Goutagny N, Latz E, Monks BG, et al. (2007) Malaria

- hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. *Proc Natl Acad Sci U S A* 104: 1919-1924.
31. Wu X, Gowda NM, Kumar S, Gowda DC (2010) Protein-DNA complex is the exclusive malaria parasite component that activates dendritic cells and triggers innate immune responses. *J Immunol* 184: 4338-4348.
 32. Barrera V, Skorokhod OA, Baci D, Gremo G, Arese P, et al. (2011) Host fibrinogen stably bound to hemozoin rapidly activates monocytes via TLR-4 and CD11b/CD18-integrin: a new paradigm of hemozoin action. *Blood* 117: 5674-5682.
 33. Nebi T, De Veer MJ, Schofield L (2005) Stimulation of innate immune responses by malarial glycosylphosphatidylinositol via pattern recognition receptors. *Parasitology* 130 Suppl: S45-62.
 34. Schofield L, Mueller I (2006) Clinical immunity to malaria. *Curr Mol Med* 6: 205-221.
 35. Baccarella A, Fontana MF, Chen EC, Kim CC (2013) Toll-like receptor 7 mediates early innate immune responses to malaria. *Infect Immun* 81: 4431-4442.
 36. Chua CL, Brown G, Hamilton JA, Rogerson S, Boeuf P (2013) Monocytes and macrophages in malaria: protection or pathology? *Trends Parasitol* 29: 26-34.
 37. Ing R, Segura M, Thawani N, Tam M, Stevenson MM (2006) Interaction of mouse dendritic cells and malaria-infected erythrocytes: uptake, maturation, and antigen presentation. *J Immunol* 176: 441-450.
 38. Leisewitz AL, Rockett KA, Gumede B, Jones M, Urban B, et al. (2004) Response of the splenic dendritic cell population to malaria infection. *Infect Immun* 72: 4233-4239.
 39. Luyendyk J, Olivas OR, Ginger LA, Avery AC (2002) Antigen-presenting cell function during *Plasmodium yoelii* infection. *Infect Immun* 70: 2941-2949.
 40. Seixas E, Moura Nunes JF, Matos I, Coutinho A (2009) The interaction between DC and *Plasmodium berghei*/chabaudi-infected erythrocytes in mice involves direct cell-to-cell contact, internalization and TLR. *Eur J Immunol* 39: 1850-1863.
 41. Perry JA, Olver CS, Burnett RC, Avery AC (2005) Cutting edge: the acquisition of TLR tolerance during malaria infection impacts T cell activation. *J Immunol* 174: 5921-5925.
 42. McCall MB, Netea MG, Hermesen CC, Jansen T, Jacobs L, et al. (2007) *Plasmodium falciparum* infection causes proinflammatory priming of human TLR responses. *J Immunol* 179: 162-171.
 43. Franklin BS, Parroche P, Ataíde MA, Lauw F, Ropert C, et al. (2009) Malaria primes the innate immune response due to interferon-gamma induced enhancement of toll-like receptor expression and function. *Proc Natl Acad Sci U S A* 106: 5789-5794.
 44. Hartgers FC, Obeng BB, Voskamp A, Larbi IA, Amoah AS, et al. (2008) Enhanced Toll-like receptor responsiveness associated with mitogen-activated protein kinase activation in *Plasmodium falciparum*-infected children. *Infect Immun* 76: 5149-5157.
 45. Fu Y, Ding Y, Zhou T, Fu X, Xu W (2012) *Plasmodium yoelii* blood-stage primes macrophage-mediated innate immune response through modulation of toll-like receptor signalling. *Malar J* 11: 104.
 46. Bettiol E, Van de Hoef DL, Carapau D, Rodríguez A (2010) Efficient phagosomal maturation and degradation of *Plasmodium*-infected erythrocytes by dendritic cells and macrophages. *Parasite Immunol* 32: 389-398.
 47. Arama C, Giusti P, Boström S, Dara V, Traore B, et al. (2011) Interethnic differences in antigen-presenting cell activation and TLR responses in Malian children during *Plasmodium falciparum* malaria. *PLoS One* 6: e18319.
 48. Adegnikaa AA, Köhler C, Agnandji ST, Chai SK, Labuda L, et al. (2008) Pregnancy-associated malaria affects toll-like receptor ligand-induced cytokine responses in cord blood. *J Infect Dis* 198: 928-936.
 49. Gbédandé K, Varani S, Ibitokou S, Houngbegnon P, Borgella S, et al. (2013) Malaria modifies neonatal and early-life toll-like receptor cytokine responses. *Infect Immun* 81: 2686-2696.
 50. Mockenhaupt FP, Cramer JP, Hamann L, Stegemann MS, Eckert J, et al. (2006) Toll-like receptor (TLR) polymorphisms in African children: Common TLR-4 variants predispose to severe malaria. *Proc Natl Acad Sci USA* 103: 177-182.
 51. Munde EO, Okeyo WA, Anyona SB, Raballah E, Konah S, et al. (2012) Polymorphisms in the Fc gamma receptor IIIA and Toll-like receptor 9 are associated with protection against severe malarial anemia and changes in circulating gamma interferon levels. *Infect Immun* 80: 4435-4443.
 52. Omar AH, Yasunami M, Yamazaki A, Shibata H, Ofori MF, et al. (2012) Toll-like receptor 9 (TLR9) polymorphism associated with symptomatic malaria: a cohort study. *Malar J* 11: 168.
 53. Leoratti FM, Farias L, Alves FP, Suarez-Mútiis MC, Coura JR, et al. (2008) Variants in the toll-like receptor signaling pathway and clinical outcomes of malaria. *J Infect Dis* 198: 772-780.
 54. Esposito S, Molteni CG, Zampiero A, Baggi E, Lavizzari A, et al. (2012) Role of polymorphisms of toll-like receptor (TLR) 4, TLR9, toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) and FCGR2A genes in malaria susceptibility and severity in Burundian children. *Malar J* 11: 196.
 55. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, et al. (2007) A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. *Nat Genet* 39: 523-528.
 56. Sam-Agudu NA, Greene JA, Opoka RO, Kazura JW, Boivin MJ, et al. (2010) TLR9 polymorphisms are associated with altered IFN-gamma levels in children with cerebral malaria. *Am J Trop Med Hyg* 82: 548-555.
 57. Greene JA, Sam-Agudu N, John CC, Opoka RO, Zimmerman PA, et al. (2012) Toll-like receptor polymorphisms and cerebral malaria: TLR2 \uparrow 22 polymorphism is associated with protection from cerebral malaria in a case control study. *Malar J* 11: 47.
 58. Sawian CE, Lourebam SD, Banerjee A, Baruah S (2013) Polymorphisms and expression of TLR4 and 9 in malaria in two ethnic groups of Assam, northeast India. *Innate Immun* 19: 174-183.
 59. Greene JA, Moormann AM, Vulule J, Bockarie MJ, Zimmerman PA, et al. (2009) Toll-like receptor polymorphisms in malaria-endemic populations. *Malar J* 8: 50.
 60. Clark IA, Budd AC, Alleva LM, Cowden WB (2006) Human malarial disease: a consequence of inflammatory cytokine release. *Malar J* 5: 85.
 61. Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, et al. (1989) Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 320: 1586-1591.
 62. Kern P, Hemmer CJ, Van Damme J, Gruss HJ, Dietrich M (1989) Elevated tumor necrosis factor alpha and interleukin-6 serum levels as markers for complicated *Plasmodium falciparum* malaria. *Am J Med* 87: 139-143.
 63. Kwiatkowski D, Hill AV, Sambou I, Twumasi P, Castracane J, et al. (1990) TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. *Lancet* 336: 1201-1204.
 64. Engwerda CR, Mynott TL, Sawhney S, De Souza JB, Bickle QD, et al. (2002) Locally up-regulated lymphotoxin alpha, not systemic tumor necrosis factor alpha, is the principle mediator of murine cerebral malaria. *J Exp Med* 195: 1371-1377.
 65. Favre N, Da Laperousaz C, Ryyffel B, Weiss NA, Imhof BA, et al. (1999) Role of ICAM-1 (CD54) in the development of murine cerebral malaria. *Microbes Infect* 1: 961-968.
 66. Rudin W, Eugster HP, Bordmann G, Bonato J, Müller M, et al. (1997) Resistance to cerebral malaria in tumor necrosis factor-alpha/beta-deficient mice is associated with a reduction of intercellular adhesion molecule-1 up-regulation and T helper type 1 response. *Am J Pathol* 150: 257-266.
 67. Nie CQ, Bernard NJ, Norman MU, Amante FH, Lundie RJ, et al. (2009) IP-10-mediated T cell homing promotes cerebral inflammation over splenic immunity to malaria infection. *PLoS Pathog* 5: e1000369.
 68. Coban C, Ishii KJ, Uematsu S, Arisue N, Sato S, et al. (2007) Pathological role of Toll-like receptor signaling in cerebral malaria. *Int Immunol* 19: 67-79.
 69. Griffith JW, O'Connor C, Bernard K, Town T, Goldstein DR, et al. (2007) Toll-like receptor modulation of murine cerebral malaria is dependent on the genetic background of the host. *J Infect Dis* 196: 1553-1564.
 70. Franklin BS, Ishizaka ST, Lamphier M, Gusovsky F, Hansen H, et al. (2011) Therapeutic targeting of nucleic acid-sensing Toll-like receptors prevents experimental cerebral malaria. *Proc Natl Acad Sci U S A* 108: 3689-3694.
 71. Zhu X, Pan Y, Li Y, Jiang Y, Shang H, et al. (2012) Targeting Toll-like receptors by chloroquine protects mice from experimental cerebral malaria. *Int Immunopharmacol* 13: 392-397.

72. Gowda NM, Wu X, Gowda DC (2012) TLR9 and MyD88 are crucial for the development of protective immunity to malaria. *J Immunol* 188: 5073-5085.
73. Togbe D, Schofield L, Grau GE, Schnyder B, Boissay V, et al. (2007) Murine cerebral malaria development is independent of toll-like receptor signaling. *Am J Pathol* 170: 1640-1648.
74. Lepenies B, Cramer JP, Burchard GD, Wagner H, Kirschning CJ, et al. (2008) Induction of experimental cerebral malaria is independent of TLR2/4/9. *Med Microbiol Immunol* 197: 39-44.
75. Kordes M, Matuschewski K, Hafalla JC (2011) Caspase-1 activation of interleukin-1 β (IL-1 β) and IL-18 is dispensable for induction of experimental cerebral malaria. *Infect Immun* 79: 3633-3641.
76. Finney CA, Lu Z, Hawkes M, Yeh WC, Liles WC, et al. (2010) Divergent roles of IRAK4-mediated innate immune responses in two experimental models of severe malaria. *Am J Trop Med Hyg* 83: 69-74.
77. Loharungsikul S, Troye-Blomberg M, Amoudruz P, Pichyangkul S, Yongvanitchit K, et al. (2008) Expression of toll-like receptors on antigen-presenting cells in patients with falciparum malaria. *Acta Trop* 105: 10-15.
78. Wiley SR, Raman VS, Desbien A, Bailor HR, Bhardwaj R, et al. (2011) Targeting TLRs expands the antibody repertoire in response to a malaria vaccine. *Sci Transl Med* 3: 93ra69.
79. Bargieri DY, Rosa DS, Braga CJ, Carvalho BO, Costa FT, et al. (2008) New malaria vaccine candidates based on the Plasmodium vivax Merozoite Surface Protein-1 and the TLR-5 agonist Salmonella Typhimurium FliC flagellin. *Vaccine* 26: 6132-6142.
80. Leal MTA, Camacho AGA, Teixeira LH, Bargieri DY, Soares IS, et al. (2013) Immunogenicity of recombinant proteins consisting of Plasmodium vivax circumsporozoite protein allelic variant-derived epitopes fused with Salmonella enterica Serovar Typhimurium flagellin. *Clin Vaccine Immunol* 20: 1418-1425.
81. Carapau D, Mitchell R, Nacer A, Shaw A, Othoro C, et al. (2013) Protective Humoral Immunity Elicited by a Needle-Free Malaria Vaccine Comprised of a Chimeric Plasmodium falciparum Circumsporozoite Protein and a Toll-Like Receptor 5 Agonist, Flagellin. *Infect Immun* 81: 4350-4362.
82. Lousada-Dietrich S, Jogdand PS, Jepsen S, Pinto VV, Ditlev SB, et al. (2011) A synthetic TLR4 agonist formulated in an emulsion enhances humoral and Type 1 cellular immune responses against GM22--a GLURP-MSP3 fusion protein malaria vaccine candidate. *Vaccine* 29: 3284-3292.
83. Crompton PD, Mircetic M, Weiss G, Baughman A, Huang CY, et al. (2009) The TLR9 ligand CpG promotes the acquisition of Plasmodium falciparum-specific memory B cells in malaria-naive individuals. *J Immunol* 182: 3318-3326.
84. Overstreet MG, Freyberger H, Cockburn IA, Chen YC, Tse SW, et al. (2010) CpG-enhanced CD8+ T-cell responses to peptide immunization are severely inhibited by B cells. *Eur J Immunol* 40: 124-133.
85. Rudra JS, Mishra S, Chong AS, Mitchell RA, Nardin EH, et al. (2012) Self-assembled peptide nanofibers raising durable antibody responses against a malaria epitope. *Biomaterials* 33: 6476-6484.