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-κB), 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-a 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-kB 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-kB, 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: 61393452664; 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

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.
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.

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

| TLR      | Location                          | Ligand                                             | Signaling adaptor |
|----------|-----------------------------------|----------------------------------------------------|-------------------|
| TLR1/2   | Plasma membrane                   | Triacyl lipopeptide, intact malarial GPI           | MAL, MyD88        |
| TLR2     | Plasma membrane                   | Peptidoglycan, LAM, hemaglutinin, phospholipomannan, | MAL, MyD88        |
| TLR2/6   | Plasma membrane                   | Diacyl lipopeptide, malarial sn-2 lyso-GPI, LTA    | MAL, MyD88        |
| TLR3     | Endosome                          | dsRNA, ssRNA (viral)                               | TRIF              |
| TLR4     | Plasma membrane and endosome      | LPS, GPI (*Plasmodium* and *Trypanosoma*), fibrinogen bound to malarial hemozoin, mann, viral envelope proteins (RSV and MMTV), *Plasmodium* peroxiredoxin | MAL, MyD88, TRIF, TRAM |
| TLR5     | Plasma membrane                   | Flagellin                                           | MyD88             |
| TLR7     | Endosome                          | ssRNA from viruses (VSV, influenza), proposed *Plasmodium* 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 *Toxoplasma gondii* possibly as heterodimer with TLR12, unpathogenic bacteria | MyD88             |
| TLR12    | Endosome                          | Undefined, likely bacterial products               | MyD88             |
| TLR13    | Endosome                          | Bacterial 23S ribosomal RNA, vesicular stomatitis virus | MyD88             |

Table Malarial ligands highlighted in bold. *Human only. #Mouse only.

---

**Figure 1: TLR Detection of Parasite Antigens.**
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 IFNγ, 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-κB 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 TLR2 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 IFNy and TNF in children exhibiting cerebral malaria, where excess inflammation is an important basis for disease, revealed an association with polymorphisms in TLR9 and IFNy 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 mice did not confer protection from CM [69,70,71], and TLR9 -blocking and inhibition of TLR9 increased survival rates of mice did not confer protection from CM [69]. In addition, specific infiltrate and cytokine production were observed [68]. Similar findings were comparable to wild type mice, but a reduction in leukocyte brain 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 mice did not confer protection from CM [69,70,71], and TLR9 -blocking and inhibition of TLR9 increased survival rates of mice did not confer protection from CM [69]. In addition, specific infiltrate and cytokine production were observed [68]. Similar findings were comparable to wild type mice, but a reduction in leukocyte brain 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 mice did not confer protection from CM [69,70,71], and TLR9 -blocking and inhibition of TLR9 increased survival rates of mice did not confer protection from CM [69]. In addition, specific infiltrate and cytokine production were observed [68]. Similar findings were comparable to wild type mice, but a reduction in leukocyte brain 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 mice did not confer protection from CM [69,70,71], and TLR9 -blocking and inhibition of TLR9 increased survival rates of mice did not confer protection from CM [69]. In addition, specific infiltrate and cytokine production were observed [68]. Similar findings 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 mice did not confer protection from CM [69,70,71], and TLR9 -blocking and inhibition of TLR9 increased survival rates of mice did not confer protection from CM [69]. In addition, specific infiltrate and cytokine production were observed [68]. Similar findings 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 mice did not confer protection from CM [69,70,71], and TLR9 -blocking and inhibition of TLR9 increased survival rates of mice did not confer protection from CM [69]. In addition, specific infiltrate and cytokine production were observed [68]. Similar findings 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 mice did not confer protection from CM [69,70,71], and TLR9 -blocking and inhibition of TLR9 increased survival rates of mice did not confer protection from CM [69]. In addition, specific infiltrate and cytokine production were observed [68]. Similar findings were comparable to wild type mice, but a reduction in leukocyte brain infiltrate and cytokine production were observed [68].
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 quality control of 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 quality control.

Acknowledgement

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

References

1. 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.
2. Krishnegowda KR, Medzhitov R, Preston-Hurlburt P, Routman TD, Hu W, Fulenchek S, Yamazaki T, Kurosaki T, et al. (2012) Role for TLR2-TLR6 heterodimer induced by binding of a tri-acylated lipopeptide. Cell 149: 1191-1195.
3. Li J, Takeda K, Hemmi H, Akira S, et al. (2005) Toll-like receptor 4 TICAM-1 that induces interferon-beta. J Biol Chem 279: 49751-49762.
4. Oshiumi H, Sasai M, Shida K, Fujita T, Matsumoto M, et al. (2003) TLR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to TLR4 that induces interferon-beta. J Biol Chem 278: 49751-49762.
5. Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshiba K, et al. (2003) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. Nat Immunol 4: 1144-1150.
6. 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.
7. 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-like receptor 3 homology domain-containing adapter molecule-1-mediated IFN regulatory factor 3 activation. J Immunol 174: 27-30.
8. Akira S, Takeda K, Hoshino K, Uematsu S, et al. (2006) Toll-like receptors: structure, signaling, and regulation of the innate immune system. Annu Rev Immunol 24: 667-707.
9. Sasai M, Takeda K, Hemmi H, Uematsu S, Hoshiba K, et al. (2003) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. Nat Immunol 4: 1144-1150.
10. 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.
11. Horng T, Barton GM, Flavell RA, Medzhitov R (2002) The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. Nature 420: 329-333.
12. Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, et al. (2002) Essential role for TIRAP in activation of the signaling cascade shared by TLR2 and TLR4. Nature 420: 324-329.
13. Yamamoto M, Sato S, Mori K, Hoshiba 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.
14. Oshiumi H, Sasai M, Shida K, Fujita T, Matsumoto M, et al. (2003) TLR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-beta. J Biol Chem 278: 49751-49762.
15. Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshiba K, et al. (2003) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. Nat Immunol 4: 1144-1150.
16. 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.
17. 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-like receptor 3 homology domain-containing adapter molecule-1-mediated IFN regulatory factor 3 activation. J Immunol 174: 27-30.
18. Akira S, Takeda K, Hoshino K, Uematsu S, et al. (2006) Toll-like receptors: structure, signaling, and regulation of the innate immune system. Annu Rev Immunol 24: 667-707.
19. Sasai M, Takeda K, Hemmi H, Uematsu S, Hoshiba K, et al. (2003) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. Nat Immunol 4: 1144-1150.
20. 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.
21. Kim Y, Zhou P, Qian L, Chuang JZ, Lee J, et al. (2007) MyD88-linking mitochrondia, microtubules, and JKNS in neurons and regulates neuronal survival. J Exp Med 204: 2063-2074.
22. Szretter KJ, Samuel MA, Gillisston S, Fucha A, Colonna M, et al. (2009) The immune adjuvant 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.
23. 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.
24. 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.
25. Sethman CR, Hawiger J (2013) The innate immunity adjuvant SARM translocates to the nucleus to stabilize lamin and prevent DNA fragmentation in response to pro-apoptotic signaling. PLoS One 8: e70994.
26. Campos MA, Almeida IC, Takeuchi O, Akira S, Valente EP, et al. (2001) Activation of Toll-like receptor-2 by glycosphosphatidylinositol anchors from a protozoan parasite. J Immunol 167: 416-423.
27. Krishnegowda G, Hajjar AM, Zhu J, Douglass EJ, Uematsu S, et al. (2005) Induction of proinflammatory responses in macrophages by the glycosphosphatidylinositol of Plasmodium falciparum: cell signaling receptors, glycosphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity. J Biol Chem 280: 8066-8061.
28. Furuta T, Imao-Omih S, Fukuda H, Kano S, Miyake K, et al. (2008) Mast cell-mediated immune responses through IgE antibody and Toll-like receptor 4 for malariar peroxiredoxin. Eur J Immunol 38: 1341-1350.
29. 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: 4928-4933.
30. 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.
31. 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: 1915-1924.

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(5): 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, Gumedze B, Jones M, Urban B, et al. (2004) Response of the splenic dendritic cell population to malaria infection. Infect Immun 72: 4233-4239.

39. Luwendy J, Olivas OR, Ginger LA, Avery AC (2002) Antigen-presenting cell function during Plasmodium yoelii infection. Infect Immun 70: 2941-2949.

40. Seixas AL, Rockett KA, Gumedze B, Jones M, Urban B, et al. (2004) Interplay of TLR signaling in cerebral malaria. Immunopharmacol Immunotoxicol 26(3-4): 392-397.

41. Perry JA, Oliver 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, Hermsen CC, Jansen T, Jacobs L, et al. (2007) Plasmodium falciparum infection causes proinflammatory priming of human TLR responses. J Immunol 178: 162-171.

43. Franklin BS, Parroche P, Alaidy MA, Lauw F, Roepert 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 cells. Infect Immun 86: 5148-5157.

45. Fu Y, Ding Y, Zhou T, Fu X, Xu W (2012) Plasmodium yoelii blood-stage parasites modulate TLR-mediated innate immune response through modulation of toll-like receptor signalling. Malar J 11: 104.

46. Bettiol E, Van de Hoef DL, Carapau D, Rodrigues 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, Bostrom S, Dara V, Traore B, et al. (2011) Interethnic differences in antigen-presenting cell activation and TLR responses in Malian children during Plasmodium falciparum infection. PLoS One 6: e18319.

48. Adegbola AA, Köhler C, Aignan Di, 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, Houngbgon 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) Toll like polymorphisms in the Fc gamma receptor IIIa and Toll-like receptor 9 gene are associated with protection against severe malaria anemia and changes in circulating gamma interferon levels. Infect Immun: 4435-4443.

52. Omar AH, Yasunami M, Yamazaki A, Shibata H, Otobe 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-Muñoz 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, TLR5, 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 DJ, van Bierno ST, Dunn C, Mephyt 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 Δ22 polymorphism is associated with protection from cerebral malaria in a case control study. Malar J 11: 47.

58. Sawian CE, Lourembam 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, Mooam AM, Vuluje A, Bockarie MJ, Zimmerman PA, et al. (2009) Toll-like receptor polymorphisms in malaria-endemic populations. Malar J 8: 50.

60. Clark IA, Budd AC, Allema LM, Cowden WB (2006) Human malaria disease: a consequence of inflammatory cytokine release. Malar J 5: 85.

61. Grau GE, Taylor TE, Molyneux ME, Wirrima JS, Vassali P, et al. (1989) Tumor necrosis factor alpha, alpha, is the principle mediator of murine cerebral malaria. J Exp Med 170: 1371-1377.

62. Favre N, Da Laperouse C, Ryffel 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.

63. Rudin W, Eustig 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.

64. Nie CZ, 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.

65. 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.

66. 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.

67. Franklin BS, Ishizuka 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: 3698-3699.
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 is independent of toll-like receptor signaling. Am J Pathol 170: 1640-1646.

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-1β (IL-1β) and IL-1β 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, Jorgland PS, Jepsen S, Pinlo VV, Diliev SB, et al. (2011) A synthetic TLR4 agonist formulated in an emulsion enhances humoral and type 1 cellular immune responses against GMZ2—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, Cockbum 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.