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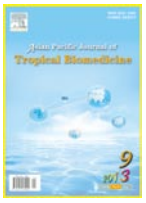

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Effect of malaria components on blood mononuclear cells involved in immune response

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PEER REVIEW

Peer reviewer

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Comments

This work is vitally important, as it helps to generate knowledge for the understanding of the immunological mechanisms induced by the parasite molecules present during malaria infection and reveal new perspectives to generate new strategies for the development of a cure for the infection.
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ABSTRACT

During malaria infection, elevated levels of pro-inflammatory mediators and nitric oxide production have been associated with pathogenesis and disease severity. Previous *in vitro* and *in vivo* studies have proposed that both *Plasmodium falciparum* hemozoin and glycosylphosphatidylinositols are able to modulate blood mononuclear cells, contributing to stimulation of signal transduction and downstream regulation of the NF-κB signaling pathway, and subsequently leading to the production of pro-inflammatory cytokines, chemokines, and nitric oxide. The present review summarizes the published *in vitro* and *in vivo* studies that have investigated the mechanism of intracellular signal transduction and activation of the NF-κB signaling pathway in blood mononuclear cells after being inducted by *Plasmodium falciparum* malaria components. Particular attention is paid to hemozoin and glycosylphosphatidylinositols which reflect the important mechanism of signaling pathways involved in immune response.

KEYWORDS

Malaria components, Hemozoin, Glycosylphosphatidylinositols, Nuclear factor kappa B, Blood mononuclear cells

1. Introduction

Malaria remains one of the most common parasitic diseases in the world and causes over one million deaths every year[1,2]. The precise molecular basis for understanding the pathogenesis and progression of malaria has been extensively studied in various ways. There are several key processes that can be utilized to explain the relationship between host and malaria parasite and the mechanisms of severe malaria development. These include the cytoadherence of parasitized red blood cells (PRBCs) to microvascular endothelial cells, which leads to vascular obstruction and cerebral hypoxia[3], a hallmark feature occurring during *Plasmodium falciparum* (*P. falciparum*) infection and excessive production of pro-inflammatory mediators in response to parasitic factors[4]. Investigating host immune response to malaria parasites and their components, previous *in vitro* studies demonstrated that *P. falciparum* hemozoin and glycosylphophatidylinositols (GPIs) were able to stimulate monocytes and macrophages, leading to the activation of signal transduction and nuclear factor kappa B NF-κB downstream signaling pathway-induced expression of pro-inflammatory cytokines, chemokines, and nitric oxide (NO)[5,6]. Researchers have reported that NF-κB appears to be a critical upstream regulator of gene expression because of its presence in the enhancer promoter regions of the pro-inflammatory cytokine gene[7-9]. In unstimulated cells, NF-κB is bound to its inhibitor, kappa B (IκB) protein, and appears in the cytoplasm as an inactive form. Following the stimulation

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of cells, IκB is firstly phophorylated by IκB kinase and then rapidly degraded by proteosome. Subsequently, activated NF-κB dimer translocates into the nucleus, where it binds to the DNA regulatory site to activate specific gene expression[10]. Although it is known that increased production of pro-inflammatory cytokines is an important process contributing to the pathogenesis of *P. falciparum* malaria, the underlying mechanisms of blood mononuclear cells in response to malaria parasites are not yet sufficiently understood. In addition, very little is known about the nature of various potential ligands of parasites and their involvement in intracellular signaling mechanisms.

The aim of the present paper is to summarize the modulation of intracellular signal transductions and NF-κB signaling pathways in blood mononuclear cells induced by *P. falciparum* hemozoin and GPIs. The works further explains how they promote the excessive production of pro-inflammatory cytokines, cytokines and NO. Understanding the mechanism signaling pathway in blood mononuclear cells induced by hemozoin and GPIs will serve as a guideline for exploring malaria pathogenesis and developing treatment for malaria in the future.

2. Effect of hemozoin on blood mononuclear cells

Hemozoin, or malaria pigment, is insoluble ferriprotoporphyrin IX crystal occurring during detoxification of heme by plasmodium parasite[11]. When PRBCs rupture, hemozoin is released into the blood and is phagocyted by host mononuclear cells such as monocytes and macrophages[11]. After hemozoin-phagocytosis, serious dysfunctions of monocytes have been reported, including repeated phagocytosis[12], bacterial killing abilities[13], oxidative bursts[14], MHC class II expression and antigen presentation[13], maturation to dendritic cells[15], and coordination of erythropoiesis[16]. In hemozoin-fed monocytes, gene expression of pro-inflammatory molecules including cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-1RA)] and chemokines [macrophage inflammatory protein (MIP)-α and MIP-β, GROα, β and γ, monocyte chemoattractant protein (MCP)-1, IL-8] was elevated[17]. Experimental data from hemozoin-fed monocytes demonstrated that hemozoin can induce extensive degradation of IκBα. Nuclear translocations of either p65 or p50, confirmed by Western blot analysis, were detected 2 h and 24 h post-phagocytosis, whereas the NF-κB complex was absent in the nuclear protein faction at the end of the phagocytic period[18]. In addition, hemozoin-triggered overproduction of TNF-α, IL-1β, and MIP-1α has been proposed to mediate induction of lysosyme releasing from human monocytes through stimulation of p38 mitogen-activated protein kinase (MAPK) phosphorylation, cytosolic IκBα phosporylation, and degradation and DNA binding of NF-κB[19].

Previous experiments investigating the mechanism of chemokine production in macrophages has shown that both *P. falciparum* hemozoin and synthetic hemozoin can induce increased mRNA levels of various chemokine transcripts, including MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCLL, and MCP-1/CCL2, in macrophages[20]. The mechanism of signal transduction by hemozoin involved extracellular signal-regulated kinases (ERK) 1/2 phosphorylation, IκBα phosphorylation, NF-κB nuclear translocations, and subsequent DNA binding of NF-κB subunits[20]. Both MEK 1/2 and ERK 1/2 phosphorylation were detected at 30 min post-stimulation and were sustained for ERK1/2 for up to 4 h during observation[20]. Subsequently, phosphorylation of IκBα rapidly occurred after 15 min post-stimulation, leading to nuclear translocations of NF-κB p50 and p65 which peaked at 1 h post-stimulation and then declined over 4 h[20]. Finally, DNA binding of both p50 and p65 NF-κB subunits was observed with the pattern of sustained NF-κB activity binding to the MIP-2 sequence up to 4 h, with transient NF-κB activity binding to the MIP-1 promoter was still detectable at 4 h post-stimulation[20].

Not only enhanced cytokine levels, but also elevated NO production, appear to be significant markers and potential mediators of disease severity. Previous studies have proposed that both native *P. falciparum* hemozoin and synthetic hemozoin can induce activation of ERK 1 and 2 pathways and binding of NF-κB to the murine inducible nitric oxide synthase (iNOS) promoter, rapidly occurring within 1 h of stimulation and returning to almost basal levels after 4 h[21]. The presence of both synthetic hemozoin and interferon (IFN)-γ has been shown to act in synergy to induce NF-κB binding to the murine iNOS gene[21]. In addition, *ex vivo* and *in vitro* studies have demonstrated that significantly elevated activity of NO synthase (NOS) was found in peripheral blood mononuclear cells (PBMCs) from children with malaria anemia and that it is inversely associated with hemoglobin levels[22,23], suggesting that hemozoin can induce NOS2 related to NO formation[22].

3. Effect of glycosylphosphatidylinositols (GPIs) on blood mononuclear cells

Glycosylphosphatidylinositol anchors of *P. falciparum* are important compounds responsible for malaria pathogenesis. They have been proposed as the major factors involved with the production of pro-inflammatory cytokines TNF-α, IL-1 and IFN-γ in macrophages[24]. Initially, the response of blood mononuclear cells to *P. falciparum* GPIs was modulated via Toll-like receptor-mediated recognition for pro-inflammatory responses that transmit signals by intracellular pathway and lead to activation of the transcription factor NF-κB[6,25,26], which in turn propagates a signal to the nucleus to regulate the expression of pro-inflammatory cytokines. Previous investigations studying immune response to malaria infection demonstrated that Toll-like receptor (TLR) 1, TLR2, and TLR4 were expressed by PBMCs from both experimentally and naturally acquired malaria infections[26,27]. In malaria infection, GPI-induced pro-inflammatory response by blood mononuclear cells, which has been reported to be involved in the activation of several signal transductions, including ERK, c-Jun n-terminal kinase (JNK), p38, protein kinase C, and protein tyrosine kinase signaling pathways[6,28]; These collaboratively mediate activation of NF-κB and nuclear translocation, contributing to the regulation of many productions of pro-inflammatory cytokines such as TNF-α, IL-12, IL-6, and NO. Previous data by Zhu *et al*. concluded

that JNK1 and JNK2 are functionally redundant for the expression of TNF, IL-6, and NO, whereas JNK2 is essential for IL-12 production[6]. However, the ERK signaling pathway is not involved in TNF and NO production[6]. For the NF-κB signaling pathway in macrophages stimulated by GPIs, it has been reported that c-Rel appears to be essential for TNF-α, IL-12, IL-6, and NO, whereas NF-κB1 is essential for IL-6 and IL-12 production but not for TNF-α and NO[6]. In addition, Tachado *et al*. reported that malaria GPI was able to induce NO release and synergize with IFN-γ in regulating NO production in both murine macrophages and human vascular endothelium through a mechanism depending on tyrosin kinase, protein kinase, and NF-κB activity[5].

4. Increased activation of NF-κB in blood mononuclear cells of malaria patients

NF-κB is a key transcriptional factor that regulates the gene expression of a number of cytokines, chemokines, and growth factors by host immune cells in response to stimulation by microbial agents[9]. Recently, NF-κB has been proposed as a major transcriptional factor related to the excessive production of pro-inflammatory cytokines after stimulation of malaria hemozoin and GPIs. However, the mechanisms underlying the role of NF-κB activation in blood mononuclear cells during malaria infection have not yet been complexly elucidated. In malaria patients, excessive production of pro-inflammatory mediators is one of the key processes contributing to malaria pathogenesis. Accumulated evidence also demonstrates that levels of endogenous pyrogens such as IL-6, IL-1β, and IL-8 are elevated in *Plasmodium vivax* (*P. vivax*) and *P. falciparum* malaria[23,29,30]. In Malawian children with severe malaria, mortality was observed to increase with increasing serum TNF-α[31], and in plasma of African children, TNF-α levels were found to be higher among those with cerebral malaria (CM) than in those with uncomplicated *P. falciparum* malaria[23]. A recent experiment involving *P. falciparum* infection in malaria-naive individuals has shown a coordinated increase in the level of pro-inflammatory cytokines, including IFN-γ, IL-12, and IL-8, in serum at the time when parasite emergence from the liver and the first appearance of parasitized erythrocytes[32]. *In vitro* studies have demonstrated that PRBCs can induce the production of TNF-α, IL-12, and IFN-γ by PBMCs of naive donors within 10 h[33]. Several experimental *in vitro* studies have proposed that activation of NF-κB in mononuclear cells was triggered by compartments of malaria parasites including hemozoin[20,21] and GPIs[5,6]. This led to up-regulation of NF-κB signaling and nuclear translocation of NF-κB. Previous investigations demonstrated that hemozoin can mediate activation of NF-κB pathways in macrophages and blood mononuclear cells via rapid phosphorylation of IκBα and subsequent NF-κB nuclear translocation to regulate enhancement of inflammatory cytokines[18,20-22]. In human monocytes fed with trophozoites and hemozoin, persistent activation of NF-κB signaling was connected with activation of matrix metalloproteinase-9 (MMP-9)[18]. In both monocytes and macrophages, GPIs of *P. falciparum* can stimulate the activation of NF-κB downstream signaling pathways, and induce expression of pro-inflammatory mediators such as TNF-α, IL-6, IL-12, and NO[5,6]. Genome-wide expression profiles in PBMCs of malaria patients illuminate up-regulation of signaling through the NF-κB pathway and enhancement of inflammatory cytokines[26]. Recently, an *ex vivo* study of PBMCs isolated from malaria patients found that NF-κB was activated in the PBMCs of *P. vivax* and uncomplicated *P. falciparum* patients on both day 0 and day 7, whereas in complicated *P. falciparum* patients, elevated NF-κB p65 activity was observed only on day 7 after treatment[34]. As observed with other diseases, increased NF-κB activation was seen in PBMCs from patients with sepsis, trauma, severe cholangitis, acute pancreatitis, diabetes mellitus, and chronic heart failure[34]. In contrast, Adib-Conquy *et al*. demonstrated that the *ex vivo* nuclear expression of NF-κB p65 and p50 heterodimer in an active form was significantly decreased in all sepsis and trauma patients on the day of admission[35].

In addition, previous investigations studying immune response of malaria infection demonstrated that TLR1, TLR2, and TLR4 were induced in PBMCs from both experimentally and naturally acquired malaria infections[26,36]. These findings suggest that activation of TLRs by parasites such as GPIs and hemozoin transmits signals by intracellular pathway[25,37], leading to activation of transcription factor NF-κB, which in turn propagates signals to the nucleus to regulate the expression of pro-inflammatory cytokines[26]. Consequently, during malaria infection, increased levels of phospho-NF-κB and nuclear translocation of NF-κB in the host immune cells are stimulated by parasite products which subsequently release and contribute to inflammatory mediators. In other conditions, it is interesting to note that increased activation of NF-κB in circulating leukocytes of malaria patients is similar to results of NF-κB analysis performed with cells derived from other compartments and tissues. Increased activation of NF-κB has previously been reported in alveolar macrophages of patients with acute respiratory distress syndrome[38]. Similarly, activation of NF-κB was found in lung mononuclear cells and lung neutrophils after hemorrhage in mice[39], and in lung and liver tissue of mice with peritonitis[40].

NF-κB p65 expression of PBMCs was significantly increased at 30 min following stimulation with serum from malaria patients with *P. vivax*, uncomplicated *P. falciparum* malaria, and complicated *P. falciparum* malaria[34]. NF-κB p65 was localized in the nucleus at 30 and 60 min after activation by serum from malaria patients using immunofluorescence with an antibody raised against NF-κB p65 to verify and demonstrate the translocation of NF-κB p65 from the cytoplasm to the nucleus[34]. Another previous study by Bierhaus *et al*. reported that serum from patients with *P. falciparum* at day 1 induced tissue factors in endothelial cells via the activation of NF-κB, resulting from induction of TNF-α in patient serum[41].

5. Decreased activation of NF-κB in blood mononuclear cells of malaria patient

Down-regulation of NF-κB could be due to two important processes. The first reason is the consequent high levels

of some immunosuppressive and anti-inflammatory cytokines such as IL-10, which acts as a negative feedback loop mechanism. The second reason is the silencing of NF-κB gene expression reported in severe systemic inflammations[27]. Recently, a previous study investigating PBMCs of malaria patients documented that decreased NF-κB p65 activity in PBMCs of patients with complicated *P. falciparum* malaria on the day of admission is linked to plasma IL-10[34]. Normally, IL-10 is produced by macrophages as well as T and B lymphocytes and plays a significant role in immunoregulation, involving negative feedback from the production of pro-inflammatory cytokines[42]. During malaria infection, increased plasma IL-10 has been reported in the serum of malaria patients on admission[43]and elevated IL-10 levels have been detected in serum of Thai patients with acute *P. falciparum* malaria prior to treatment, with normal levels returning after treatment[44]. Moreover, transforming growth factor (TGF)-β appeared to act as an immunosuppressant, contributing to inhibition of NF-κB activation[45]. TGF-β mRNA and protein were expressed in CM post-mortem brain tissue from Malawian children infected with *P. falciparum* malaria[46]. TGF-β was also elevated in the CSF of Vietnamese patients with CM[47]. Furthermore, Thai patients with malaria had low levels of serum TGF-β, which were noted to increase after treatment with artesunate and mefloquine[48]. A reverse correlation between plasma IL-10 and NF-κB has been reported in PBMCs of non-survivors with sepsis[35] and severe cholangitis[49]. Therefore, excessive production of IL-10 and TGF-β may act as a negative feedback loop mechanism contributing to inhibition of NF-κB activation and nuclear translocation and may be related to decreased levels of NF-κB in the early phase of malaria infection in PBMCs of patients with complicated *P. falciparum* malaria on the day of admission. In addition, the reduction of activated NF-κB may result from malaria parasite clearance and improvement of clinical symptoms as well as patients' immunity. These patient groups recovered to a normal state of malaria parasite clearance in blood circulation, which may lead to the absence of mediators from the malaria parasite to stimulate the PBMCs and turnover of NF-κB to an inactive state. In contrast, Punsawad *et al* reported NF-κB p65 activity increased in complicated *P. falciparum* patients on day 7 compared to the day of admission. Several factors that may be associated with the increased level of NF-κB in PBMCs of complicated patients include the contributing effects of other complications during malaria if it is prolonged such as pulmonary edema, acute renal failure, anemia, and pneumonia, which may stimulate the activation of NF-κB in PBMCs and impair immune regulations. Persistent anemia after the treatment of complicated malaria may be associated with prolonged activation of macrophages and prolonged interferon-γ activity[23]. However, these possible mechanisms for increasing the level of NF-κB in PBMCs of complicated malaria patients should be explored in future studies. In addition, as a potent antimalarial agent artemisinin was recently shown to inhibit nuclear translocation of NF-κB in the human astrocytoma cell line (T67), resulting in prevention of degradation of IκBα protein[50]. These findings are in agreement with those from a previous study which demonstrated that antiparasitic treatment of patients with *P. falciparum* malaria reduces the ability of patient serum to induce tissue factor in cultured ECs by decreasing NF-κB activation[41].

6. Conclusion

The data discussed in the present review summarizes the achieved *in vitro* and *in vivo* studies on the mechanism of signal transductions via dependent NF-κB signaling pathway in blood mononuclear cells including monocytes and macrophages, as well as PBMCs, after simulation by *P. falciparum* malaria components, especially hemozoin and GPIs. Data in published literature demonstrates that hemozoin and GPIs were able to induce this mechanism depending on MEK1/2, ERK1/2, p38 MAPK, JNK, protein C, protein tyrosine kinase, and NF-κB activity, especially p50 and p65 mediated by IκBα phosphorylation and degradation, subsequently leading to regulation of excessive production of pro-inflammatory cytokines, chemokines, and NO. For better understanding of the signal transduction signaling pathway, efficient molecular techniques such as Western blotting, real-time polymerase chain reaction (real-time PCR), gene knockout, DNA microarray, and proteomics will help verify the biology of blood mononuclear cells stimulated by malaria products. In addition, a better understanding of the effects of malaria components on blood mononuclear cells will serve as a guideline for designing further experiments for the precise signal transduction induced by malaria components and developing new malaria treatment.

Conflict of interest statement

I declare that I have no conflict of interest.

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Comments

Background

Malaria remains a global public health threat. The immune system plays a dual role in malaria, by providing life-saving immunity against the parasite, but also by causing lethal complications in a number of patients. Cytokines, chemokines and proteases are key players in the immunopathological complications, possibly immunomodulation mechanism may be involved. Recent advances in immunology could accelerate efforts to unravel the mechanisms of acquired immunity to malaria.

Research frontiers

This paper describes the modulation of intracellular signal transduction and NF-κB signaling pathways in blood

mononuclear cells induced by *P. falciparum* hemozoin and glycosylphophatidylinositols (GPIs).

Related reports

Some work describes methods for enhancing the immunogenicity that involves activation of NF-κB by transgenic expression of a intracellular signaling molecule, NF-κB kinase-inductor (NIK). NIK enhances immune responses toward a T helper 1 immune response with increased IgG2a levels, T cell proliferation, IFN-gamma production, and cytotoxic T lymphocyte responses. These findings define NIK, and possibly other inducers of NF-κB activation, as a potent adjuvant strategy that offers great potential for genetic vaccine development.

Innovations and breakthroughs

The hemozoin, and GPIs are molecules with immunoregulatory capacity by altering the expression of cytokines, chemokines, and molecules that directly and indirectly affect the signaling pathway of NF-κB during malaria infection. This paper is a review of the mechanisms involved in the alteration of this signaling pathway in peripheral blood mononuclear cells.

Applications

The review summarizes the mechanism of intracellular signal transduction and activation of the NF-κB signaling pathway in blood mononuclear cells after being inducted by *P. falciparum* malaria components. A better understanding of the effects of some molecules of *P. falciparum* on signaling pathways in mononuclear cells could set the tone for the development of new therapeutic agents.

Peer review

This work is vitally important, as it helps to generate knowledge for the understanding of the immunological mechanisms induced by the parasite molecules during malaria infection and reveal new perspectives to generate new strategies for the development of a cure for the infection.

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