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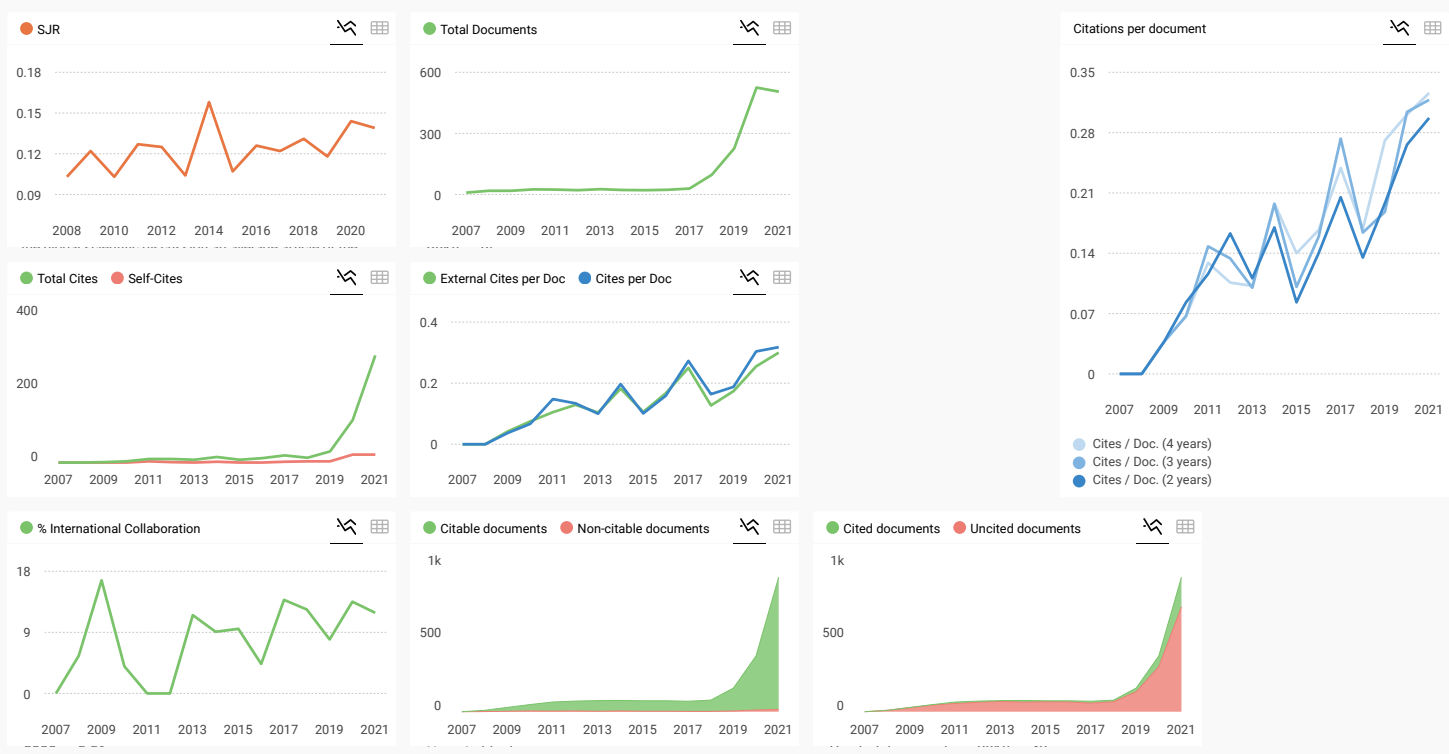
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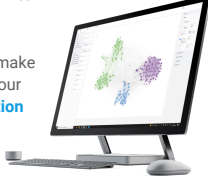
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REVIEW ARTICLE

Protein Encodes *Plasmodium berghei* Vaccine Candidates: A Review

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ABSTRACT

Background: Malaria has become a serious threat in many developing countries, including Indonesia. Malaria infection without proper treatment can cause death. *Plasmodium berghei* (*P. berghei*) is a malaria parasite that mostly infects rodents, and is often used as a model for in vivo antimalarial research in mice. Giving the right vaccine can be one of the efforts to control and eliminate malaria. **Objectives:** The purpose of this article is to review the protein coding for *P. berghei* as a vaccine candidate. **Review:** Antibodies are components of the immune system that play a role in the humoral immune response. Expression of Pb51, P47, PbVaC, PSOP25 genes in *P. berghei* are selected antigens against malaria parasites, has the ability to block malaria through chemotherapy and immunoprophylaxis interventions efficiently as a potential malaria vaccine candidate. **Conclusion:** The protein coding for *P. berghei* has good antigenicity as a candidates for malaria vaccine. The mechanism of blocking the transmission of parasites to mosquitoes, induction of the functional immune system and the effectiveness of malaria vaccines require further studies.

Keywords: Malaria, *Plasmodium berghei*, TBV, Pbs51, P47

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Plasmodium malariae have infected many humans (4) (5). *P. falciparum* is the malaria species most at risk and can cause death in humans (6). In addition to infecting humans, *Plasmodium* sp can also infect monkeys and rodents.

INTRODUCTION

Malaria has become a global health threat, including in Indonesia. Based on data of WHO in 2020, there were 241 million cases of malaria worldwide, and mortality reached 627,000. The mortality rate continued to decline in the 2000-2019 period, from 869,000 in 2000 to 558,000 cases in 2019. As an endemic country according to Arbani (1991), the problem of malaria in Indonesia is often experienced by residents living in rice fields close to the forest (1). In 2021 the incidence of malaria reached 94,610, a decrease compared to the previous year 2020, which was 226,364 (2).

Malaria is caused by parasites of the genus *Plasmodium* belonging to the phylum Apicomplexa (3). In general, *Plasmodium* species such as *Plasmodium falcipari*, *Plasmodium vivax*, *Plasmodium ovale*, and

P. berghei is a malaria parasite in rodents that is commonly used to study pathology (7)(8) in addition to being used as an in vivo antimalarial research model in mice (9). Research by Rodrigues & Gamboa (2006) used mice as experimental animals in the development of malaria drugs (10). *Plasmodium* is transmitted through the bite of a female Anopheles mosquito and infects erythrocytes. At the end of the infection cycle, erythrocyte cell lysis causes severe anemia and internal organ damage (4)(11)(12).

Malaria can be controlled with artemisinin-based combination therapy (ATC), and indoor insecticide spraying, but insecticide is less effective because it can cause parasite resistance to mosquito insecticides (13) (14). The provision of natural medicines is also used as an effort to prevent malaria. Many studies have

evaluated compounds for this species, but the parasite has developed resistance to certain drugs. Another effort that can be used to break the chain of malaria is by giving vaccines.

MALARIA IN RODENTIA

P. berghei is a species of malaria parasite that is commonly used to study the pathology of malaria and the immune system against infection with these parasites. Previous studies have reported that malaria caused by infection with *P. berghei* is analogous to humans and primates. *P. berghei* has similar surface proteins that play a role in the invasion of red blood cells seen from several aspects such as structure, physiology, morphology and life cycle. *P. berghei* infection can cause cerebral complications in experimental animals such as cerebral malaria in humans infected with *P. falciparum*.

The classification of *P. berghei* belongs to Kingdom Protista, Phylum Apicomplexa, Class Aconocid, Order Haemosporida, Family Plasmodiidae, Genus Plasmodium, and Species: *Plasmodium berghei*.

MORPHOLOGY OF *P. berghei*

Several stages can be found in infected rodent blood, including the trophozoite, schizont and gametocyte stages (15). The trophozoite form has a red nucleus and blue cytoplasm. There are two forms of gametocytes of *P. berghei*, namely macrogametocytes and microgametocytes. Macrogametocytes are oval in shape and contain a clump of nucleus and granules, whereas microgametocytes are pea-shaped containing a shiny nucleus and scattered granules of smaller size. The schizont form is a *Plasmodium* stage that undergoes asexual division, there are dark and clear rough spots. These spots are damage cytoplasm due to ongoing cells division

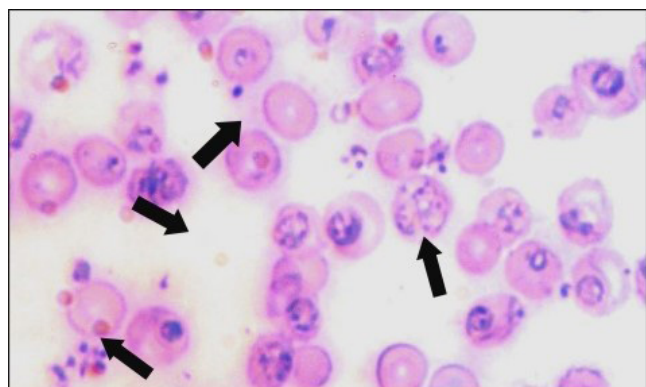


Fig. 1: *Plasmodium berghei* (16). Arrows indicate the shape of trophozoites *P. berghei* on thin blood smear with giemza staining.

IMMUNE RESPONSE TO MALARIA INFECTION

Mice infection begins with the injection of parasite-

infected erythrocytes into host mice. The parasite develops into a schizont stage and then the schizont ruptures releasing the merozoite stage, hemozoin and glycosylphosphatidylinositol (GPI) which are found on the surface of the merozoite. All three play a role as a pathogen associated molecular pattern (PAMP). Macrophages have a receptor called a toll-like receptor (TLR) which functions to recognize GPI toxins. The binding between GPI and TLR will increase the secretion of tumor necrosis factor-alpha (TNF- α) (17).

P. berghei ANKA invades erythrocytes via the ligand PBANKA-1332700 at the merozoite stage. Glycophorin C (GPC) is a receptor on mouse erythrocytes. GPC is homologous to GPC in human erythrocytes for the *P. falciparum* EBA-140 ligand (18). The innate immune response is aimed at controlling parasite growth by enhancing anti-parasitic cell-mediated immunity. However, due to the excessive proinflammatory response to malaria infection, dendritic cells shift antigen presentation from pro-inflammatory to anti-inflammatory T cell subsets in the adaptive immune response, namely from Th1 to Th2 phenotype to induce B cells to differentiate into plasma cells that can produce antibody. Thus, a balanced pro and anti-inflammatory response of Th1/Th2 can prevent pathogenesis and enhance protective humoral immunity against malaria, an unbalanced response contributing to pathogenesis. Thus, the innate immune response contributes to either protective immunity or pathogenesis (17). Parasites are very fast in multiplying, innate defense is the first to respond to cytokines against malaria parasites, TNF-cytokines as the initial product play a role in protecting the development of malaria to become more severe (2).

The host immune response to intraerythrocytic malaria parasites will activate macrophages through the classical pathway, so that the levels of the proinflammatory cytokine TNF- increase (19). At low levels TNF- is protective against the host, but at high levels it has a damaging effect on the tissues around the infection (20). Excessive TNF- secretion will damage endothelial cells, cause vascular occlusion, reduce blood flow, and increase endothelial permeability. The host will fight infection by increasing levels of anti-inflammatory cytokines, namely IL-10 and suppressing the secretion of proinflammatory cytokines IL-23. The IL-10 is a regulator that controls the work of TNF- α , IFN- γ , and IL-12 and protects tissues by preventing excessive inflammation (19). IL-10 secretion is one indicator of the success of the immune response through a balance between protection from parasites and immunopathology (21)(22). IL-10 is secreted by several cells such as B cells, macrophages, dendritic cells, CD4+ CD8+ T cells, Th1, Th17, Treg cells, and Foxp3+ CD4+ Treg cells (23). IL-2 complex consisting of 3 chains of CD25, CD122 and CD132, plays a role in antigen recognition, increasing the proliferation and differentiation of other immune cells, such as NK cells and B cells. through Fas and stimulates

regulatory T cell activity (24). The Treg cells that control malaria infection are CD4+ and CD25+ (25). However, Erythrocytic stage malaria parasite infection sometimes induces a poor immune response that can lead to severe pathology (26)(27). A subpopulation of malaria-specific CD4+ T cells that produce IL-27 in response to T Cell Receptor (TCR) stimulation (28).

It is known that IL-27 exhibits anti-inflammatory activity during *P. berghei* infection in mice (28). The immunosuppressive cytokine IL-27 is elevated in plasma from many septic patients and has been shown to inhibit Th17 cell differentiation (29). IL-27 is able to attenuate many autoimmune diseases by limiting the differentiation of naive CD4+ T cells into Th17 cells (29). IL-17 from macrophages attracts CCL2/7 dependent macrophages, which promotes invasive macrophage supply to the spleen (30). A reliable supply of macrophages is essential for eradication of blood-stage malaria parasites. Analysis of cytokine production according to anemia status showed significantly higher IL-17 production in children with mild malaria. In the early stages of infection, high IL-17 production can prevent severe malaria. However, in cases of acute malaria, overproduction of IL-17 induces SMA (31). IL-23 induces IL-17 via macrophages. Macrophages stimulated by IL-23 express IL-23 receptors and produce IL-17 (30). IL-17 is an inhibitor of the proliferation of human hematopoietic progenitors (32). A study in mice has identified a clear role for IL-10, namely controlling the inflammatory response and preventing tissue damage (23). The balance between proinflammatory and anti-inflammatory cytokines determines the degree of parasitaemia, level of anemia, clinical severity, presentation and outcome (33). IL-17 overproduction induces SMA (31). IL-23 induces IL-17 via macrophages. Macrophages stimulated by IL-23 express IL-23 receptors and produce IL-17 (30). IL-17 is an inhibitor of the proliferation of human hematopoietic progenitors (32). A study in mice has identified a clear role for IL-10, namely controlling the inflammatory response and preventing tissue damage (23). The balance between proinflammatory and anti-inflammatory cytokines determines the degree of parasitaemia, level of anemia, clinical severity, presentation and outcome (33). IL-17 overproduction induces SMA (31). IL-23 induces IL-17 by macrophages. Macrophages stimulated by IL-23 express IL-23 receptors and produce IL-17 (30). IL-17 is an inhibitor of the proliferation of human hematopoietic progenitors (32). A study in mice has identified a clear role for IL-10, namely controlling the inflammatory response and preventing tissue damage

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***Plasmodium berghei* CODING PROTEIN AS A VACCINE CANDIDATE**

Vaccines are designed to enhance the immune system by inducing and sustaining immune responses against target pathogens and activating memory B cells to avoid future infections (34). Malaria vaccines target the life cycle of the parasite (35). The sporozoite and liver stages of the pre-erythrocytic phase are designed to protect populations in low endemic areas. Asexual blood stage and sexual stage malaria vaccines not only provide direct protection against clinical disease by inducing immunity, but also have the advantage of reducing infection. Transmission blocking vaccine (TBV) that target the mosquito's sexual stage and midgut antigens are aimed at inducing immunity and stopping malaria infection. TBV focuses on stage-stage vaccines that infect mosquitoes directly and will prevent the spread of malaria in the community. TBV can prevent malaria transmission through the induction of antibodies against antigens present at the sexual stage of the parasite (43).

Recent advances in malaria control are goals for malaria endemic areas (42). Currently developing mosquito-stage antigen to stop malaria transmission (SSM-VIMT) to support elimination initiatives. The first clinical trial with Pfs25-EPA is currently underway in endemic settings. Another alternative to prevent malaria transmission is to use monoclonal antibodies that inhibit transmission. The most potent monoclonal antibodies exhibit full blocking

Table 1. Coding proteins and their role in malaria infection

Gene	Role in malaria infection	Source
Pb51	possesses excellent immunogenicity and antibodies against this protein inhibited both asexual proliferation in RBCs as well as transmission of the parasites to the mosquitoes	Wang et al, 2017 (36)
PSOP25	effectively block the formation of ookinetes in vitro and transmission of the parasites to mosquitoes	Zheng et al, 2017 (37)
PBCS, PbVaC	infecting and developing in human hepatocytes but not in human erythrocytes, and inducing neutralizing antibodies against parasites.	Mendes et al, 2018 (38)
P47	reduced oocyst density	Douti et al, 2020 (39)

activity at micrograms per milliliter and reach possibly feasible blocking concentrations in humans (42).

The genome sequences were taken from several *Plasmodium* species in PlasmoDB. Nucleotide base sequences or sequencing results are aligned through contig using ClusterW. The effect of immunization on mosquito infectivity was evaluated by feeding directly to *P. berghei*-infected mice (36)(39). In the malaria parasite rodents *P. berghei* encodes a hypothetical 51-kDa protein, and is thus referred to as Pb51 (36). In this study, Wang et al (2017), 2 groups of experimental animals given recombinant Pb51 (rPb51), antibody titers were tested by ELISA. Antibody titers from serum samples on days 14, 19 and 44 after immunization showed an increase (36).

Plasmodium fertilization occurs in the midgut of the mosquito, and the zygote grows into an oocyst (40). A transmission inhibitory vaccine (TBV) is needed to prevent the transmission of the malaria parasite from humans to mosquitoes. Most TBV relies on host antibodies that are ingested during blood administration, along with *Plasmodium* parasites, which bind to proteins on the parasite's surface and block transmission by inhibiting parasite development. Putative secreted ookinete protein 25 (PSOP25) is a highly conserved ookinete surface protein, and has been shown to be a promising new TBV target. PSOP25 is present on the surface of the mature zygote, retort, and ookinete. In vivo, rPSOP25 antibodies can reduce oocyst density by 63.3% (37). Other studies (39) reported that passive immunization with rP47 doses of 100 and 50 g/mL anti-Pbs47 IgG reduced oocyst density by 77 and 67%, respectively. Purified Pbs47-specific IgG significantly reduced oocyst density by 88 and 77%, respectively. The amount of antibody injected was based on the weight of the mice, assuming the average total blood volume of the mice was about 80 mL/kg (41).

Another platform that can be used as a candidate for *P. berghei* vaccine is the sporozoite-based platform. *P. berghei* vaccine sporozoite-based platform is used as a candidate for human malaria vaccine because Pb can infect human hepatocytes but cannot infect blood stages (38). Whole-sporozoite (WSp) exhibits a high degree of immunity. The WSp malaria vaccine uses sporozoites from *P. berghei* as a cross-species immunization agent. Mendes et al (2018) research showed that PbVac elicits a cross-species cellular immune response and can inhibit sporozoite invasion in human and mouse hepatocytes. He reported that PbVac is safe and induces a functional immune response in preclinical studies, which require clinical testing and development (38).

CONCLUSION

The proteins coding for *P. berghei* has good antigenicity as candidate for malaria vaccine. The mechanism of

blocking the transmission of parasites to mosquitoes, induction of the functional immune system and the effectiveness of malaria vaccines require further studies.

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