IMMUNOGENICITY OF THE MEROZOITE SURFACE PROTEIN-1 (MSP-1) OF HUMAN *PLASMODIUM* SP.

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ABSTRACT

Malaria is a major cause of mortality and morbidity globally. Great efforts have been made in the prevention and the elimination of malaria, especially in controlling the malaria vector, the mosquito. Another promising approach would be the development of malaria vaccines. Malaria vaccine studies can be focused on the pre-erythrocytic-stage antigens and the blood-stage antigens, and on the transmission blocking agents targeting the malaria gametocytes. The blood-stage antigens are the leading candidates in malaria vaccine development, as the blood-stage parasites are responsible for causing symptomatic malaria. Human acquired immunity largely targets on blood-stage antigens. This review focuses on one of the most extensively studied blood-stage antigen, the merozoite surface protein-1 (MSP-1), specifically on its evaluation and immunogenicity in rodents and primate models, and its safety and immunogenicity in human clinical trials.

Keywords: *merozoite surface protein***,** *phase trials, Plasmodium, protective immune responses, vaccination*

Background

Malaria remains as one of the important infectious diseases which lead to high global mortality and morbidity annually. According to the World Malaria Report in 2013 (1), there are approximately 207 million clinical cases of malaria each year resulting in 670,000 deaths. About 2.5 billion people, approximately 40% of the world's population, are at risk. There are five common *Plasmodium* sp. that naturally infect humans: *P. falciparum, P. vivax, P. malariae, P. ovale*, and *P. knowlesi*. More than 90% of the global malaria mortality cases occur in sub-Saharan Africa as the majority of the infections in that area is caused by the highly malignant species *P. falciparum* (2, 3). Most of the victims are children under 5 years old. *Plasmodium vivax* has been described as the benign form of malaria, yet many case reports showed that vivax malaria remains responsible for severe clinical manifestations and mortality in vivax-endemic areas (4). Knowlesi-infection is widely distributed in South East Asian countries (5-9) and could lead to hyperparasitemia in a short period as *P. knowlesi* has the fastest life cycle of 24 hours among the human *Plasmodium* sp. (10). There are limited literature reviews available for *P. malariae* and *P. Ovale,* and this could possibly be due to their low prevalence and milder clinical manifestations compared to the other human *Plasmodium* sp.

The life cycle of *Plasmodium* consists of an exoerythrocytic cycle which takes place in the liver, an erythrocytic cycle in the blood circulation and a sporogonic cycle in the mosquito vector. In the erythrocytic cycle, the steps involved are the release of merozoites from the infected hepatocytes, the invasion of merozoites into the erythrocytes, the development and maturation of trophozoites in the erythrocytes, and the release of new merozoites with the rupture of the erythrocytes. This blood-stage life cycle repeats every 24 to 72 hours, with the period dependent on the species of *Plasmodium*. Clinical illness only occurs in this stage. After repeated exposures, naturally acquired immunity against malaria could develop, predominantly targeting the blood-stage parasites (11). In immunization studies using animal models, a protective immune response is elicited when animals were immunized with blood-stage antigens, particularly merozoite antigens (12, 13). Therefore merozoite proteins, either located within the apical organelles or on the merozoite surface, are the leading blood-stage vaccine candidates. To date, the vaccine studies in human trials have been carried out with merozoite surface protein-1 (MSP-1), merozoite surface protein-2, (MSP-2) (14), merozoite surface protein-3 (MSP-3) (15), apical membrane antigen-1 (AMA-1) (16, 17), erythrocyte-binding antigens-175 (EBA-175) (18) , glutamate-rich protein (GLURP) (19), serine repeat antigen

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(SERA) (20), circumsporozoite protein (CSP) (21), and sporozoite surface protein (SSP). Among these candidates, merozoite surface protein-1 (MSP-1) is one of the most extensively studied.

MSP-1 is a high molecular mass protein, of ~185 to 225 kDa which fixes at the merozoite surface membrane of the *Plasmodium* parasite via the glycosyl-phosphatidyl-inositol (GPI) anchor (22, 23). MSP-1 undergoes two steps of processing by proteases and cleaves into a number of fragments. The first processing occurs during the rupture of schizonts, when the merozoites differentiate and are released from an infected erythrocyte. The MSP-1 precursor polypeptide cleaves into four major fragments of different sizes: 83 kDa (MSP-1_{83}), 30 kDa (MSP-1_{30}), 38 kDa (MSP-1_{38}), and 42 kDa (MSP-1_{42}) (24). During the invasion of merozoites into the new erythrocytes, the second processing further cleaves MSP-1_{42} into two fragments, 33 kDa (MSP- 1_{33}) and 19 kDa (MSP-1_{19}) (25). The MSP-1_{33}, corresponding to the N-terminal region of MSP-1_{42}, sheds from the surface in a fully soluble form (26, 27), whereas membrane-bound MSP-1_{19} remains anchored to the merozoite membrane by the GPI tail attached to the C-terminal residue and will be carried into a new red blood cell (26, 28).

Malaria infected individuals who have significantly higher IgG level against N-terminus of MSP-1 are usually asymptomatic compared to the subjects who have acute malaria, demonstrating that anti-MSP-1 antibodies are associated with clinical malarial protection and a reduction of an infection risk (29, 30). Studies have also shown that anti-MSP-1_{42}, anti-MSP-1_{33} and anti-MSP-1_{19} could be detected in most of the malaria infected samples, indicating that MSP-1 is one of the immunodominant antigens that could be useful in malaria vaccine development or seroepidemiological screening (31-35). Antibodies directed against MSP-1_{19} and MSP-1_{42} can interrupt merozoite invasions, and may also inhibit MSP-1 processing (36-39). MSP-1_{42} and MSP-1_{19} are the two fragments that have been most studied, especially in *P. falciparum*. MSP-1_{19} is responsible for humoral immunity responses and a considerable number of studies show that anti-MSP-1_{19} antibody plays a role in the protection from symptomatic disease. Children with a naturally acquired immune response to *Plasmodium* MSP-1_{19} are significantly associated with a resistance towards malaria infection and clinical manifestations (40), while pregnant women with anti-MSP-1_{19} antibodies are protected against placental infection and infection in infants (41).

This paper aims to provide an overview on the immunogenicity of MSP-1 and its proteolytic fragments, particularly MSP-1_{42} and MSP-1_{19}. The main issues to be discussed include cell mediated immune response, humoral immune response, protective effects of these fragments in rodent and primate models and human trials. The potential and limitation of MSP-1 as a malaria vaccine candidate will also be discussed.

Immunogenicity of MSP-1 in rodent models

Before a target antigen could be used in vaccine development, immunization study with animal models is a crucial step to validate the immunogenicity and immunoprotectivity of the target antigen. Rodent models are often chosen as the preliminary animal model due to the ease of handling. In MSP-1_{42} and MSP-1_{19} immunization studies using rodent models, animals were found to be variably protected during a challenge with live *Plasmodium* parasites. Immunogenicity studies by Dutta *et al.* (42) in a mice model showed that *Escherichia coli* (*E. coli*)-expressed *P. vivax* MSP-1_{42} induced specific antibody production and lymphocyte proliferation. Protective cytokines, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10) and interferon-gamma (IFN-γ), were detected in the immunized mice. A similar study was carried out on *E. coli*-expressed *P. falciparum* MSP-$1_{42,}$ and the rabbit-raised anti-MSP-1_{42} antibodies significantly inhibited the merozoite invasion, while the mice which were passively immunized with anti-MSP-1_{42} IgG were protected during a challenge with *P. berghei*/*P. falciparum* chimeric line that expresses *P. falciparum* MSP-1_{19} (43). Cheong *et al.* (32) also demonstrated that mice immunized with *E. coli*-expressed *P. knowlesi* MSP-1_{42} exhibited significantly higher levels of IFN-γ, IL-2, IL-4, IL-10 compared to the negative control mice. It is important to take note that MSP-1_{42} exists in nature as two distinct major allelic forms, and immune response produced towards this protein may be greatly affected. Co immunization with *E. coli*-expressed *P. falciparum* and *P. vivax* MSP-1_{19} stimulated specific antibody responses against both antigens and the elicited humoral response lasted up to one year after immunization (44). Sachdeva *et al.* (45) also found that both *E. coli*-expressed *P. vivax* MSP-1_{42} and MSP-1_{19} induced specific antibody responses and T-cell responses with six different adjuvants in immunized mice, and high levels of immunoglobulin G1 (IgG1), IL-4, interleukin-5 (IL-5) and IFN-γ were detected. Parween *et al*. (46) showed that the immunogenicity of recombinant *P. falciparum* MSP-1_{19} and *P. vivax* MSP-1_{19} was strongly enhanced when the recombinant proteins were coated on Gold Nanoparticles formulated with alum. The raised anti-PfMSP-1_{19} antibodies could inhibit an *in vitro* merozoite invasion. As opposed to MSP-1_{42}, MSP-1_{19} is highly conserved but may not provide as much immune response as the longer MSP-1_{42}.

Immunogenicity of MSP-1 in primate models

Immunization studies in primate models are believed to better resemble the regulation of human immune responses. Numerous immunization studies of MSP-1_{42} and MSP-1_{19} in primate models demonstrated encouraging results, as most of the MSP-1_{42} and/or MSP-1_{19}-immunized non-human primates were significantly protected when challenged with live malaria parasites. For instance, Rogers and co-workers immunized *Macaca mulatta* with DNA plasmids encoding four *P. knowlesi* antigens including MSP-1_{42}, and they found that a few of the immunized macaques

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were sterilely protected, while the mean parasitemia in the other macaques was significantly lower than the control macaques during a challenge with *P. knowlesi* sporozoites (47, 48). A significantly lower parasitemic level was detected in the rhesus monkeys immunized with *E. coli*-expressed *P. vivax* MSP-1_{42} compared to the negative control group upon a challenge with *P. cynomolgi, a P. vivax*-closely related *Plasmodium* sp., blood stage parasites (49, 50). On the other hand, *A. nancymai* vaccinated with *E. coli*-expressed *P. falciparum* MSP-1_{42} was highly protected during a lethal *P. falciparum* challenge (51-53), and the protective effect was stronger than the baculovirus-expressed *P. falciparum* MSP-1_{42} (54). Moreover, specific antibodies and antigen-specific T-cell responses with the production of IFN-γ were also detected in *M. mulatta* which were immunized with DNA plasmid encoding *P. falciparum* MSP-1_{42} (55). Before a human trial, pre-clinical analysis on *P. falciparum* MSP-1_{42} formulated with adjuvant AS02A or alum was performed in *M. mulatta* macaques and both vaccines were shown to be safe and highly immunogenic (56). MSP-1_{42} formulated with other adjuvants AS01B, AS05 and AS08 were also tested in rhesus. All these formulations were found to be safe and immunogenic. AS01B formulation induced a strong Th1 response compared to AS02A which induced a balanced Th1/Th2 response (57). Partial protection was detected in *Saimiri boliviensis* monkeys upon immunization with yeast-expressed *P. vivax* MSP-1_{19} (58, 59). On the other hand, vaccination of yeast-expressed *P. falciparum* MSP-1_{19} also conferred protection against a lethal challenge of *P. falciparum* in *Aotus vociferans* monkeys, and the raised anti-MSP-1_{19} antibodies could inhibit the secondary processing of MSP-1_{42} (60). Efficacy of baculovirus-infected insect cells-expressed *P. falciparum* MSP-1_{42} and *Saccharomyces cerevisiae* (*S. cerevisiae*)-expressed *P. falciparum* MSP-1_{19} were compared in an *A. nancymai* monkey model. A significantly higher protection level was observed in insect cells-expressed *P. falciparum* MSP-1_{42}-vaccinated group compared to *S. cerevisiae*-expressed *P. falciparum* MSP-1_{19}-vaccinated group, and the protection was associated with corresponding antibody levels in the immunized monkeys (61).

MSP-1 in human trials

Besides animal models, efficacy of MSP-1, including MSP-1_{42} and MSP-1_{19} fragments, as a vaccine candidate has also been tested in human trials. Most of the efforts for the development of malaria vaccines and human trials are still focused on *P. falciparum*. Results indicated that MSP-1 formulated with different adjuvants conferred different levels of protection in human clinical trials. Sheehy *et al.* (62) evaluated the *P. falciparum* MSP-1 in a Phase Ia clinical trial and induction of exceptionally strong T-cell responses was detected. *P. falciparum* MSP-1 was tested together with MSP-2 and ring-infected erythrocyte surface antigen (RESA) as a three-component blood-stage vaccine, formulated with Montanide ISA720, in ten male adults (63) and 120 children (64) in a malaria endemic area of Papua New Guinea. The vaccine was found to be safe for use in an already immune population, and MSP-1 was shown to be the most immunogenic molecule in that vaccine cocktail, as good cellular response with an increased level of IFN-γ and an increase in geometric mean antibody titres against MSP-1 was detected.

The C-terminal MSP-1_{42} has also been tested in human trials besides that of the full length MSP-1. Phase I human vaccine studies by using *P. falciparum* MSP-1_{42} formulated with adjuvant AS02A (FMP1/AS02A) have been carried out in USA with 15 adults. The vaccine was shown to be safe and created minimum reactogenicity with no severe adverse effects in all subjects. A high titre of parasite-reactive anti-MSP-1 antibodies was induced and 80% of immunized-subject sera reached the minimum functional inhibitory level of 15% inhibition in parasite growth inhibition assay (65). The same vaccine was evaluated in the falciparum-malaria endemic areas, including western Kenya (66) and Mali (67), with 40 adult volunteers in a Phase I trial. The safety and tolerability levels of FMP1/AS02A were high, and the vaccine was highly immunogenic and a statistically significant antibody response was detected. Another Phase I trial was conducted in 135 Kenyan children of ages 12 years to 47 months, and the induced-immune response was dosage-dependent (68). However, a Phase II trial with 400 Kenyan children indicated that FMP1/AS02A may not be a promising candidate for monovalent malaria vaccine, with an overall vaccine efficacy of 5.1% only (69).

Besides AS02A, MSP-1_{42} was tested with a few other different adjuvants. The adjuvant Alhydrogel was formulated to *Plasmodium falciparum* MSP-1_{42}, with both 3D7 and FVO alleles, and evaluated in 60 volunteers in USA. The results showed that although the cytokines IFN-γ, IL-2, IL-5, IL-10 and IL-13 were detected in the vaccinated-volunteers, addition of other immunostimulants to both vaccines were needed as the raised anti-MSP-1_{42} antibodies were insufficient to inhibit parasite growth up to protection level (70, 71). Ellis *et al.* (72) mixed the FVO and 3D7 of *E. coli*-expressed *P. falciparum* MSP-$1_{42,}$ formulated with Alhydrogel and novel adjuvant CPG 7909, in order to induce immune responses that recognized the major antigenic polymorphisms. A Phase I trial was carried out in 60 healthy adults. A high safety profile was demonstrated and the encouraging result showed that the sera of MSP-1_{42}/CPG 7909-immunized volunteers had an average of 14%, ranging from 3% to 32%, inhibition activity in the parasite growth inhibition assay. Recently, two Phase I clinical studies on MSP-1_{42} (FVO) formulated with adjuvant AS01 were conducted in 26 adults in USA and 30 adults in Kenya (73). The sera of only a few vaccinated-volunteers significantly inhibited parasite growth *in vitro*. However, the raised-antibodies in USA volunteers exhibited better cross-reactivity to heterologous MSP-1 alleles than the previously tested MSP-1_{42} (3D7) vaccine.

P. falciparum MSP-1_{19} was combined with domain 3 of apical membrane antigen 1 (AMA1) as a chimeric protein (PfCP-2.9) in human vaccine studies (74, 75). This chimeric

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vaccine was formulated with adjuvant Montanide ISA720 and tested in 52 healthy adults (76) and 70 healthy Chinese adults (77), separately. The results demonstrated tolerability and immunogenicity of the formulation, yet optimization evaluations are needed to reduce the reactogenicity. No functional activity against the parasite was observed. Human trials with MSP-1 of other human *Plasmodium* sp. have not been carried out (78, 79).

Conclusion

Development of malaria vaccines by using MSP-1 encounters numerous challenges. Knowledge about the real functions of this antigen and its interactive mechanisms with human host cells are limited. The approaches of vaccine development to target on functionally important domains/epitopes on MSP-1 are difficult. Human malaria *P. falciparum* and *P. vivax* cannot infect mice. Therefore, vaccine studies of human *Plasmodium* MSP-1 in non-human models , especially in rodent models, are unable to completely represent the safety, efficacy and immunogenicity of the vaccine targets in human, as there are distinct differences between rodents and human in the immunity regulation and pathogenic responses towards malaria. Most of the non-human primate vaccine studies on MSP-1 alone indicated that only a small percentage of monkeys was protected (58, 59, 61), either partially or completely, during a challenge of live *Plasmodium*, while in human vaccine trials, although specific antibodies have been induced, yet the titres were insufficient to neutralize the parasites *in vitro*. Hence, immunostimulants are needed in order to induce a higher level of protective immune responses. The low level of protection elicited by this single antigen vaccine is an impetus to develop multi-antigen vaccines. Nonetheless, the highly immunogenic MSP-1 should remain as one of the potential candidates for blood-stage malaria vaccine design (32, 35, 66, 67). Further investigations and evaluation are needed.

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