

# Why Is It So Difficult To Develop A Malaria Vaccine?

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Challenges To Malaria Subunit Vaccine Design

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## INTRODUCTION

Despite more than a century of research efforts to eradicate malaria, the disease remains a major, growing threat to the public health and economic development of countries in the tropical and subtropical regions of the world. There are an estimated 300–500 million cases and up to 2.7 million deaths from malaria each year (WHO, 2007). In Sub-Saharan Africa, children under five years of age account for 90% of all deaths due to malaria (WHO, 2007). Human malaria is caused by infection with intracellular parasites of the genus *Plasmodium* that are transmitted by *Anopheles* mosquitoes (reviewed in Sherman, 1998). Of the four species of *Plasmodium* that infect humans, infection with *Plasmodium falciparum* (*P. falciparum*) is the most lethal. Due to increasing resistance of *P. falciparum* to anti-malarial drugs and insecticides, studies suggest that the number of malaria cases may double in 20 years (WHO, 2007).

The discovery of a vaccine against malaria represents an urgent medical need for the vast population living in areas where malaria is endemic. The ideal vaccine would be cheap, extremely safe, induce life-long immunity, be active against all strains of the parasite and result in nearly complete interruption of the malaria life cycle by vaccine-induced immune responses. While promising results have been obtained, particularly with subunit vaccines, progress toward a malaria vaccine has been slow, owing in part to extensive genetic diversity in candidate vaccine antigens. As with other genetically diverse pathogens such as the influenza virus and HIV, vaccines based on polymorphic malaria proteins may not elicit responses against all variants of the target antigen circulating in the parasite population and could rather lead to an increased frequency of variants not targeted by the vaccine. This paper focuses on the different challenges that such antigenic polymorphism poses to malaria subunit vaccine design.

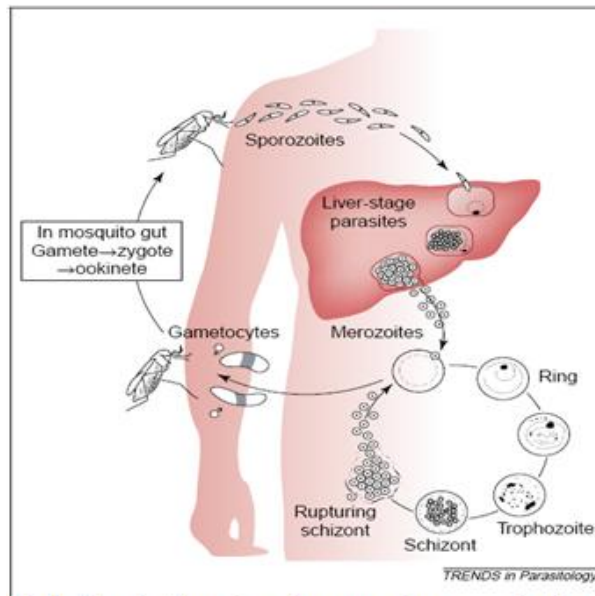
## **SUBUNIT VACCINE DESIGN**

In subunit vaccination, partial or complete antigens are identified from a pathogen's proteomic complement and used to induce protective immunity to the whole pathogen on vaccination. The approaches being followed for malaria subunit vaccine development are aimed by means of vectored, recombinant or synthetic fragments to induce anti-parasite effects against the different phases of the parasites life cycle (Fig. 1).

The parasite life cycle is composed of a sexual stage inside the mosquito and an asexual stage inside the human host (Sherman, 1998). Firstly, a mosquito infected with the malaria parasite bites a human cell and passes cells called sporozoites into the human's bloodstream. Sporozoites travel to the liver where they each undergo asexual reproduction; each nucleus splits to form two new cells, called merozoites. Merozoites enter the bloodstream and infect red blood cells (RBCs). In RBCs, merozoites grow and divide to produce more merozoites, eventually causing the RBCs to rupture. Some of the newly released merozoites go on to infect other RBCs. Some merozoites develop into sex cells known as male and female gametocytes. Another mosquito bites the infected human, ingesting the gametocytes. In the mosquito, the gametocytes mature and male and female gametocytes undergo sexual reproduction, uniting to form a zygote. The zygote multiplies to form sporozoites, which travel to the mosquito's salivary glands. If this mosquito bites another human, the cycle begins again.

Sporozoites in transit from the site of mosquito inoculation to the liver are targeted by pre-erythrocytic vaccines and their effect is to prevent infection and impact clinical disease (Good, 2005). Merozoites leaving infected liver cells then which travel between ruptured liver cells and RBCs, or between a ruptured RBC and a fresh one are targeted by bloodstage vaccines whose effect is to lessen the disease. Whole infected RBCs or parasites ingested by the mosquito

vector are targeted by mosquito-stage vaccines. A further blood-stage subunit strategy is to induce antibodies to neutralize parasite toxins (Good, 2005).



**Fig. 1: Vaccine Strategies.** Current vaccine strategies aim to block the malaria parasite life cycle at various stages as depicted in the figure: sporozoites travelling to the liver; within the hepatocyte; merozoites invading red blood cells; infected red blood cells; gamete fertilization within the mosquito; and zygote development (Good, 2005)

## **CHALLENGES TO SUBUNIT VACCINE DESIGN**

Different malaria parasite populations express antigenically different alleles of particular genes that have evolved overtime i.e. they are said to exhibit antigenic polymorphism: a phenomenon that is the underlying challenge to subunit vaccine development. In the presence of immunity to a specific parasite strain, parasites within a population expressing variant alleles that are not recognized by the immune system would be at a selective advantage to expand. Thus, antigenic polymorphism makes it difficult, slow and sometimes impossible for natural immunity to malaria to develop even after years of endemic parasite exposure. And if immunity should develop, it tends to be specific only to the particular strain that had infected the individual (Good, 2005). Complete immunity to all parasite strains within the population would require exposure to the many parasite variants within the population. As a result of this, individuals living in a malaria-endemic country quickly become re-infected following a previously treated infection with malaria (Matuschewski et. al, 2007).

Antigenic polymorphism is the most troublesome hurdle to overcome in the development of effective malaria vaccines. During the life cycle of *P. falciparum*, several *P. falciparum* polymorphic antigens are exposed to the human immune system. Of these, apical membrane antigen 1 (AMA1), merozoite surface protein (MSP-1) and circumsporozoite protein (CSP) are of particular interest in vaccine development.

## Polymorphism of AMA1 and MSP1

Epidemiological data suggest that naturally acquired antibodies targeted against merozoite proteins (AMA1 and MSP1) contribute to the acquisition of protective immunity in malaria-endemic areas. Thus, although AMA1 and MSP1 are leading subunit vaccine candidates, their antigenic polymorphism continues to thwart vaccine development.

AMA1 is a merozoite protein found in the organelles of merozoites and on the merozoite surface. The extracellular domain of the molecule consists of 546 amino acid residues (Dutta, et al., 2007). There are three subdomains, I, II, III, demarcated by disulfide bonds. Sequences of AMA1 from different *P. falciparum* isolates have been published and analysis of these sequences reveal that AMA-1 occurs as distinct allelic variants that differ by a number of point mutations (Dutta, Lee, H, & E., 2007). In subdomain I, there are at least 41 polymorphic nucleotides and at least 49 different nucleotide substitutions; only three of the mutations are synonymous. Domains II and III have 9 polymorphic sites, with no synonymous mutations (Dutta et. al., 2007).

Immunization with AMA1 induces antibodies that inhibit parasite invasion of merozoites, conferring protection in animals (Dutta et. al., 2007). However, in the rodent malaria challenge model, polymorphism of AMA1 has been unequivocally linked to vaccine failure in that immunization with one specific AMA1 allele did not confer immunity to other AMA1 alleles (Good, 2005). In an attempt to overcome this polymorphism problem, a number of groups are following a co-immunization strategy by combining two AMA1 strains in a single vaccine. Antibodies generated by the bi-allelic 3D7+FVO vaccine: a vaccine that combines the 3D7 and FVO parasite strains, show high-level inhibition of both the vaccine alleles (Dutta et. al., 2007). Given this success, a number of AMA1 3D7+FVO vaccines have progressed to efficacy human trials. However, given the enormous diversity of the AMA1 alleles, combining just two alleles in a vaccine may not be enough at targeting other alleles within the population. For this particularly vaccine to be effective across different human populations, the antibodies elicited by

the vaccine must be cross-reactive thereby being able to target different alleles other than the 2 alleles incorporated in the vaccine mixture. In addition, the vaccine mixture must also be able to account for the human immune system as a selective pressure and should thereby be able to target the important “escape” residues. This would allow the vaccine candidate to be effective over a long period of time regardless of whether the human immune system has selected for a slightly different allelic variant.

Gathering information about the extent of global haplotype diversity within AMA1 in terms of the immune system as a selective pressure and in terms of different human populations should be a critical component in the vaccine development process. Such information would provide researchers the ability to rationally select different haplotypes over others for the multi-allelic mixture approach in vaccine design.

MSP1-19, the 19 kDa C-terminus of the major merozoite surface protein, MSP1, and the longer fragment, MSP1-42, are also leading vaccine candidates (Good, 2005). Merozoite surface proteins are typically functional proteins that bind to glycophorin A on the surface of RBCs thereby allowing RBC invasion (Sutherland, 2007). The MSP1 protein is synthesized as a 190 kDa precursor, which undergoes proteolytic cleavage into four fragments that remain on the surface of the merozoite as a glycosylphosphatidylinositol-anchored complex. Prior to erythrocyte invasion, the entire MSP-1 complex is shed, except for the C-terminal 19 kDa (MSP-119), which remains on the merozoite’s surface as it enters the erythrocyte (Sutherland, 2007). MSP-119 contains two epidermal growth factor (EGF)-like domains which are thought to play a role in erythrocyte invasion (Alonso et. al, 2005). Naturally acquired antibodies to MSP-119 can inhibit erythrocyte invasion by preventing the secondary processing that releases this fragment from the rest of the MSP-1 complex (Sutherland, 2007), and are associated with protection from clinical malaria in field studies. MSP-119 has a highly conserved sequence with direct repeats that are thought to be restricted to the short domain known as block 2 (Fig. 2) (Biggs et. al,

1991). Significantly, block 2 repeats are those targeted by *P. falciparum*-inhibitory monoclonal antibodies and naturally acquired antibodies associated with clinical immunity in humans (Chen et. al, 2000). MSP-119's highly conserved sequence, along with its hypothesized critical function, makes it an attractive vaccine target. However, the MSP-119 region also contains six non-synonymous single nucleotide polymorphisms (SNPs): one in the first EGF-like domain corresponding to amino acid position 1644 (Q/E) and five in the second EGF-like domain corresponding to positions 1691 (K/T), 1699 (S/N), 1700 (N/S), 1701 (G/R), and 1716 (L/F) (Alonso et. al, 2005). Most of these polymorphic sites alternate with the conserved sequences in the molecule.

These nucleotide polymorphisms result in several challenges to anti-MSP1 vaccine design. Firstly, just as with AMA1, they provide a risk that MSP-1 vaccine-elicited immune responses may be variant specific, and will thus not provide protection against all parasite genotypes encountered within a given population. This is because, as previously mentioned, sequence polymorphism in MSP-1 is so considerable. For example, although MSP-1 has been found to have certain conserved sequences within the molecule, these are actually found to alternate with variable unconserved sequences. This sequence arrangement makes the conserved sequences difficult antibody targets (Takala et. al, 2007; Alonso et al., 2005). Candidate anti-MSP1 vaccines currently in clinical development target the 42-kDa MSP1 fragment. Candidates targeting the 19-kDa MSP1 fragments are in preclinical development (Takala et. al, 2007).

Since the function of MSP1 is unclear, the exact impact of MSP1 polymorphisms on anti-MSP-119 immunity remains controversial. On the one hand, studies have demonstrated cross-reactive antibody responses between MSP-119 alleles (Takala et. al, 2007). On the other hand, studies have also shown some differential recognition, particularly of amino acids in the second EGF-like domain (Takala et. al, 2007). Furthermore, although under certain conditions, recombinant MSP119 can induce immunity in monkeys and in mice, a high antibody titer

appears to be essential in conferring protection suggesting that an ideal vaccine would stimulate an immune response that recognizes all these strain-specific antigens (Sutherland, 2007).

However, little is known about the distribution of MSP-1 in parasite populations in malarial regions, or about how this distribution changes over time. Information about the prevalence and dynamics of vaccine antigen polymorphisms in a population being targeted for malaria vaccines is important because it informs choices about which MSP-119 haplotypes to include in future vaccine formulations, and will allow more accurate interpretation of the efficacy of current formulations of MSP1-based vaccines being tested in clinical trials. For example, the FMP1 vaccine antigen is based on the C-terminal 42 kDa of MSP-1 from the 3D7 strain of *P. falciparum* (MSP-119 haplotype ETSSRL) (Angov E et al, 2003). In the Malian group examined in this study and in other malaria endemic areas, this haplotype was found to be actually not the most common haplotype circulating in the parasite population (Ferreira MU, et. al, 2003, Qari et. al, 1998, Sakihama N et. al, 1999). Instead, the FVO (QKSNGL) and FUP (EKSNGL) haplotypes were identified to be the most prevalent and the FUP (EKSNGL) haplotype had the highest frequency in Vietnam (Ferreira MU, et. al, 2003), Kenya (Qari et. al, 1998), and Thailand (Sakihama N et. al, 1999). If immunity conferred by such monovalent vaccines is allele specific, a vaccine with high allele-specific efficacy would have low overall efficacy in populations where the target allele is in the minority (Angov et al., 2003). Without an understanding of the distribution of vaccine target haplotypes, this scenario could result in the premature abandonment of a promising vaccine that could be modified (perhaps by including additional target alleles) to be more universally protective (Takala et al).

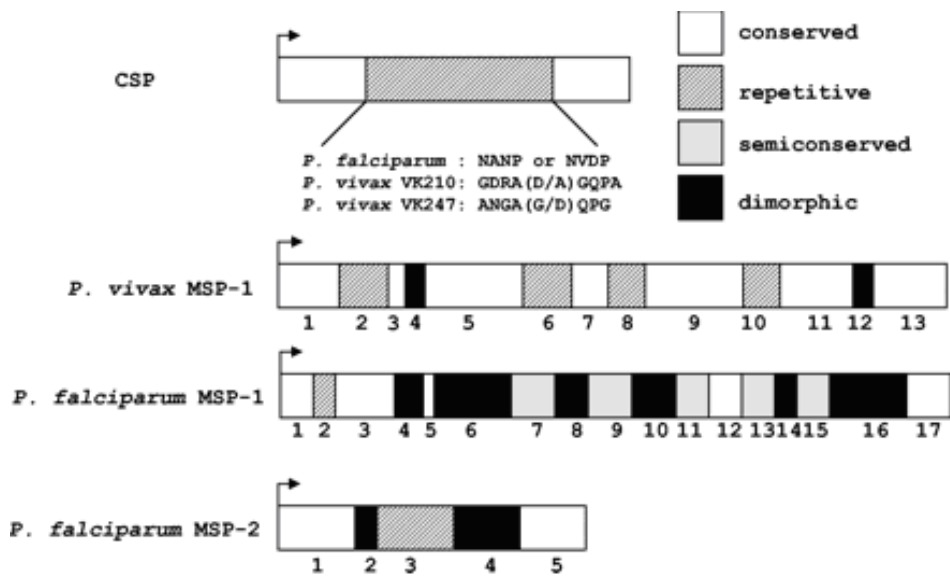


FIG. 2. Schematic representation of three major malarial surface antigens. Amino acid sequences of CSP repeat motifs are shown for *P. falciparum* and the two variants of *P. vivax*. MSP-1 of *P. falciparum* was divided into 17 blocks according to the levels of interallele sequence divergence (111). MSP-1 of *P. vivax* was divided into 13 blocks (87). MSP-2 of *P. falciparum* was divided into five blocks (107); the central repeats differ both between and within dimorphic families.

## Circumsporozoite Protein (CSP) & RTS, S/ AS02A

The leading sporozoite/liver-stage vaccine candidate is circumsporozoite protein (CSP).

This abundant protein forms a dense coat on the circumsporozoite; the form of the parasite that is released into the human bloodstream upon the bite of a mosquito (Good, 2005). This protein is involved in specifically binding to heparin sulfate proteoglycans on the hepatocytes during invasion. The CS proteins from all species of *Plasmodium* are similar in overall size (400 amino acids) and domain organization and include a signal peptide, a central region composed mostly of amino acid repeat motifs, and a C-terminal GPI membrane anchor (Alonso et al., 2005). The repeat motifs in the *P. falciparum* CS protein comprise 37 tandem repeats of the tetrapeptide Asn-Ala-Asn-Pro (NANP) interspersed with four copies of Asn-Val-Asp-Pro (NVDP). The function of the CS repeats is unknown, but they may fulfill a structural role, with repeats from neighbouring CS molecules interlocked to form a sheath surrounding the parasite. CSP antibody responses to the CS protein are primarily directed against immunodominant B-cell epitopes in the central NANP repeat region of the protein. Despite the abundance of the CSP, its use as a target in vaccine design is problematic (Alonso et al., 2005).

Firstly, antibody production alone due to B cells has been found to be inefficient in preventing infection by *P. falciparum* (Sherman, 2005). This is because permanent high levels of antibodies are needed to prevent infection in a relatively short time; only one single sporozoite of approximately 1000 infecting sporozoites can escape blocking antibodies and may lead to infection and progression of the disease (Sherman, 2005). This requirement for a high antibody titer is thought to arise as a result of the tandem repeats in CSP. The repeats provide immunodominant B cell epitopes: individuals who have had repeated infections with malaria have high concentrations of antibodies to these regions in serum (Good, 2005). Nonetheless, these individuals are still susceptible to new infections thereby suggesting that antibodies that

contribute to a lesser extent to protection against malaria are produced during infection with malaria. Together with the generation of antigen diversity, tandem repeats may play a pivotal role in immune evasion. The presence of polymorphic repeats in antigens that are not targets of protective immunity, affects affinity maturation of antibodies and thus masks critical epitopes, thereby resulting in antibodies that do not target the critical epitopes. Tandem repeats also induce T cell-independent B cell activation by cross-linking their surface immunoglobulins and suppressing antibody responses to important adjacent regions (Alonso et al., 2005).

Secondly, apart from the highly conserved B-cell epitopes, CSP contains highly polymorphic T cell epitopes. These polymorphisms in the T-cell epitope can completely abrogate recognition by T cells (Urban, 1999). Since T cell recognition depends on the primary structure of proteins rather than protein conformation, which is often recognized by antibodies, substitution of only one amino acid in a protective T cell epitope results in the failure to activate protective T cells. This is because the adaptive immune system of humans requires proper T-cell communication with other components of the immune system (such as dendritic cells) in order to ensure a pathogen is properly killed and cleared from the body (Urban, 1999). This evasion through polymorphism is critical and can be considered to be a kind of a human immune system suppression mechanism employed by the parasite. It has been exhibited in many cases of malaria infection in patients (Urban, 1999).

The immunodominant B-cell epitopes of NANP central repeat peptides formed the basis of the first synthetic and recombinant subunit malaria vaccine candidates tested in human clinical trials. Moreover, the current leading malaria vaccine candidate already in clinical trials, called RTS, S/ AS02A (GlaxoSmithKline Biologicals) is based on two recombinant proteins, one (RTS) containing amino acids 207–395 of the CS protein from the 3D7 clone of *P. falciparum*, which includes 19 copies of the NANP repeat, fused to the amino terminus of the hepatitis B surface antigen (HBsAg), and the second (S) being the 226 amino acid HBsAg, formulated

together with AS02A adjuvant (Alonso et. al., 2005). The AS02 consists of an oil-in-water emulsion and incorporates the immunostimulants monophosphoryl lipid A and the saponin derived QS21. The hepatitis B surface antigen is an important vaccine component because it has been proven to be highly immunogenic in animal models and is considered to be a commercially viable expression system (Matuschewski, 2007). Adjuvants, such as AS02, are pharmacological or immunological agents that are meant to amplify the effect of vaccines while having few if any direct effects when given by themselves.

Challenge studies conducted at the Walter Reed Army Institute of Research (WRAIR) showed that the RTS, S/AS02 vaccine completely protected approximately 40% of the participants against experimental sporozoite challenge in Mozambique (Vekemans J et. al, 2008). In most of those volunteers that received RTS, S but who developed parasitemia after experimental challenge, parasitemia was delayed by 48 h or longer compared with the non-vaccinated controls. Modeling studies showed that such a delay in parasitemia corresponds to at least a 95% reduction in the burden of liver-stage parasites. The RTS,S/AS02 formulation seems to resolve the problem of immune suppression during malaria infection by inducing high levels of anti-CS and anti-HBs antibodies, and by activating IFN- $\gamma$ -producing T cells (Vekemans J et. al, 2008). While immunological readouts have failed to identify a clear correlate of protection, there appears to be a consistent association between higher (vs lower) IgG and CD4 T-cell responses against CS (Vekemans J et. al, 2008).

Studies in malaria-experienced adults in The Gambia demonstrated that the vaccine prevented 34% of infections over a 15-week surveillance period but vaccine efficacy appeared to wane within that period. An efficacy of 47% was observed over 9 weeks during the next malaria transmission season, following a booster dose. Ongoing surveillance will enable more precise assessment of duration of protection (Bojang et. al, 2001).

Genotype analysis of parasite strains in RTS,S-vaccinated study participants who were not fully protected and developed parasitemia, and in the control population, both in the Gambian adult study, as well as in the study in Mozambican children, showed that vaccine-induced protection was not strain specific (Vekemans J et. al, 2008). This possibly eliminates the main problem posed by antigenic polymorphism to vaccine design i.e. the problem of a vaccine candidate targeting only one allele within the population such that only partial immunity is developed resulting in the possibility that same individual may become infected with the other parasite alleles within the population.

The results from phase trials with RTS/S/AS01 seems to overcome some of the most troublesome hurdles of malaria vaccine design suggesting that a vaccine for malaria is imminent. Due in part to the success of the phase 1 and 2 trials of the vaccine, the Malaria Vaccine Initiative at PATH and GlaxoSmithKline Biologicals committed more than \$100 million for multicenter, phase 3 trials of adjuvanted RTS, S starting in 2008. By the end of 2008, the lead RTS, S formulation will enter phase 3, multicenter licensure trials in Africa, and could be licensed within the next five years (Vekemans J et. al, 2008). .

## CONCLUSION

Variability in the genome of the human malaria parasite, *Plasmodium falciparum*, is key to the parasite's ability to cause disease and overcome therapeutic interventions such as drugs and vaccines. Elucidating the extent of genetic variation in the malaria parasite will therefore be central to decreasing the malaria disease burden. A thorough analysis of the malaria genome sequences will allow the discovery of thousands of new genes and proteins. This would allow researchers to characterize the factors leading to their maintenance or emergence thereby unveiling complex processes where the parasite genetic diversity interacts with the environment. In such cases, the balance of natural selection and demographic processes seem to play an important role in defining when, how and where a given phenotypic trait will be maintained, dispersed or disappear.

The tremendous possibility for diversity within the malaria parasite demonstrates the importance of determining the genetics of pathogen populations before starting vaccine trials (Dutta et al., 2007). Without this information, a vaccine's ability to prevent infections against specific parasite strains cannot be determined accurately and potentially useful vaccines might be abandoned if they are tested in inappropriate populations (Dutta et. al, 2007). Importantly, baseline information of this sort from which vaccine developers can work with, will also allow vaccine developers to detect any vaccine-induced changes in the pathogen population that might affect the long-term efficacy of their vaccine.

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