Vaccines for malaria – prospects and promise

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Malaria has remained a major global health problem and despite serious efforts for many years, a successful malaria vaccine has remained elusive. Main reasons among many are highly complex life cycle of the parasite, extensive polymorphism of key malaria proteins and lack of correlates for malaria immunity, and unavailability of a suitable animal model. However, these hurdles notwithstanding, many experimental malaria vaccines have been developed for carrying out clinical trials. Some of these trials have already shown promising results and there is a hope, more than ever before, that at least a partially effective malaria vaccine may be available in the foreseeable future. Experimental pre-erythrocytic and erythrocytic stage vaccines have been developed beyond the laboratory research stage. Pre-clinical and efficacy studies in a few cases have given a new hope for the development of at least a partially protective vaccine.

Keywords: DNA vaccine, malaria, vaccine development.

MALARIA is a disease caused in humans by four species of parasites of genus *Plasmodium, P. falciparum, P. vivax, P. ovale* and *P. malariae*. Malaria is one of the major causes of mortality and morbidity worldwide, affecting nearly 40% of the world's population and accounting for about 3–5 million deaths and more than 500 million new cases annually[1]. Besides widespread drug resistance in the parasite and insecticide resistance in the anopheles mosquito vector, inadequate infrastructure measures for delivery of control measures, uncontrolled population growth and increased mobility of non-immune populations to endemic areas have also contributed to the alarming situation posed by malaria today[2–4]. More recent estimates suggest that under the present scenario, development of either new anti-malarial drugs or of an effective vaccine that can generate parasite-specific protective responses, are of utmost importance. Historically, vaccines have been one of the most cost effective and easily administered means of controlling infectious diseases, but no licensed vaccines exist yet for malaria. A multitude of difficulties has hampered the development of vaccines for malaria; complex multi-stage life cycle involving a vast array of receptor–ligand interactions during invasion, incomplete knowledge of the effector mechanisms involved in malaria immunity, and last but not the least, lack of suitable experimental models to test the efficacy of new vaccine strategies and extrapolatable *in vitro* correlates of protection have all contributed significantly towards the slower than desirable pace of malaria vaccine development. Immunity to malaria takes several years exposure of recurring infection and illness and this acquired immunity is only partially effective and results in mild, sometimes asymptomatic infections with the persistence of parasites[5,6]. This immunity is short-lived and highly stage and strain-specific, owing largely to the capability of the parasite to alter critical antigenic structures rapidly[7,8]. For these reasons, development of malaria vaccines is a highly complex exercise and although in recent times much attention has been paid to this field, the progress in malaria vaccine development has been slow.

Understanding the life cycle of the parasite is fundamental to all efforts to develop malaria vaccines. Parasite species that cause malaria are transmitted inclusively by a few mosquito species of the genus *Anopheles* and the prevalence and capacity of the disease to spread are closely related to the biology of the vector[9]. The parasite undergoes a complex process of differentiation and growth inside the mosquito. Thus, following a blood meal from an infected human in which female mosquito ingests gametocytes, fertilization occurs in the mosquito midgut leading to the formation of zygote. This zygote further differentiates into an ookinete that penetrates the midgut wall and develops into an oocyst. Thousands of sporozoites are produced within the oocyst, which make their way to the salivary glands where they mature. The transmission cycle is completed when an infected mosquito prepares for its next blood meal and injects sporozoites into the human host and in less than 30 min, the sporozoites enter hepatocytes[10,11]. A single sporozoite can develop over the next 5–8 days into 30,000–40,000 merozoites and maintain the erythrocytic stages. The parasite develops inside the red cell over a period of 48 h and ranging from 6 to 24 merozoites develop inside each of red cell. Following the rupture of the infected red cell, each merozoite can continue the life cycle by invading another red cell. All clinical manifestations of malaria occur during erythrocytic stages of the parasites. The sexual stage is initiated when some erythrocytic parasites differentiate into male and female gametocytes, which are then taken up by the mosquito during a blood meal, to begin another round of life cycle.

Pre-erythrocytic vaccines are designed to target sporozoites or schizont-infected liver cells and thus prevent the

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release of primary merozoites from infected hepatocytes. Preclinical studies in rodents and humans immunized with radiation-attenuated sporozoites indicated that this may be achieved by antibodies that target sporozoites and block their ability to infect liver cells or by cell-mediated responses that kill parasite-infected hepatocytes before the release of infectious merozoites[12–15]. Relative to the number of circulating blood stage parasites that result in clinical disease, the number of sporozoites or liver stage schizonts is quite small; however, the window of opportunity to interrupt the pre-erythrocytic stages must be 99% effective to translate into sterile immunity in non-immune individuals. A single sporozoite that evades vaccine-induced immunity can, in principle, lead to full fledged infection. Blood stage and gametocyte stage vaccines are mostly based on production of antibodies to proteins exposed on the surface of the parasite.

Although scientists have been working for long to develop a malaria vaccine, and have shown that such an effort has a scientific rationale, many complex challenges need to be addressed, well beyond the scope and capabilities of individual scientists or one scientific group. A Malaria Vaccine Technology Road map developed after extensive inputs from experts has set out the strategic goal of developing a malaria vaccine by 2025 that is more than 80% efficacious against the clinical disease and lasts longer than four years, and as a landmark, to develop and license a first generation malaria vaccine by 2015 with a protective efficacy of more than 50% against severe disease and death, and lasts for at least one year[16].

The Road Map has particularly emphasized the need of developing standard immunological assays and reagents so that immune response to experimental vaccines can be compared meaningfully. The need for standardizing vaccine clinical trial design with a hope to compare data resulting from different trials, possible determination of protection correlates, use of modern tools to characterize the biological function of proteins, identification of novel potential vaccine candidates and to better understand host–parasite interactions are highlighted as some of the other critical issues in the development of a malaria vaccine. For the vaccine development, per se, malaria scientists still do not have a methodology for choosing the most appropriate vaccine candidate antigen, and therefore, there is an urgent need for providing a robust rationale platform for this purpose. Other issues like the ability of producing stable and pure form of vaccine candidate(s), formulation of antigen(s) with available adjuvants, ability to scale-up production process and the kind and duration of the immune response produced are no less important. Clearly, if these are the current major concerns of the malaria vaccine community then it also becomes apparent that the present malaria vaccine development efforts are essentially empirical. Organizationally, preclinical and clinical trial studies are tedious jobs which, when possible, require very different effort and skills that usually are not available with the malaria scientists. That being so, a recent WHO review of the portfolio of candidate malaria vaccines currently in development has identified as many as 80 vaccines at the preclinical development stage out of which more than 30 malaria vaccines have entered clinical testing and at least three have gone as far as phase IIb trials[17].

Since it is not practical to produce malaria parasites on a scale required for a live or attenuated vaccine, almost all efforts in malaria vaccine development are focused on the design and delivery of subunit vaccines. However, subunit vaccines based on a single antigen or a few B and T cell epitopes have several inherent problems, like low immunogenicity of the target proteins, quality, quantity and duration of the immune responses need, for an appropriate adjuvant (most recombinant malaria proteins are poor immunogens) and stringent requirements for quality control in the production of the recombinant vaccines. In this regard, DNA-based vaccines are exciting prospects, in which several of the above issues are a lot simpler to deal with[18]. Several viral vectors which can be used to deliver a DNA vaccine construct and many DNA and recombinant viral subunit vaccines have been developed[19–21]. However, while the DNA-based vaccines have been found to induce high T-cell immune responses, the antibody responses in humans have been generally poor[18,22].

As mentioned earlier, a number of subunit vaccines have been developed against the pre-erythrocytic stage, blood stage and sexual stage of the parasite. In addition, an altogether different anti-disease vaccine, based on the well-characterized *P. falciparum* malaria toxin, glycophosphatidyl inositol (GPI) supposed to be responsible for the clinical disease, has also been developed and tested in human[23]. The malaria vaccine development area has been the focus of several excellent reviews[17,24–32]. The present review is not intended to re-review the various efforts in malaria vaccine development, but rather the focus here will be on the vaccine candidates that have progressed to and beyond the design, production and preclinical studies. Also, since the transmission-blocking vaccines are subject of a separate review in this issue (**page 1535**), this review will focus mainly on the pre-erythrocytic and the blood-stage vaccines.

Pre-erythrocytic vaccines

These vaccines are expected to target sporozoites or schizont-infected liver cells and thereby prevent the release of primary merozoites from infected hepatocytes. There is evidence that antibodies against sporozoites can block their entry into liver cells and that cell-mediated response can kill specifically infected hepatocytes. Protective immune response against pre-erythrocytic stages must be entirely foolproof, since escape of a single or a few

sporozoites will lead to establishment of malaria infection. Of the many characterized sporozoite stage proteins, the circumsporozoite protein (CS)[32–38] and thrombospondin-related adhesive protein (TRAP, also known as sporozoite surface protein 2 (SSP-2) are the two most well-characterized vaccine target antigens against which immune responses are most clearly associated with protection[32–45]. Several candidate vaccines based on these two antigens have been designed, produced and taken to clinical trials and are at various stages of further development but two of these have made it to phase II field trials and will be discussed here in some detail.

The RTS,S/AS02 vaccine

The most advanced malaria vaccine in clinical development is the RTS,S/AS02 vaccine, jointly developed by Glaxo-SmithKline (GSK) and Water Reed Army Institute of Research (WRAIR)[46–48]. The ability of hepatitis B surface antigen (HBsAg) to form particles was central to the design of RTS,S, in which DNA encoding the well-characterized sequences of the CS, including a part of the central repeats (NANP) and other B and T cell epitopes from the C-terminal part of the protein, were fused to hepatitis B surface antigen DNA. Co-expression of the fused DNA with unfused HBsAg in yeast, followed by purification of the product from yeast cells, provided spontaneously assembled multimeric particles (RTS,S), which are adjuvanted with AS02, an adjuvant formulation containing an oil in water emulsion of de-acylated monophosphoryl lipid A and QS 21. Formulations of RTS,S with other adjuvants were also developed and tested but were not found as effective as with AS02.

A large number of adult volunteers were vaccinated with RTS,S/AS02, both malaria-naïve and malaria-exposed and showed no significant abnormalities[49]. Most of the reported symptoms were mild and transient and in general terms the RTS,S/AS02 vaccine was found to be safe for further human trials[46,49,50]. The RTS,S/AS02 vaccine induced strong humoural response against the CS epitopes as well as against induced HBsAg. The vaccine also elicited a strong lympho-proliferative response and $CD4^+$ T cells specific to both CS and HBsAg epitopes. Initial efficacy studies with RTS,S/AS02 were carried out in malaria-naïve individuals using the sporozoites challenge model[47] in which the immunized individuals are subjected to bites of infectious mosquito carrying the parasites of the same strain as the one from which the CS sequence is derived. In these controlled phase II studies, RTS,S/AS02 showed an efficacy of 30–85% against the homologous challenge. In a phase IIb, randomized controlled field trials with RTS,S semi-immune adult males in Gambia, the overall efficacy was 34% ($P = 0.014$) during the 15-week follow-up period. However, estimated efficacy which was 71% during the first nine weeks of follow-up decreased rapidly to 0% in the last 6 weeks of the surveillance period. However, the vaccine efficacy was not strain-specific[51,52].

Further trials in children in Gambia and Mozambique showed the vaccine to be safe and led to a phase IIb proof of concept efficacy study in children in Southern Mozambique. First six months follow-up showed that immunization with RTS,S/AS02 provided with 29.9% ($p = 0.004$) reduction in the risk of clinical malaria, delayed time to first infection by 45% ($p < 0.0001$) and reduced incidence of severe malaria by 57.7% ($P = 0.019$)[53]. A further follow-up study of this trial showed that the partial protection observed during the first six months continued to last for at least 18 months[54]. This is a significant finding that addressed the crucial issue of the durability of protective immune response as a result of vaccination with a subunit candidate vaccine, and confirms the potential of malaria vaccines as one of the control tools for public health. It has been planned to produce large batches of this vaccine for further development, alone or combination with other candidate vaccines.

Pre-erythrocytic subunit DNA vaccines

There is clear evidence from a large number of studies that T-lymphocytes are involved in controlling pre-erythrocytic malaria infection in the host[55–60]. In humans, there is evidence that HLA class I-restricted T cells are linked with protection from severe malaria[59–61]. Also, the well established irradiated sporozoite induced immunity is essentially associated with cellular responses[14,15]. Based on several studies in animal models, most efforts in developing pre-erythrocytic stage vaccine have mainly focused on inducing cellular immune responses to specific malaria antigens. Also, since DNA-based vaccines are generally considered more suitable for inducing cellular responses, a number of DNA viral vector-based malaria vaccines have been designed and studied[18,19,62–65]. The major advantages of different DNA-based vaccines are the ease of production, stability and the fact that no adjuvant is necessary for DNA vaccinations[18]. As it seems, DNA vaccines are efficient in priming the host but they do not boost efficiently. To overcome this, a heterologous prime-boost approach in which, vaccination is carried out at first with DNA, followed by vaccine viral vectors, encoding the same antigen, has been suggested[65,66]. The two viral vectors used in DNA-based malaria vaccines are modified vaccinia virus Ankara (MVA) and attenuated poxvirus (FP9)[45,66].

A vaccine termed as ME-TRAP has been developed and tested in humans[66,67]. This vaccine consists of full length *P. falciparum* TRAP of strain T9/95, fused to a string of 20 previously characterized T-cell and B-cell epitopes. Epitopes but not the full-TRAP was recoded to a mammalian codon bias and a series of sequential small

clinical trials in healthy malaria-naïve adult volunteers indicated that (i) vaccines were well tolerated and, (ii) no antinuclear antibodies were detected after vaccination. Also, the prime-boost (DNA–MVA) immunization produced 5–10 fold higher than the T-cell responses induced by the DNA vaccine or the recombinant vaccine alone. Importantly, some degree of efficacy was also observed following a sporozoite-challenge model[68]. However, randomized controlled trial of the efficacy of DNA and ME–TRAP followed by MVA and ME–TRAP, carried out in 372 adult volunteers in Gambia, showed no evidence for the efficacy of this vaccine[67]. The lack of efficacy observed in field trials with a vaccine that showed partial protection in a laboratory-based challenge assay suggests that it will be risky to develop any experimental vaccine for clinical trials, if it has not shown complete protection in laboratory-based challenge assays.

Notable among other CS-based vaccine is the one being developed by Apovia Inc. that also uses the ability of hepatitis B surface antigen to assemble as particles[69,70]. Out of the several constructs that were designed and tested for immunogenicity in small animals, the one that contains three NANP repeats, and a CS-based universal T-cell epitope attached to HBsAg, and assembles as virus particles during expression in *E. coli*, was found to be most suitable for phase I clinical trials carried out in the USA and Europe. This vaccine, named as ICC-1132, formulated in alum and Montanide ISA 720, was found to be safe and well tolerated and is being developed further[69].

A number of DNA vaccines based on pre-erythrocytic antigens have been developed and phase I trial conducted, but it seems that no attempt was made to assess their protection ability by using the sporozoite-challenge model[71]. DNA vaccines consisting of a single antigen like CS and its combination with other pre-erythrocytic stage antigens like TRAP, LSA-1, LSA-3 and EXP-1 (MuStDO5) have been developed at the Naval Medical Research Centre, USA[72]. Results of clinical trials showed that while these DNA vaccines are safe and well tolerated, they induced no detectable antibody response to any of the antigens. Although most volunteers did show T-cell responses, there was no evidence of any protection by the vaccines upon homologous sporozoite challenge[73,74]. A totally synthetic polyoxime malaria vaccine containing B-cell and a universal T-cell epitope was found to elicit immune responses in volunteers of diverse HLA types[75]. A malaria vaccine consisting of a long synthetic peptide containaing B-cell epitopes in an oil-based adjuvant induced CD 8(+) and CD 4(+) T-lymphocyte responses in humans[76,77]. However, in none of these studies was any evidence of protection against the sporozoite challenge reported and it is not clear if these constructs are being pursued further with vigour.

Besides the CS and TRAP, several other liver stage antigens, including LSA-1, LSA-3 and exported antigen 1 (EXP-1) have been characterized as vaccine candidate antigens. This has enabled researchers to carry out prime-boost studies using different combinations of these antigens alone or with the CS protein[72]. Vaccine constructs based on both LSA-1 and LSA-3 have been developed but have not moved beyond phase I trials.

Blood stage vaccines

There are two lines of arguments that build rationality for a blood stage vaccine. First, individuals living in endemic areas do develop immunity to malaria infection upon repeated exposures, and second, transfer of antibodies from those living in endemic area to malaria-naïve individuals protected them from infection[6]. However, the exact nature of the target antigens of these immune responses and immunological correlates of protection in the blood stage are far from being clear[25–27]. These factors and unavailability of a suitable animal model of human malaria and the lack of an established human challenge model for the blood stages are major impediments in the development of a blood stage vaccine.

Merozoite surface, as a major interface between host and pathogen, is an obvious target for the development of a blood stage malaria vaccine. Proteins on the merozoite surface are exposed to the immune system (although only for a short time), before and during invasion of erythrocyte. A number of merozoite surface proteins have been characterized and almost all of them are being considered as potential vaccine candidates[17,25,27,28,77]. Merozoite surface protein-1 (MSP-1), a large 200 kDa protein, is found on the surface of the merozoites of all malaria species. MSP-1 has been shown to undergo proteolytic processing and to be involved in the invasion of erythrocytes by merozoite. The C-terminal fragments remain attached to the merozoite surface through a GPI-anchor, and a 19 kDa fragment has been located inside the invaded erythrocyte. MSP-1 and, in particular, its C-terminal fragments have been the basis for the design of several blood stages vaccines[77]. However, it has been shown that some antibodies to MSP-1 can block the ability of malaria protective antibodies to the same antigen[78,79]. Studies involving the inhibitory monoclonal antibodies and immunization experiment with recombinant proteins have suggested that the C-terminal fragments of MSP-1 are the targets for protective immune responses, and therefore both, a 42 kDa ($MSP\text{-}1_{42}$) and 19 kDa ($MSP\text{-}1_{19}$), are being developed as vaccine candidates by several workers[17]. Prominent among other merozoite-surface proteins are MSP-2, MSP-3, MSP-4, MSP-5, MSP-9, AMA-1, Pf, EMP-1, EBA-175, etc. which are also being developed as vaccine candidates[1,25,28,30,80].

Synthetic peptide vaccine SpF66

The first blood stage vaccine to be tested in humans, based on polymeric synthetic peptides, consisting of a

number of epitope sequences from the blood as well as sporozoite stage protein, called SPf66, was developed in Columbia. A number of field trials carried out in Columbia, Equador and Tanzania indicated a significant reduction in malaria morbidity but further trials of the same vaccine failed to reduce clinical disease in different locations in Latin America and Africa resulting in termination of its further development[81–85]. The development and trials with SPf66 highlighted the complex nature and variability in field trials of malaria vaccine. Although SPf66 did not finally show the efficacy that was reported from earlier trials, it certainly showed that subunit malaria vaccines can be designed, produced and taken to field trials and in many ways paved the way for future design and field trials of malaria vaccine.

A combination blood stage vaccine (MSP-1, MSP-2, RESA)

A blood stage vaccine based on three recombinant blood stage malaria antigens, namely, MSP-1, MSP-2 and a portion of the Ring Infected Erythrocyte Surface Antigen (RESA, also known as Pf155), adjuvanted with Montanide ISA 720 has been developed[86–88]. Results of two phase Ia vaccine trials conducted to test safety and immunogenicity revealed that all the three antigens elicited both antibody and T-cell responses. No antigenic competition was observed and the volunteers receiving a mixture of antigens showed similar response to those receiving three antigens at separate sites (ASI). Phase-I safety trials in adult male volunteers living in a highly endemic area of Papua New Guinea showed that the vaccine combination B formulation was safe in an already immune population and that it induced significant cellular responses, especially for MSP-1 and RESA[87]. The combination-B vaccine was then tested in children of 5–9 years of age. In this study, a careful de-escalation design was introduced since neither the adjuvant nor the antigens had been previously tested in children. The results of this double blind, placebo-controlled trial provided evidence of the tolerability and safety of the vaccine in children living in malaria-endemic areas. However, adverse events were frequently observed (more than 60%) but most of them were local and mild. The fact that there were no serious or severe adverse events in these trials suggested that a potent adjuvant like Montanide ISA 720 could be considered safe. Although the study was not designed to seriously investigate mechanisms of protection, the results of immunogenicity showed an enhanced antibody response to all the three antigens in the vaccine[88]. Considering the safety and efficacy in reducing parasite density, further efficacy studies using different combinations of the three proteins in children are being planned. These results are significant in that they show that different antigens produced by recombinant methods cannot only be made, but also that they can be mixed to make a cocktail vaccine, without any interference with immune responses to the individual components.

MSP-1-based vaccines

The main strategy of MSP-1-based vaccine is to achieve the goal of production of antibodies directed against the C-terminal of MSP-1 (refs 77, 89–99). As mentioned earlier, most efforts in this direction have been focused on the production of recombinant MSP-1_{42} or MSP-1_{19} in their correctly folded confirmations; the C-terminal region of MSP-1 contains a number of disulphide linkages and their correct folding is crucial for obtaining protective immune responses[79]. Immunization with MSP-1_{42} and MSP-1_{19} provides protection in mice and monkeys[90,97,98]. A substantial population of antibodies generated through immunization with MSP-1_{42} is directed towards MSP-1_{19} portion of MSP-1_{42}, and invasion-inhibiting activity in human immune serum is associated with anti MSP-1_{19} antibodies, and *in vitro* these antibodies inhibit invasion of erythrocytes by merozoites[79,92]. The exact mechanism of invasion of inhibition by these antibodies is not clearly understood and it is known that some anti MSP-1 antibodies can interfere with the protective effect. It seems that the fine specificity of anti MSP-1_{19} is crucial in determining their protective ability and may well be an issue in the design of MSP-1-based subunit vaccines[93,94].

There has been a lot of debate on the choice of the two C-terminal fragments of MSP-1 (MSP-1_{42} and MSP-1_{19}) but both are being developed as vaccine candidates[95–97]. The issue with MSP-1_{42} is that it is polymorphic and exists in two distinct allelic forms and the concern is that one form of MSP-1_{42} may not provide protection against the other. A vaccine that utilizes a combination of the two major allelic forms (CFVO and 3D7 forms) has been developed and its formulation in alum has been taken to phase I trial. A recombinant MSP-1_{42}-based vaccine developed at the WRAIR in collaboration with GSKbio and formulated with AS02, was found safe in two phase Ia clinical trials[95]. However, a phase IIa challenge study failed to show any protection from infection in humans vaccinated with this MSP-1_{42}-based vaccine. Phase I and phase II clinical trials with the same vaccine in Kenya have also been completed and the results of these efficacy trials are keenly awaited.

Immunization with MSP-1_{19} in mice and monkeys has provided complete protection even though it has been argued that MSP-1_{19} contains lesser number of T-cell epitopes than MSP-1_{42}. However, MSP-1_{19} is highly conserved and antibody response to MSP-1_{42} immunization is primarily focused on the MSP-1_{19} region, suggesting that it is anti MSP-1_{19} antibody response that is crucial for protection[91,96]. Methods to prepare correctly folded MSP-1_{19}

have been developed at the International Centre for Genetic Engineering and Biotechnology (ICGEB) and University of Hawaii[96–99]. A vaccine based on the full length MSP-1 is also being developed at the University of Heidelberg[100].

MSP-3 and GLURP

Merozoite surface protein-3 (MSP-3) and glutamate-rich protein (GLURP) have been characterized as the two proteins that are targeted by human IgG antibodies which can inhibit parasite growth in a monocyte-dependent manner, both in *in vitro* and *in vivo* conditions[7,101,102]. Levels of MSP-3 and GLURP-specific cytophilic IgG1, and IgG3 antibodies were shown to be associated with a reduced risk of malaria infection[102,103]. The major B-cell epitopes that are targets of human cytophilic antibodies have been identified in MSP-3 and it is remarkable that immuno dominant, both B- and T-cell epitopes, in MSP-3 are highly conserved[104,105]. A vaccine based on a long synthetic peptide containing three B-cell epitopes and three T-cell epitopes from MSP-3, formulated and adjuvanted with alum and Montanide ISA 720 was shown to be safe and well tolerated in human in a phase I trial. Interestingly, alum was as effective as Montanide ISA 720 in the induction of antibody-dependent cellular inhibitor (ADCI) mediating antibody response. The antibodies developed in human volunteers in response to immunization, inhibited parasite growth in *in vitro* and *in vivo* assays[106]. Based on a similar strategy, a 128 amino acid synthetic peptide vaccine containing well characterized B- and T-cell epitopes has been developed as a vaccine construct and phase I trials with both alum and Montanide 720 have been completed. A fusion protein derived from GLURP and MSP-3 has been produced in *Lactococus lactis* and its immunogenicity tested in small animals and saimiri monkeys. In animals, antibody response to both the constituent proteins was observed[107,108]. Clinical trials with MSP-3-GLURP hybrid proteins are being planned[108,109].

Apical membrane protein-1

AMA-1 is another prominent blood stage antigen that is being considered as a vaccine candidate. Exact function of this microneme-associated, transmembrane protein is unclear but immunization with recombinant AMA-1 has provided protection in mice and monkeys[110,111]. Even though AMA-1 is significantly polymorphic, vaccine constructs based on an octodomain have been designed[110,111]. A recombinant AMA-1 vaccine, adjuvanted with AS02 has been developed by WRAIR together with GSKbio and taken to phase I clinical trials[17,30]. A chimeric fusion protein of PfAMA-1 and PfMSP-1_{19}, called *P. falciparum* chimeric protein 2 (PfCP–2.9), has been developed at the Second Military Medical University, Shanghai. The fusion protein, produced in high yields in yeast *(P. pastoris*), elicited antibodies to both the components in small animals and monkeys. Following immunogenicity experiments in animals, phase I trials have been undertaken with a Montanide 720 formulation[17].

Malaria vaccine development at ICGEB, New Delhi

The Malaria Group at ICGEB, New Delhi has undertaken efforts to develop vaccines for both *P. vivax* and *P. falciparum* malaria. Given that *P. vivax* and *P. falciparum* account for around 50% of malaria cases each, vaccines for both parasite species will be needed to tackle the problem of malaria in India effectively. *P. vivax* is absolutely dependent on binding the Duffy antigen receptor for chemokines (DARC) on human red cells for invasion[112]. The receptor-binding domains of *Plasmodium vivax* Duff Binding Protein (PvDBP) have been mapped to its N-terminal conserved cysteine-rich region referred to as region II (PvRII)[113]. Antibodies against PvRII are expected to block the parasite invasion of erythrocytes. Methods to produce recombinant PvRII in its correctly folded conformation have been developed[114–116]. The process has been transferred to an industrial partner for production of clinical grade recombinant PvRII under cGMP for use in human clinical trials to test the safety, immunogenicity and efficacy of a vaccine based on PvRII.

P. falciparum primarily uses sialic acid residues on glycophorin A as receptors for invasion[112]. Interaction with sialic acid/glycophorin A is mediated by the 175 kDa erythrocyte binding antigen, EBA-175 (ref. 112). Receptor-binding residues for sialic acid/glycophorin A lie in the N-terminal conserved cysteine-rich region, referred to as PfF2, which shares homology with PvRII (ref. 11). Antibodies raised against PfF2 have been shown to block erythrocyte invasion *in vitro* and immunization in non-human primate models has yielded partial protection against blood stage challenge[117–119]. Both $PfMSP1_{19}$ and PfF2 are leading candidates for malaria vaccine development. The vaccine for *P. falciparum* malaria being developed at ICGEB contains a physical mixture of recombinant $PfMSP1_{19}$ and PfF2. Immunogenicity studies in small animals have demonstrated that immunization with the recombinant $PfMSP1_{19}$ and PfF2 mixture elicits high titre invasion inhibitory antibodies against both antigens with no evidence for suppression of immune responses against either antigen (Chitnis and Chauhan, unpublished results). Methods to produce recombinant $PfMSP1_{19}$ and PfF2 have been developed. Both proteins are expressed in *E. coli*. While recombinant $PfMSP1_{19}$ is soluble and correctly folded when expressed in *E. coli,* recombinant PfF2 accumulates in inclusion bodies and requires refolding into its native conformation. Methods to produce recombinant $PfMSP1_{19}$ and PfF2 have been

developed and transferred to an industrial partner for production of the vaccine antigens under cGMP conditions. The safety, immunogenicity and efficacy of the vaccine will be tested in a series of phase I and II trials in the next few years.

Conclusion

Malaria vaccine development is a complex and rigorous process which begins with the identification of candidate antigen(s) through basic research, including the methods for preparation of the antigen, adjuvant selection and immunogenicity and toxicity in animals. This follows GMP grade manufacture planning for preclinical and clinical trials, which are carried out in different phases. Clinical trials are complex, require careful planning and are expensive. Most malaria vaccines are being developed in countries, where there is no malaria but the clinical trials necessarily need to be carried out in malaria-endemic areas, making the logistics of these trials even more demanding; developing countries like India may be more relevant. It might be more relevant and rewarding in the long term, to create infrastructure for development, production and field trials of vaccines for diseases like malaria, in developing but disease-endemic countries like India, among others.

An ideal malaria vaccine would promise to be safe, highly effective and provide long-term immunity. It would also be stable, easy to administer, inexpensive to manufacture and affordable in poor malaria-endemic countries. However, from the existing knowledge and experience, it is more likely that the first generation malaria vaccine will be partially protective, although safe but not entirely free of small side effects and provide protective immunity for a limited period of time. These vaccines will be expensive to manufacture and not easily affordable by those who will need them the most, without the help of donor funds.

Even though malaria vaccine development is complex and a long-drawn affair, the last ten years or so have seen substantial increase in financial support and enhanced coordination in vaccine production and field trials. This being so, the situation is far from satisfactory, let alone ideal. Most malaria researchers in academic settings are realizing that malaria subunit protein-based vaccines would require strong, human compatible adjuvants, other than alum, since most malaria antigens produced so far have shown poor immunogenicity in alum. Lack of suitable and readily available adjuvants is a problem that needs to be addressed urgently; the adjuvants developed by private companies may not always be easily available. With the *P. falciparum* genome now being available, it is believed that many more novel antigens can be characterized. While this may be true, it is also almost certain that this alone will not hasten the process of malaria vaccine development. The real hurdles are GMP grade production of vaccine antigens, lack of challenge models, complex nature of clinical trials and, last but not the least, huge costs involved in field trials. However, with some of the major donor agencies/governments providing the required financial support, clinical trials with several vaccine candidates will be planned and carried out. It is hoped that at least partially efficacious malaria vaccine will be available in the next 10 years and that it will form the basis for the development of other more effective malaria vaccines.

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