Malaria vaccine developments

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Large gains in the reduction of malaria mortality in the early 20th century were lost in subsequent decades. Malaria now kills 2–3 million people yearly. Implementation of malaria control technologies such as insecticide-treated bednets and chemotherapy could reduce mortality substantially, but an effective malaria vaccine is also needed. Advances in vaccine technology and immunology are being used to develop malaria subunit vaccines. Novel approaches that might yield effective vaccines for other diseases are being evaluated first in malaria. We describe progress in malaria vaccine development in the past 5 years: reasons for cautious optimism, the type of vaccine that might realistically be expected, and how the process could be hastened. Although exact predictions are not possible, if sufficient funding were mobilised, a deployable, effective malaria vaccine is a realistic medium-term to long-term goal.

We summarise the life cycle of malaria, describe the differences between naturally acquired and vaccine induced immunity, discuss the relevance of the elucidation of the Plasmodium falciparum genome sequence to vaccine development, and highlight some vaccine candidates that have reached clinical evaluation.

Some history
Which statement speaks to your instinct: Plutarch’s “history repeats itself” or Robert Walpole’s “anything but history, for history must be false”? Your answer could determine your level of optimism in malaria vaccine development. 1973 saw the first report of human protection from malaria by vaccination.19 However, the vaccination consisted of the bites of about a thousand mosquitoes infected with malaria parasites that had been X irradiated.16 This demonstration was obviously unlikely to be a practical means of mass vaccination. For about 20 years, progress occurred mainly in experimental models rather than in human vaccine trials.20,21 Much speculation and excitement was generated by the Spf66 candidate vaccine, despite uncertainty about how such a construct could work. Eventually, phase III trials showed that Spf66 lacked efficacy.22–23 During the past 5 years, many candidate vaccine approaches have been tested in clinical trials (table).24 Many potential candidate vaccines now warrant preclinical assessment.

The life cycle of P falciparum
A female anopheline mosquito requires a blood meal for egg production (figure 1). During such a meal, a mosquito infected with P falciparum will inject five to 20 sporozoites,25,26 which invade hepatocytes within minutes. Sporozoites migrate through several hepatocytes before entering one; this is the start of the liver stage.27,28 The sporozoite and liver stages are the pre-erythrocytic parts of the life cycle. Over an average of 6.5 days, we inject five to 20 sporozoites, which invade hepatocytes within minutes.
erythrocyte, and starts a 48-h cycle of replication. Replication is followed by schizont rupture and invasion of new red blood cells—the blood stage of malaria. The blood stage culminates either in death of the human host or control by the immune system. Some merozoites differentiate into male or female gametocytes, which can be ingested by an anopheline mosquito. Fertilisation occurs within the mosquito midgut, leading to completion of the life cycle, with sporozoites migrating to the salivary glands, and becoming infective.

Subunit vaccination

The production of live, attenuated, or killed inactivated vaccines is not practical for many diseases. In subunit vaccination, part or complete antigens are identified from a pathogen’s proteomic complement, which can induce protective immunity to the whole pathogen on vaccination. The hepatitis B vaccine is an effective recombinant protein subunit vaccine. This vaccine was designed to induce the maximum antibody (humoral) immune response. Unfortunately, proteins vary greatly in their immunogenicity. Moreover, induced antibodies must have the correct avidity (ability to bind), specificity, biological activity, and be produced at a high enough titre to block infection. Increased understanding of antigen processing, adjuvants, and their effects on innate immunity, genetic engineering techniques, and novel delivery systems are gradually increasing antibody immunogenicity. Insufficient duration of induced immune responses remains a difficulty. Also, recombinant protein subunit vaccines are generally poor at induction of effector T-cell responses, such as CD8+ cytotoxic T lymphocytes, that are necessary for...
elimination of intracellular pathogens such as liver-stage malaria parasites.

The newest generation of subunit vaccines are DNA based.30,31 DNA sequences from *P. falciparum* parasites have been inserted into plasmid DNA molecules (DNA vaccines) or various recombinant attenuated DNA viruses (recombinant viral vaccines) to generate candidate vaccines.32,33 DNA vaccines are taken up by host cells, protein is expressed, and T-cell epitopes bound to HLA molecules prime naïve T cells to form memory T-cell populations.3 Recombinant viral vaccines work in a similar way, but actively infect cells and express the recombinant malaria proteins before aborting infection.34 DNA and recombinant viral subunit vaccines can induce high levels of effector T-cell immune responses, although antibody responses have been poor in clinical trials.4,14

Assessment of T-cell responses has been revolutionised by the enzyme-linked immunospot (ELISPOT) assay and the tetramer assay.35–37 ELISPOT is a highly sensitive means of quantitatively detecting functional antigen-specific T cells. Tetramer assays allow detailed characterisation of antigen-specific T cells. These advances in assays, together with those of subunit vaccination in malaria, raise the possibility of identifying robust antibody and T-cell immune correlates of protection or, in other words, of understanding how partly effective vaccines provide their level of protection. Such an understanding should allow tailoring of vaccine design around immune correlates of protection to systematically improve vaccine efficacy—a process dubbed iterative vaccine development.

### Natural and vaccine induced immunity

Natural exposure to *P. falciparum* gradually elicits, in human hosts, short-lived strain-specific malaria immunity: first to severe disease and death, and then to mild disease.40 Repeated infections are required to maintain immunity, which is both antibody and T-cell based, although evidence is most clear for antibody-mediated immunity to blood-stage malaria.41,42 Exactly which of the 5300 antigens encoded by the *P. falciparum* parasite produces the key protective immune responses is not known, although some evidence implicates about 20. Immunity acquired in malaria-endemic areas is likely to be mediated by an integration of low to moderate responses to many antigens. Immunity to one stage of the parasite is restricted to that part of the life cycle; this complicates vaccine development—although sporozoite and liver-stage immunity overlap to some extent. However, proteomics techniques have detected antigens thought to be specific to one stage of the life cycle to be present at other stages.43

The aim with most vaccines is to induce antibody and T-cell responses to one or a few antigens, but for effective vaccination these will need to be of greater magnitude, duration, and strain-transcendence than in naturally acquired immunity. Antigenic variation occurs in some important blood-stage malaria antigens, and there is a possibility that vaccination could select for escape mutants, but this is less of a concern than with viruses such as HIV-1. T-cell responses have been neglected, in particular for blood-stage vaccine development; which responses are necessary is little known or understood, except for the need to produce T-cell help for an antibody response. An integrative, ambitious, long-term approach is to use a cocktail of many antigens to attempt to mimic natural immunity, but this could lead to a complex and costly product.45

### Pre-erythrocytic vaccines

The ideal vaccine for this stage would induce high titres of functional antibodies against sporozoites to prevent all parasites entering the liver stage, and induce potent cytotoxic T-lymphocyte immunogenicity against the liver stage to kill infected hepatocytes, while not harming the human host. The lead candidate vaccine of this type is RTS,S—a recombinant protein vaccine.46 Hepatitis B surface antigen DNA was fused to DNA encoding a large part of the best characterised pre-erythrocytic malaria antigen, the circumsporozoite (CS) protein.47,48 When expressed in yeast, the fusion product (RTS) binds hepatitis B surface antigen (S) to form RTS,S particles. These particles are mixed with an adjuvant, AS02—a mixture of decacylated monophosphoryl lipid A, QS21, and an emulsion—and given intramuscularly on two to three occasions. RTS,S vaccination induces high titre antibodies to CS and to hepatitis B, and gives 30–60% protection against parasites of the same strain as the vaccine in a sporozoite challenge model.49 In this model, vaccinees from developed countries (USA and Europe) are bitten by five mosquitoes infected with the 3D7 strain of *P. falciparum*, which is sensitive to chloroquine. Volunteers are monitored closely by malaria blood smears or PCR techniques, and treated promptly once blood stages are detected by microscopy.50

Proof of the efficacy of RTS,S/AS02 followed years of iterative development of CS-based vaccines—trials either with no challenge component, or resulting in very limited protection.51–53 Several adjuvants used with the RTS,S construct were far less protective than AS02. In a randomised controlled field trial of three-dose RTS,S in Gambian adults, vaccine efficacy was 34% (p=0.014) during the 15-week surveillance period, but with 71% efficacy in the first 9 weeks and 0% in the next 6 weeks.54 Protection was not strain-specific.43 Although the duration of efficacy was short, RTS,S is the first pre-erythrocytic vaccine to show clear protection against natural *P. falciparum* infection. Development of RTS,S has been accelerated by the Malaria Vaccine Initiative, which is funding an efficacy trial of RTS,S in children aged 1–4 years in Mozambique. Phase-I trials of varying doses of RTS,S in children aged 1–11 years have already been done in The Gambia and Mozambique.

Several other pre-erythrocytic candidates have reached the clinical evaluation stage: ICC-1132 is being tested in different formulations in the USA, Germany, and the UK. ICC-1132 is a hepatitis B core particle, genetically engineered to include a region of CS for high titre antibody induction. High titres of biologically active CS antibody have been noted in preclinical studies,44 and clinical trials have started.

Another approach is heterologous prime-boost vaccination. Two different vaccine vectors encoding the same antigen are given sequentially. Viral vectors can be given first (priming) or second (boosting); DNA vaccines are efficient priming vaccines but do not boost efficiently.55 Three carriers have been clinically tested: DNA; modified vaccinia virus Ankara (MVA); and attenuated poxvirus FP9, once used to vaccinate chickens against fowlpox.56 The insert includes thrombospordin-related adhesive protein (TRAP), a well characterised pre-erythrocytic antigen, and a string of T-cell epitopes (called ME for multiple epitope); these ME-TRAP vaccines are given in prime-boost sequence—ie, DNA then MVA, or FP9 then MVA.55,57 This approach has induced high T-cell responses and some protection, manifest by a substantial delay to parasitaemia in sporozoite challenge studies.58 A randomised controlled trial of the efficacy of
DNA and ME-TRAP followed by MVA and ME-TRAP has been completed in The Gambia with 372 adult volunteers. MVA encoding the CS protein and given before or after RTS,S is being assessed in phase I and IIa studies in the UK.

Intense efforts have been made to develop effective DNA-based vaccines to the liver stage and blood stages. Various DNA vaccines, each encoding a pre-erythrocytic antigen, have undergone phase-I studies. A mixture of five pre-erythrocytic DNA vaccines has been administered in phase-I studies, but no evidence of protection was noted in sporozoite challenge tests. DNA vaccines require viral boosting to induce strong T-cell immunogenicity in macaques as well as in human beings; antibody induction in human beings is generally very low after DNA vaccination, by contrast with some animal models.

Other CS-based candidate vaccines that have been tested in phase-I studies include a multiple antigen peptide, a type of synthetic delivery system, which induced strong antibody responses; a polyoxime construct, containing a universal T-cell epitope; and a long synthetic peptide in an oil-based adjuvant, which induced detectable antibody and CD4+ and CD8+ T-cell responses with a good safety profile.

Blood-stage vaccines: invasion and complication

There are two possible classes of blood-stage vaccine: anti-invasion and anticomplication. A vaccine that could prevent invasion of red blood cells by merozoites would prevent malaria disease. Development of such vaccines has been hampered by the lack of an established human challenge model, by the limitations of available animal models, and by unclear immunological correlates of protection. Merozoite surface protein-1 (MSP-1) is the most well characterised antigen involved in invasion, and is the basis of several candidate vaccines. However, vaccine development has been complicated by the discovery of parallel pathways for invasion, and by the elegant demonstration that some antibodies to MSP-1 can block the activity of malaria-protective antibodies. In a small efficacy study in Papua New Guinea, a blood-stage vaccine incorporating the antigen MSP-2 and two other blood-stage antigens reduced parasite density in vaccine recipients. Participants were protected most from infection with the vaccine strain of malaria, suggesting that for polymorphic antigens such as MSP2, a vaccine including just one allelic form of the antigen is not likely to give sufficient protection.

A recombinant viral vaccine, NYVAC Pf-7 (P falciparum-7), has been developed that encodes seven antigens from various life-cycle stages. Results of a sporozoite challenge study of NYVAC Pf-7 showed encouraging delays in time to parasitaemia, and some antibody and cytotoxic T-lymphocyte immunogenicity, but this candidate has not been further developed. An anti-invasion vaccine based on MSP-1 known as falciparum malaria protein (FMP-1) is being clinically assessed and has progressed quickly to an adult phase-I study in western Kenya.

Two blood-stage candidates, glutamate rich protein (GLURP) and MSP3, have been clinically assessed in Europe. A key issue with all such protein candidates is the identification of a safe, immunogenic adjuvant, since the traditional adjuvant, alum, seems to be insufficiently immunogenic for many malaria proteins. Additionally, vaccines with an alum adjuvant induce a Th2 response, rather than the generally more desirable Th1 response. Induction of biologically-relevant antibodies is a further challenge, and it is uncertain how often this will require a native conformation of the recombinant protein.

Another approach to blood-stage vaccine design has been suggested by the demonstration that vaccine induced T-cell responses to blood-stage antigens can be protective in animal models, and by the finding that human volunteers can be protected against infection by immunisation with low doses of blood-stage parasites that do not induce detectable antibodies. Development of blood-stage vaccine models, and the increasing availability of new antigens, should lead to a growing number of clinical studies of blood-stage candidate vaccines.

Sequestration of P falciparum by adherence to vascular endothelial cells in the brain, kidneys, and placenta is an important cause of severe malaria. The PfEMP-1 antigen (erythrocite membrane protein-1), the main ligand for such adherence, is being researched as a vaccine candidate. However, its high degree of variability, rapid rate of antigenic variation, and high copy number within each parasite complicate vaccine development, although some researchers think that use of a conserved part of the antigen could be a promising approach. At schizont rupture, inflammatory mediators are released, leading to many severe manifestations of malaria disease. The P falciparum glycosyl phosphatidyl inositol (GPI) molecule is a lead candidate for this mediator, the so-called malaria toxin. Immunisation with P falciparum GPI protected mice from severe disease manifestations on malaria challenge, although this finding was not reproducible by other investigators, and the pathway from this work to an effective clinical vaccine is unclear.

Sexual-stage vaccines: the altruistic vaccine

Induction of antibodies to gametocyte antigens can prevent fertilisation in the mosquito; as well as its blood meal, the mosquito ingests antibodies that block fertilisation. As a result, assessment of the efficacy of gametocyte vaccines is possible with a simple ex-vivo assay. Mosquitoes are fed on gametocytes with or without the addition of human serum samples from vaccinated volunteers. The US National Institute for Allergy and Infectious Disease Malaria Vaccine Development Unit plans clinical assessment of a P falciparum gametocyte candidate vaccine, PfS25, a recombinant protein. There is little commercial funding for sexual-stage vaccine candidates, since they have no market in developed countries. Such vaccines could, however, contribute to malaria control, especially if linked with other interventions. A sexual-stage vaccine consisting of an antigen not expressed in human beings during natural infection would not select for escape mutants. Therefore, combination of such a vaccine with a blood-stage or pre-erythrocytic vaccine could prevent potential immune selection. Sexual-stage vaccination would not protect vaccinated individuals from disease but would protect communities from infection.

Vaccine development in the post-genomic era

Results of whole-genome sequencing indicate that there are probably 5300 P falciparum antigens. Genome databases can be used for identifying hundreds of candidates for vaccination. However, the number of possible antigens is not rate-limiting for malaria vaccine development. Identification of antigens does not help solve some key problems in malaria vaccine development: how to combine multiple antigens without interference or competition. Post-genomic antigen identification should generate a wealth of information of long-term value to
vaccine development, but solving other problems could be a faster means to developing an effective vaccine. Clearly, diversion of funding from clinical development of the well characterised antigens already available would be counterproductive. A distinction can be made here between vaccine and drug development, in which there are likely to be shorter-term promising applications of genome sequence information.

**Discussion**

Development of an effective and deployable malaria vaccine seems technically feasible in the view of most malaria researchers. New vaccine delivery methods and adjuvants could continue to increase the antibody and cellular immunogenicity of subunit vaccination. The rate of clinical assessment of candidate malaria vaccines is increasing; in the past 5 years, the number of groups doing such research has increased from three to 11. Careful clinical expansion is needed to translate immunogenicity into efficacy against malaria parasites in people resident in malaria-endemic countries. Artificial challenge models and improved in-vitro assays should speed up this process. However, development of an effective vaccine also requires research into antigenic polymorphism, duration of efficacy, and means of antigen combination. A practical limitation is the lack of worldwide Good Manufacturing Practice (GMP) manufacturing facilities for some new technologies such as recombinant viral vaccines.

An effective vaccine is urgently needed. Efficacy studies often have to progress through adults and children aged 1–5 years before reaching their target age group of infants (figure 2). There will probably be a need for combination vaccines, and therefore vaccine development efforts of several groups will almost certainly have to be combined. Although one candidate vaccine has moved from first use in human participants to a phase-I trial in developing countries within months, a greater challenge is speeding the progression from demonstrated efficacy to licensing of a vaccine. Currently, it can take more than a decade between first demonstration of high-level efficacy of a new vaccine and licensing for use in young children. The availability of trained, motivated, local investigators to do efficacy studies is a further limiting factor. Funds are needed to train and support developing country investigators to work with sponsors and take a leading role in vaccine development.

The cost of vaccines should be considered before large-scale efficacy trials are planned. Estimation of cost is complicated by the unpredictable but anticipated decrease in price of a vaccine over time. The establishment of a global purchase fund could be essential to spur industrial interest in late-stage vaccine development. Increasing numbers of trials will result in increasing numbers of study participants who should be followed up in the long term. However, funding rarely exists for more than 1–2 years per trial; thus, the best way to maintain long-term follow-up is to do sequential trials in the same setting, and to include demographic surveillance infrastructures. As occurred in The Gambia in phase-III trials of hepatitis B and *Haemophilus influenzae* type b vaccines, a plan should be made in conjunction with local governments for provision of vaccine to the country or region participating in key prelicensing field trials. Increasingly, cessation of vaccinations once such a trial has ended is seen as unacceptable if the intervention has been shown to work.

Informed consent is a complex issue in efficacy studies. In many rural African settings, community consent is as important as individual consent, and rates of literacy can be poor. The American-European-Japanese ICH-GCP (International Committee on Harmonisation-Good Clinical Practice) guidelines are moving towards the status of law in much of the developed world. The guidelines were drawn up by regulatory authorities and pharmaceutical companies with little contribution from developing countries. GCP consent forms may be very
Figure 3: Endpoints in malaria vaccine field trials

A trial in adults can detect 40% efficacy against infection but not disease with only 300 participants, even in moderate transmission settings. The higher the transmission intensity the smaller the necessary sample size. About 1000 children aged 1–5 years are needed to measure efficacy against mild malaria, whereas 5000 such children would be required to measure efficacy against severe malaria, and about 20 000 against death. The more clinically relevant the endpoint, the larger and more complex the trial, but the more likely the trial would be to change public-health policy locally.

detailed, in part for the legal protection of sponsors. In rural Gambia, for example, the local consensus (of lay Gambian and Gambian Government ethics review board members) is that ICH-GCP-compliant consent forms are not always appropriate. Complex trials must be clearly explained to participants; Gambian experience is that by repeated delivery of complex messages with reinforcement throughout the study, adequate understanding is possible, but this undertaking is far from trivial. In particular, the fact that the vaccine being tested is not known to protect against malaria must be stressed throughout the consent procedure and the study.

If funding continues to increase in line with recent trends, a malaria vaccine candidate could, in the next decade, be proven to have sustained efficacy in infants, young children, or both. The next step would be to do large trials in various epidemiological settings, perhaps including other interventions such as insecticide-treated bednets. These trials should be designed with severe disease or death as an endpoint, and with sufficient sample size to convince local policymakers and international organisations of its worth (figure 3). If an effective vaccine is licensed, public-sector funding will be needed to deliver the product to African infants. Organisations such as the Global Alliance for Vaccines and Immunisation; the Global Fund to Fight AIDS, Tuberculosis and Malaria; or a dedicated purchase fund could support widespread vaccination in the medium term.

Conflict of interest statement
AVSH is a co-founder of, and consultant to, Oxinnon Pharmaceuticals, which is developing prime-boost vaccines for therapeutic applications using MVA. No conflicts declared by VSM or MFG.

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