

Progress and challenges for malaria vaccines

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Malaria causes much physical and economic hardship in tropical regions, particularly in communities where medical care is rudimentary. Should a vaccine be developed, it is the residents of these areas that stand to benefit the most. But the vaccine, which has been promised to be 'just round the corner' for many years, remains elusive. It is important to ask why this is so, when effective vaccines exist for many other infectious diseases. What are the reasons for the slow rate of progress, and what has been learned from the first clinical trials of candidate malaria vaccines? What are the remaining challenges, and what strategies can be pursued to address them?

Four species of malaria infect humans, which raises an initial question of how many vaccines are needed. Studies in the 1950–60s of sequential heterologous infections in malaria therapy patients, such as *Plasmodium vivax* after *Plasmodium falciparum*¹, and cross-species challenge experiments in the 1970s using the irradiated sporozoite vaccine² showed that protection against several species will be difficult to achieve with a single vaccine. Accordingly, species-specific vaccines for *P. falciparum* and *P. vivax* — the two parasites that contribute most heavily to the malaria burden — are being developed, keeping open the possibility of combining the antigens into a single formulation sometime in the future. Once successful vaccines have been developed against *P. falciparum* and *P. vivax*, it should be relatively straightforward to create similar vaccines for *Plasmodium malariae* and *Plasmodium ovale*.

The *P. falciparum* parasite deserves particular attention because of the variety and severity of disease syndromes that it causes (see review in this issue by Miller *et al.*, pages 673–679). Several risk groups are found among those living in endemic areas who are subject to repeated *P. falciparum* infections: infants and young children suffer particularly from life-threatening anaemia, older children from an induced coma³, and primigravida women from severe disease related to placental sequestration⁴. For each of these groups, an anti-morbidity vaccine, possibly tailored to the underlying pathophysiology, would be of great benefit. Malaria-naïve travellers, either crossing international borders or travelling from malaria-free to malaria-endemic areas in their own countries, constitute another risk group, and are susceptible to severe disease after acquiring their first infection; for this group, it is important to prevent malaria infection altogether.

Although practical considerations of both development and production costs favour a single vaccine for *P. falciparum*, the different risk groups and vaccine requirements have generated at least three approaches for this species alone (Box 1): an anti-infection vaccine aimed at protecting malaria-naïve travellers or residents of low endemic areas from becoming infected; an anti-disease/anti-mortality

vaccine aimed at children, pregnant women and migrants living in endemic areas; and an anti-mosquito-stage vaccine aimed at preventing the transmission of malaria from one person to another⁵.

In this review, we discuss the challenges faced in developing these vaccines, why we believe that success is likely, the strategies being pursued and the progress to date, including some encouraging results from clinical trials. We outline the tasks that remain and how they are being addressed by a worldwide effort.

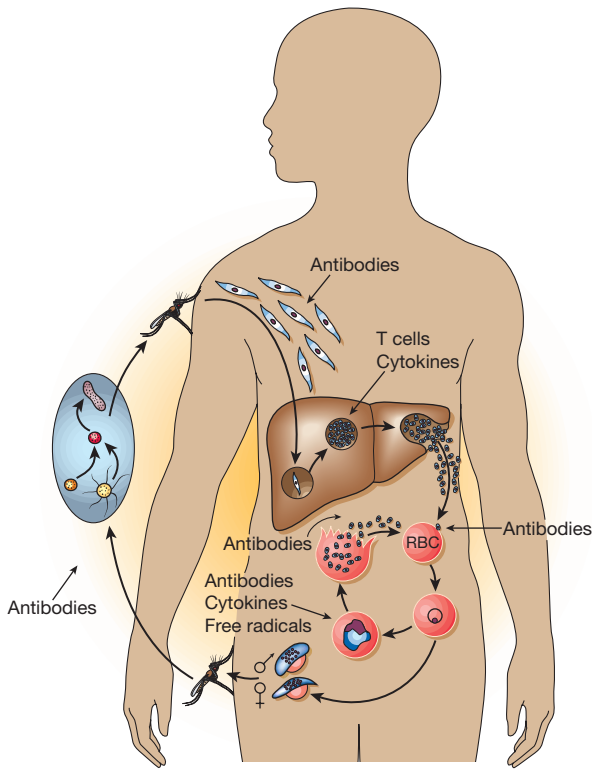
Attacking a chronic infection

To appreciate the constraints of curtailing malaria infection with a vaccine, consider that the parasite that can establish a chronic infection in an immunocompetent host, successfully evading all branches of the immune system. This contrasts with most acute infectious diseases, for which it is relatively straightforward to reproduce the sterile immunity that follows natural infection. There are several immune responses that restrict parasite growth, but the parasite persists. Indeed, the parasite benefits from immune responses if they lead to chronic infection and thereby enhance transmission to the mosquito. As yet, none of the immune responses identified in humans robustly predicts protection from infection or from disease, nor do we know for sure which branches of the immune system will succeed ultimately in eradicating the parasite. Thus, the plan to harness immune responses to destroy the parasite presents a conundrum and requires clever approaches that short-circuit the mechanisms of immune evasion.

Complicating the picture, the different stages of the parasite express different antigens, and a vaccine effective in killing liver-stage parasites may not inhibit the growth of blood-stage parasites. Many parasite proteins exhibit polymorphism, which potentially limits the effectiveness of any vaccine not incorporating distinct variants of antigen. For example, a single parasite clone contains roughly 50 different copies of the gene for the variable surface antigen PfEMP1 (*P. falciparum* erythrocyte membrane protein 1). During a chronic infection, each successive wave of parasitaemia expresses a new variant surface antigen, thus

Box 1

Parasite stages under attack



Current approaches to malaria vaccine development can be classified according to the parasite stages that are targeted:

1. Vaccines directed against sporozoites and/or liver stages (collectively termed pre-erythrocytic stages) are designed to prevent blood-stage infection and thereby avoid all manifestations of disease (anti-infection vaccines).
2. Vaccines directed against asexual blood stages are designed to reduce clinical severity (anti-morbidity/mortality vaccines).
3. Vaccines directed against mosquito stages are designed to halt development in the mosquito (transmission-blocking vaccines). Protective mechanisms of immunity are shown for each stage.

In reality, the effects that can be anticipated for each type of vaccine overlap broadly: a pre-erythrocytic-stage vaccine, even if not 100% effective, could reduce transmission and morbidity — the latter is predicted by the reductions in morbidity and mortality associated with the use of insecticide-impregnated bednets; a highly effective blood-stage vaccine could eliminate blood stages as soon as they emerge from the liver, thereby curtailing both infection and transmission; and a transmission-blocking vaccine could reduce population-wide malaria infection rates and malaria-associated morbidity.

allowing parasite multiplication despite the presence of antibodies directed against the preceding parasitic wave⁶. The sheer number of malaria proteins — estimated to be at least 5,000–6,000 — presents a perplexing choice for the designer of a subunit vaccine (see review in this issue by Hoffman *et al.*, pages 702–709). On the other side of the parasite–host equation, humans exhibit striking heterogeneity in their immune response, depending on their type of human leukocyte antigen (HLA) and other factors (Fig. 1). There is also a bewildering array of other genetic traits, such as haemoglobin type and red cell polymorphisms, that control susceptibility to and have been

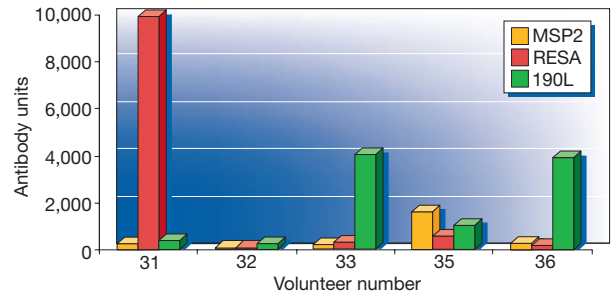


Figure 1 Diversity of immune responses in humans to malaria antigens. Data from five consecutive volunteers (volunteer numbers 31–36; 34 was a placebo) receiving a mixture of 50 µg of each of three malaria antigens, the 190L fragment of MSP1, MSP2 and RESA, in Montanide ISA720, followed by a boost of 20 µg of each antigen³⁰. A similar diversity of responses was seen in volunteers who received the three antigens separately. Tasks for vaccine developers include increasing the immunogenicity of malaria antigens through new formulations and approaches, and designing vaccines that induce protective immune responses in everyone, despite diverse genetic backgrounds.

selected by malaria⁷. Given these complexities, is it possible to make an effective vaccine?

Why a vaccine is feasible

Several studies have shown that malaria vaccines are feasible. First, immunization with irradiated sporozoites protects or partially protects rodents⁸, monkeys⁹ and humans^{2,10,11} from being infected by sporozoites. In humans, immunization through the bites of more than 1,000 irradiated, infected mosquitoes, in batches of a few hundred bites over several months, confers sterilizing protection against sporozoite challenge in more than 90% of vaccine recipients. Although the immune mechanisms underlying this protection are not known, evidence indicates the importance of T-cell responses directed against parasite proteins expressed on the surface of infected hepatocytes¹².

So far we have not identified a single dominant immune response in irradiated sporozoite-immunized hosts¹³. This suggests, but does not establish, that protection may be mediated by the summation of many modest immune responses against the large variety of antigens presented by this attenuated-organism vaccine. Although irradiated sporozoites delivered by mosquito bite are unlikely to be a practical vaccine, their effectiveness indicates that a multivalent pre-erythrocytic (sporozoite and liver)-stage vaccine might induce sterile immunity. This type of anti-infection vaccine would prevent all manifestations of disease and would be especially suited for travellers or others exposed to relatively low intensity transmission, but may also have an important role in limiting malaria-related morbidity and mortality in those living in endemic areas.

Second, people infected repeatedly by malaria develop ‘naturally acquired immunity’ (NAI), which protects against clinical disease¹⁴. But if their parasites are eliminated through radical drug cure, these individuals can become re-infected, which indicates that NAI does not include absolute anti-infection immunity¹⁵. If we could reproduce NAI with a vaccine (that is, use a vaccine to hasten the transition from malaria-naïve to clinically immune), we could shelter recipients from malarial disease. Antibody responses to blood-stage parasites constitute part of NAI, because passive transfer of purified immunoglobulins from immune individuals protects children^{16–18}. Thus, another avenue for designing a vaccine is to generate antibody responses to blood-stage parasite antigens. This type of vaccine, which would suppress blood-stage parasites and prevent disease, would be especially suited to those exposed to intense

transmission and could be used either alone or as a complement to a pre-erythrocytic-stage vaccine. The efficacy of such a vaccine for particular target groups might be improved by tailoring its composition to the underlying pathophysiology, such as the inclusion of components that block placental sequestration in a vaccine designed for women considering pregnancy.

Third, immunization studies show that vaccines already in hand can protect against malaria infection in animals models and in humans. Notably, consistent protection against experimental sporozoite challenge has been achieved in naive humans vaccinated with a recombinant anti-sporozoite vaccine based on the circumsporozoite protein — the principal surface antigen of sporozoites¹⁹. Protection has been achieved routinely in new world monkeys (*Aotus* and *Saimiri*) against blood-stage challenge with *P. falciparum* by vaccination with the antigen merozoite surface protein 1 (MSP1)²⁰. Both types of vaccine, anti-sporozoite and anti-blood-stage, have provided evidence of protection in field trials (see below), although the efficacy of these vaccines is still too low and the duration of protection still too short to be of practical value.

Last, studies have shown success in protecting mosquitoes against infection by *P. falciparum* and *P. vivax*. In these studies, the gametocyte-containing blood that is used as the infection source for the mosquito is mixed with serum from animals immunized with prototype human anti-mosquito-stage (transmission-blocking) vaccines^{5,21}. Thus, the efficacy of all three types of vaccine against human parasites — aimed at the sporozoite stage, blood stage and mosquito stage — has been proved in principle.

Strategies for vaccine design

Given that there is evidence supporting the feasibility of all three types of vaccine, what is the best strategy for designing them? What antigens should be included, and how should they be formulated? Certain principles can be defined that guide development.

First, we should choose antigens that meet specific criteria. Antigens are favoured as candidates if they are accessible to the immune system, induce protective immune responses in animal models, and either lack antigenic diversity or have at least limited diversity. Antigens such as PfEMP1, which show considerable

diversity but are potentially outstanding vaccine targets, will require a means by which to overcome that diversity — for example, by focusing on conserved functional domains²². Parasite receptors that mediate the binding of merozoites to erythrocytes, or of infected cells to endothelial cells, are important candidates. Some of these interactions are regulated by redundant pathways that may need to be blocked simultaneously (see review by Miller *et al.*, pages 673–679). Consideration of the parasite biology may lead to new antigens, such as proteins that are expressed in the mid-gut of mosquitoes and have therefore not evolved under human immune selection pressure, but which could be the targets of antibodies taken up in the mosquito's blood meal. Current candidate antigens are described in Table 1.

Second, we should combine antigens from different stages. Because of the stage specificity of antigen expression, the escape of even small numbers of parasites into the blood of a non-immune individual after immunization with a pre-erythrocytic-stage vaccine will result in clinical malaria. In recent experiments, naive volunteers challenged with 100–1,000 ring-infected erythrocytes (roughly 30–300-fold fewer than released from a single sporozoite-infected hepatocyte) had unaltered rates of the parasite during the pre-patent period and also developed clinical malaria at the expected threshold density of parasitaemia²³. But if a pre-erythrocytic vaccine were combined with a second line of defence — namely, a component designed to limit the growth of asexual blood stages — then vaccinated individuals with break-through blood-stage infection would be able to reduce further the risk of significant clinical illness. As another example, a combination of an anti-mosquito-stage vaccine with a blood- or pre-erythrocytic-stage vaccine would decrease the probability of resistant clones that emerge in vaccinated hosts spreading through the population.

Third, we should combine several antigens from a single stage. There are several arguments in favour of multivalency. (1) If the protection induced by each antigen is independent of that afforded by its companions, and if each is unable to induce sterile immunity on its own, then the potency of the vaccine will increase with more antigens. Few data support the supposition of independence, however, and problems with interference among antigens, if encountered, would have to be offset by the benefits of including several antigens or

Table 1 Candidate vaccine antigens

Stage and process	Antigens*	Primary mechanism
Sporozoite		
Hepatocyte invasion	CSP ⁷³ , TRAP/SSP2 ⁷³ , STARP ⁷⁴ , SALSA ⁷⁴	Antibody binding to antigens expressed on surface of sporozoite: Prevent binding interactions required for invasion Enhance splenic clearance or complement mediated lysis
Hepatic stages		
Growth, schizogony	CSP ⁷³ , TRAP/SSP2 ⁷³ , LSA1 ⁷³ , EXP1 ⁷³ , LSA3 ⁷⁵ , STARP ⁷⁴ , SALSA ⁷⁴	T-cell recognition of antigens expressed on surface of infected hepatocyte ⁷³ : Release soluble immune mediators (IFN- γ) resulting in intracellular parasite death Lyse infected hepatocyte directly (CTL)
Merozoite		
Erythrocyte invasion	MSP1 ²⁰ , MSP2 ³⁰ , MSP3 ⁷⁶ , MSP4 ⁷⁷ , PfEBA175 ⁶⁷ , PvDABP ⁷⁸ , AMA1 ⁷⁹ , SERA ⁸⁰ , GLURP ²⁸ , Pf155/RESA ³⁰ , RAP1 ⁸¹ , RAP2 ⁸¹	Antibody binding to parasite antigens: Agglutinate or prevent release of merozoites ⁸² Block invasion into erythrocytes ^{22,83,84} Induce monocytes to release soluble immune mediators, killing parasites (antibody-dependent cellular inhibition) ^{18,23} Facilitate phagocytosis ²³
Erythrocyte stages (asexual)		
Growth, sequestration	PfEMP1 ^{6,85} , rifins ²⁴ , Pf332 ²⁴	Antibody binding to antigens expressed on surface of infected erythrocyte: Enhance splenic clearance or complement mediated lysis Interfere with parasite nutrition and growth Induce antibody-dependent cellular inhibition Prevent or reverse endothelial adherence, thereby enhancing splenic clearance and reversing a key pathogenic mechanism
Erythrocyte rupture	Glycosylphosphatidylinositol (GPI) anchors ⁸⁶	Antibody binding to GPI resulting in prevention of toxic effects ⁸⁶
Mosquito stages		
Fertilization, oogenesis	Pfs25 ^{5,86} , Pfs28 ^{5,26} , Pfs48/45 ^{5,26} , Pfs230 ^{5,26}	Antibody binding to parasite antigens: Inhibit exflagellation and fertilization Complement induced lysis of gametes and zygotes Neutralize ookinete function

*Antigen names in bold type are in clinical development. Antigens have been listed according to the broad category of vaccine in which they are most likely to be included. However, many antigens are expressed in several stages (for example, LSA3 is expressed in blood stages as well as the liver, and most merozoite antigens are probably expressed in liver stages). Thus antigens in one category may also be targets in other stages.

by alterations to vaccine design that eliminate the interference. (2) Many leading vaccine candidates (MSP1 and apical membrane antigen 1 (AMA1)) are polymorphic, and several forms of each antigen may be required to provide protection against parasite diversity²⁴. (3) The immune response developed by each person is unique (owing to polymorphisms in HLA, other genetic traits and previous exposure); therefore mixtures of antigens may be required to ensure that each person has a reasonable chance of generating a sufficient response, particularly for genetically restricted T-cell responses. (4) Combining antigens may facilitate the development of a single vaccine that protects against more than one species of malaria. (5) The emergence of parasites that are simultaneously resistant to all immune responses induced by a multivalent vaccine is less likely than the emergence of parasites that are resistant to a single component, which could potentially prolong the useful life of the vaccine relative to that of a monovalent formulation.

Fourth, we need to keep vaccines as simple as possible. Optimizing the immunogenicity of individual components in a mixture is likely to be difficult, particularly as the complexity increases. For protein-based vaccines, the total amount of protein that can be injected in one vaccine is limited by the formulation and possibly by the frequency of adverse events; in addition, malaria vaccines will have to be inexpensive and the cost rises quickly with complexity. Vaccines using naked DNA or delivery systems such as replicons offer an attractive way of increasing the antigenic diversity within these constraints.

Last, we must induce the right kind of immune response. Biological characteristics of each parasite stage determine which immune responses are optimal for parasite destruction. For example, CD8-expressing T-cell and/or CD4-expressing T-cell type 1 responses may be essential for eliminating intracellular liver-stage parasites²⁵; by contrast, transmission-blocking vaccines must induce high, sustained amounts of antibody to neutralize antigens in the mosquito gut^{5,26}. Blood-stage vaccines also must generate high concentrations of antibody for protection, but may also need to induce other effector mechanisms, such as antibody-dependent cellular inhibition^{27,28}. Particular adjuvant systems may be needed to drive responses in one direction or the other, and this might constrain the composition of a particular vaccine.

So far, antigen choice has been dominated by the arbitrary order in which antigens have been identified. This is now changing with the sequencing of parasite genomes and an increasing understanding of parasite biology. We view the sequencing of the *P. falciparum* genome as a great boon to vaccine developers, because it should allow selection of the very best candidate antigens. Methods for screening the hundreds of proteins expressed during a particular parasite stage for those that induce protective immune responses are already being developed (see review by Hoffman *et al.*, pages 702–709).

These 'guiding principles' clearly involve compromises between conflicting demands. For example, the need to keep vaccines as simple as possible must be balanced against the need to cover antigenic diversity. Many researchers are proposing that a complex vaccine will be required, because this is the 'safe' assumption. Should one or a few antigens prove to elicit a universal protective response, vaccine development would be accelerated considerably.

Clinical trials of malaria vaccines

Over the past 15 years, a series of phase 1/2 vaccine trials has been reported using synthetic peptides or recombinant proteins based on malarial antigens. Engers and Godal²⁹ report that there have been roughly 40 trials up to 1998. Most trials were directed against sporozoites or liver stages, in which the use of experimental mosquito challenges allows rapid progress through phase 1 to phase 2a preliminary efficacy studies. Anti-sporozoite vaccines tested have included completely synthetic peptides, conjugates of synthetic peptide with proteins such as tetanus toxoid to provide T-cell help, recombinant malaria proteins, particle-forming recombinant chimaeric constructs, recombinant viruses, and bacteria- and DNA-

based vaccines. Several trials of asexual blood-stage vaccines have used either synthetic peptide conjugates or recombinant proteins, and there has been one trial of a transmission-blocking vaccine — recombinant Pfs25 (ref. 26; and unpublished data from National Institutes of Health (NIH) Pfs25 clinical trial). A recurring theme is the difficulty of obtaining a sufficiently strong and long-lasting immune response in humans even though the same vaccine preparation is often strongly immunogenic in test animals³⁰. Various strategies have been designed to overcome this limitation, including the exploration of potent immune-stimulatory conjugates or adjuvants to boost the human response. An interesting approach is the *in situ* expression of the vaccine through attenuated strains of *Salmonella*³¹.

Vaccines directed against the circumsporozoite protein covering the sporozoite illustrate the frustrating quest for a consistently protective immune response in humans. Early studies with both recombinant proteins, peptide conjugates and recombinant protein conjugates could elicit anti-circumsporozoite antibody, provided marginal protection in phase 2a studies but provided no protection in field studies³². More recently, collaboration between Glaxo-SmithKline and the US Army has produced a chimaeric protein, consisting of a fusion between the circumsporozoite protein and the hepatitis B surface antigen (HBsAg), which is expressed in yeast¹⁹. In the presence of unmodified HBsAg, the mixture forms typical HBsAg particles (the RTS,S vaccine). Although much more immunogenic than recombinant circumsporozoite protein, RTS,S is still incapable of eliciting a protective response when used with conventional adjuvants, but elicits a protective response in about half of vaccine recipients with the AS02 (formerly SBAS2) experimental oil in water adjuvant, which also contains monophosphoryl lipid A (3D-MPL) and the saponin QS21. Other recent approaches to a circumsporozoite vaccine have used synthetic peptide technology to generate immunogens containing both T- and B-cell epitopes with an endogenous lipophilic adjuvant³³.

Only a few *P. falciparum* vaccines have been tested in field trials. This area has been dominated by the synthetic peptide vaccine SPf66, which is absorbed on alum and is directed at blood-stage parasites³⁴. Initial results of trials in children and adults residing in relatively low endemic regions of South America and children in a highly endemic area of Tanzania suggested that this vaccine might delay the time to a first clinical malaria episode, but subsequent trials in infants in The Gambia and Tanzania, children in Thailand and adults in Brazil failed to show efficacy. In a recent meta-analysis of nine trials involving 9,800 volunteers, Graves and Gelbrand³² concluded that there was no evidence of efficacy for SPf66 in Africa but that it caused a modest reduction of malaria attacks in South America.

Two other vaccines that have recently undergone field trials show promise. In a collaboration with the Medical Research Council (MRC) unit in The Gambia, a trial of the RTS,S vaccine in adult Gambian males resulted in a significant reduction in the rate at which they were infected after vaccination, although the protection was not long-lived³⁵. A combination of three asexual blood-stage antigens (the 190L fragment of MSP1, one form of the polymorphic MSP2 antigen and a portion of the RESA or ring-infected erythrocyte surface antigen)³⁰ has been tested in children aged 5–9 years in the Wosera district of Papua New Guinea. Designed to determine the parasitological outcome, this trial detected significant decreases in the parasite density and the frequency of parasite episodes greater than 1,000 per microlitre, and a major switch in the MSP2 genotype of parasites³⁶.

Although protein-based vaccines are promising and are furthest along the pathway towards malaria vaccine development, alternative technologies such as DNA-based vaccines may prove particularly amenable to multivalent formulations and ultimately less expensive to produce, store and deliver than conventional vaccines (ref. 37; and Box 2). Progress with DNA vaccines has been rapid, and studies have demonstrated the following. (1) Protection against *P. yoelii*

sporozoite challenge in BALB/c mice immunized with a plasmid encoding the *P. yoelii* circumsporozoite protein in 1994 (ref. 38). (2) Improved protection in mice with a two-plasmid vaccine in 1996 (ref. 39). (3) Complete protective efficacy of DNA prime/poxvirus boost regimens in mice in 1998 (ref. 40). (4) Safety of DNA and induction of genetically restricted, antigen-specific, cytotoxic T lymphocyte (CTL) responses in humans vaccinated with a plasmid encoding *P. falciparum* circumsporozoite protein in 1998 (refs 41,42). (5) Induction of CD8-expressing and CD4-expressing T-cell-dependent interferon- γ responses by ELISpot (enzyme-linked immunospot) assay in humans immunized with the same plasmid in 2001 (ref. 43). (6) Partial protection of rhesus monkeys against *P. knowlesi* sporozoite challenge with a four-plasmid cocktail boosted by poxvirus constructs encoding the priming malarial antigens in 2001 (ref. 44; and W. O. Rogers, unpublished data). (7) Similar success with *P. cynomolgi* challenge of rhesus monkeys immunized with a epitope string derived from *P. falciparum* fused with the cholera toxin B subunit in 2000 (ref. 45). A DNA vaccine including a string of CTL epitopes from six different *P. falciparum* pre-erythrocytic antigens fused to the full-sequence thrombospondin-related adhesion protein (TRAP) from *P. falciparum*, also administered using a plasmid-prime, poxvirus-boost approach, has been in clinical evaluation in Oxford since 1999 and in The Gambia since September 2000, with encouraging results⁴⁶.

DNA plasmids encoding malaria antigens have generated antibody responses in animal models^{38,47-50}, but not in humans as yet⁴¹. The interferon- γ responses shown in humans are appropriate for liver-stage vaccines designed to effect intracellular killing, but are probably insufficient for vaccines designed to neutralize circulating sporozoites or blood-stage parasites. Many new modalities to improve expression and immunogenicity and to extend the breadth of the immune response to DNA-based vaccines are now being tested. These include replacing native genes with synthetic genes using codon frequencies optimized for host expression⁵¹, adding sequences such as the Fc region of immunoglobulin that increase antigen uptake by dendritic cells⁵², absorbing plasmids onto microparticles⁵³ to increase antigen uptake by dendritic cells, particle-mediated gene transfer (gene gun)⁵⁴ or jet injection⁴³ to enhance plasmid delivery, and co-stimulation with plasmids encoding cytokines, such as granulocyte/macrophage colony-stimulating factor⁵⁵⁻⁵⁷, or other immunostimulatory molecules such as CpG motifs⁵⁸ or an interleukin-2/immunoglobulin fusion protein⁵⁹.

Of the various approaches, the one that is most potent in improving protection in animal models is heterologous vaccination, in which DNA plasmids are used to prime specific immune responses and virus constructs^{44,50,54,60} or recombinant proteins⁶¹ are used to boost these responses. Several viral vectors are under development, including avian and mammalian recombinant poxviruses^{62,63}, non-replicating and replicating recombinant adenoviruses⁶⁴, and alphavirus replicons⁶⁵. Clinical studies of DNA priming followed by either recombinant poxvirus or protein boosting are in progress at various centres. Although DNA (plasmid) vaccines on their own are currently not sufficiently immunogenic or protective, if immunogenicity can be improved then DNA-based technologies should allow the development of effective malaria vaccines.

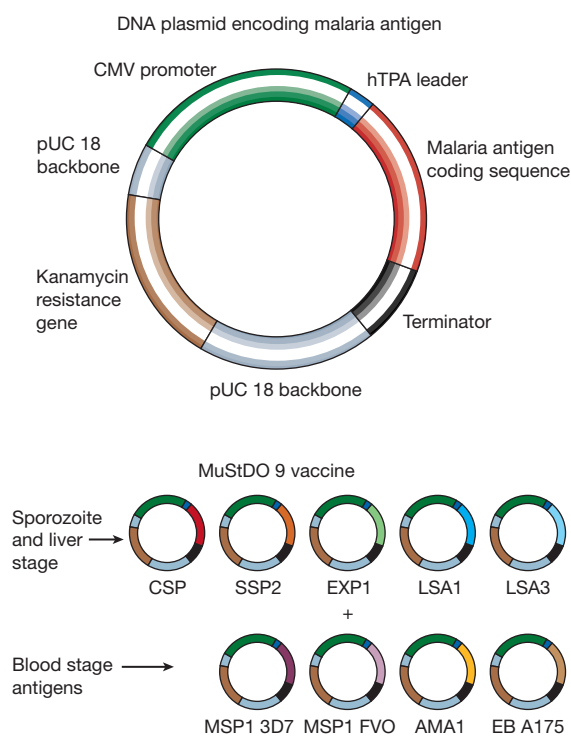
Challenges in vaccine development

Our progress in identifying antigens and the demonstration that several of these can elicit protective immune responses give us reason for optimism. Nevertheless, a substantial investment of resources is needed to improve the degree and duration of protection and to bring vaccines to licensure. But perceptions concerning the economics of developing, manufacturing and distributing vaccines to a global market that comprises some of the world's poorest people limit our ability to mobilize these resources, especially from the private sector.

Typically, pharmaceuticals have been developed through a process that involves screening large numbers of compounds for

Box 2

Cocktail to soothe tropical fevers?



Supercoiled rings of double-stranded DNA containing malaria genes are injected, taken up by muscle cells and translated into malarial proteins. These proteins are processed and presented by antigen-presenting cells, inducing immune responses designed to inhibit the developing malaria parasites. The MuStDO 9 vaccine^{87,88} consists of five plasmids encoding proteins from the sporozoite and liver stages of *P. falciparum*, CSP, SSP2, EXP1, LSA1 and LSA3, and four encoding proteins from blood stages, MSP1 (two alleles representing the major antigenic families), AMA1 and EB A175. The two-tiered design is based on the rationale that parasites escaping the pre-erythrocytic-stage defence will be suppressed by a second line of immune responses directed at blood stages, thus decreasing the likelihood of serious illness in vaccinated individuals with breakthrough blood-stage infections. Each gene in the expression cassette has been synthesized using codon frequencies that are consistent with mammalian patterns. At present, naked DNA vaccines are not sufficiently immunogenic to protect humans against malaria, and require boosting by recombinant viral constructs or recombinant proteins to achieve measurable efficacy. New formulations and designs aiming to increase plasmid uptake by dendritic cells, improve expression and strengthen antibody responses may remove this requirement. So far, DNA plasmids encoding malarial antigens have been administered to roughly 80 healthy adult volunteers in the United States by the US Navy and about 50 healthy adult volunteers in Britain or The Gambia (mainly in heterologous prime-boost regimens with modified vaccinia virus Ankara) by the Oxford University group (A. V. S. Hill, personal communication) and seem to be safe and well tolerated⁴¹.

activity and picking lead candidates for further development. For malaria we now have at least 5,000 candidate proteins and multiple variants of many of these, and although we have *in vitro* and animal models that give indications of a candidate's importance, we lack validated models that reliably predict what protection a vaccine formulation will give in humans. Views differ on the contribution

Box 3

Malaria vaccines in or close to clinical trials

1. Pre-erythrocytic vaccines RTS,S and TRAP/SSP2, and blood-stage vaccines MSP1_{42-3D7} and AMA1-3D7 by a collaboration between the US Army Walter Reed Army Institute of Research (WRAIR), USAID and GlaxoSmithKline.
2. An RTS,S phase 2b trial in Gambian adults and a phase 1 trial in Gambian children being undertaken by the MRC unit in The Gambia, in collaboration with GlaxoSmithKline and with support from WRAIR, the University of Oxford, and The London School of Hygiene and Tropical Medicine.
3. Plasmid DNA, modified vaccinia virus Ankara and an attenuated fowlpox strain encoding the multi-epitope-TRAP insert, by a collaboration between the University of Oxford and the MRC, with phase 1/2a trials in Britain and phase 1 trials in The Gambia⁴⁶.
4. Phase 1/2a studies of multistage DNA vaccine combinations ('MuStDO') in the United States and Ghana by a collaboration between the US Navy and USAID^{87,88}.
5. Phase 1/2a studies of modified vaccinia virus Ankara and a fowlpox strain encoding the circumsporozoite protein by the University of Oxford.
6. Vaccines based on the merozoite surface protein MSP1₁₉ at the Institut Pasteur.
7. Two phase 1 trials by the European Malaria Vaccine Initiative with protein vaccines made by peptide synthesis (MSP3, GLURP), followed by a phase 1 trial of Pichia-expressed recombinant AMA1.
8. Phase 1 studies of *P. vivax* and *P. falciparum* transmission-blocking protein vaccines Pvs25 and Pfs25 by the NIH Malaria Vaccine Development Unit.
9. Phase 1 studies of a pre-erythrocytic-stage vaccine that is based on a virus-like particle comprising a modified hepatitis B core particle and circumsporozoite protein-specific T- and B-cell epitopes by a collaboration between Apovia, New York University, Malaria Vaccine Initiative, NIH and the University of Maryland.

Several other antigens are at an advanced state of development and many phase 1 and some phase 2 clinical trials could reasonably be expected in the next 1–2 years. These include major polymorphisms of AMA1, MSP1₄₂ and MSP2 that explore different expression systems, and single forms of other antigens such as MSP4, RAP2, EBA175 and the *P. vivax* Duffy-binding protein.

that can be made by new and old world primate models to screen candidate vaccines^{66,67}. Even where primate models assist antigen selection, the development of useful formulations and vaccine regimens will require several human phase 1 and phase 2 trials, and screening for protection in humans is difficult.

Screening anti-morbidity vaccines in human populations in endemic areas imposes many restraints. First, producing multiple test vaccines is a big task. Second, these vaccines are designed primarily for children, and using children for a primary efficacy screen contributes to the complexity of the trial design. Last, decisions need to be made regarding the end point of the trial. Both for ethical reasons and to diminish sample size requirements, surrogate markers of efficacy may be chosen.

For example, in the Papua New Guinea trial discussed above³⁶, parasite density was used as the test of efficacy. This required group sizes of 30 with a 12-week follow up to give reasonable power of detecting a 30% decrease in parasite density. In the same population⁶⁸, groups of a few hundred would have been required to see a significant decrease in febrile cases if the vaccine caused a 30% decrease in malaria-related fever. In this region, the frequency of severe disease or death from malaria is too low to use as a useful end point, even if this could have been justified ethically. In much of Africa — which has an infant mortality of about 100 in 1,000 live births⁶⁹ — depending on the accuracy with which a cause of death can be diagnosed, group sizes of many thousands to tens of thousands would be needed to use mortality as an end point, and this is not feasible at an early stage of vaccine development. Because there are gaps in our understanding of the progression of pathology from parasitaemia to death (see review by Miller *et al.*, pages 673–679), our choice of end point for early efficacy studies is associated with a risk of either discarding a good vaccine because it fails to give an imperfect correlate of protection in early stage testing or wasting scarce resources by taking a poor vaccine through extensive clinical testing.

In contrast to anti-morbidity vaccines for infants or other high risk groups, anti-infection vaccines designed for malaria-naive travellers and residents of low endemic regions face more stringent efficacy constraints, but should be relatively simpler to test. Experimental sporozoite challenge of vaccinated, naive volunteers has become a standard way of early stage testing of vaccines for travellers, especially pre-erythrocytic-stage vaccines⁷⁰, and a blood-stage challenge has also been developed as an alternative for testing blood-stage vaccines²³.

In principle, mosquito-stage vaccines should have the simplest development path, because there is a relatively straightforward test for assessing the impact of vaccine-induced antibodies on the transmission of parasites to mosquitoes, allowing down-selection to take place at an earlier stage of development²⁶. The eventual proof that such a vaccine is effective under field conditions will require large trials in which a high proportion of the population is vaccinated and whole villages are randomized to treatment groups.

Meeting the challenge

The development of vaccines for the billion people living in endemic countries will require a different scheme from that of more commercially attractive vaccines. The public sector will need to provide the 'push' to facilitate vaccine technology development and early-stage testing and also the 'pull' of guaranteed markets to encourage pharmaceutical firms to license and manufacture vaccines⁵.

The manufacturing capacity to meet global demands may be relatively modest, depending on the type of vaccine. Most of the world's need for a vaccine to prevent serious disease and death in infants will be in sub-Saharan Africa where the live birth rate is 26 million per year. For a recombinant protein vaccine, roughly 1 kg of each protein component would be needed each year, depending on the number and size of the doses. Even with the non-optimized expression systems that are being used to produce prototype vaccines, this could be met by a single plant capable of fermenting 200 litres per week, which is well within the capacity of biotechnology companies in more industrialized malaria-endemic countries in which national considerations, economics and marketing strategies make local production attractive.

Currently, the biggest bottleneck is the capacity for producing clinical grade material for protein-based vaccines and for performing field trials for all types of vaccine. One of the most encouraging recent developments has been the substantial expansion in government and philanthropic interests to address this need, including the founding of the European Malaria Vaccine Initiative⁷¹ and the creation of the Malaria Vaccine Initiative at the Program for Appropriate Technology in Health through a grant from the Bill & Melinda Gates Foundation⁷². The US NIH has developed new programmes for the development and testing of malaria vaccines through its extramural programme and through the creation of an intramural Malaria Vaccine Development Unit. These new developments join several continuing programmes, such as those of the World Health

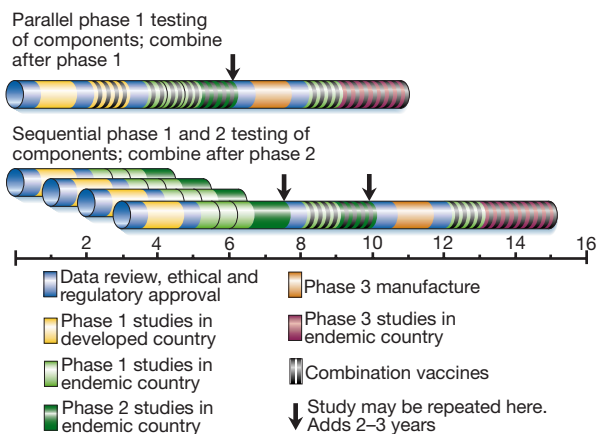


Figure 2 Timetable of clinical trials. Shown is an optimistic and simplified view of the timetable to completion of phase 3 (pre-registration) trials for a hypothetical tetravalent malaria vaccine starting from the time that clinical grade material is available. Each stage in the process requires review and approval by regulatory bodies. Initial phase 1 safety, immunogenicity and dose escalation testing in adults in the country of manufacture will generally need to be repeated in endemic countries in adults, children and infants, and these trials must run sequentially for safety reasons. The vaccine is then tested for efficacy, and if successful will move to larger phase 3 studies before registration, to confirm its efficacy and safety. Phase 3 trials test the vaccine made under the same conditions that will be used for final manufacture; before they can commence, the final manufacturing process and plant need to be established. Two schemes are examined here. The first combines the four components of the vaccine after the initial phase 1 trial (combination vaccine shown with banding). The second combines the vaccine only after each component is shown to be efficacious. Parallel phase 1 testing takes less time than sequential testing, but requires more resources. Early combination takes fewer resources in field studies, but may require separate studies to show that all components are required (not shown on time line).

Organization, US Agency for International Development (USAID) and the US Department of Defense. Several sites in endemic areas such as The Gambia, Ghana, Kenya, Mali, Mozambique, Papua New Guinea and Tanzania are suitable for vaccine testing.

A potential capacity for vaccine testing has been created through the development of the African Malaria Vaccine Testing Network and its programme of training Africans in clinical trial capability. In addition, many endemic countries are making significant contributions from their scarce resources through the support of staff and facilities for malaria research in general and for vaccines in particular. Despite these developments, progress remains limited by resources, and this limitation will become more acute as vaccine testing programmes gain momentum. Put into perspective, the public support for development of malaria vaccines is roughly tenfold less than the support for a vaccine for HIV — a disease of comparable global impact.

In this setting, several groups have phase 1/2 testing programmes either underway, or have clinical grade immunogens prepared with phase 1 trials commencing in the near future. These developments are summarized in Box 3.

When will there be malaria vaccines?

On the assumption that at least some of these vaccines will be efficacious, when can we expect the vaccine? Even under the most optimistic scheme of unlimited resources (Fig. 2), it will still be many years from now, requiring iterative testing of improved combinations and formulations until sufficient efficacy is obtained. In the meantime there is much to do to ensure that when the vaccines are available, health infrastructures are in place to deliver integrated

programmes that will use the vaccines effectively. That may be as much of a challenge as the vaccine itself. □

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