Review

Progress in the development of recombinant and synthetic blood-stage malaria vaccines

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Summary

The use of asexual blood-stage proteins as malaria vaccines is strongly supported by experimental data directly implicating antibodies induced by these antigens in parasite clearance and protection from re-challenge. The selection of blood-stage antigens is based on their ability to interfere with the pathogenesis of clinical malaria by reducing parasitemias. These vaccines could complement other vaccines aimed at preventing infection, such as those targeted at pre-erythrocytic or mosquito stages of the parasite. Asexual blood-stage vaccines may reduce disease by blockade of red blood cell invasion, inhibition of parasite growth in red cells or interference in cytoadherence of infected red cells. Clearance of blood-stage parasites is dependent primarily on antibody-mediated mechanisms, but CD4 T cells may also play an important role in help for B cells and probably have a direct effector function in the clearance of blood-stage parasites. Since asexual blood-stage parasites reside within erythrocytes, they are accessible to immune clearance mechanisms only for a short time, which imposes special requirements on vaccines. For example, immunity that induces high titers of antibody will be required. Antigenic variation and extensive polymorphism of malarial proteins also needs to be addressed. Several recombinant antigens derived from blood-stage proteins have moved beyond basic research and are now poised for phase I trials in endemic countries. In this review we discuss the state of asexual blood-stage vaccines, focusing on recombinant antigens from *Plasmodium falciparum*. The significance of polymorphism and antigenic variation, the relevance of parasite immune evasion mechanisms, the need for reliable measures of successful intervention and new adjuvants are reviewed. Results from trials of asexual blood stage vaccine that support the continued effort to develop these antigens as key ingredients of multi-component, multistage malaria vaccines are documented.

Key words: malaria, vaccine, blood stage, recombinant antigen, *Plasmodium falciparum*, clinical trials, antigenic polymorphism.

Introduction

Vaccines are one of the most cost-effective and logistically feasible means of disease control and have remarkable success in the control of many infectious diseases (Andre, 2003). Notable examples are the eradication of smallpox, the virtual eradication of polio, and the striking reduction in the prevalence of measles in the western hemisphere (Bonanni, 1999). Although vaccines for malaria will be more complicated than anti-viral vaccines because of the complex biology of the parasite, extensive antigenic polymorphism, distinct immune-evasion strategies and the ecology of malaria vectors, there is accumulating evidence to suggest that anti-malaria vaccines can be developed successfully (Good et al., 1998; Richie and Saul, 2002). Epidemiological data and modeling studies indicate that vaccines would reduce the morbidity and mortality from malaria and have a significant impact on impoverished regions of the world (Sachs and Malaney, 2002).

Successful vaccination against the malaria parasite entails overcoming special hurdles, because the parasite can establish a chronic infection within an immunocompetent host. Although repeated natural infections confer immunity against severe disease, this immunity is normally species/strain specific, dependent on continuous boosting by regular infections, and usually short-lived. Thus the vaccine is required to generate 'super-natural' immunity. Additionally, different stages of the parasite express a different repertoire of antigens, and many proteins of the parasite exhibit remarkable polymorphisms. Host genetic determinants, for example, specific polymorphisms in immune response genes (Hill, 1999; Troye-Blomberg, 2002), may profoundly influence the immunity towards individual antigens

Despite these challenges, there are several lines of evidence supporting the feasibility of a vaccine: the age-related acquisition of immunity against severe clinical malaria in endemic regions (Bull et al., 1998; Bull and Marsh, 2002); the

ability of passively transferred antibodies from immune adults to protect against natural and challenge infections with *P. falciparum* (Bouharoun-Tayoun et al., 1990; Cohen et al., 1961; McGregor, 1964); induction of protective immunity with defined antigens in animal models (reviewed in Kumar et al., 2002); in experimental models, the ability of adoptive transfer of B cells from immune donors into B cell-deficient mice to clear parasites (von der Honarvar et al., 1996); and the demonstrated reduction of parasite density in the only Phase 2b trial to date, that of a recombinant asexual blood-stage vaccine (Genton et al., 2002; Saul et al., 1999).

Blood-stage vaccines against *Plasmodium falciparum* are aimed at preventing complications of disease, such as cerebral malaria or anemia. Both *P. falciparum* and *P. vivax* can cause severe anemia, but only *P. falciparum* causes the many complications of cerebral malaria, hypoglycaemia, metabolic acidosis and respiratory distress. *P. falciparum* is responsible for the great majority of deaths and, for this reason, most effort has been devoted to *P. falciparum* vaccines. Therefore, we have limited this discussion to these vaccines.

Many strategies have been tested, including recombinant proteins, synthetic peptides, DNA with or without prime-boost strategies, and progress made in some of these areas are reviewed in other articles in this issue. In this review we focus on progress made in the development of *P. falciparum* blood-stage antigens in the form of recombinant or synthetic protein vaccines.

Immunology and pathogenesis of the asexual blood stages of *P. falciparum*

An examination of the mechanisms of merozoite invasion of the erythrocyte reveals immune evasion strategies employed by the parasite and potential targets for vaccines. Invasion of erythrocytes follows a well-defined and rapid sequence of events at the red blood cell (RBC) surface. First, the merozoite interacts with the RBC surface and re-orients the apical region towards the membrane (Aikawa et al., 1978; Dvorak et al., 1975). The contents of the apical organelles (rhoptries and micronemes) are expelled and a moving junction is formed between the merozoite and RBC membrane. Finally, the parasite enters a vacuole formed by invagination of the RBC membrane and membranes secreted from the parasite. This process is completed in a matter of minutes. Three organelles are present on the invasive (apical) end of the parasite (rhoptries, micronemes and dense granules). Receptors that mediate invasion of RBCs by merozoites and invasion of liver by sporozoites are found in micronemes, on the cell surface, and in rhoptries, and accumulating evidence suggests that these molecules are the major targets of neutralizing immune responses (Miller et al., 2002).

The location of these receptors within organelles provides some protection to the parasite from antibody-mediated neutralization, since exposure to antibody occurs only between release from one RBC and invasion of another. This provides a short window of opportunity for antibodies to bind and neutralize merozoites. Once inside RBC, some parasite molecules make their way to the RBC surface, while others remain in membranous vacuoles within the cell (Miller et al., 2002). Since the RBC lacks Class I and Class II major histocompatibility complex (MHC) antigens, the blood-stage parasites are also protected from effector T-cell responses, which are dependent on Class I and II antigen-presentation pathways. Only after rupture of the RBC at the ring stage are the antigens internal to the parasite directly exposed to antibodies.

Immunity against blood-stage antigens is thought to involve both antibodies and T cells although, in humans, better evidence exists for antibody-mediated immunity (Good et al., 1998). Epidemiological studies in malaria-endemic regions support the notion that antibody responses to blood-stage parasites contribute to naturally acquired immunity. Sera from humans living in hyperendemic regions contain antibodies that prevent red cell invasion by targeting antigens on merozoites (Bull and Marsh, 2002). Direct evidence for a protective function of antibodies comes from passive transfer of purified immunoglobulins from 'naturally immune' individuals into partially immune children, which was found to produce rapid clearance of parasites in recipient children even when the antibodies did not block growth *in vitro* (McGregor, 1964). These sera undoubtedly contained high levels of antibodies against the variant RBC surface antigen, PfEMP1, which would not kill *in vitro*, but would prevent sequestration of mature parasites *in vivo*, resulting in their splenic destruction. These results are consistent with the observed effects of anti-PfEMP1 antibodies induced in *Aotus* monkeys challenged with *P. falciparum* (Baruch et al., 1996). In humans, sera from immune individuals have high titers of antibodies against erythrocyte membrane protein 1 (PfEMP1) in addition to other surface and internal merozoite antigens, so investigators have speculated that PfEMP1 is a target for antibody-mediated immunity (Miller et al., 2002). Different domains of this erythrocyte surface-expressed antigen mediate cytoadherence of parasitized RBC through interactions with specific receptors, for example, CD36 (on endothelial cell surfaces) and chondroitin sulfate A (on placental syncytiotrophoblasts) (Miller et al., 2002). PfEMP1 is encoded by the large and diverse *var* gene family, which plays a key role in clonal antigenic variation. Immune pressure results quickly in the emergence of parasites expressing different variant genes. However, recent studies indicate that some functional domains of the molecule may induce a degree of cross-variant immunity (Baruch et al., 1997).

In addition to the direct effect of human antibody on parasites, *in vitro* incubation of infected erythrocytes with IgG from immune human donors and monocytes from a naïve donor kills the parasite within the RBC. This antibody-dependent cellular inhibition (ADCI) associated killing of the parasite is mediated by transferable soluble factors in macrophage supernatants (Bouharoun-Tayoun et al., 1995). Cytophilic antibodies to merozoite antigens have been implicated *in vitro* in assays with human macrophages and *in*

vivo in SCID mice (Badell et al., 2000). ADCI may provide an alternative or complementary strategy for antibody-mediated parasite clearance following vaccination.

Independent of antibody, T-cell lines and clones can adoptively transfer protection, suggesting that CD4 T cells can control blood-stage parasites (Amante and Good, 1997; Brake et al., 1988). Studies in animal models have also implicated T-cell-mediated (or antibody-independent) mechanisms in immunity against blood-stage malaria parasites (for a review, see Wipasa et al., 2002). These studies have shown that clones (Brake et al., 1988) and polyclonal populations of T cells (Amante and Good, 1997) can adoptively transfer protection, apparently in the absence antibodies, suggesting that CD4 T cells can control blood-stage parasites. Speculation continues about the mechanisms underlying the CD4 T-cell-mediated control of parasites. Cytokines and other direct effector mechanisms like nitric oxide and $\gamma\delta$ T cells have been implicated in parasite clearance by a number of studies (Hirunpetcharat et al., 1999; Seixas et al., 2002). However, conclusions from rodent studies should be extended to human malaria cautiously, because there are clear differences in the relative importance of antibodies and T-cell effector function for parasite clearance between different rodent malaria species and hosts. While most of the evidence for involvement of T cells has come from murine studies, recent data from humans showing that repeated low grade infection induces sterile immunity in the absence of detectable antibodies suggests that T-cell-mediated protection also operates in humans (Pombo et al., 2002).

Recent investigations have yielded new insights into the role of T cells and innate immunity in clearance of blood-stage parasites (Perlmann and Troye-Blomberg, 2002). Evidence comes from experiments demonstrating that infection with some parasite species in rodents can lead to anergy and apoptosis of parasite-specific CD4 T cells (but not cells of other specificities) (Xu et al., 2002). This could be an additional parasite strategy to inhibit host immunity. Other mechanisms of immune impairment by the malaria parasite have also been described, such as inhibition of dendritic cell maturation and activation by the parasite (Urban et al., 2001).

Vaccine candidates currently under development

Most blood-stage vaccines have focused on antigens responsible for red cell invasion, antigens that are either expressed on or associated with the surface of the merozoite (e.g. MSP1, MSP2, MSP3 and GLURP) or in apical organelles (e.g. RAP1/2, AMA1 and EBA175). The design of these vaccines employs a combination of mechanisms to ameliorate clinical disease, including direct attack on erythrocyte-stage parasites, blockade of erythrocyte invasion, inhibition of cytoadherence, induction of antibody-dependent cellular inhibition (ADCI) and possibly, neutralization of malarial toxins (Schofield, 2002). The recent recognition of the importance of T-cell help in the generation of long-term antibody responses should spur the development of vaccine strategies that enhance cellular immunity (Good, 2001). Although immunity induced by antigens expressed in asexual blood stages of the parasite does not achieve complete clearance of the parasite, or sterile immunity (Hoffman et al., 1987), repeated infections and the associated re-exposure to these antigens are likely to provide a natural boost to vaccine-specific immunity. Unlike vaccines for pre-erythrocytic stages, the goal of erythrocytic (asexual blood stage) vaccines would, therefore, be to either reduce the parasite load by preventing invasion of red cells or parasite replication/growth after invasion or to prevent clinically apparent complications, such as cerebral malaria. In other words, asexual blood-stage vaccines would act as a disease-ameliorating vaccine (Moorthy and Hill, 2002).

Among the recombinant blood-stage antigens that have been proposed for development as candidate vaccines, the leading asexual (erythrocyte) stage antigens, merozoite surface protein 1 and apical membrane antigen 1 (AMA1) are expressed in all species of *Plasmodium*, with homologues in rodent and simian parasites, thus making it possible to test these vaccines in animal models, although the models are often imperfect representations of human infections (Anders and Saul, 2000). Several other candidate antigens that are expressed during the erythrocytic stage of *P. falciparum* have also been tested in animal models (rodent or non-human primates challenged after immunization with asexual blood-stage parasites) and a number of these are at various stages of development as vaccine antigens, either alone or as components of multi-antigen vaccines (Table 1).

Clinical trials of recombinant vaccines against asexual blood-stage malaria

A meta-analysis of malaria vaccines trials to date noted that 18 trials incorporating 10 971 participants aged between 1 month and 86 years have been conducted with pre-erythrocyte and blood-stage vaccines (Graves and Gelbrand, 2003). Merozoite surface proteins are a major focus of research for blood-stage vaccines and the favored candidates for future trials are MSP1 (in the form of $MSP1_{19}$ or $MSP1_{42}$) and AMA1. Other merozoite proteins (MSP3, MSP4, MSP5 and RAP2 and GLURP) are at an earlier stage along the vaccine development path. Many phase I or II clinical trials in humans have been performed using blood-stage antigens either alone or in combination with pre-erythrocytic antigens (Graves and Gelbrand, 2003). The most extensive experience in humans has been with SPf66, a combination of erythrocytic antigens (Patarroyo et al., 1987) that has shown mixed results, making it difficult to justify more trials. Among vaccines based on blood-stage antigens, one that has moved on to phase II trials is Combination B, a mixture of three recombinant blood-stage antigens, *P. falciparum* RESA and fragments of two merozoite surface proteins ($MSP1_{190L}$, consisting of the relatively conserved blocks 3 and 4 of MSP1 fused with a universal T-cell epitope derived from the circumsporozoite protein of *P. falciparum*, and the near full-length MSP2 sequence of the 3D7

Table 1. *Asexual blood-stage antigens under development as vaccines*

Antigen	Localization and processing	Function	Effect of gene disruption/deletion[1]	Best evidence of protection	Level of vaccine development	Conclusions from human trials	Selected references
MSP1	Merozoite surface; cleaved into four fragments during schizogeny; final cleavage during invasion	Involved in inter-merozoite and merozoite–RBC interactions	Lethal; cross-species complementation possible between *P. falciparum* and *P. chabaudi*	Protection from challenge in NHP	Phase I/II (MSP1$_{190L}$) Phase I (MSP1$_{42}$)	Reduction in parasite density; responsible component not identified No data available	Genton et al., 2002; Holder, 1996; Stowers and Carter, 2001
AMA1	Micronemes; move to surface membrane of merozoite	Unknown	Lethal	Protection from challenge in NHP	Phase I	No data available	Deans, 1984; Hodder et al., 2001; Stowers et al., 2002
SERA	Parasitophorous space; binds to merozoite and schizont surface	Papain-like enzyme with presumed protease activity	Lethal	Vaccine trials in rodents and *Aotus*	Unknown	Not published	Aoki et al., 2002; Inselburg et al., 1993
MSP2	Merozoite surface	Unknown	Not reported	Protection from challenge in NHP	Phase II	Reduction in parasite density; apparent selection pressure on MSP2 suggested a role in protection	Genton et al., 2002; Graves and Gelbrand, 2003
MSP3	Merozoite surface	Unknown	Decreased invasion; decreased expression of ABRA	Protection from challenge in rodents and NHP ADCI	Phase I	No data available	Hisaeda et al., 2002; Oeuvray et al., 1994a
MSP4/5	Merozoite surface	Unknown	Not reported	Protection in rodents	Unknown	No data available	Kedzierski et al., 2002
EBA-175	Microneme	RBC binding *via* glycophorin A	Stable switching to alternative invasion pathway	Protection from challenge in NHP	Trials anticipated	Not applicable	Jones et al., 2001; Sim et al., 1994
RESA	Microneme	Unknown	Not reported	Protection from challenge in NHP	Phase I/II	Reduction in parasite density; responsible component not identified	Berzins et al., 1991; Collins et al., 1986; Genton et al., 2000
RAP1 and RAP2	Rhoptry	Unknown	Knockout of RAP1 survives; RAP2 knockout is lethal	Partial protection from challenge in NHP	Unknown	Not applicable	Collins et al., 2000; Ridley et al., 1990
GLURP	Parasitophorous vacuole; binds to merozoite surface	Unknown	Not reported	ADCI	Unknown	No data available	Oeuvray et al., 2000; Theisen et al., 2001
pfEMP1	Red cell surface	Binding to RBC, endothelial cells and syncytio-trophoblasts	Viable	Vaccine trial in monkeys (using CIDR)	Requires proof of principle	Not applicable	Baruch et al., 2002; Leech et al., 1984

[1]Data from Cowman et al. (2002) and publications cited therein.
NHP, non-human primates; ADCI, antibody-dependent cellular inhibition; RBC, red blood cells; ABRA, acidic–basic repeat antigen; CIDR, cysteine-rich interdomain region; HLA, human leucocyte antigen.

cloned line) formulated in an oil-based adjuvant. This vaccine has undergone five human trials, including a phase IIa/b trial, and although there was no reduction in episodes of clinical malaria, a significant reduction in parasite density was observed in the vaccines (Genton et al., 2002). Fortunately, the bottleneck in the production of clinical grade material for other blood-stage antigens, a major impediment to vaccine development, is resolving and a slate of new antigens are on the verge of clinical trials.

A phase I trial of a vaccine based on $MSP1_{19}$ (FVO and 3D7 strains of *P. falciparum*) fused to CD4 T-cell epitopes from tetanus toxoid concluded that the vaccines were immunogenic but had a sufficiently high rate of adverse reactions to warrant alternative formulations (Keitel et al., 1999). $MSP1_{42}$ formulated in ASO2 went into phase I trials in the US and Kenya (Heppner et al., 2001; Lee et al., 2002; Stoute et al., 1998) and *E. coli*-produced $MSP1_{42}$ and RTS,S combined with $MSP1_{42}$, in the United States (Gordon et al., 1995). Other phase I and II trials are planned for vaccines based on the C terminus of MSP1. An AMA1 vaccine comprising two allelic forms (clones 3D7 and FVO) is in phase I testing. A chimeric molecule that includes $MSP1_{19}$ and AMA1 is likely to be tested soon in China (reviewed in Genton and Corradin, 2002). Thus, it is likely that several recombinant blood-stage vaccines will undergo safety and immunogenicity studies in malaria-endemic regions within the next year.

Challenges for the development of recombinant blood-stage vaccines

Assays to predict protection

Animal malarias, such as those of mice and non-human primates, do not provide good models for human malaria for the purpose of investigating immunity induced by the asexual blood stage, and it is not possible to perform experimental human challenge with blood-stage parasites when assessing the protective efficacy of blood-stage vaccines. Therefore, there has been great interest in developing *in vitro* tests of immunity with the ability to predict protection after immunization with blood-stage antigens. This is a key issue in testing the immunogenicity and efficacy of blood-stage vaccines for malaria. Assays that have been used to test the immunogenicity of antigens include ELISA antibody titers, growth inhibition assays (GIA), and in some laboratories, ADCI activity (Druilhe and Bouharoun-Tayoun, 2002). While high ELISA antibody levels against pre-erythrocyte antigens have been found to correlate with protection (Egan et al., 1993), the relationship of these measures to protection has not been defined for blood-stage antigens. Similarly, *in vitro* measures of parasite growth inhibition have not been conclusively demonstrated to reflect the ability to kill the parasite *in vivo*. Correlation of the ADCI assay with immune status has yielded inconsistent results – some investigations support the idea that ADCI reflects *in vivo* mechanisms of antibody-mediated protection (Bouharoun-Tayoun et al., 1990; Druilhe and Bouharoun-Tayoun, 2002; Shi et al., 1999) and others have failed to confirm this concept

(Rzepczyk et al., 1988). As more antigens move towards efficacy testing in phase II trials, it will be useful to identify good correlates of protective immunity, to aid in the decision making for moving candidates along the development path.

Formulations and immunogenicity of malaria vaccines

The short time of exposure of the merozoites to antibodies between release from one infected RBC and attachment/entry into another means that very high antibody levels are necessary to block entry (Saul, 1987), levels that may not be achieved with alum. Therefore, considerable effort is being devoted to the testing and development of new adjuvants for asexual blood-stage antigens (Daly and Long, 1996; Hui and Hashimoto, 1998). The adjuvant formulation has two functions: enhancing the delivery of antigens to the immune system, e.g. by generating a depot of antigen and direct antigen-independent stimulation, often through pattern-recognition receptors, such as Toll receptors (Cox and Coulter, 1997).

Substances that have been used as adjuvants include aluminium salts, water-in-oil emulsions, oil-in-water emulsions, immune-stimulating complexes (ISCOMs), liposomes, saponin, bacterial toxins and recombinant cytokines. However, there is considerable variability in the immune-enhancing effects of a given adjuvant, depending on the antigen and experimental model. Thus, it is difficult to predict the efficacy of an adjuvant in humans based on experimental animal models. Furthermore, the incidence of severe or serious adverse reactions to an adjuvant is unpredictable – often the most immunogenic adjuvants are also the most reactogenic, limiting the choice of acceptable antigen-adjuvant combinations (Daly and Long, 1996; Gordon, 1993). Ideally, therefore, many combinations should be tested in human phase I trials. The choice of adjuvants available for use in humans include alum, MF59, Montanide ISA720, Montanide ISA51 in combination with MPL, QS21 and CpG as immunostimulators. However, the only adjuvants with a track record in humans are alum and MF59. ASO2 that was used in the RTS,S vaccine (Bojang et al., 2001), an MF59-like oil-in-water adjuvant containing MPL and QS21, is also being used with MSP1.

There is a dire need for a single-platform formulation, usable with a number of antigens, because the immunogenicity of each antigen in humans is strongly influenced by the formulation used. However, when an antigen formulated with a new adjuvant goes to field trials, a difficult choice often has to be made between enhanced immunogenicity and the risk of late adverse reactions, because the late appearance of serious reactions can stop a promising vaccine program. The identification of appropriate formulations with low risks of adverse reactions, important as it is now, will become vitally important when multiple antigens are deployed in multistage combination vaccines.

Antigenic polymorphism and development of vaccines

An attractive approach in dealing with the problem of antigenic variation is to include multiple antigens in the

vaccine. Multivalency in the design of blood-stage vaccines offers several advantages. First, each antigen may induce protection independent of the others included in the vaccine. The additive effect of the immunity induced by each component may result in substantial immunity, even if each antigen is insufficient on its own. Second, mixtures of antigens will help induce immunity in genetically heterogeneous populations (e.g. because of polymorphisms in HLA, or other genetic traits); this is particularly true for genetically restricted T-cell responses. Third, combining antigens may facilitate the development of a single vaccine that protects against more than one species of malaria. Additionally, the emergence of parasites that escape vaccine-induced immunity is much less likely with a multivalent vaccine than with a single-component vaccine. However, mixtures of antigen carry the risk of interference between antigens. Mixtures may also increase the likelihood of local reactogenicity, which may increase as the amount of antigen in the vaccine increases.

ADCI may provide a means of generating cross-strain protection, since macrophages activated by the antibodies to one variant may be able to kill parasites in RBC infected by other strains or variants, thus providing density-dependent control of mixed infections, provided the key antigens from at least one isolate in the mixture are recognized by the appropriate antibody. Additionally, since the concentration of IgG required for ADCI may be lower than those needed for blockade of merozoite invasion, this mechanism of parasite inhibition may still be useful for antigens that are not highly immunogenic. Merozoite antigens that have been demonstrated to have ADCI activity include MSP3 (Oeuvray et al., 1994b), SERA (Aoki et al., 2002) and GLURP (Theisen et al., 2001). Since ADCI, once initiated, appears not to be strictly dependent on continuing antigen–antibody interactions, parasite killing *via* ADCI can continue after merozoite invasion and entry into the RBC, and is not restricted to a small time-window in the same manner as direct antibody-mediated blockade of invasion. The exploitation of ADCI-mediated clearance of the parasite may be a useful strategy for vaccines against blood stages.

Antibody-independent mechanisms may also be useful for blood-stage vaccines. Rodent studies showed that CD4 T cells can control parasite growth in an antibody-independent manner (Hirunpetcharat et al., 1999; Weidanz et al., 1988), and human T cells demonstrate parasite growth inhibition *in vitro* by production of nitric oxide and other small reactive molecules (Good and Doolan, 1999). In humans, repeated infections with low doses of blood-stage parasites followed by drug cure resulted in resistance to re-challenge, despite the lack of development of antibodies against the parasite or RBC surface (Pombo et al., 2002). This suggests that T cells may also play a role in resistance against malaria in humans, as has been observed in mouse malaria with *P. chaubaudi*/*P. vinckei* (Hirunpetcharat et al., 1999; Xu et al., 2002). However, there are practical hurdles to cross before vaccines relying on T-cell mechanisms can be developed. For example, selection of epitopes is not straightforward since animal models may lack

the correct T-cell host specificities. Furthermore, the T-cell responses generated in the mouse models are pro-inflammatory, often leading to death of the mice from immunopathology, and this needs to be resolved before human trials could commence.

The need for more clinical trials

The large number of blood-stage antigens identified for development and the timely selection of antigens for multicomponent vaccines requires testing of multiple formulations in phase I and phase II trials. Since the first multicomponent vaccine with a blood-stage antigen, SPf66 (Patarroyo et al., 1988), 13 more human phase II trials have been published, 11 using SPf66 (Greenwood and Alonso, 2002). The numbers of human clinical trials ongoing at present are too few to allow rapid progress in the selection of the best antigens. The capacity for testing vaccines in endemic regions is being expanded by efforts such as the development of the African Malaria Vaccine Testing Network/African Malaria Network and programs for training African scientists in clinical trials. However, progress will become limited by resources, and several fundamental issues pertinent to clinical trials remain unresolved. These include identification of clinical and laboratory measures of malaria-specific morbidity and of clinical protection, the need for better infrastructure to conduct these studies, and the necessity of large study populations (thousands of subjects in phase III trials) when assessing the impact of these vaccines on population-level morbidity and mortality (Kwiatkowski and Marsh, 1997). Factorial and clustered designs may help in reducing the operational difficulties of large study groups and thereby aid the feasibility of clinical trials (Greenwood and Alonso, 2002), but the continuing investment of national and international funding agencies will be critical for the malaria research community to overcome these challenges.

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