REVIEW ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2005 ISSN 1595-689X VOL 6 NO 2
AJCEM/2004047/2523

http://www.ajol.info/journals/ajcem

PROSPECT AND PROGRESS OF MALARIA VACCINE DEVELOPMENT

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Malaria kills one child every 30 seconds in Africa. The development of a safe vaccine remains an urgent unmet need which could greatly control and even lead to the eradication of the disease. The success recorded in the recent vaccine trials have given some ray of hope that a safe and effective vaccine against malaria will soon be produced. In this article, we bring together important published information on the status of *malaria vaccine development* and reviewed some field trials and the obstacles as well as prospect for effective malaria vaccination.

Keywords: Malaria vaccine, prospect, review

INTRODUCTION

Malaria causes more deaths than any other parasitic disease and is probably the most important pathogen for children under 5 years of age in sub-Saharan Africa. It is estimated that every 30 seconds one child in Africa dies from malaria (1). About 4 billion people in approximately 90 different countries are at risk of developing the disease and up to 500 million cases of malaria occur each year (2). Most disease and deaths are due to *Plasmodium falciparum*, although *P. vivax* is important throughout Asia and Latin America (3).

One strategy that could prove very effective in the control of malaria but is taking some time to come to fruition is a malaria vaccine (3). Four species of malaria infect humans, which raise an initial question of how many vaccines are needed (4). Although practical consideration of both development and production favour a single vaccine for *P. falciparum*, the different risk groups and vaccine requirements have generated at least three approaches for this species alone (4). An anti-infection vaccine aimed at protecting malaria-naive travelers 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 vaccine from one person to another (5).

Successful vaccination against the malaria parasite entails overcoming special hurdles, because the parasite can establish a chronic infection within an immunocompetent host. Host genetic determinants, for example, specific polymorphisms in immune response genes, may profoundly influence the immunity towards individual antigens. (6). Despite these challenges, there are several lines of evidence supporting the feasibility of a vaccine.

TYPES OF MALARIA VACCINE

Pre-erythrocyte vaccines

A pre-erythrocytic vaccine would either prevent invasion of hepatocytes by sporozoites or kill parasites within infected

hepatocytes (2). They are designed to target sporozoites or schizont-infected liver cells and thus prevent the release of primary merozoites from infected hepatocytes (7). Research has also focused on the liver stage; a synthetic peptide representing region I bound specifically to hepatoma cells in vitro and antibodies to the region inhibited invasion of hepatoma cells by sporozoites (8). Thus a sequence in the CSP has been identified as a binding ligand and is being examined along with a second sporozoite surface protein (SSP2), which has sequence homologous to region II in CSP (9).

Naked DNA is also being developed as a delivery strategy for malaria as well as other diseases and may be particularly useful as a means to induce $CD8^+$ T cells (10). In 1994 protection from malaria in mouse model was demonstrated with a DNA vaccine encoding *P. yoelii* (11).

i. RTS, S/AS02A

Currently, this pre-erythrocytic stage vaccine is based on the CS protein of the 3D7 clone of *P. falciparum* and it is the malaria vaccine candidate for which clinical development is most advanced. (7). While many candidate malaria vaccines have been developed over the past 25 years, the RTS,S/AS02A vaccine is the first that has demonstrated a significant capability to protect human adult volunteers against an experimental infection with the malaria parasite (7).

The recent results also indicated that the vaccine induces protection against malaria in children one to four years old in Africa. Although the 57.7% reported vaccine efficacy against severe disease is less than that of classical childhood vaccines, which is often greater than 80%, the outcome of the trial is very encouraging for the future of malaria vaccines because it is the first demonstration of any efficacy against severe malaria in children (12).

ii. ICC-1132 CS/hepatitis B core particle

This is a CS-based particle vaccine that uses the highly immunogenic hepatitis B core antigen (HBcAg) as a delivery platform (13). Three Phase 1 clinical trials in healthy, malaria-naive adults are underway. Phase 1 trials in the United Kingdom and Germany have shown the vaccine to be safe and well tolerated (7).

iii. Pf CS 282-383 long synthetic peptide

This clinical grade vaccines based on CS and other protein were produced as a result of advances in the manufacturing of long synthetic polypeptides antigens (7). Based upon promising pre-clinical studies in mice and *Aotus* monkeys immunized with formulations alum or Montanide ISA 720, the vaccine formulations were well tolerated and induced antibodies that reacted with intact sporozoites, especially in the volunteers that received the Montanide ISA 720 formulation. A new dose and adjuvant finding Phase 1 study of Pf CS 282-303 formulated with GSKBio AS02A , or Montanide ISA 720 adjuvants is currently underway (7).

iv. Multi-epitope TRAP

Investigators constructed this novel vaccines consisting of plasmid DNA or recombinant attenuated live viral vectors (14). Trial studies have been conducted in healthy malaria-naive adults in the United Kingdom to identify prime-boost vaccination regimens that optimize the cellular-mediated immune response and generate protective immune responses (7). Some of the tested regimens provided a prolongation of pre-

patent period, while other regimens also completely protected a portion of volunteers against heterologous strain sporozoite challenge. The safety of these vaccine candidates was investigated in adults and children in The Gambia (2).

v. Plasmid DNA vaccines

Investigators have developed a vaccine platform that uses combinations of plasmid DNA vaccines that permit targeting of one or more *P. falciparum* antigens (15). The Phase 1 study of the CS construct when injected as a single vaccine showed it to be safe and well tolerated, but poorly immunogenic for antibody production (none detected), while most subjects developed cell-mediated immune responses, including antigen-specific cytolytic T cell responses and $CD8^+$ ELISPOT IFN- production (16).

vi. Peptide CS vaccine for *P. vivax*

This vaccine is composed of three long peptides representing different fragments of the central and flanking regions of the common type *P. vivax* CS protein (17). A Phase 1 clinical trial in 69 malaria-naive healthy adult showed the vaccines were safe and well tolerated. Immune responses are being evaluated to assist in the design of Phase 2 (9).

Blood stage vaccines

A second strategy for the development of a malaria vaccine is to target immune responses against the asexual stage (blood stage) of the parasite. Effective vaccination with a blood stage vaccine would either prevent invasion of red cells or prevent complications, such as cerebral malaria, anaemia, renal failure and all the manifestations of severe malaria in pregnancy (18).

These complications, which occur only in *P. falciparum* infection, are likely due to cytoadherence of PfEMP-1 expressed on the surface of infected red cells, to CD36 or intercellular adhesion molecule-1 (ICAM-1) on microvascular endothelial cells or chondroitin sulphate A or other glycosaminoglycans in the placenta (19). Although PfEMP1, which was cloned in 1995 (20), is the prime target for an 'anti-complication' vaccine, creating such a vaccine is a very difficult challenge due to its extraordinary variation and clonal switching, the characterization of which has now begun (21).

The principal target of current asexual stage vaccine development is the merozoite, the stage that is initially released from the infected hepatocyte and rapidly invades and replicates in circulating red blood cells (9). Erythrocyte invasion is an energy-dependent, active process that requires the merozoite to contact, adhere, and orient to the red blood cell membrane. During invasion, the membranes fuse, thereby permitting the parasite to become internalised without otherwise damaging the red blood cell (7).

This complex process is rapid (taking only seconds) and involves a number of parasite proteins that are located on the surface of the merozoite and that are thus transiently accessible to circulating antibodies (8). The most well studied antigens include merozoite surface protein 1 (MSP-1), MSP-2, MSP-3, and apical membrane antigen 1 (AMA-1). Antibodies to these molecules are reported to block invasion of merozoites, except for MSP-3, in which they trigger a monocyte-mediated effect. MSP-1, AMA-1, and MSP-3 have been

produced as candidate malaria vaccines and have been shown to protect non-human primates from uncontrolled asexual stage parasitaemia when administered with Freund's complete adjuvant (9).

i. Merozoite surface protein

Investigators have produced a C-terminal *P. falciparum* MSP-1 vaccine as a lyophilized recombinant antigen expressed in *Escherichia coli* (7). The safety and immunogenicity of the vaccine has been assessed in two clinical trials conducted in malaria-naive individuals and has been successfully completed, the vaccine is scheduled to begin Phase 1 studies in Kenyan children 1–4 years old living in a malaria-endemic region of western Kenya (22). A phase I trial in Washington DC produced sero-conversion in 9 out of 16 volunteers immunised with the higher dose, but with hypersensitivity problems after the third dose (23).

ii. Apical Membrane Protein (AMA-1)

While AMA-1 is an attractive candidate antigen, there is considerable antigenic polymorphism that could limit its impact in the field (7). Data from animal studies indicate that antisera raised against one form of the molecule that efficiently block the growth of the homologous parasite are less efficient at blocking the growth of heterologous parasites. This has led to the production or clinical grade antigens from two variants of the *P. falciparum* AMA-1: AMA-1(3D7) and AMA-1(FVO) (24).

iii. AMA-1/MSP-1 chimera

This combination contains conserved portions of both AMA-1 and MSP-1 proteins in an attempt to avoid the problems associated with sequence polymorphism (25). A Phase 1 trial in malaria-naive healthy adults is underway with a Montanide ISA 720 formulation (7).

iv. Merozoite surface protein III

A remarkable feature of this vaccine is the full conservation of the MSP-III critical epitopes. These epitopes were selected on the basis of data indicating that they were targets of human cytophilic antibodies that interact with monocytes to mediate antibody-dependent cell-mediated parasite killing (26). A Phase 1 dose ranging study was recently completed and showed that the formulations were safe; well tolerated with alum, and mildly reactogenic with Montanide ISA 720 (7).

v. Glutamate-rich long synthetic peptide

Glutamate-Rich Protein (GLURP) is a 128- amino acid synthetic peptide vaccine produced using the partial sequence from GLURP (7). A Phase 1 trial in normal healthy malaria-naive volunteers has been recently concluded using vaccine formulated with alum and Montanide ISA720. Local reactogenicity was noted with both preparations including swelling at the contralateral injection site with subsequent injections (7).

vi. SPf66

This is the only malaria vaccine to undergo extensive field trials in Latin America, Africa, and Southeast Asia. It a synthetic peptide cocktail developed in Colombia (27). In field trials, the vaccine, under conditions of high as well as low malaria transmission, gave partial protection in some studies and none in others (28). An analysis of all published trials to date in various age groups and epidemiologies concluded that SPf66 had a combined efficacy of 23% (29).

Transmission-blocking vaccines

Preclinical studies conducted over the past decade have clearly demonstrated that antibodies directed against several sexual stage antigens are capable of preventing the development of infectious sporozoites in the salivary glands of *Anopheles* mosquitoes, thereby suggesting that so-called transmission blocking vaccines (TBV) might be an effective weapon against malaria (7).

More than half a dozen prefertilization target antigens have been discovered some notable ones include Pf230, Pf48/45, and Pf11.1 (9). Studies have suggested that antibodies to Pfs230 may be complement dependent, and therefore inclusion of such an antigen in a vaccine may require an appropriate adjuvant to induce antibodies of the right isotype (9). A caveat is that in *P. vivax, transmission*-blocking antibodies, produced as a result of natural infection, may enhance transmission when present in low dilution (30).

i. Plasmodium vivax s25 and P. falciparum s25

The purification and characterization of this candidate vaccine has just recently been achieved. (7). A formulation adjuvanted with aluminum hydroxide is currently in a Phase 1 clinical trial. Similar preclinical data have been obtained with Pfs25 expressed in *P. pastoris*, setting the stage for a Phase 1 trial with this vaccine candidate (7).

Multistage, multivalent vaccines

There is a good case that malaria vaccines should be multistage and multivalent. As already discussed, a vaccine directed against one stage, if not fully effective, has no activity against later stages in the life cycle, hence, a malaria vaccine is expected to be more effective if it contains antigens against more than one stage of the parasite.

i. NYVAC-Pf7

This is a genetically engineered, attenuated vaccinia virus, multistage, multicomponent *P. falciparum* vaccine that includes the transmission-blocking Pfs25; the pre-erythrocytic antigens CSP, SSP2/TRAP, and liver-stage antigen 1; and the asexual blood-stage antigens MSP-1, AMA-1, and SERA. (9) This recombinant vaccine has been through phase I-IIa safety and efficacy studies, but the results in terms of protection were disappointing.(31).

OBSTACLES TO VACCINE DEVELOPMENT

Antigenic variation, diversity, and immune evasion represent one of the greatest hurdles to the development of both natural immunity to malaria and an effective subunit vaccine. (3). It is known, that a monoclonal antibody specific for one allelic variant of *P. yoelii*, MSP-119, does not recognize other variants (32). One vaccine strategy to overcome this obstacle would be to combine all the known allelic sequences together in a vaccine. The success of such a strategy would depend on how many epitopes would need to be combined and whether the immunogenicity of the individual epitopes would be preserved when mixed with many other epitopes. Such strategies are being attempted for malaria as well as for other pathogens (3).

The parasite protein on the erythrocyte surface (PfEMP1) performs the critical function of binding the parasite to the endothelium to protect the infected erythrocyte from sequestration in the spleen (19). Epidemiological data have demonstrated that anti P*f*EMP – 1 antibodies

provide protection against disease (33). However despite this apparent role in the development of antimalarial immunity, the use of P*f*EMP – 1 in vaccine development is hampered by the extensive polymorphism in the var gene family (34).

PROSPECTS

The foregoing gloomy picture of malaria and the armory of methods to combat its ravages, has led to conclusion that vaccination against *P. falciparum* and *P. vivax* is the method of intervention with the greatest potential to reduce the morbidity and mortality associated with severe malaria in areas of intense transmission (9). As with antimalarials, there is little commercial incentive for industry to invest in development of vaccines against any human parasite, including malaria (4). A malaria vaccine represents a major challenge, even with unlimited resources to devote to the task.

To have a large impact, a vaccine must be cheap, safe effective and easy to administer. The slow progress to date is an indication of the magnitude of the task and the lack of industrial interest to invest in development of vaccines against any human parasites, including malaria (9). In the decades after 1950, several dominant ideas set the stage for vaccine research. First it was argued that antibody was the mechanism of immunity, but now both cellular and humoral immunity, are now known to control the immune response and that both play critical roles. Another major development was the ability to culture *P. falciparum* parasite in vitro, making malaria research possible in any laboratory in the world.

Over the past five years, there has been increased funding from public-private partnerships and the result has been encouraging. There has been a remarkable increase in the quality and number of candidate vaccines entering clinical trials and considerable optimism that major progress is being made. Indeed, there are currently three vaccine candidates (RTS, S/AS02A, MVA-ME TRAP, and MSP-1/AS02A) being studied. The recent success recorded in the phase 2b testing of RTS, S/AS02A among 1-4 year old children in Maputo Province, southern Mozambique is a welcome development (12). There is increasing involvement on the part of industry that brings crucial pre-clinical experience, manufacturing know-how, and clinical development capabilities that will significantly increase the chances of success.

The not-so-good news is that the investigators in developing countries where the disease is ravaging are not with strong clinical and experimental development skills. More importantly, there are clearly insufficient financial and personnel resources identified to date to support the complete development of the most promising candidate vaccines. Indeed, the clinical development of one such candidate that underwent promising field studies in Papua New Guinea was recently abandoned largely due to lack of sufficient financial and industrial resources (35). The way forward will require that researchers will continue to refine target product profiles to be very clear on where and how the vaccines will be used and that there be greater public-private partnership involvement in these discussions.

Malaria vaccine development is at an exciting and very promising stage; it is essential that the momentum achieved to date be sustained in the years ahead.

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