THE QUEST FOR A MALARIA VACCINE: CLEARING THE AIR

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ABSTRACT
Malaria has continued to be a life-threatening scourge predominantly in sub-Saharan Africa. Attempts to eradicate it in the past as failed and it is now re-emerging, at a very fast rate, in areas hitherto not known to inhabit the parasite. 40 % of the global population is at risk of malaria, with around 300 million persons, mainly children of 6 months to 5 years, suffering from malaria yearly. This situation is made possible by loop-holes in global malaria control programme; excruciating poverty; population movements; conflicts; environmental and climate changes. Malaria parasites are becoming resistant to drugs and mosquitoes are showing resistance to chemical means of control. Recognizing the failures of past initiatives, and in the light of emerging data on the potentials of vaccines in a sustained attack on malaria, malaria vaccine is increasingly being embraced as a solution to the menace of malaria. Presently, no malaria vaccine is licensed, but with funding from the Wellcome Trust in the U.K., and from the U.S., as well as grants from the Bill & Melinda Gates Foundation, research has reached a heightened peak and vaccine candidate, targeted at one or more stages of the Malaria parasite's life-cycle and could interfere successfully with infection, and parasitemia in the host or vector, or even block the manifestations of clinical disease, are currently in different trial stages and leading to a successful development of a vaccine for malaria. In reviewing the different malaria vaccine candidates presently under development, we can conclude, based on data on percentage efficacy, that the RTS, S/AS02A - a pre-erythrocytic vaccine remains the leading candidate towards our search for a malaria vaccine.

Keywords: Malaria parasites, Malaria vaccine, Mosquitoes, RTS,S/AS02A Vaccine.

INTRODUCTION
Malaria is a parasitic mosquito-borne disease transmitted to man through the bite of infected female Anopheles mosquitoes. More than 40 species is known (Weller, 2003). Other documented routes of transfer of parasite to man are through blood transfusion; sharing of needles (Kitchen and Chiodini, 2006; Raffenot *et al*. 1999), and through congenital means (from mother to her unborn infant before or during delivery) (de Pontual *et al.* 2006). However, the disease that results from such a transfer of the parasite into man is termed 'malaria', (Ross, 1898**;** Mueller, 2007**).**

Malaria is an ancient scourge of humanity and continues to claim lives in substantial part of the world which includes malaria endemic areas and in areas with imported malaria. Children between the ages of 6 months to 5 years are most affected in sub-Saharan Africa (Breman, 2001). The initial successes of chloroquine as an anti-malarial drug led the World Health Organization (WHO) to launch a plan in 1955 of eradicating malaria and early successes in some areas of the globe were dramatic, with malaria reduced to very low levels in certain countries (Wernsdorfer, 1980). However, in the face of practical constraints, loop-holes in anti-malaria programmes; excruciating poverty; regional conflicts; health sys-

tem failures in many countries of sub-Saharan Africa as well as migration and worsening environmental changes, the campaign began to lose force (Aylward *et al.,* 2000; Sachs, 2002; WHO Factsheet No 94, 2007). Malaria was soon to be re-established (Sharma, 1996). Today, malaria has not only returned to areas where it was once controlled, but has entered new areas, and is known to cause as many as 10% of all deaths in children in these areas (Mehta, 2007).

With the failure of in treatment and eradication of the disease due mainly to evolving of drug-resistant parasites, a malaria vaccine becomes imperative.

LIFE CYCLE OF MALARIA PARASITE – *PLASMODIUM FALCIPARUM.*

When an infected man is bitten by a mosquito, the mosquito sucks up blood and also takes in the gametocytes form of the blood-borne parasite (Fig. 1). One week after this bite, the gametocytes-form of the parasite is transforms into sporozoites **(1)** (inside the mosquito). When the mosquito bites another man, the sporozoites, mixes with the mosquito's saliva and is injected into this next person. Inside this next bitten victim, the sporozoites are carried to the liver where they infest the hepatocytes **(2)** and mature into hepatic schizonts **(3)**. This stage is termed the Exo-erythrocytic cycle **(A)** (Marsh, 1999).

When infected hepatocytes ruptures and schizonts are released into the blood (4), they infest the erythrocytes **(5)** and transforms into immature trophozoites within the erythrocytes. Further maturation turns it into mature trophozoites or gametocytes **(7)**. The mature trophozoites develop into erythrocytic schizonts inside the erythrocytes. The erythrocytes ruptures and schizonts are released to cause the fever that is associated with malaria illnesses. Severe anaemia, respiratory distress, altered consciousness, seizures, hypoglycaemia, cardiac and renal failure and even death are possible at this stage (Marsh, 1999). The erythrocytic schizonts are also capable of re-infecting other erythrocytes at this stage **(6)**. The gametocytes develop into male (microgametocytes) and female (macrogametocytes) **(7)**. This is the Erythrocytic cycle **(B)** (CDC, 2006)**.**

When a mosquito bites an infected man, it sucks up microgametocytes and macrogametocytes **(8)** commencing the sporogonic cycle **(C)**. Inside the mosquito stomach, microgametocyte penetrates the macrogametocytes **(9)** and generates zygotes which become motile and elongated to form an **ookinetes (10)**. These invade the mosquito's mid-gut wall and develop into oocytes **(11)**. These oocytes develop further and rupture, releasing sporozoites **(12)** which migrate to the mosquito's salivary glands. These sporozoites are passed onto any one bitten by the mosquito **(1)** perpetuating the malaria parasite's life cycle (CDC, 2006).

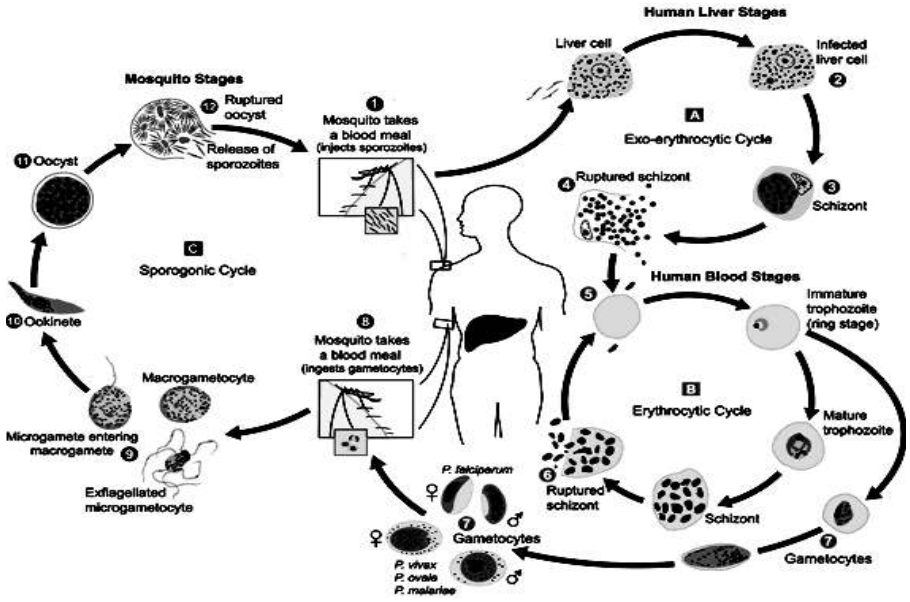

Fig 1: Schema of the life cycle of malaria.

Source: Centre for Disease Control and Prevention. (2006). Available at http://www.cdc.gov/malaria/

The epidemiology of malaria differs geographically. Malaria causes a huge health problem in Africa, Asia, Central America, Oceania, and South America. About 40% of the world's population lives in malaria endemic areas (WHO Factsheet No 94, 2007) (Fig1). About 300-500 million cases of malaria is recorded yearly, resulting in 1-2 million deaths (most of which are young children). Most deaths resulting from malaria occur in children younger than 5 years. Older children and adults also suffer from mild malaria, which may progress to severe malaria, especially when there are predisposing factors. Pregnancy, especially in primigravidae, causes major changes to maternal immune system, most marked in the feto-maternal placental unit. Malaria parasites can massively adhere to the placental syncitiotrophoblast through specific receptor-ligand interactions (Rogerson *et al.,* 2007). Consequences for mother and baby can be dramatic leading to maternal mortality, miscarriage, low birth weight or foetal death.

P. vivax shows a very wide geographical range. It is prevalent in temperate, sub-tropics as well as in the tropics. However, *P. falciparum* remains the commonest species in the tropics and subtropics. *P. malariae* is patchily present in the same areas as *P. falciparum*, but much less common. Whereas *P. ovale* is found mainly in tropical Africa, and less commonly in West Pacific.

Malaria endemicity indicates the level of malaria in a given region, whereas malaria ***epidemic*** refers to periodic or sharp increase in levels of malaria. The presence of peripheral parasitemia has been used to determine the degree of malaria endemicity in certain community. Such information is put together by random community-based samples. WHO, in the 1950s, suggested the use of spleen rates (% of children with enlarged spleen) as a proxy of malaria endemicity, and based on both the parasite and spleen rates, malaria endemicity can be grouped into hypoendemic, mesoendemic, hyperendemic and holoendemic (Table 1).

Fig 2**. Malaria endemic areas of the world, 2001**
Source: http://www.malaria.am/eng/docs/malaria/epidemiology/epidemiology.gif

Table 1. Classification of endemicity

Type	Spleen rates	Parasite rates	Description
Hypoendemicity	<= 10% of children aged 2-9 years	<= 10% of children aged 2-9 years but may be higher for part of the year	Areas with little transmission and the effects, upon the general population is unimportant during the average year,
Mesoendemicity	11-50% of children aged 2-9 years	11-50% of children aged 2-9 years	Found typically in rural communities of subtropical zones. Wide geographical variations in transmission risk exist here.
Hyperendemicity	Constantly > 50% in children aged 2-9 years; also high in adults (>25%)	Constantly > 50% among children aged 2-9	These are areas with seasonal intense transmission; immunity is however insufficient in all age groups
Holoendemicity	Constantly > 75% in children aged 2-9 years, but low in adults	Constantly > 75% among infants aged 0-11 months	There is intense transmission which results in considerable level of immunity outside early childhood

Source: http://www.malaria.am/eng/docs/malaria/epidemiology/epidemiology.gif

EVOLUTIONARY RESILIENCE OF PLASMODIUM FALCIPARUM

Plasmodium falciparum has demonstrated the capability, through the development for multiple drug-resistance parasites, of evolutionary change. This enables pharmaceutical treatments that are effective in reducing the reproduction rate but not halting it to exert a high selection pressure, thus favouring the development of resistance. The process of evolutionary change is one of the key considerations necessary when considering potential vaccine candidates. The development of resistance could cause a significant reduction in efficacy of any potential vaccine thus rendering useless a carefully developed and effective treatment.

The use of synthetic anti-malaria drugs in the 20th century altered the *P. falciparum* species through the selection of drug-resistant strains. Chloroquine resistance in nearly all malaria-infected-regions has since been linked to mutations in the *P. falciparum* protein PfCRT, a molecule that functions as a transporter in the parasite's digestive vacuole membrane (Fidock *et al.,* 2000). Studies on PfCRT mutations have shown that chloroquine resistance started in four distinct geographic foci (Wellems and Plowe, 2001; Wootton *et al.,* 2002). The period between the appearance of resistance in *P. falciparum* and in *P. vivax* is thought to be in-line with genetic discoveries that revealed different mechanisms of drug resistance in the two species (Nomura *et al.,* 2001).

In *P. falciparum* malaria, it is known that parasites evade immune destruction by altering antigenic and adhesive properties of infected erythrocytes. This is due to expression and export of effector proteins such as PfEMP1s, Maurer's cleft proteins and FIKK kinases, containing a conserved protein export motif, called a VTS or PEXEL motif into the

host cell (Baruch *et al.,* 1995; Marti *et al.,* 2004; Bhattacharjee *et al.,* 2006 Winzeler, 2008). After infection, malaria parasites form a parasitophorous vacuole in host cells and afterwards, a tubovesicular network to promote protein trafficking. Trafficking is likely to go in both directions, with both import of nutrients and export of proteins involved in immune evasion occurring. In erythrocytic stages of the life cycle parasites establishes a tubovesicular network within the erythrocyte and exports effector proteins across the parasitophorous vacuole (PV) into the cytoplasm of the infected erythrocyte (Marti *et al.,* 2004; Bhattacharjee *et al.,* 2006). Some of these proteins, such as PfEMP1s, eventually reach the surface of infected erythrocyte where they have a role in antigenic variation and immune evasion.

In the liver stages of a malaria infection, circumsporozoite protein (CSP) (an abundant surface protein associated with pre-erythrocytic phases of parasite) and potentially other effector proteins may pass through the cytoplasm and eventually reach the nucleus (N). These proteins interfere with import of nuclear factor-κB (Kumar *et al.,* 2006), a protein needed for activation of the human immune response, and may have other roles controlling hepatocyte physiology.

A transient over-expression of CSP in HeLa cells may have effect on host transcription, down-regulating many genes associated with immune signalling and up-regulating other genes associated with cell adhesion and also apoptosis (Kumar *et al.,* 2006). Parasites can grow for many days in the liver without triggering apoptosis or inflammatory response. Once matured in the hepatocytes, and ready to be released, the infected hepatocytes 'round up', losing their adherence property (Taylor *et al.,* 2006). Active immune modulation also occurs in parasitic infections, such as *schistosomiasis*, and is likely to be a sign of a successful pathogen. Indeed, a secreted protein kinase affecting gene expression has recently been identified as a major virulence factor in *Toxoplasma gondii*, a closely related apicomplexan parasite (Saeij *et al.,* 2007; Baniecki *et al.,* 2007).

In individual parasites, PfEMP1 is an exclusively expressed product from one of about 50 different *var* genes within the genome (Su et al., 1995; Chen *et al.,* 1998; Scherf *et al.,* 1998). Parasites spontaneously changes expression from one *var* gene to another, producing different PfEMP1 molecules and altering the antigenic properties of infected cells (Smith *et al.,* 1995). Such changes gives rise to anti-genetically diverse sub-populations that continually challenges the human immune system (Newbold, 1999). In field populations, vast numbers of *var* gene repertoires are present among different parasites (Kyes *et al.,* 1997). The capacity for generating new antigenic forms is comparable to that of another human parasite, the African trypanosome (Rudenko, 1999), and represents a critical survival strategy that has evolved under continuous pressure from host defences (Freitas-Junior *et al.,* 2000). There is evidence for co-evolutionary cycles of selection and adaptation in gene-for-gene struggles between the parasite and the host. The protective effect shown by the human lymphocyte antigen (HLA) HLA-B53 type may be linked to an immune response to the *P. falciparum* liver-stage antigen LSA-1 (Hill *et al.,* 1992). Studies on immune responses to parasite LSA-1 polymorphisms shows that the prevalence of parasite polymorphisms is affected by HLA types in human populations (Plebanski *et al.,* 1999). It has also been suggested that sickle-cell traits can influence the genetic structure of parasite populations. Distributions of the *P. falciparum* genes encoding the merozoite surface proteins Msp-1 and Msp-2 have been reported to be connected with the presence of HbS trait in some regions (Ntoumi *et al.,* 1997; Konate *et al.,* 1999). *P. falciparum*-infected erythrocytes are also able to adhere to the endothelial cells of microvasculature and are sequestered within tissues The parasites thus avoid passing through the spleen thereby avoiding destruction.

TYPES OF TREATMENT AVAILABLE

Malaria Deterrence Kits:

Wearing long-sleeved clothing and use of insect repellents to prevent infection.

Use of N, N-Diethyl-meta-toluamide (DEET) on skin to prevent transmission of the parasite through mosquitoes bite.

Use of bed nets treated with permethrin.

Chemoprophylatic treatment with anti-malarials in patients travelling to endemic areas.

Mosquito coils containing insecticide to keep mosquitoes away.

Insecticide sprays designed to kill mosquitoes.

MALARIA TREATMENT (MEDICATION)

Anti-malaria drugs are designed to act at different stages of the life cycle of the parasite (Fig 5). Sporontocidal drugs blocks the development of oocysts in mosquito, decreasing malaria transmission. Hypnozoitocidal drugs are designed to kill dormant hypnozoites in the liver, preventing the relaps of infection. Tissue schizonticidal drugs affect early stages of parasite the development in liver, before the release of merozoites into the blood. Blood schizonticidal drugs kills parasites in erythrocyte, ending clinical attacks, and finally, Gametocytocidal drugs are designed to destroy sexual forms of the parasite in human, decreasing transmission.

Choosing a form of treatment for malaria is usually influenced by the type of species causing the infection. While some species rarely may exhibit chloroquine resistance (e.g. *P. vivax*), others may have a hypnozoite stage eradicated only by primaquine or tafenoquine. In all, the parasite sensitivity must be considered. It has been established that *P. falciparum* has evolved multi-drug resistance in many regions of the world. Also to be considered in the choice of drug, is the host's degree of immunity. There are classes of drug acceptable for treatment of "semi-immune" patients, but unacceptable for non-immune patients. The risk-benefit of the drug adverse effects and treatment benefits must always be made before initiating therapy.

P. falciparum drug resistance is a common occurrence in malaria endemic areas such as Africa, therefore, standard anti-malarias such as chloroquine and antifolates (sulfadoxine-pyrimethamine) have become ineffective. This increasing prevalence of drug resistance and a high likelihood of resistance development to new agents have led to the development of combination therapy as a standard for treating *P falciparum* infection worldwide. Artemisinins, a new class of anti-malaria agent, belongs to these newly forms of regimens. Most are available in most African countries, however, combination drugs such as the Artemisinins Combination Therapies (ACT) (Artesunate and amodiaquine (Coarsucam and ASAQ); Artemether and lumefantrine (Coartem Riamet, Amatem and Lonart); Artesunate and sulfadoxine/pyrimethamine (Ariplus and Amalar plus); Dihydroartemisinin-piperaquine (Duo-Cotecxin, Artekin); and Pyronaridine and artesunate (Pyramax) remain highly efficacious (WHO, 2010).

THE FUTURE ROLE OF VACCINES

With data showing an increasing mortality and morbidity, the imperative for a malaria vaccine becomes intense. The global problem associated with malaria is ever increasing due to evolving drug-resistant and insecticide-resistant. The disease is now re-emerging in regions that were previously safe. The world is becoming less well prepared to handle the malaria scourge. The current morbidity and mortality patterns are set to continue unless an effective control method are developed. The increasing weight on the public health infrastructure and economy of malaria endemic regions has long been a cause for concern, making alternatives treatment options and prevention strategies a research priority. It is therefore hoped that a successful malaria vaccine would help reduce and eventually eradicate the disease caused by malaria globally.

POTENTIAL TARGETS OF VACCINES

Parasites have been known to be more complex organisms than bacteria and viruses. Despite the large number of vaccines presently available, there are none designed to target parasitic infections. This, coupled with the unique life-cycle of the malaria parasite, is creating developmental hurdles. It should be noted however that theoretically, each developmental stage could have a vaccine developed specifically to target the parasite at that stage.

The first stage in the life cycle, following a mosquito bite is a relatively short "pre-erythrocytic" or "hepatic" phase. A potential vaccine for this stage must be able to protect against sporozoites invasion and also be able

to block the development of parasites in the liver by inducing the production of cytotoxic T-lymphocytes capable of destroying infected liver cells.

The second is termed the "erythrocytic" or blood phase. A vaccine for this stage should be able to block merozoite multiplication or invasion of the red blood cells. The lack of MHC molecule expression on the surface of erythrocytes has however complicated this means. Another means could be to try to inhibit processes leading to erythrocyte adherence to blood vessel walls.

The last phase of the life cycle with a potential for target by a vaccine is the "sexual stage". While this stage would not provide any protection to individuals bitten by mosquitoes, it however has the potential to stop further transmission of parasite by preventing gametocytes from producing multiple sporozoites in the mosquito gut wall. It therefore could be employed at eliminating the parasite from regions of low prevalence or to block the development and spread of vaccine-resistant parasites. This type of transmission-blocking vaccine could be potentially very important.

PRE-ERYTHROCYTIC" or "HEPATIC" PHASE (SPOROZOITE)

Antibodies that block hepatocyte invasion
Antibodies that kill the sporozoite via complement fixation or opsonization

Infected hepatocyte

CTL mediated lysis
CD4+ help for the activation and differentiation of CTL
Localized cytokine release by T cells or APCs

ASEXUAL ERYTHROCYTIC PHASE

- Localized cytokine release that directly kills infected erythrocyte or intracellular parasite
- Antibodies that agglutinate the merozoites before schizont rupture
- Antibodies that block merozoite invasion of RBCs
- Antibodies that kill iRBC via opsonization or phagocytotic mechanisms
- Antibodies engulfed with the merozoite at time of invasion which kill intra-erythrocytic parasite

SEXUAL ERYTHROCYTIC PHASE (Transmission blocking)

Antibodies that interfere with fertilization
Antibodies that inhibit transformation of the zygote into the ookinete
Antibodies that block the egress of the ookinete from the mosquito midgut (Doolan and Hoffman, 1997)

This section provides a review of *P. falciparum* malaria vaccine candidates that are presently in human clinical development, and selected candidates in preclinical development.

MALARIA VACCINE CANDIDATES CURRENTLY IN CLINICAL DEVELOPMENT

PRE-ERYTHROCYTIC STAGE VACCINES

The possibility of immunization against malaria with pre-erythrocytic vaccine was first shown experimentally through the demonstration of protection against malaria infection by irradiated sporozoites (Nussenzweig *et al.,* 1969; Clyde, 1975; Rieckmann *et al.,* 1979). Immunization with irradiated sporozoites induced both humoral and cellular immune responses which reduced the hepatocytes infectivity and eradicated parasite-infected hepatocytes (Hoffman *et al.,* 2002). The ultimate aim of pre-erythrocytic vaccines is to prevent the initiation of blood-stage infection. However, non-sterile protection could occur and recent data shows that the immune response could reduce the burden of merozoite release from the liver (Bejon et al 2005), meaning a delayed initiation of blood-stage infection. This delay could empower the immune system to better control the blood-stage infection (Alonso *et al.,* 2005).

The CS antigen has been shown to be the main target of protective immunity in the irradiated sporozoite immunization model (Kumar *et al.,* 2006). This 412-amino acid protein is uniformly distributed on the surface of sporozoites. CS is also found on the membrane of early liver schizonts as well as in the cytoplasm and nucleus of infected liver cells. The first description of the single copy gene coding for CS protein was done in 1984 (Enea *et al.,* 1984). Recently, investigators have shown the main role of CS in the metabolic

processes favourable to intra-hepatocytic parasite growth through intra-nuclear influence of host-cell gene expression (Singh *et al.,* 2007). This may include down regulation of NF-κB-mediated inflammation.

RTS,S/AS

RTS,S is a leading malaria vaccine candidate under development for the past 20 years by GlaxoSmithKline (GSK) Biologicals (Paediatric Clinical Development of RTS,S) in partnership with the Bill and Melinda Gates Foundation funded PATH-Malaria Vaccine Initiative (MVI), since 2001. RTS,S is a recombinant protein technology; two polypeptides (RTS and S) are co-expressed in *Saccharomyces cerevisiae.* Part of the CS protein of the 3D7 clone of *P. falciparum* is joined to the N terminus of hepatitis B surface antigen to create RTS molecule. A 19-tetrapeptide repeat motif (NANP) joined to the C-terminal portion of the protein is added to the part from the CS protein which is expressed. RTS and S spontaneously assemble to create virus-like particles within the yeast cell, (Vreden *et al.,* 1991).

Phase I/IIa sporozoite studies in humans showed that the vaccine's efficacy was dependent on the formulation. This was demonstrated by adjuvanting RTS,S with AS02 (Stewart *et al.,* 2006). Studies conducted at the Walter Reed Army Institute of Research (WRAIR) proved the RTS,S/AS02 vaccine to have completely protected about 40% of participants against sporozoite challenge. The RTS,S/AS02 combination produced a high level of anti-CS and anti-HBs antibodies, which activated IFN-γ-producing T cells. However, immunological readouts did not identify a clear pattern of protection, even though there was a consistent association link between higher (vs lower) IgG and CD4 T-cell responses to CS. It was soon suggested that persons with high titres of anti-CS antibodies seem better protected than persons with low antibodies titre (Aponte *et al.,* 2007). While a study of co-administering RTS,S with a DTPw/Hib EPI vaccine is going on in Tanzania, there was however a parallel clinical study at WRAIR showing that another adjuvant, AS01, could further potentiate the RTS,S-conferred protection. Compared to AS02, the AS01 adjuvant produced higher levels of anti-CS antibodies as well as CS-specific activated T cells. There was a correlation of a higher protection, following a sporozoite challenge with the AS01 adjuvant even though the safety profile is similar for both (Polhemus, 2006; Lell, 2007). Plans aimed at integrating RTS,S/AS01 in EPI recently began, with immunology studies to plan out necessary immunization schedules and doses.

Multiple Epitope, Thrombospondin-related Adhesion Protein-, CS- & LSA1-Expressing Fowl pox 9 & Modified Vaccine Ankara

Investigators at the University of Oxford are exploring strategies of heterologous prime-boost vaccines. A multi-epitopes (ME) string (containing B-cell, CD4 and CD8 T-cell epitopes derived from six sporozoite and/or LSAs, including CS, LSA-1 and LSA-3, joined to the thrombospondin-related adhesion protein (TRAP; T9/96 strain) serves as the candidate antigen), and is delivered as a DNA plasmid or is expressed by recombinant attenuated viruses – fowl pox strain (FP9) or modified vaccine Ankara (MVA).

This antigen has been shown to produce high T-cell responses, as well as delayed parasitemia and, in few persons, complete protection in sporozoite challenge studies (Moorthy *et al.,* 2003; Webster *et al.,* 2005). Even then, human adult studies failed to demonstrate protection against malaria.

MVA and FP9 polyprotein constructs in combination with CS and LSA1 antigens are being researched at the University of Oxford in conjunction with the European Malaria Vaccine Initiative (EMVI) and Wellcome Trust support.

Liver-stage Antigen-1

Liver-stage antigen-1 is a pre-erythrocytic antigen expressed during the liver stage of *P. falciparum* infection. Research on this is hindered by the fact that there is no identified homolog in murine or simian plasmodia, however, data from malaria-endemic regions and from vaccination with irradiated sporozoites (Kurtis *et al.,* 2001), revealed a link between levels of anti-LSA1 IgG and ma-

laria protection.

WRAIR in collaboration with GSK and MVI are developing a LSA1-based vaccine candidate (designated FMP011). Testing this vaccine in combination with AS02 and AS01 adjuvant systems in Phase I/IIa trials showed good safety profile and was immunogenic, however, that did not link-up with protection against infection or a delay in parasitemia (Epstein *et al.,* 2007).

Liver-stage antigen-3

Liver-stage antigen-3 was identified by screening a DNA library clone coding for pre-erythrocytic proteins gotten from humans and monkeys immunized with irradiated sporozoites (Daubersies *et al.,* 2000). Partial protection against sporozoite was revealed in monkeys and mice (Daubersies et al., 2000; Sauzet *et al.,* 2001). The Pasteur Institute in Paris (France) is presently working on this candidate vaccine in different ways which include producing long synthetic peptides and a lipopeptide formulation.

ERYTHROCYTIC STAGE VACCINES

Blood-stage Antigens

Interest is presently pointing toward the possibility of a vaccine against the blood stage form of malaria parasite. Immunity against clinical malaria acquired over periods of natural exposure in malaria-endemic countries is a pointer to this, even though protection is quite short lived (Gupta *et al.,* 1999). Cell-mediated mechanisms of protection are also thought to be important (Pombo *et al.,* 2002).

The merozoites are the main target of any anti-blood-stage vaccine. Indeed, the merozoites are exposed to blood immune factors during its short, extracellular passage into the erythrocytes expressing several surface proteins as well as several enabling it to evade the body's immunity. On the erythrocyte surface are blood-stage parasite antigens, but the hyper-variable nature of these surface proteins makes antigen selection difficult. The high level of polymorphic variability; the redundant nature of several blood-stage proteins; and the conformation-dependant nature of the antigens make it difficult to develop.

Merozoite Surface Protein-1

Both blood- and liver-stage merozoites express merozoite surface protein-1 (MSP1). Epidemiological studies suggest that naturally acquired anti-bodies targeted against MSP1 plays a role in the age-related development of protective immunity in malaria-infested regions, making this antigen a potential vaccine candidate (John *et al.,* 2004). Obstacles to the development of anti-MSP1 vaccine include sequence polymorphism, however, candidate anti-MSP1 vaccines targeting the 42-kDa MSP1 fragment and the 19-kDa MSP1 fragments are in preclinical development currently.

MSP1-42

The Infectious Diseases (NIAID) at NIH and the Malaria Vaccine Development Branch (MVDB) of the National Institute for Allergy has produced two 42-kDa MSP1-based malaria vaccine candidates. The first from the 3D7 clone and the other from the FVO clone. Anti-bodies gotten from testing with the 3D7- and FVO-MSP1-42 showed the induction of antibodies against conserved antigen epitopes and to be cross-reactive by IFA (Epstein *et al.,* 2007). The vaccine was well tolerated and produced antibodies that recognized both native parasite-protein antigens contained in the vaccine, but it failed to show significant impact during *in vitro* parasite growth inhibition (Withers *et al.,* 2007).

Apical Membrane Antigen-1

Apical membrane antigen-1 (AMA1) is a type 1 integral membrane protein, synthesized late in merozoite development. It is initially stored in micronemes (secretory organelles in the merozoite apex) before it is redistributed on the merozoite surface prior to erythrocyte and hepatocytes invasion, AMA1 is possibly linked to hepatocytes invasion (Silvie et al., 2004). AMA1-based vaccine candidates would therefore have the potential of being non-stage specific.

Apical membrane antigen-1 has been shown to be protective. Inhibitory anti-AMA1 antibodies can block merozoite entry into erythrocytes *in vitro,* however, blocking can be strain specific. Clusters of polymorphisms surrounding conserved, putatively functional,

hydrophobic region have been shown, suggesting AMA-1 as a protective antibody target (Pizarro *et al.,* 2005). In spite of successful animal model trials, the very many antigenic variants constitute a problem in human vaccine development.

AMA1-C1

Investigators at MVDB have produced a combination candidate vaccine called AMA1-C1, made from equal quantities of two variants of AMA1 from 3D7 and from FVO clones. Both are expressed in *Pichia pastoris,* and mixed together (Stowers *et al.,* 2002). Phase I studies of AMA1-C1 adjuvanted with aluminium hydroxide has been conducted in adults. An adequate safety profile was demonstrated however, only moderate levels of GIA blocking were observed.

Combination vaccines

RTS,S-based Combination Vaccines

Aimed at improving the efficacy of the RTS,S-based vaccines, RTS,S/AS02 combined to prior PfCSP DNA vaccination or CS-expressing adenovirus 35 or followed by CS-expressing live MVA is been studied. A multistage, multi-antigen recombinant vaccine based on RTS,S and MSP-1 from the 3D7 strain is under investigation at WRAIR (Heppner *et al.,* 2005).

PEV 301, PEV 302

A candidate malaria vaccine based on synthetic peptides displayed on surfaces of reconstituted influenza virosomes is been studied by the Molecular Immunology unit of the Swiss Tropical Institute in cooperation with Pevion Biotech Ltd and the Institute for Organic Chemistry (University of Zürich, Switzerland) . Currently, two candidates, one comprising a CS-like sequence (PEV 301), and another, an AMA1 like sequence (PEV 302), (Mueller *et al.,* 2003) are been evaluated. The aim of this combination is to try and combine different antigenic peptides and develop a multi-antigen, multistage vaccine.

A Phase I and Phase lla study was conducted at the University Hospital of Basel and the Centre for Clinical Vaccines and Tropical Medicine, at Oxford University respectively. A rapid age de-escalation Phase Ib study is planned for Mali.

Combination B: MSP1, MSP2 & RESA Combination

A vaccine candidate combining MSP-1, MSP-2 and the ring-stage infected erythrocyte surface antigen (RESA) adjuvanted with Montanide has been developed. In Phase IIa challenge study, the combination vaccine could not produce a decrease or delay in parasitemia, however, a later phase I/IIb study in children in Papua New Guinea revealed decreased parasite density in some volunteers compared with controls (Genton *et al.,* 2002), However, this was mainly restricted to parasites that expressed the 3D7 allelic form of MSP-2. This is the first blood-stage vaccine to show an appreciable vaccine efficacy. Subsequent vaccine candidates will include the opposite dimorphic form of MSP-2, FC27.

Vaccine against pregnancy-associated malaria

The ability of parasite-infected erythrocytes to cling to vascular endothelium directly correlates with the severity of the malaria. This interaction is facilitated by the PfEMP1 protein family, encoded by the *var* gene family. The *var* genes, coding for highly variable PfEMP1 proteins demonstrates a uniquely high degree of polymorphism. This is known to contribute to parasite's pathogenicity, particularly marked in placental malaria where one particular variant of PfEMP1 called VAR2CSA, is involved in parasite sequestration in the placenta bed through interaction with placental chondroitin sulfate A (CSA) (Chen *et al.,* 2004). Multigravidae women are able to acquire anti-PfEMP1 immunity through successive pregnancies and are able to control pregnancy-associated malaria.

A candidate vaccine based on PfEMP1 antigens for the prevention of pregnancy-associated malaria is been developed. However, the multi-variable nature of this antigen becomes a challenge for it to be developed.

GAMETOCYTE STAGE VACCINES

Transmission-blocking Vaccines

Antibodies directed at the gametocyte stage of the parasite have been shown to occur in humans, but its ability to reduce transmission is

unclear (Graves *et al.,* 1990). Investigating this would involve feeding a mosquito with mixture of infective gametocytes incubated with serum from someone vaccinated. A post -fertilization antigens Pvs25 (*P. vivax*), and Pfs25 (*P. falciparum*), expressed as a recombinant protein in *S. cerevisiae,* are currently under evaluation. An analog of Pfs25 (TBV25H) adjuvanted with aluminum hydroxide, was shown to be immunogenic, however, it only could cause a sub-optimal degree of transmission-blocking activity when tested in man (Malkin *et al.,* 2005).

DISCUSSION

There is yet to be a licensed malaria vaccine, but many vaccine candidates are currently being studied, with each aimed at one or more potential targets that could be successful employed to block the infection of a host or vector; or prevent the manifestations of clinical aspect of the infection by down-regulating parasitaemia.

The ideal malaria vaccine should be cheap, extremely safe, induce life-long immunity, be active against all strains of the parasite and result in nearly complete interruption of the malaria life cycle by vaccine-induced immune responses (Tarantola *et al.,* 2007). Unfortunately, the hope of creating a malaria vaccine of that kind remains elusive.

Present malaria vaccine developmental strategies are directed at reducing the risk of infection and blocking or reducing the release of merozoite from the liver; controlling the erythrocytic merozoite reproduction cycle to reduce malaria transmission (Moorthy *et al.,* 2007; Malaria vaccine technology roadmap 2006).

It is also important to define the correlates of vaccine-induced protection which would potentiate vaccine development efforts and enable studies of important immunological readouts instead of parasitological or clinical end points. Plans aimed at characterizing the protective immunological responses demonstrated how important the role of humoral and T-cell responses in pre-erythrocytic models and in the blood stage (Sun et al., 2003; Good *et al.,* 2005). Merozoite surface protein (MSP)1, MSP3, apical membrane antigen (AMA)1 and liver-stage antigen (LSA)1 are all very good targets for protective immunity in vaccine development, but the importance of an individual immune markers of protection in vaccine research is yet to be seen. Measuring the blocking effect of antibodies on sporozoite infectivity, which may be good for the future of vaccine research is now possible (Bouharoun-Tayoun *et al.,* 1990; Haynes *et al.,* 2002; Kumar *et al.,* 2004), even though, the advantage of this is yet to be proven by clinical studies (Giersing *et al.,* 2006).

Selecting a good system is important in developing a vaccine. A vaccine meant to target several antigens would need to be delivered to the several targets through different means so as to produce effects. The use of adjuvant or specialised delivery system is meant to direct the vaccine to the specific target. An adjuvant is generally thought of as a substance used in combination with an antigen to produce a better and robust immune response than the antigen alone can produce, and in most cases, consist of easily identifiable components of micro-organisms easily recognised by the innate immune system cells. However, due to the diverse nature of compounds with this potential on the immune system, it has been difficult to classify adjuvant into specific groups.

The efficiency of all of the malaria vaccines candidates developed to date shows that the use of an adjuvant is key in determining any protection derived against malaria. Options so far identified for use in combination with a malaria vaccine, include mycobacterial cell walls, liposomes, monophosphoryl lipid A and squalene.

CONCLUSION

The challenges facing the development of a successful malaria vaccine are still very many. These include the need to cope with the different antigenic types and the need to stimulate a life-long response that is boosted by natural infection processes. A vaccine-induced sterilising immunity that is not boosted by exposure to malaria becomes risky to an individual when eventually, the vaccine-induced immunity wanes. Presently, we do need vaccines that can be incorporated into national immunisation programs as part of a coordinated, holistic approach to malaria con-

trol.

Attempts at employing recombinant protein antigens in controlled expression system is failing as a result of the fact that many of the antigen targets for the malaria vaccine strategies are all functional proteins that are structurally complex and with their functions highly dependent on their structures. Under selective immunological pressure, genes encoding for specific functional proteins have been known to display diverse sequence variation. It has also been shown that blood-stage proteins exhibit what is termed redundancy in function which has not helped in checking merozoites entry into erythrocytes.

Use of live-attenuated viral vectors and parasites to broadly activate the immune system, have been researched upon, but we know that large-scale production, complexity and cost would be a problem. Safety in immuno-compromised patients and the risk associated with reverse mutation is also a problem to this area of research.

Developing a malaria vaccine based on the radiation- or genetically attenuated sporozoite strategy has many hurdles to overcome. Limitations of this strategy include the unavailability of an *in vitro* sporozoite culture system. The fact that this strategy also depend on human blood or animal products for its production as well as the need to manually generate sporozoites from mosquito salivary glands and cryopreservation it, makes this a cumbersome process. Except all of these can be overcome in the quest to mass produce it, it sure would continue to remain as a research option.

In the face of the bleak picture painted above, no research in malaria vaccine development will ever be considered useless considering the global threat that malaria disease poses to human. Nevertheless, recent years have been marked by important developments in the field of malaria vaccines. There have been remarkable successes with the RTS,S vaccine research. This malaria vaccine candidate keeps moving towards eventual licensing, by demonstrating very encouraging results and creating positive hope as a future malaria vaccine development.

A robust industrial partner, GSK Biologicals, has shown commitment to the project for more than 20 years and, since 2001, in the context of a public-private partnership with PATH-MVI, a new business model has emerged for vaccine development for low-profitable markets.

RTS,S efficacy will be determined by a phase lll multicenter study across various parts of Africa, and key data on its efficacy and possibly mortality and side effects would guide its implementation strategy. This we hope would drive reforms in the present EPI schedule.

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