Challenges in malaria control in Sub-Saharan Africa: the vaccine perspective

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Abstract: Malaria is a life-threatening disease of public health importance, especially in sub-Saharan Africa. It is estimated that about 500 million cases of malaria occur annually and among these 1 million die annually. Children below five years and pregnant women are the most vulnerable groups. Several malaria control measures have been applied such as environmental improvements, use of insecticide impregnated nets, residual indoor spraying, early case detection and treatment with effective antimalarial drugs. However, the adaptation of vector and parasite has so far limited the effect of these interventions. The emergence of resistance against drugs and insecticides requires in response a steady stream of new interventions. Up to the beginning of this millennium, most sub-Saharan African countries have been using chloroquine (CQ) as the first-line antimalarial drug, which had to be replaced with sulphadoxine-pyrimethamine (SP) after resistant parasites had rendered CQ ineffective. Currently the first line treatment of malaria consists of combination therapy which includes an artemisinin derivative. The current approach appears robust but history has taught us to be alert and to expect resistance to emerge. There is a pressing need to develop and deploy complimentary strategies. Adding a protective vaccine to the existing control tools for malaria holds great promise yet no malaria vaccine has ever been licensed despite a large number of attempts. The complexity of malaria parasites and the ability of the parasite to suppress and evade immune responses are formidable challenges. Fortunately, there are several promising antimalarial vaccine candidates in the development pipeline. The most promising vaccine candidate is RTSS which is currently tested in various countries in sub-Saharan Africa, including two sites in Tanzania. There is a hope that malaria vaccines could be developed and deployed in malaria endemic communities. This article highlights the challenges of developing and deploying malaria vaccines.

Key words: malaria, control, vaccine, development, challenges, Sub-Saharan Africa

Introduction

Malaria is one of the biggest global health problems especially in Sub-Saharan Africa where *Plasmodium falciparum* accounts for more than 90% of malaria burden (Breman, 2001; Roll Back Malaria, 2005). The burden caused by the disease is difficult to estimate because the majority of clinical cases are not confirmed and the malaria burden changes over time. It is generally believed that each year 300-500 million malaria cases occur globally which may result in more than 1 million deaths (Snow *et al.*, 2005). The majority of malaria related mortality is in young children residing in sub-Saharan Africa. Besides young children pregnant women especially primigravidae are at high risk for malaria with potentially damaging effects for the mother and foetus.

Patterns of malaria transmission and disease vary markedly between regions and even within individual countries. This diversity of malaria is the result of variations between malaria parasites, mosquito vectors, ecological conditions, socioeconomic status and access to effective health care and preventive services (RBM, 2005). Several epidemiological tools are used in estimating patterns of malaria transmission intensity, which include parasite prevalence, spleen rates, entomological inoculation rates (EIR) and

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serological estimates of exposure to *Plasmodium falciparum.* Malaria endemicity is classified as hypoendemic (low endemicity) if parasite rates are 0-10%, mesoendemic (moderate endemicity) if parasite rates are 10-50%, hyperendemic (high endemicity) if parasite rates are 50-75% and holoendemic (very high endemicity) if parasite rates are above 75% (White 1996).

The life cycle of malaria parasites

Five species of malaria parasites affect man, *P. falciparum, P. malariae, P. vivax, P. ovale and P. knowlesi* (Cox-Singh 2008, White 2008). For the survival of these parasites mosquitoes and human hosts are required. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Sporozoites circulate for a few minutes before they infect liver cells (hepatocytes), and mature into schizonts. Repeated nuclear divisions of the parasite result in 30000 uninucleate merozoites within 6-7 days. After this initial replication in the liver (exo- erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites invade red blood cells within few seconds. Inside the red blood cells, merozoites feed on haemoglobin and transform into the uninucleated ring stage. These trophozoites divide and mature into schizonts. Infected red cells disappear from the circulation and adhere to the endothelial cells of various internal organs, a process known as sequestration. Nuclear division continues until 16-32 merozoites are formed, break out of the cell and invade uninfected erythrocytes. In the absence of an effective immune response and drug treatment this process continues over extended periods. The emergence of the merozoites is accompanied by release of toxic substances and hence the erythrocytic stage is responsible for the fever associated with malaria. (http://www.cdc.gov/malaria/biology/life-cycle.htm).

Some parasites differentiate into sexual erythrocytic stages. If male (microgametocytes) and female (macrogametocytes) gametocytes, are ingested by an *Anopheles* mosquito during a blood meal they can undergo multiplication in the mosquito (sporogonic cycle). While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

Pathogenesis of malaria

The pathogenesis of malaria is determined by the complex interplay between virulence factors of the parasite and the defence systems of the human host within underlying behavioural factors that are influenced by geographic and socio-economic conditions (Miller *et al.*, 2002).

Besides man-made interventions, human beings can be protected against malaria through innate mechanisms and adaptive immunity. Innate mechanisms include natural immunity and genetic factors such as haemoglobinopathies (e.g. sickle cell disease, Glucose-6-Phosphate Dehydrogenase, thalassaemias) (Tripathy & Reddy 2007). Acquired immunity to malaria can be divided into anti-disease and anti-parasite immunity. Anti-disease immunity is characterised by the ability to control manifestations of malaria morbidity despite relatively high parasitaemia and usually precedes anti-parasite immunity (Riley *et al.*, 1994). Anti-parasite immunity is characterised by the ability to inhibit parasite multiplication and control parasitaemia at low densities. Partial anti-parasite immunity usually developes only after several years of endemic exposure (Good, 2005).

Parasitic factors include antigens that enable the parasite to invade uninfected red blood cells and enhance cytoadherence which can facilitate sequestration and thus evasion of destruction by the spleen. Malaria parasites within the human host parasites are under evolutionary pressure, and have to evade the immune response of the host by hiding within cells, by inducing immunosuppression or by antigenic variation (Gupta *et al.*, 1994; Marsh & Snow 1997; Kyes et al., 2001). In areas of highly seasonal transmission, the episodes of malaria occur in a short period of the year following the rainy season. In order to

survive the dry season, the parasites have to establish long lasting infections to survive for months in the human host until the following transmission season (Riley *et al.*, 1994; Elhassan et al.,1995). In holoendemic areas, transmission is stable over most of the year; the challenge to the parasite is widespread acquired immunity of the human host. The success with which *P. falciparum* evades the immune control are testified by the occurrence of continued super-infections with new parasite strains, the paucity of sterile immunity, the establishment of chronic asymptomatic parasitaemia, and the recrudescence of the parasite (Riley *et al.*, 1994). The evasion strategies include parasite-derived proteins on the surface of the infected erythrocytes, such as variant surface antigens (var genes), which have the ability to undergo antigenic variation (Anders 1991). Thus, in human host, infections can become chronic with a low-grade but relapsing parasitaemia lasting up to 3 years in *P. falciparum* (Riley *et al.*, 1994).

Merozoites released after schizont rupture have to evade the human immune response to survive for brief periods spent in the bloodstream to invade a suitable red cell. To establish an infection it is critical for the parasite population to infect a sufficient number of red cells to survive clearance in the spleen. To maximise the probability of proliferation it is equally important not to overwhelm and kill the host, yet low sufficient time to proliferate in the host in order to generate the maximal number of sexual forms (Gupta *et al.*, 1994).

The expression of antigenically different epitopes by parasite of identical genotype provides the antigenic variation which allows parasites to evade the human immune response. Another source for antigenic variation is genetic recombination in addition to mutation (Kyes *et al.*, 2001). The diversification of antigens allows parasites to infect a host exposed to parasites but naïve in regard to the variant genotype, as immunity is species-and strain-specific. Several surface antigens such as PfEMP1, RIFINS, STEVOR and SURFINS which are involved in the antigenic variation of *P. falciparum* have been indentified (Baruch *et al.*, 1995; Newbold *et al.*, 1997; Chen *et al.*, 1998; Winter *et al.*, 2005; Garcia *et al.*, 2005). To date about 5268 proteins encoding malaria parasites genes have been recognised. More variant parasite antigens are likely to be identified and characterised in the future.

Transmission intensity, disease and immunity

At high rates of transmission, malaria mainly affects children under five years (Kitua *et al.*, 1997; Ellman *et al.*, 1998; Snow & Marsh 1998). In children less than two years of age morbidity and mortality is frequently the result of severe malaria and anaemia. At lower levels of transmission, morbidity and mortality shifts to older children who present with cerebral malaria (Snow et al., 1994). At very low transmission levels, little if any immunity is acquired and all age groups are at risk (Marsh & Snow 1999). At endemicity levels too low for the development of protective immunity, there is a direct relationship between the transmission intensity and incidence of clinical malaria. In contrast at higher transmission levels only a fraction of infections result in clinical episodes (Marsh & Snow 1997).

Clinical manifestations

The clinical features of malaria are notoriously non-specific, especially in children. Infection with plasmodia can be asymptomatic or can manifest itself with a spectrum of presentations ranging from mild fever to death.

The asymtomatic malaria infection is characterised by the presence of asexual blood stage parasites in the peripheral blood in an individual without obvious symptoms of malaria. This is common in intense malaria transmission sites (Riley *et al.*, 1994; White 1996).

In non-severe (uncomplicated) malaria vague symptoms may occur before the development of acute paroxysms of high fever and chills. Other common symptoms include headache, general weakness, vomiting, diarrhoea and anaemia. These symptoms and signs of malaria are associated with the rupture of red blood cells when merozoites mature and their release into the blood stream that triggers a host immune response. The cytokines and reactive oxygen intermediates are responsible for the fever, chills, sweats, weakness, and other systemic symptoms associated with malaria

(White, 1996; Marsh, 1999). The classical presentation of malaria described as periodic fever patterns are not observed unless the illness is left untreated for many days, a situation that may happen in resource limited communities but unlikely to be encountered in research settings. More commonly malaria patients are found to have pyrexia, tachycardia, and tachypnoea. Hepatosplenomegaly is a common finding in chronically or recurrently infected individuals especially in endemic communities. Other findings may include jaundice and pallor.

The clinical attacks due to *P. falciparum* are associated not only with haemolysis of infected but also uninfected red cells. In addition the production of red blood cells is suppressed (dyserythropoiesis), contributing to anaemia as a common feature of malaria that is also aggravated by high malaria parasite densities (Menendez *et al.*, 2000).

Severe (complicated) malaria may occur in some cases. Almost all severe forms of malaria are caused by *P. falciparum*. It is rare for *P. vivax* or *P. ovale* infections to produce serious complications or death in Sub-Saharan Africa (Trampuz *et al.*, 2003). The major complications of falciparum malaria include cerebral malaria, pulmonary oedema, acute renal failure, severe anaemia and a bleeding diathesis. Acidosis and hypoglycaemia are the most common metabolic complications. Complications can develop rapidly and progress to death within hours.

Several hypotheses have been proposed to explain the pathophysiology of severe malaria complications. The leading explanation is that the sequestration of the infected RBC in the peripheral blood vessels causes reduced perfusions of the brain, lung, and kidney leading to cerebral malaria, respiratory distress syndrome and renal failure, respectively. Alternatively or perhaps additionally, inflammatory processes may play a major role in the clinical manifestations of malaria. The inflammatory mediators include glycosylphosphatidylinositol, nitric oxide and inflammatory cytokines, such as tumour necrosis factor, interferon-gamma and interleukins (IL1 and IL6). One proposed mechanism is that by up regulating the expression of endothelial adhesion molecules TNF and other pro-inflammatory cytokines promote sequestration (Kwiatkowski & Perlmann 1999). There is evidence that high TNF levels are associated with the life-threatening complications of malaria (McGuire *et al.*, 1998). Respiratory distress syndrome has been linked with lactic acidosis, which is a common cause of severe malaria and mortality (Marsh & Snow 1997). Furthermore, hypoglycaemia occurs due to increased glucose consumption accelerated by a large number of parasites and decreased availability of glycogen due to the inability of patient to eat and dysfunctional gluconeogenesis (White 1996; Marsh 1999; Greenwood *et al.*, 1991; Chen *et al.*, 2000).

Approaches to malaria diagnosis

Clinical criteria

Effective treatment of malaria cases relies on prompt and accurate diagnosis. In malaria endemic areas and travellers returning from such areas clinicians have to maintain a high index of alertness. In endemic areas without adequate laboratory facilities, all fever episodes are diagnosed as clinical malaria and treated presumptively. However, the clinical features of malaria overlap with those of other common childhood diseases. Therefore, such practice often results in over-treatment with antimalarial drugs, increased operational costs and potentially under-treatment of bacterial infections with similar presentation (Tarimo et al., 2001;Reyburn *et al.*, 2004).

Conventional light microscopy

A Giemsa stained thick and thin blood films remain the mainstay of laboratory diagnosis of malaria, since they allow to screen for presence and quantification of malaria parasites and identification of species (Hanscheid 1999). However, microscopic examination requires a laboratory setting with skilled personnel, electricity, microscopes, and an hour processing time from blood collection to reporting of results. Unfortunately, at the peripheral levels of health care systems in many malaria endemic countries, such requirements are often not met (Hanscheid 1999; Makler *et al.*, 1998).

Alternative laboratory diagnostic methods

Several alternative methods have been developed

and applied in the diagnosis of malaria. They include rapid dipstick immunoassay, fluorescent microscopy of parasite nuclei stained with acridine orange, polymerase chain reaction and flow cytometry. A variety of simple and rapid diagnostic tests have been developed for accurate and reliable malaria diagnosis in settings that lack adequate laboratory facilities. These rapid tests detect major antigens derived from malaria parasites using immunochromatographic assays and yield results within 5 to 15 minutes (Wongsrichanalai *et al.*, 2007, Murray *et al.*, 2008). The commonly targeted antigens are the histidine-rich protein 2 (HRP2) and the parasite lactate-dehydrogenase (pLDH). There are challenges to the use of rapid diagnostic tests especially in high transmission settings where the majority of residents are parasitaemic. Parasitaemic patients may be "sick with malaria in addition to other diseases" or "sick due to malaria". Since dipsticks detect the presence of antigens, individuals may remain to have positive diagnostic tests several weeks after clearance of parasites, especially if HRP2 based rapid tests are used (Swarthout *et al.*, 2007). Even if rapid malaria test results are available, clinicians tend to rely on their clinical judgement and not on test results (Reyburn *et al.*, 2007). It may not be enough to provide rapid diagnostic tests, training and experience are essential for more widespread reliance on rapid diagnostic test results.

In fluorescent microscopy, capillary filled with 50-100 microlitre blood is centrifuged and examined under fluorescent microscopy. Three setbacks have been reported: (i) high cost of capillaries and equipments, (ii) difficulties in species identification and quantification and (iii) technical problems, including broken capillaries and inability to store for future references. Another method is polymerase chain reaction (PCR) which has a lower detection threshold than even well performed Giemsa stained blood films (Brown *et al.*, 1992). Because the PCR method is more sensitive it can be useful for the detection of parasitaemia in slide negative patients. While this method holds promise for research purposes it is not used in routine clinical practice due to its cost, time requirements and reproducibility (Hanscheid & Grobusch 2002). Flow cytometry offers reliable, automated counts of parasites but this is offset by a rather low sensitivity of 0.005% (10-times inferior to Giemsa-stained film) owing to background noise caused by stained RNA in reticulocytes (Janse & Van Vianen 1994).

Malaria control measures

The entomological approaches are directed at reducing the burden of mosquito vector or contact between the human host and the vector. These approaches include: environment management (reducing breeding sites), residual insecticide spraying (e.g. pyrethroids, DDT) and use of insecticides treated materials (Mnzava *et al.*, 1993; Mabaso et al., 2004; Roberts et al., 2004; Barnes *et al.*, 2005). Recently it has been shown that use of insecticide treated nets by majority of the entire population has a community protection effect for both users and non-users (Killeen *et al.*, 2007).

Another important tool is directed to the control of parasites once they have infected the human host. These include case management with effective antimalarial drugs, presumptive treatment of infants (IPTi) or pregnant women (IPTp) (Schellenberg et al., 2001; Massaga et al., 2003; Shulman et al., 1999; Parise *et al.*, 1998; Wolfe et al., 2001).

Malaria vaccine strategies and targets

Since a significant proportion of anopheline mosquitoes have developed resistance to currently available insecticides and the life-threatening *P. falciparum* parasites have developed resistance to most of the previously effective antimalarial drugs, malaria vaccines are thought to be a possible complimentary option. Although no malaria vaccine has yet been licensed, progress has been made in the development of malaria vaccines (Greenwood & Alonso, 2002). In the current post-genomic era, an increasing number of antigens are recognised opening opportunities to develop vaccine candidates.

Convincing evidences for malaria vaccines

Accumulating evidence suggests that it should be possible to develop a vaccine against malaria (Moore *et al.*, 2002; Hoffman & Epstein 2002; Moorthy & Hill 2002) (Box 1). First, human populations residing in malaria endemic areas

acquire protective immunity against disease, (Christophers, 1924; Cohen *et al.*, 1961). Second, immunoglobulin purified from the blood of immune adults from endemic regions can passively transfer protection against *P. falciparum* (McGregor et al., 1963; Sabchareon *et al.*, 1991). Third, clinical studies carried out since the 1970's demonstrated that experimental vaccination with attenuated sporozoites can effectively immunize patients against a subsequent malaria infection (Clyde et al., 1975; Edelman *et al.*, 1993). Fourth, animal models of malaria clearly substantiate the potential for induction of protective immunity with defined vaccines (Baruch et al., 2002; Koning-Ward et al., 2003). Last but not least, recent clinical trials of the RTS,S vaccine candidate have reported significant, though limited, efficacy (Alonzo *et al.*, 1995, 2004; Bojang *et al.*, 2001).

Box 1: Evidence in favour of malaria vaccines

1. Residents of malaria endemic areas develop natural immunity to malaria
2. Immunoglobulin serotherapy is efficacious
3. Vaccinations with attenuated sporozoites prevent malaria infection
4. Animal models substantiate induction of protection
5. Clinical trials with RTSS (MVI/GSK) appear successful

Barriers to deployment of malaria vaccines

As shown in box 2, despite scientific evidence that malaria vaccines can be produced, there remain formidable barriers (Reed *et al.*, 2006). *Plasmodium* parasites express more than 5200 proteins during their life cycle (Reed *et al.*, 2006). Each protein may represent a theoretical target for malaria vaccine candidate but few will have the characteristics of a useful vaccine target making the identification of the useful antigens rather complex. The human host immune response to malaria infection is variable and the identification of effector mechanisms remains elusive (Keenihan *et al.*, 2003).

Box 2: Barriers to development and deployment of malaria vaccines

1. *P. falciparum* express >5200 antigens during their life cycle and the identification of useful antigens is difficult
2. The targets and effector mechanisms responsible for acquired immunity are not identified
3. Pre-clinical and clinical evaluation complex (medical, ethical, logistic considerations)
4. Limited research capacity in malaria endemic countries
5. Equity and accessibility of the vaccine to vulnerable groups in resource-poor communities
6. Un-attractive commercial market

Approaches to malaria vaccines

Despite of the aforementioned barriers, several approaches have been applied in the development of a malaria vaccine, which might be used in malaria endemic settings. Figure 1 shows several approaches to malaria vaccines development conveniently divided into pre-erythrocytic, erythrocytic, transmission blocking and combinations of the above (Moore *et al.*, 2002; Moorthy *et al.*, 2004). An extensive account of the portfolio of malaria vaccine candidates in different phases of research and development is shown in the following website: (http://www.who.int/vaccine_research/documents/en/malaria_table.pdf).

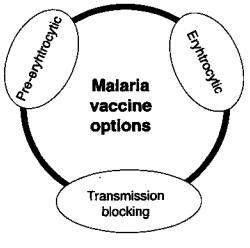

Figure 1: Targets of malaria vaccines.

Pre-erythrocytic malaria vaccine candidates

Pre-erythrocytic malaria vaccine candidates prevent infection of erythrocytes by sporozoites or eradicate infected liver cells, through production of high titres of functional antibodies against sporozoites to prevent parasites entering the liver stage, and induce potent cytotoxic T-lymphocyte immunogenicity against infected hepatocytes, while not harming human host. Current attempts to develop candidates are focused on recombinant or synthetic expression of a part of the

circumsporozoite protein (CSP), at a region that consists of short repeated sequences of amino acids. Such vaccines will be in high demand by travellers and non-immune residents of malaria endemic sites (Moore et al., 2002; Moorthy *et al.*, 2004; Genton *et al.*, 2003).

The failed candidate vaccine SPf66 was thought to be a pre-erythrocytic vaccine (Leach *et al.*, 1995; Alonso *et al.*, 1994; Masinde *et al.*, 1998). The currently leading candidate in this group is known as RTS,S and has already undergone successful field trials in The Gambia and Mozambique (Bojang *et al.*, 2001). This candidate is currently in field trials (Phase 2), in Gabon, Ghana, Kenya, Mozambique and Tanzania. Vaccinations with irradiated sporozoites have been shown to be highly protective, however, experts have been sceptical whether industrial production of irradiated sporozoites could be feasible (Luke & Hoffman 2003). Due to the persistence of the developers and the recent availability of funds for malaria vaccines this candidate is receiving more attention. Clinical trials with irradiated sporozoites are likely to start in sub-Saharan Africa in the near future.

Asexual blood-stage (erythrocytic) malaria vaccine candidates

This approach targets the parasite in the circulation and is aimed at mimicking the acquisition of natural immunity to malaria by either preventing invasion or sequestration (Genton *et al.*, 2003) . The candidate should trigger the production of functional antibodies which prevent merozoite invasion into erythrocytes and enhance lysis of merozoite-infected erythrocytes. Leading candidates are Glutamate-Rich Protein and Merozoite Surface Proteins (Dodoo *et al.*, 2000; Cavanagh *et al.*, 2004). Several candidates are in various stages of field trials in malaria endemic countries in Africa. MSP3 trials sponsored by African Malaria Network Trust (AMANET) are carried out in Burkina Fasso, Tanzania and Mali.

P. falciparum adhere to vascular endothelial cells as an evasion mechanism from the immune system particularly the spleen. In this category, the variant surface antigen, particularly *P. falciparum* erythrocyte membrane protein1 (PfEMP1), is under investigation as a vaccine candidate. It is anticipated that antibodies against PfEMP1 could inhibit the cytoadherence, reduce parasite biomass and the related morbidity (Jensen et al., 2004; Lusingu *et al.*, 2006; Magistrado *et al.*, 2007). This approach is in the preclinical stages of research and development.

Transmission blocking malaria vaccine candidates

These vaccine candidates are designed to block the part of malaria lifecycle within the mosquito host by acting against the sexual stages of malaria parasites. The immunised individual is not protected against malaria but will no longer contribute to the transmission of malaria. Transmission blocking vaccine candidates elicit antibodies against surface antigens on extra-cellular parasites that have emerged from the erythrocytes in the mid-gut of a mosquito after a blood meal. Gametocytes in the immunised human host are not affected and hence they will not provide protection to the infected host (Genton *et al.*, 2003). Antibodies against the gametes prevent fertilization or destroy the gametes or zygotes within 5-10 minutes of entering the mosquito's midgut. Antibodies against ookinetes act 12-24 hours later to prevent them entering the midgut and forming sporozoites, which could eventually infect another host. Leading candidate antigens are pre-fertilization (Pfs230 and Pfs48/45), post-fertilization (P25 and P28) and chitinase (peritrophic matrix (PM)) (Riley *et al.*, 1995; Arakawa et al., 2005; Sauerwein 2007)

Multi-stage malaria vaccine

Current approaches of malaria vaccine development target single antigens; but even the currently most successful candidate, RTS,S affords only limited protection. There is hope that there could be synergism or at least an additive effect from combining vaccines which induce immune responses to different stages of the life cycle. Such multivalent vaccines could be cocktails of the discussed candidates, combined DNA vaccines or purified recombinant proteins (Moorthy *et al.*, 2004; Collins *et al.*, 2005; Ockenhouse *et al.*, 1998). Such multivalent vaccines have to overcome additional challenges including possible competition between antigens, compatibility of

antigen presentation system, the technical difficulties to scale up production, the complexity of evaluating incremental improvements and the associated regulatory hurdles.

Conclusion

Despite the accepted need and great promises there is still no licensed malaria vaccine. All vaccine development is a high risk venture which can result in the dismissal of a previously promising candidate at any stage of development. Yet malaria vaccines have their special challenges. This is partly due to the pathogen which is genetically much more complex than bacteria or viruses (Reed et al., 2006). A number of vaccine candidates are in development yet it is difficult to know which the best candidate is. Different groups claim that various antigens are the best suited and efforts are under way to test pre -erythrocytic, erythrocytic and transmission blocking antigens. Competition, rivalries and insufficient funding may have contributed to the overall lack of success in malaria vaccine development. It is also not clear whether protective mechanisms are humoral, cellular or both. The duration of protective immunity appears variable suggesting that booster doses of promising candidates will be necessary (Tsuji et al., 2001). In addition, immunological memory acquired through repeated exposure may be lost if antigenic stimulation ceases. In theory the introduction of protective vaccines could therefore render populations at risk once immunity is waning.

Another challenge is the absence of a suitable animal model such that vaccine developers are forced to evaluate vaccine candidates early on human volunteers which raise ethical and regulatory issues. While safety and immunogenicity (Phase I and II) studies can be conducted in non-endemic settings the proof that the vaccine can protect against malaria requires a setting with high malaria endemicity and an infrastructure which can satisfy the requirements of regulatory agencies. The relative scarcity of sites with an adequate infrastructure and malaria endemicity has driven investment into sites which can potentially evaluate the protective efficacy of malaria vaccine candidates in phase 3 trials. A further challenge is the choice of clinical case definition and end-points which will be accepted by regulatory agencies. It is interesting that recently WHO consultation group agreed parameters required and appropriate definitions for malaria vaccine end-points (Moorthy *et al.*, 2007).

With the license of malaria vaccine candidates a new set of barriers will arise. Vaccine production has to be scaled up which besides the technical challenges requires massive financial investment. The big pharmaceutical companies have the required technical expertise to produce adequate amounts of vaccines to cover the potential demand in sub-Saharan Africa but require assurance that their investment will bring competitive returns. Financial mechanisms are required to make vaccines available where they are needed. Ultimately the populations at risk will not only have to accept the malaria vaccines they will have to fight for the deployment of such vaccines.

Currently, promising malaria vaccine candidates being tested include RTSS, MSP1, MSP3, GLURP and AMA1. The development of these candidates has been supported by various organisations such as Malaria Vaccine Initiative (MVI) at PATH, United States Aids Agency (USAID), European Malaria Vaccine Initiative (EMVI), AMANET and malaria Clinical Trials Alliance (MCTA). Private-public partnerships have accelerated malaria vaccine candidates entering clinical trials. The roadmap to a malaria vaccine hopes to license a malaria vaccine by 2015 which affords 80% protection. Researchers from malaria endemic countries have to be involved in each stage of development of a malaria vaccine if widespread deployment of the vaccines is to become a reality.

There is a widespread misunderstanding that a malaria vaccine will replace other malaria control efforts. It is not realistic to expect that a malaria vaccine candidate will be 100% protective, that all members of community agree to be vaccinated, and protection is live long. More realistically, a vaccine will protect 80% or less of the vaccinated individuals for a few years after which immunity will be waning. Not all members of malaria endemic communities will agree to be vaccinated making complete vaccine coverage impossible. Furthermore the basic reproductive number of

malaria is unusually high in comparison to any infectious disease. This means that a single case of malaria can result in a very large number of secondary cases. No malaria vaccine on its own can reduce transmission sufficiently to eliminate malaria. However the addition of promising vaccine candidates with the currently available tools for malaria control such as insecticide treated nets, environmental management and prompt diagnosis and treatment using effective antimalarial drugs could drive malaria transmission to very low levels and allow people in Tanzania and many other parts of sub-Saharan Africa to live free of malaria.

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