Invited Review

Malaria as a Public Health Problem and Status of Vaccine Development

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ABSTRACT

Malaria remains one of the most important infectious diseases worldwide considering that 40% of the world s population live in health risk areas and millions of febrile episodes due to malaria infection occur annually in children under the age of five in Africa alone and almost 3 million patients, primarily children, die each year. Among the various measures envisaged, to contain the disease, the concept of a vaccine to protect humans against malaria appears particularly attractive. The development of an effective malaria vaccine represents one of the most important approaches to provide cost-effective intervention, in addition to currently available malaria control strategies. Here, we review malaria as a public health problem and the status and promise in malaria vaccine development.

Keywords: Malaria, public health, vaccine development

INTRODUCTION

Malaria is one of the most important infectious diseases in the world and the scourge of many developing countries. There are an estimated 300-500 million cases of malaria each year, resulting in over 1 million deaths, mainly of children under five years in sub-Saharan Africa.[1] Furthermore, tens of millions of non-immunes have been highlighted by the impact of malaria on military operations and wars, and catastrophic natural disasters such as famines and floods. During the twentieth century, the United States military forces lost more person days to malaria than to bullets in every operation conducted in a malarious-region. It is only natural therefore that major efforts are underway to develop an efficacious malaria vaccine.

The parasite has a complex life cycle. Sporozoites are inoculated by the Anopheles species mosquitoes that feed on humans from dusk until dawn. The extracellular sporozoites are present in the bloodstream for <1 hour. They invade hepatocytes where a single, uninucleate sporozoite develops during 2 to 10 days, depending on the plasmodium spp. (5 to 6 days for *P. falciparum*), to a mature liver stage schizont. The liver stage parasite ruptures releasing up to 30,000 uninucleate merozoites, each of which can invade an erythrocyte, and subsequently develop during 2ˇ3 days (43 to 48 hours for *P. falciparum*) to a mature erythrocytic stage schizont with 10ˇ30 merozoites. When the infected erythrocyte ruptures, it liberates parasite-derived toxins and merozoites, and the released merozoites invade other erythrocytes. In some instances the merozoite will develop within the

erythrocyte to the sexual stage called gametocytes. When a mosquito ingests erythrocytes containing gametocytes, the parasites develop in the midgut of the mosquito to gametes that are released from the erythrocytes. This is followed by fertilisation of the female by the male gamete leading to zygote formation, and subsequently ookinete, oocyst, and sporozoite development. Only the asexual cycle is associated with pathology.

MALARIA AS A PUBLIC HEALTH PROBLEM

Malaria has been estimated to represent 2.3 % of the overall global disease burden and 9 % in Africa, [2] ranking third among the infectious disease threats, after pneumococcal acute respiratory infections (3.5%) and tuberculosis (2.8%). In 1990, malaria was responsible for the loss of 31,705 000 disability-adjusted life-years (DALYs). The disease is often linked to the movement of refugees or population seeking work and to environmental change, including forestry, mining and water development projects.[3] By the year 2000, the current WHO Global Malaria Control Strategy aims to reduce malaria mortality by at least 20% in at least 75% of the affected countries. This strategy recognises that multiple varied transmission and operational drug-resistant patterns worldwide lead to different epidemiological patterns/ paradigms of malaria illness for which certain risks are particularly important and certain approaches to control are more likely to succeed than others.[4] Because malaria represents a moving target, control programmes need to identify and monitor epidemiological patterns.[5] However, pregnant women and children under five years continue to constitute two of the most important risk groups. To control malaria, better ways must be found to apply existing control methods, improved tools must be developed and solutions need to be identified to circumvent and combat emerging problems including social/political unrest, the widespread resistance of malaria parasite to existing drugs [6] and the potential change in the distribution and incidence of malaria due to anthropogenic climate change.[7]

The cost of physical intervention methods intended to interfere with the transmission of the disease such as bednets and window screens is often prohibitive and such measures are not highly effective. This is compounded by and the availability and cost of prophylactic drugs which precludes their use by many of the individuals who need them the most. Moreover, the emergence of drug-resistant parasites means that many of the prophylactic drugs that were effective in the past are no longer useful, and many of the newer generation drugs are associated with rare but significant side-effects, such as fatal heart rhythms (halofantrine), fatal skin disease (pyrimethamine/sulfadoxine), neurological disturbances (mefloquine), or gastrointestinal distress (doxycycline). The increase in insecticide-resistant vectors that transmit malaria, and the undesirable environmental impact of those insecticides shown to be most effective means chemical interventions are frequently not useful in combating the disease. These factors emphasise the urgent need for the development of an effective malaria vaccine.

MALAYSIAN SCENARIO

Incidence of Malaria continues to decrease from almost 60,000 cases in 1995 to only 6154 in 2004 (Table 1). In 2004, Sabah, followed by Sarawak, had the majority of cases in Malaysia,

Table 1. Immune mechanisms related to vaccines directed against malaria parasite stages
in the life cycle.

Stage/target	Immune response/ effector mechanism	Rational for approach
Pre-erythrocytic stages		
1. Sporozoite 2. Hepatic forms	Antibodies CD4+ and CD8+ T cells, including CTL activity and cytokine secretion	Sporozoite secretion limited to minutes. Parasite develops inside hepatic cells, which can express antigens and MHC molecules recognised by T cells.
	Antibodies	Cell surface expression of parasite antigens.
Asexual blood stage		
Merozoite/infected erythrocytes	Antibody-includes the inhibition of red blood cell invasion, ADCC activity; inhibition of cytoadherence, anti-toxin activity, anti-cytokines or reduction of free radicals.	No MHC molecules expressed on infected erythrocytes. Anti-disease vaccine can be directed to cytokine-inducing processed parasite antigens or toxins.
Sexual stage		
Gametocyte/gamete/ aokinete	Antibody ˘ includes blocking activity pre-and/or post-fertilisation in the mosquito.	Sporogonic development is a crucial target; no host MHC molecules involved in this stage of parasite development.
	Cell-mediated immunity-cytokines?	No MHC molecules expressed on infected cells.

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CTL, cytolytic T lymphocytes; MHC, major host-compatibility complex.

with less than a quarter in Penisular Malaysia, with Pahang and Johor topping the list (Ministry of Health 2004) (Table 2). With regard to malarial parasite distribution, *Plasmodium vivax* (51.46 %) still predominates followed by *P. falciparum* at 40.56% whilst only 5.35% are *P. malaria*. (*P. ovale* has not been detected in Malaysia).[8]

CLINICAL IMPLICATIONS

Children face the greatest risk of malaria complications with the highest mortality in those below 5 years [10] whilst in pregnant women, the risk of prematurity, abortion or stillbirth exists.[11] The classic symptoms of malaria are high fever with chills and rigours, sweating and rigours; depending on the infection species, fever appears every or every third day. Anaemia and thrombocytopenia are common with pallor and jaundice that may occur due to hemolysis.
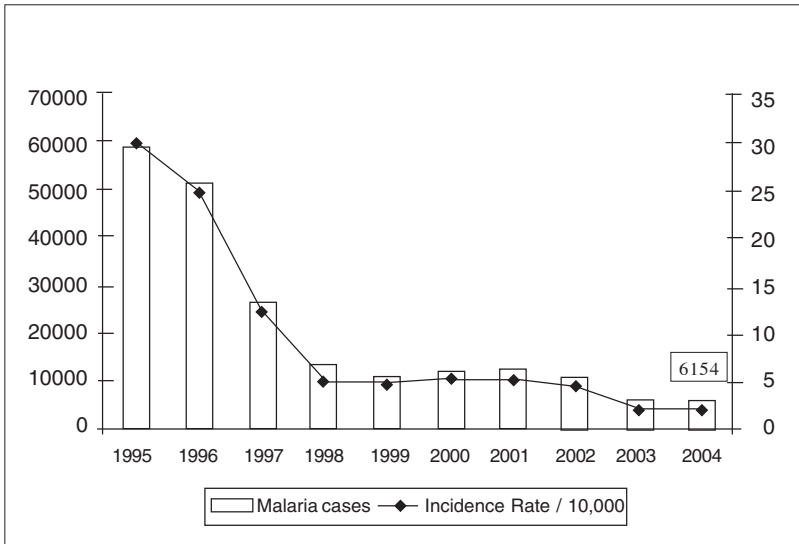
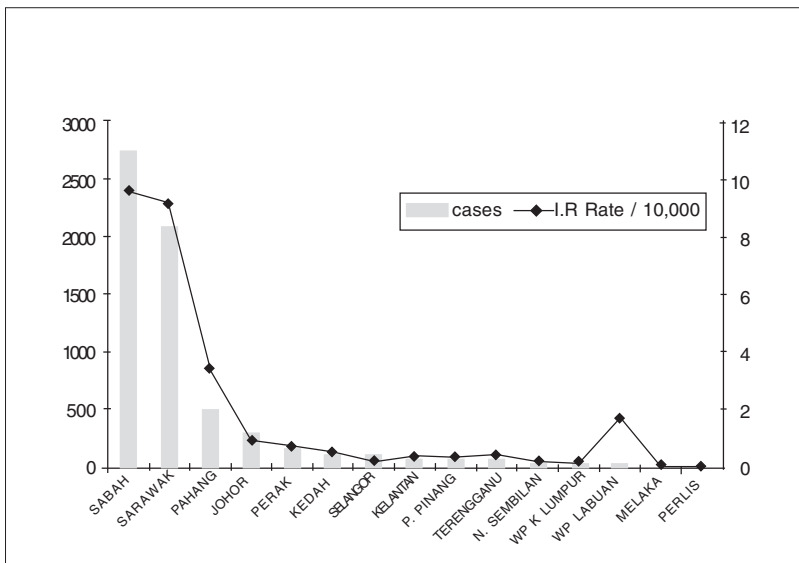**Figure 1.** Incidence and incidence rate of malaria in Malaysia, 1995 - 2004

Figure 2. Malaria cases and incidence rate by geographical area (state), 2004

Infection with *P. falciparum* is potentially fatal, with the severe form manifesting with any of the following *viz*, cerebral malaria, hypoglycaemia (quinine associated), non-cardiogenic pulmonary edema, renal failure and even vascular collapse.[9] Therefore the emergence of *P falciparum* resistance compounds the treatment issue even further. It has been reported that in Penisular Malaysia, 28.5 % of falciparum malaria was resistant to chloroquine but by mid-1990s, it had risen to about 60 %, according to Hakim *et al*. [12] Even more worrying is that a combination of Fansidar (sulpadoxine & pyrimethamine) and chloroquine encounterd 50-60 % resistance according to 2001 records in Malaysia.

In view of the difficulty of treating falciparum malaria, a strategy to combat rapid resistance has to be formulated, with combination therapy using drugs with differing anti malarial mechanisms. The most widely advocated combination is ACT s Artemesinin based combination therapies. Artemesinin reduces gametetocyte carriage and is thus a good transmission blocking agent; at the same time it is efficacous, rapid acting and well tolerated. [13] Existing combinations include Artemether-lumefantrine and Artesunate-mefloquine. Another strategy to circumvent the problem of resistance arising from existing drugs is to search for suitable vaccines, an imperative that has become even more critical.

VACCINATION PROSPECTS

The primary goal of malaria vaccine research is to develop and field a vaccine that prevents the majority of naïve recipients from developing any clinical manifestations of disease after exposure, and to prevent the development of severe disease and death in those individuals who become ill by limiting the effects of blood stage infection.[8] The first step should be to develop a vaccine designed to prevent any clinical manifestations of malaria completely and the populations that would benefit from such a vaccine would be travelers, deployed military personnel, and non-immune individuals living in non-malarious areas of countries with malaria. The second is for a vaccine designed to prevent mortality and severe morbidity, by limiting the effects of blood stage infection by reducing parasite replication, reducing cytoadherence, and/or inhibiting the effects of toxic materials released by the parasite.[14] The populations that would benefit from this are individuals, primarily children, residing in malarious areas.

The development of a vaccine against malaria presents formidable obstacles in terms of parasite biology and host immune responses. During its complicated life cycle, the parasite exists extracellularly in the blood stream of the host, within cells that express major histocompatibility complex (MHC) molecules (erythrocytes), and within the mosquito vector. The antigens that are the targets of protective immune responses are expressed at different stages of the parasite s life cycle, and different arms of the immune system are required to attack the parasite at these different stages.[15] Moreover allelic and antigenic variation of the parasites and variant disease expression based on transmission dynamics and genetic background of the host are well established. These factors suggest that only a similarly complex vaccine may provide sustainable protection against malaria.

The development of an effective malaria vaccine represents one of the most important strategies for providing an efficacious cost-effective addition to the currently available malaria control interventions. Recently the relative cost-effectiveness of a hypothetical

vaccine against *Plasmodium falciparum* compared to currently available interventions for use in high risk groups in Africa has been estimated. A malaria vaccine that could be implemented through the existing Expanded Programme on Immunisation (EPI), with costs, schedule etc, that would reduce the overall childhood mortality by 30% or more, with a duration of a three-year immunity or more, would represent a very cost effective intervention strategy. Approximately USD 1 ˇ 20 per healthy year of life could be saved. However, as no single intervention tool is likely to represent a panacea for malaria, an effective vaccine would be applied together with other appropriate cost-effective methods.

Over the past decade, there has been considerable progress in the identification of vaccine candidate antigens and their genes but, owing to the complexity and cost of vaccine development and the relative lack of commercial interest, only recently have they entered clinical trials. Moreover, appropriate *in vitro* functional assays for predicting protection have been difficult to identify and validate and they exist only for evaluating the potential of transmission blocking vaccines to inhibit parasite development in the mosquito. Indeed even the membrane-feeding assay for measuring functional transmission blocking activity remains to be validated in the field. Immune mechanisms thought to be involved in conferring protection to the different stages of malaria parasite are given in Table 1. It should be noted that vaccines directed at the asexual blood stage antigens may constitute anti-disease vaccine and that the objective of such vaccine is to reduce severe morbidity and mortality resulting from malaria, and not necessarily to provide protection against infection (i.e, sterile immunity) in the vaccinated individual. Indeed it is extraordinarily rare for sterile immunity to malaria to be ever developed in nature.

Immunisation of experimental animals with irradiated sporozoites induced complete protection against subsequent challenge with the plasmodial parasite in question. Based on these observations, human volunteers were subjected to multiple mosquito bites with *P. falciparum* or *P. vivax* infected, irradiated mosquitoes. The vaccinated individuals became resistant to sporozoite challenge, thus proving the principle that experimental immunisation of humans against malaria is feasible. Unfortunately, to date, the technology involved in executing the immunisation process using irradiated sporozoites does not lend itself to a practical, cost effective vaccination strategy. Although *in vitro* culture systems have been developed for the continuous culture of *P. falciparum* parasites and provision of asexual blood stage parasites and gametocytes, production of sufficient quantities for vaccination purposes is not feasible. Genetic manipulation may create new opportunities by transforming parasites to allow for continuous culture in the absence of red blood cells. In subsequent studies, several purified native and recombinant antigens, e.g. Merozoite Surface Protein-1 (MSP-1), Apical Membrane Protein-1 (AMA-1) or Serine-rich Protein (SERA) have been found to induce strain-specific immunity in experimental animals.

Trials of malaria vaccines have been carried out in an empirical way, with the focus in the 1980s being on antibodies. For the past 15 years, vaccine technologies have advanced considerably. [16] Examples of malaria vaccine technologies employed in clinical trials are shown in Table 2.

Table 2. A brief summary of various malaria vaccines and examples of clinical trials

Stage of plasmodium	Antigens	Salient features	Examples of clinical trials
Pre-erythrocytic	Irradiated sporozoites, Circum-sporozoite Protein (CSP) or peptides, Liver Stage Antigens-1 (LSA-1)	Stage/species specific; antibody blocks infection of the liver; large immunising dose required; can abort an infection.	C.S.P. Vaccine: Kenyan study concluded that CSP vaccine-induced antisporozoite antibody is not protective. Encouraging results have been reported with a CSP-Hbs Ag Hybrid vaccine (US Army and SKB)
Merozoite and Erythrocytes	Erythrocyte Binding Antigen (EBA-175), Merozoite Surface Protein 1 & 2 (MSP 1 & 2); Ring Infected Erythrocyte Surface Antigen (RESA); Serine Repeat Antigen (SERA); Rhoptery Associated Protein (RAP); Histidine Rich Protein (RAP); Apical Membrane Antigen-1 (APM-1)	Specific for species and stage; cannot abort an infection or prevent invasion of erythrocytes, thus reducing severity of infection.	Against *P. vivax* blood stage infection, MSP-1 in block co-polymer adjuvant with T-helper epitope, the yeast expressed P2 P30 PV20019 recombinant vaccine offers partial protection in Saimiri monkeys.
Gametocytes and Gametes	Pfs 25, 48/45 k, Pfs 230	Prevents infection of mosquitoes; Antibody to this antigen prevents either fertilisation or maturation of gametocytes, zygotes orookinete; is of use in endemic areas but not suited for travelers; antibody blocks transmission cycle.	In a 1997 study, [17] it was shown that sera which mediate gamete lysis contain IgG1 and IgG3 antibodies to gamete surface proteins. Thus, Pfs230 is a major target of C¹fixing antibodies.
Combined Vaccine (cocktail) or Patorraya Vaccine	SPf 66 (based on pre-erythrocytic and asexual blood stage proteins of Pf)	Based on incorporation of antigens from different stages into one vaccine to produce an immune response, blocking all stages of the parasite development.	Fields trials of Spf 66 in Latin America and Africa showed a protective efficacy ranging between 38.8 and 60.2 % against *P.falciparum.*

CONCLUSION

In the next decade, malaria researchers will develop new, postgenomic resources to move the field forward both in its basic understanding of plasmodium species biology and its applied work aimed at control and prevention of the disease. A list of what must be done is obviously to (i) understand the elements of *Plasmodium* parasites that enable them to cause disease and to survive in their hosts and vectors, and to understand which features of host-parasite, vector-parasite and vector-host relationships present new opportunities to develop drugs, vaccines and diagnostics; (ii) understand the molecular basis of variation in phenotypes among malaria parasite strains and species; (iii) understand the key gene products that are involved in the life of malaria parasites of humans, in stages occurring in both the host and the mosquito vectors; and (iv) ensure that members of the malaria community worldwide can use the new tools and knowledge that become available. Comparative and genome-wide analytical approaches have been made increasingly efficient through work in *Plasmodium* species, animal model systems and human population studies. All these will help to answer the scientific questions on malaria.

ACKNOWLEDGEMENT

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