Malaria vaccine: Are we anywhere close?

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Malaria has been known to students of human disease from the dawn of history. Observers had followed the association between water collections and the prevalence of the disease since early times. The discovery by Ronald Ross, in Secunderabad, of the role of the female Anopheles mosquito in sustaining the infection has become a part of medical lore. The subsequent researchers solved the complex life cycle of the malarial parasite.

Malaria is seen in all the continents to a certain extent. While infections caused by P. vivax, P. malariae and P. ovale are rarely life threatening or overwhelming, P. falciparum infections can be fatal due to the peripheral vascular localisation of the mature trophozoites. The placental and juvenile infections by P. falciparum are associated with high mortality, particularly in Sub Saharan Africa. Elsewhere the blockage of cerebral and splanchnic vasculature by parasitised erythrocytes gives rise to the manifestations of Malignant Tertian Malaria. The complex sequence of reactions that result in the clinical manifestations are well described.

At present malaria is endemic between the Tropics of Cancer and Capricorn. The density of infection is the result of the interplay of a number of factors. These include the prevalent parasite species, vector transmission efficiency, availability of good primary health care and the economic development of the population. Probably the worst combination is seen in Africa where P.falciparum accounts for a majority of the infections. There, Anopheles gambiae is the major vector, primary health care is relatively poor, and the countryside has the poorest communities of the world. This region accounts for nearly a million deaths a year predominantly in infants and pregnant women. Malaria is responsible for declining Gross Domestic Product and perpetuation of poverty.

The use of insecticides to interrupt the transmission cycle promised a lot. In fact, the World Health Organisation launched the Malaria Eradication Programme the world over in the early 1950s. Spraying of diclophan (DDT) in the homes brought about a dramatic decline in the incidence rates. In fact, in some temperate areas the infection was truly eliminated. However, rising drug and insecticide resistance, among other factors led to a resurgence of malaria. In India, the low level of infection in 1965 resurfaced to an annual incidence of 6.4 million in 1976. The worsening trend continues despite the use of newer drugs, better insecticides and a slow but steady improvement in health infrastructure, particularly in Africa.

The remedies to reverse the present situation are: 1) better sanitation, engineering solutions to mosquito breeding 2) proper town and house planning 3) drug research along with the development of newer insecticides and 4) vaccine.

The first two options are highly capital intensive and it is unlikely that the states endemic for malaria would be in a position to afford them.

It is nearly a decade since any new antimalarial drug was developed. The publication of the complete genome of P. falciparum has opened up the research field. The major stumbling block, however, is the lack of funds. The low paying capacity of malaria victims makes drug development unattractive for pharmaceutical firms. Efforts are being made to remedy the situation by setting up organizations like the Medicines for Malaria Venture, Roll Back Malaria consortium and with the help of philanthropic organisations like the Bill and Melinda Gates Foundation. However, the issues of delivery at an affordable price and ensuring easy availability still remain. At best the drug initiatives would be medium term measures since the parasite would develop resistance sooner or later. Even simple methods aimed at decreasing mosquito bites like impregnated bednets have not been fully effective due to leaching of the insecticide during wash and development of pyrethroid resistance by the mosquito.

Development of a malaria vaccine would, therefore, be a long term measure that would ultimately be cost effective too. The development of an effective vaccine will be dealt with in this review.

Immunity in Malaria

Repeated infection by the malarial parasite has been shown to elicit a level of immunity. This immunity can be passively transferred by gamma globulins across geographic borders and even across species. However the molecular specificities of the protective immunoglobulins is not well understood. The modes of action of the antibodies probably are: interruption of schizogony by interference of merozoite attachment to red cells and Antibody Dependent Cytolysis (ADCC) by CD8+
lymphocytes. This immunity is practically never sterilising. It is shortlived (even in the original donor), unless it is constantly reinforced.\(^\text{12}\)

Fresh malarial infection does elicit a polyclonal antibody response but is not accompanied by significant immunity as evidenced by the persistent susceptibility to infection in the presence of circulating antibodies.\(^\text{13}\)

In Africa there is high infant mortality due to malaria that decreases when surviving children grow older. In fact, in that continent this group and pregnant women are the major victims of the infection. While the younger children die of overwhelming infection, the older children too die (in much smaller numbers) due to the vascular occlusion by \(P. falciparum\) infected red cells.\(^\text{14,15}\)

In the human body the parasite is free in the bloodstream only for brief spells, once when the sporozoite travels from the point of inoculation to the hepatocyte, again when the hepatic merozoite gets discharged into the blood and when fresh red cells are infected during erythrocytic schizogony. This gives the antibodies little chance to act. It is now evident that the major defence is cell-mediated, acting on the infected hepatocytes. In this respect the CD8\(^+\) cytotoxic lymphocyte (CTC) is the most important. It eliminates the infected hepatocyte by releasing Interferon-\(\gamma\) (IFN-\(\gamma\)) which induces nitrile oxide that destroys the cell.\(^\text{16}\)

The malarial parasite expresses different proteins during the different stages of its life cycle so that a vaccine developed towards one stage is effective against that stage only (stage specificity of vaccines). In addition, there is considerable antigenic variation during the erythrocytic stages ensuring that some merozoites are unaffected by the circulating antibodies during their brief extra-erythrocytic transit. Interestingly, antibody against gametocyte antigens are effective in the gut of the infected mosquito in preventing the ookinete from penetrating the mosquito midgut and developing into the oocyst.\(^\text{17-19}\)

Placental malaria has been a particularly difficult infection that causes considerable morbidity in Africa. It is particularly severe in primigravida. The infection which occurs in the presence of substantial pre-existing immunity is caused by the adhesion of \(P. falciparum\) infected red blood cells to chondroitin sulphuric acid and hyaluronic acid. A coating of immunoglobulin appears to facilitate the binding. The antibodies which develop during a prior pregnancy appear to protect against infection in subsequent ones. Thus, the incidence of placental malaria is lower in subsequent pregnancies. While placental infection by malaria parasites does not appear to be a problem in India there is evidence that some of the highly endemic regions in the tribal belt may have this type of infection.\(^\text{20}\)

**Vaccines**

The feasibility of passive and active immunisation was demonstrated in the 1970s. The methods could not be used practically. Passive immunity depended on the availability of immunoglobulin from immune persons in endemic areas. Active immunisation was achieved using irradiated sporozoites. There was the development of antibodies as well as cell mediated immunity directed against the hepatic stage. In mouse experiments apart from a dominant role for CD8\(^+\) T cells, CD4\(^+\) T cells and \(\gamma\delta\) T cells and Natural killer cells have also been shown to play a part. Cytokines too appear to play a significant part. Unlike natural immunity this acquired immunity is sterilising.

The requirement of sporozoites from nearly 1000 mosquitoes to be administered repeatedly to generate evanescent (6-10 months duration) active protection imposed a seemingly insurmountable obstacle.\(^\text{21}\) However, the success of *in vitro* cultivation of sporozoites opens up the possibility of resurrecting this proved procedure. Of course, prolonging the immune status would be necessary.\(^\text{22}\)

Candidate vaccine antigens have been described from all the stages of the parasite. The *pre-erythrocytic stage* vaccines such as the RTS,S/AS02 hold out hope of achieving sterilising immunity and are eminently suited for immunisation of travellers.\(^\text{23}\) In the case of frequently infected persons in an endemic area there is a fear that there may be some enhancement of the infective process due to variations in the antigenic makeup.\(^\text{24}\)

The hepatic stage vaccines are based on antigens expressed on the infected hepatocyte. They may be from the sporozoite or the merozoite. The vaccine would be designed to boost the cell mediated immunity to achieve intracellular parasite death and lysis of the infected hepatocyte by the CTL process. The vaccine would have the same advantages as the sporozoite vaccine.

**Erythrocytic vaccines**

The next stage that would merit attention is the merozoite released after schizogony. This stage is vulnerable at the time of exiting from the erythrocyte and before it penetrates the new host. Antibodies can prevent both the events. There is a considerable antigenic drift in each cycle and there would be the need of using a number of antigens.

The parasite inside the erythrocyte is normally regarded as being in a protected environment. However, there is some evidence that it is capable of imbibing proteins from the blood.\(^\text{25}\) At the same time parasite specific antigens appear on the surface of the infected red cell. This offers a target for antibody attack which may induce complement mediated lysis or opsonise the parasitized red cell in the spleen. In the case of \(P. falciparum\) infection the fatal outcome is due to the sequestration of the infected red cells in the peripheral blood vessels. This process is due to the specific action of parasite derived red cell surface changes reacting with receptors in the endothelium. The resultant cellular damage releases vast quantities of cytokines which cause the death. Prevention of the attachment of the parasitized erythrocytes to the endothelium would
achieve a decrease in the morbidity. This appears to be in the realm of possibility.26

Mosquito Stage
The gametocytes ingested by the female Anopheline mosquito are capable of being attacked by antibodies in the mosquito gut. Antibodies taken in during the blood meal have been shown to inhibit exflagellation and fertilization. Even after fertilization the ookinete is inhibited from penetrating the mid gut and producing the oocyst. The antigens in the mosquito stages are probably not as variable as the ones in human blood and would probably be more easy to tackle. However the vaccines against the sexual stages are of little benefit to the infected subject. They certainly are of value in preventing the parasite from completing its life cycle, thereby decreasing the parasite load in the community. For this reason this vaccine is called the altruistic vaccine. Obviously, it will have to be combined with a vaccine more useful to the individual.

Recent reviews5,6,14,17 detail the antigens that are being examined for their usefulness as vaccine candidates. In the past two decades likely candidates have emerged roughly at the rate of one a year. The favoured proteins are the circumsporozoite protein (CSP), thrombosporidin related adhesive protein (TRAP) and liver stage antigens from the erythrocytic stages, spf66, a synthetic peptide, native synthetic and recombinant peptides from merozoite specific proteins (MSP 1 and 2), apical membrane antigen-1 (AMA-1), ring infected erythrocyte surface antigen (RESA), Pfalciparum erythrocyte membrane protein-1 (PfEMP-1) and others from the erythrocyte stages are under evaluation to Phase III trials. A number of constructs have been taken to human trials with two vaccines reaching the Phase III. However, to-date none of the vaccines have consistently shown protection in the field. The ones to have reached Phase III are single antigen asparagine-alanine-asparagine-proline (NANP) repeat in the circumsporozoite protein and an NANP combined with blood stage antigens including MSP-1. The former has shown no significant protection while the latter (SPf66) promised much initially but failed in a larger field trial in Gambia and Tanzania in Africa, Thailand in Asia and Brazil in South America.

The recent collaborative effort between Glaxo-Smithkline and the US Army (RTS, S) has produced a chimeric protein combining the circumsporozoite protein and Hepatitis B surface antigen (HbsAg) which has been immunogenic in animals when used with Freund’s adjuvant but not with the conventional adjuvants permitted in humans. A new permissible adjuvant AS02 has shown some activity, the vaccine candidate is undergoing further studies. The results from two other candidates under trial in Gambia and Papua New Guinea are reviewed by Richie and Saul.27 They show some protection. It remains to be seen whether they will live up to their promise.

The Future

The demonstration that the important immune mechanism in Malaria is cell mediated immunity (CMI) and the discovery of DNA vaccines has generated a flurry of activity. Some of these constructs have shown good results. The DNA constructs have been fused with pox viruses and cholera toxin sub-unit B. They have used the prime-boost strategy and early results have been encouraging. However, there is some way to go before they can be seriously considered.

The experience so far indicates that single antigen constructs would not succeed. Multiantigen constructs with antigens from the pre-erythrocytic and erythrocytic stages with transmission blocking antigens would be required to significantly influence the prevalence in the highly endemic regions. Addition of antigen(s) that would induce immunity to interfere with the adhesion of P. falciparum schizonts to the endothelium would be useful in decreasing mortality. In a recent symposium (India - US Symposium on Infectious Disease Research and Development, Jan 2004) Thomas Richie presented the initial data on a combination DNA vaccine termed CSFAM for CSP, Sporozoite Surface Protein-2 (SSP2/TRAP), LSA1, Apical Merozoite Antigen 1 and MSP 1 in pox viruses. The constructs have shown impressive results that need to be confirmed.

The most promising development in the field is publication of the genomes of Homo sapiens,28 Plasmodium falciparum29 and Anopheles gambiae30 - the three corners of the fatal triangle of African malaria. This would certainly open up immense possibilities. Use of sophisticated procedures like microarrays, gene analyses and proteomics will throw up a large number of vaccine candidates. Will they see us through? That is the crucial question.

It has been the experience of workers in the field that the vaccine adjuvants permitted for human use (only alum at present) are not as effective as Freund’s adjuvant in animal experiments. Malaria vaccine research has demonstrated the need for developing better adjuvants. One such innovation has been the adjustment AS02 which is in use with the RTS,S vaccine undergoing humans trials. There is scope for looking further in this field.

Vaccines against P. vivax have not received the same priority as in the case of P. falciparum. The heavy burden of BT malaria in terms of morbidity and the importance of the infection in travellers suggests that there is a need for research in this area. A major constraint has been the non-availability of an in vitro culture method.

It has been estimated that a useful malaria vaccine is several years away. That may be so but the point to note is that the objective is achievable. The major constraint is finance. Even this is now forthcoming in the public domain. It is now being realised that if Africa is to be saved the twin scourges of malaria and HIV/AIDS these have to be tackled vigorously. The latter has a more global connotation and is already being pursued vigorously in a mission mode. Malaria needs to be tackled similarly.

Once a suitable vaccine becomes available it will have to be used as a part of the general immunization programme in the areas where P. falciparum infections are holoendemic (e.g. Af-
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References

23. Read Andrew. quoted in Malaria - from Infants to Genomics to Vaccines, by Long CA, Hoffman SL. Science 2002;297:945-7.

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