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Where Are We in the Quest for Vaccines for Malaria?

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1. Potential Sites for Vaccine Action in Malaria

Malaria is a threat to almost half the world's population. It is a major health problem in tropical countries, where approximately 300 million malaria cases occur annually. In Africa alone, almost 1 to 2 million children die of the disease every year. More than 50 years ago, attempts to develop a malaria vaccine were initiated but gave way to searches for new drugs during World War II and to the antimosquito programme that followed. The overwhelming success of that malaria control programme, which used chloroquine as a chemotherapeutic agent and DDT as an insecticidal, was so impressive that by 1961 it had resulted in a rapid decline of scientific research and training of malaria workers. Unfortunately, the recent resurgence of malaria in many areas of the world has caused the malaria control programme's initial success and momentum to be replaced with a gradual, unrelenting battle against malaria. The alarming resurgence of malaria is due to the development of resistance in the malignant species of the human malaria parasite *Plasmodium falciparum* to antimalarial drugs and of its mosquito vector to insecticides. Chloroquine- and multiple-drug-resistant strains of *P. falciparum* are spreading in most of the tropical countries of the world. It is equally alarming that new and novel antimalarial drugs, especially for prophylaxis, are not likely to become available in the near future (Peters 1983) and that no new insecticides superior to DDT and economically usable by poor malaria-endemic countries are on hand.

Since it has become obvious that neither insecticides nor antimalarial drugs alone are sufficient to control the disease, great hopes have been placed on the development of effective malaria vaccines to supplement existing and future novel malaria control measures. Consequently, research activities toward developing an immunological solution to malaria have accelerated over the last decade. The purpose of this article is to summarise the present status of malaria vaccines. The following references are given for the reader who is interested in the historical perspective and detailed account of the technical aspects of experimental malaria vaccines (Brown 1969; Carter & Gwadz 1980; Cochrane et al. 1980; Cohen & Mitchell 1978; Heidrich 1986; Miller et al. 1984; Mitchell 1984, 1989; Nussenzweig & Nussenzweig 1986; Perlmann & Wigzell 1988; Perrin et al. 1988; Siddiqui 1980).

Malaria is caused by a protozoan parasite of the genus *Plasmodium*. The 4 species *P. falciparum, P. vivax, P. malariae* and *P. ovale* naturally infect humans. The species responsible for the highest mortality rate, especially among nonimmune children, is *P. falciparum*. Malaria infection in humans is initiated by injection of 'sporozoites' into the bloodstream by the bite of an infected female *Anopheline* mosquito. Within a few minutes, the sporozoite disappear from the bloodstream and in-

vade liver hepatocytes. Within the liver cells, sporozoites mature and multiply to produce merozoites which are released into the bloodstream and invade erythrocytes. Within the erythrocytes, they subsequently develop into ring, trophozoite and finally, schizont stages. At the schizont stage the erythrocyte ruptures. This liberates the merozoites which continue to invade red blood cells thus perpetuating the 'erythrocytic cycle' of the parasite's growth. This phase of the lifecycle of the malaria parasite is responsible for the clinical signs and symptoms of malaria. Some of the merozoites that invade the erythrocytes differentiate into sexual forms of male and female gametes. These forms are taken into the female *Anopheles* mosquito gut during a blood meal. In the mosquito gut the fertilisation of female gametes by male gametes takes place. The resultant zygotes pierce their way onto the gut wall and undergo asexual multiplication resulting in the production of sporozoites which lodge in the salivary gland of the mosquito. The cycle of transmission is complete as the mosquito engages in further blood meals.

At present there are 3 distinct malaria vaccines which are the focus of intensive research. The first type is a sporozoite vaccine which aims to block entry of sporozoites into liver hepatocytes. This would eliminate the initiation of infection. The second type is a merozoite vaccine which aims to block invasion of merozoites into erythrocytes. As mentioned above, this stage of the lifecycle is responsible for the morbidity and mortality of the disease. A merozoite vaccine would prevent the disease or significantly alleviate its course of infection. The third type is a transmission-blocking vaccine which is aimed at the sexual stages of the parasite lifecycle. This vaccine would not prevent infection or disease in vaccine recipients but would block or alleviate the spread of the disease within the human population.

The rationale for the development of malaria vaccines is based on the fact that immunity to malaria is acquired in nature but it develops slowly, after years of repeated infections in individuals living in holoendemic areas. This means that children between the ages of 6 months and 5 years are most susceptible to severe clinical disease. By adulthood, infected individuals show little parasitaemia and rare clinical symptoms. However, the acquired immunity is incomplete in that parasitaemia may continue although illness ends. It is also short-lasting because without reinfection, immunity wanes. It appears that a major reason for the slow development of malaria immunity is the considerable antigenic diversity of some of the major immunogens occurring in different strains within each parasite species (Walliker 1983). Other important features of malaria immunity are that it is species- and stage-specific. In other words, a person may be immune to *P. falciparum* but susceptible to *P. vivax* and vice versa. Similarly, a person immune to sporozoite infection will be susceptible to merozoite or blood-induced infection and vice versa. These features have important bearings on the development of effective malaria vaccines. For a malaria vaccine to succeed, it must improve on nature by generating a rapid and long lasting immunity.

1.1 Sporozoite Vaccine

Sporozoites are highly immunogenic. High levels of antisporozoite antibodies can be found in people living in endemic areas. x-Ray–irradiated sporozoites of a mouse malaria parasite were shown to induce protection against sporozoite challenge (Nussenzweig et al. 1969). Similar evidence showed that small numbers of human volunteers were protected against malaria by immunisation with attenuated, x-ray–irradiated sporozoites (Clyde et al. 1973).

The sporozoite surface is covered by a circumsporozoite (CS) protein. This protein has been identified as a protective immunogen, and has been the focus of intensive investigation as a sporozoite vaccine candidate (Ballou et al. 1985; Nussenzweig & Nussenzweig 1984, 1986; Zavala et al. 1985). In humans, there is indirect evidence that antibodies directed against the CS protein may play a role in the acquisition of immunity against malaria in endemic areas (Del Giudice et al. 1987; Nardin et al. 1979) and in fact the gene coding for the CS protein has been cloned for several plasmodia species

(Dame et al. 1984; Enea et al. 1984; Lockyer & Schwarz 1987). The immunodominant epitope of the *P. falciparum* CS protein is a repetitive sequence of the 4 amino acids asparagine-alanine-asparagine-proline (NANP). The NANP repetitions cover the surface of mature sporozoites. Protection against sporozoites is mediated at least in part by antibodies directed against this dominant epitope (Zavala et al. 1983, 1986). Due to various technical reasons, no direct vaccination experiments have been conducted in the *P. falciparum*-monkey model using native *P. falciparum* CS protein. However, based on indirect evidence that antibodies to NANP repetitions inhibit sporozoite invasion, this immunogen has been the basis of two human vaccine trials.

1.2 Merozoite Vaccine

As mentioned above, asexual blood stage multiplication initiated by the invasion of merozoites into erythrocytes is responsible for the mortality and morbidity associated with malaria. Therefore, the development of vaccine(s) against this stage has been the focus of intense investigation. The landmark discovery of a method for the continuous *in vitro* cultivation of *P. falciparum* has given researchers the essential parasite material and stimulation for these rigorous investigations (Trager & Jensen 1976). Earlier expectations of developing a merozoite vaccine that is able to induce sterile immunity has undergone realistic modification. The goal now is to develop a malaria vaccine which is able to transform the immune system of nonimmune individuals (6-month to 6-year-old children from malarious endemic areas) into that of adults having experienced repeated malaria infections.

A number of asexual blood stage antigens (major merozoite surface protein, ring-infected erythrocyte surface protein, rhoptry protein, parasitophorous vacuole protein, transferrin receptor protein, glycophorin binding proteins, cytoadherence and knob proteins) have been identified as possible vaccine candidates (Brown et al. 1985; Camus & Hadley 1985; Delplace et al. 1985; Holder & Freeman 1982; Holder et al. 1985; Leech et al. 1984; Marsh & Howard 1986; Perlmann et al. 1984; Perrin & Dayal 1982; Ravetch et al. 1985; Rodriguez & Jungery 1986; Udeinya et al. 1983). The most studied of the merozoite antigens of *P. falciparum* are the major merozoite surface protein (PMMSA) and the ring-infected erythrocyte surface protein (RESA). The precursor of the major merozoite surface antigen has a molecular weight ranging from 185 to 200kD depending on the strains of *P. falciparum*. It is a glycoprotein which is synthesised throughout schizogony and transported at the surface of the schizonts (Holder & Freeman 1982). The RESA antigen is synthesised in trophozoites and accumulates in micronemes of merozoites. Following invasion, it becomes associated with the membrane of ring-infected erythrocytes and has a molecular weight of 155kD (Brown et al. 1985; Perlmann et al. 1984). The gene encoding for PMMSA (gp195) and RESA proteins has been cloned for several strains of *P. falciparum* (Anders 1986; Chang et al. 1988; Favaloro et al. 1986; Holder et al. 1985; Lyon et al. 1986; Mackay et al. 1985; Weber et al. 1986).

The successful immunisation of *Aotus* monkeys against *P. falciparum* using the merozoite stage antigen (Mitchell et al. 1977; Siddiqui 1977) was an important finding in establishing the *Aotus* monkey as an appropriate animal model to evaluate the efficacy of various *P. falciparum* vaccine candidates. More recently, a number of laboratories have shown that immunisation of *Saimiri* monkeys (Hall et al. 1984; Perrin et al. 1984) or *Aotus* monkeys (Patarroyo et al. 1987; Siddiqui et al. 1987) with a 'native' merozoite surface protein (gp195) isolated from *in vitro* cultured blood-stage parasites induces partial to complete immunity against a lethal *P. falciparum* challenge. Subsequent vaccination studies using subunit *P. falciparum* vaccines (synthetic and/or recombinant proteins) induced partial protection in *Saimiri* (Cheung et al. 1986) and in *Aotus* monkeys (Collins et al. 1986; Herrera et al. 1990; Holder et al. 1988; Patarroyo et al. 1987).

1.3 Transmission-Blocking Vaccines

Significant progress has been made in identifying specific targets for antigamete immunity in a number of malaria species, including the human

malaria parasites *P. falciparum* and *P. vivax* (Quakyi et al. 1987; Rener et al. 1983; Vermeulen et al. 1985). Also, the gene encoding of one of these target proteins (25kD) has been cloned (Kaslow et al. 1988) enhancing the possibility of sexual stage vaccine development. However, two problems may hamper progress: the report of antigenic diversity in sexual stage antigens (Graves et al. 1985) and that antigamete sera with low titres may enhance the gamete fertilisation (Mendis et al. 1987).

2. Clinical Trials with Malaria Vaccines

In 1987 and 1988, three clinical trials were conducted, two with *P. falciparum* sporozoite vaccines and the third with a *P. falciparum* merozoite vaccine. A 12 amino acid synthetic peptide $(NANP)_3$ was conjugated to tetanus toxoid, adjuvanted with alum, and administered in 3 doses at monthly intervals to 35 individuals (Herrington et al. 1987). Three with the highest antibody titre and 4 controls were challenged with sporozoites. All 4 controls and 2 of the 3 vaccinated individuals developed parasitaemia and were treated with drugs; the third vaccinated person was completely protected. The other sporozoite vaccine tested simultaneously was a recombinant polypeptide containing the NANP repetition 32 times and linked to 32 amino acids of the tetracycline resistance gene (R32tet32). The vaccine was adjuvanted in alum and administered to 15 volunteers (Ballou et al. 1987). Six individuals with the highest antibody titre and 2 controls were challenged with sporozoites. Two volunteers had a delayed onset of parasitaemia and 1 volunteer remained completely free of parasites.

The first human trial of a blood stage vaccine was conducted in Colombia (Patarroyo et al. 1988). The vaccine consisted of 3 synthetic peptides (83.1, 55.1, and 35.1:N-terminal peptides of gp195); alum was used as an adjuvant. Five individuals were immunised. By ELISA, 2 volunteers appeared to have preimmune malaria antibodies. Following challenge, these two individuals and a third volunteer demonstrated controlled parasitaemia, while the remaining 2 individuals and all 4 controls developed parasitaemia and received drug treatment.

Although limited immunogenicity and protection have been demonstrated with no overall correlation between humoral or cellular immunity and protection, results of these clinical trials have nevertheless been encouraging in the sense that the first generation of synthetic and recombinant malaria vaccines have been found to be safe in humans. This limited success has also served to accelerate research towards the development of more effective vaccines. Our inability to culture *P. falciparum* on a large scale mandates that a practical malaria vaccine be a recombinant protein or a synthetic peptide-carrier complex. Poor immunological response and protection obtained in these clinical trials are partly due to the inherent biological nature of the immunogen, synthetic and recombinant proteins. It has been a great challenge to attempt to overcome this obstacle. However, within the last few years intensive investigations have been pursued to understand the possible mechanisms of acquired immunity, evasion mechanism(s) of parasites to the immunological response of hosts, vaccine presentation and role of adjuvants, antigenic diversity and its implication on design of vaccine, and MHC restricted responses to malaria vaccine epitopes. Progress towards surmounting these obstacles is summarised below.

3. Requirement for T Cell Epitopes in the Vaccine Molecule

Although the mechanisms of protective immunity against malaria are not fully understood, there is now ample evidence to indicate that humoral immune responses (Cohen et al. 1961; Lew et al. 1988; Majarian et al. 1984; Roberts et al. 1977; Weinbaum et al. 1976), cell-mediated immune responses and the pivotal role of the T cell (Brake et al. 1986, 1987; Weidanz & Long 1988) are implicated in potentiation of protective malaria immunity. In malaria immunity, T cells play a crucial role in regulating antibody production. They also give rise to antibody-independent cellular immu-

nity (Allison & Eugui 1983). Thus, much effort has been directed recently towards the definition of T cell–activating structures suitable for incorporation into malaria vaccines (Troye-Blomberg & Perlmann 1988).

The search for T cell epitopes within the CS protein, gp195 and RESA proteins, the 3 prime malaria vaccine candidates, has accelerated in recent years. Several T cell epitopes have been identified within the CS proteins of *P. berghei* (Romero et al. 1988) and *P. falciparum* (Good et al. 1987, 1988a). In the *P. berghei* mouse model, a high degree of protection against challenge has been demonstrated by passive transfer of cytotoxic T cell clones into mice (Romero et al. 1989). Indirect evidence that $CD8^+$ T cells are required for effector immunity against sporozoites was demonstrated earlier (Weiss et al. 1988). Tam et al. (1990) incorporated T and B epitopes of the CS proteins of *P. berghei* in a chemically defined synthetic vaccine which could induce high antibody titres and protective immunity against sporozoite challenge in mice. Impressive progress has also been made in identification of T cell epitopes within the Pf155 protein (Perlmann et al. 1988) and gp195 protein (Crisanti et al. 1988; Sinigaglia et al. 1988) – the two prime asexual blood stage candidate vaccines. It is significant that the epitopes recognised by human T cells reside within the conserved region of these immunogens. This has important implications for designing an effective merozoite vaccine.

4. Requirement for a Safe and Effective Adjuvant for Malaria Vaccines

As mentioned above, two *P. falciparum* sporozoite vaccines and a merozoite vaccine with alum as an adjuvant were found to be safe in humans but with limited immunogenicity and protective success. In contrast, the merozoite vaccine given in conjunction with Freund's complete adjuvant (FCA) induced complete protective immunity in monkeys (Patarroyo et al. 1987; Siddiqui et al. 1987). Why is FCA more effective than alum as an adjuvant in malaria vaccines? FCA has been shown to be the most potent inducer of both humoral and cell-mediated immune responses while alum has been shown to stimulate humoral responses but is deficient in inducing cell-mediated responses (Bomford 1980; Edelman 1980).

In light of general poor immunogenicity of synthetic or recombinant polypeptide antigens (Arnon 1984; Audibert et al. 1982), it is becoming recognised that the immunogenicity of malaria subunit vaccines can be enhanced by incorporating strong adjuvants (Miller & Good 1988; Siddiqui & Tam 1988). This adjuvant must be as potent as FCA in inducing both humoral and cell-mediated immune responses and at the same time be as safe as alum – the only adjuvant acceptable for human use. The development of such adjuvants has been pursued vigorously for the last decade (Bomford 1989). Several analogues of MDP, such as murabutide, MTP-PE, B30-MDP as well as lipid A and especially combinations of these adjuvants, have been identified as potential effective adjuvants (Hui et al. 1990). Some of these adjuvants are already undergoing phase I (safety) testing in humans. For example, phase I testing for a lipid A product with marked reduced pyrogenic activity has been completed (Vosika et al. 1984) and preliminary results regarding its efficacy for a malaria sporozoite vaccine candidate R32tet32 have been encouraging (Richards et al. 1988).

5. Antigenic Diversity in Candidate Vaccine Antigens

There is growing evidence of significant diversity in candidate malaria vaccine antigens both at the serological and molecular levels. Recent review articles have discussed this subject in detail (Anders et al. 1989; Kemp et al. 1987; McBride et al. 1985; Walliker 1989). Good et al. (1988a) reported polymorphism within a major T cell domain of the *P. falciparum* CS protein. This disturbing observation has been extended to another human malaria parasite, the CS protein of *P. vivax* (Rosenberg et al. 1989). In contrast, T cell epitopes have been recognised within the conserved region of *P. falciparum* merozoite vaccine candidate gp195 (Crisanti et al. 1988; Sinigaglia et al. 1988). However,

there is no evidence that the conserved region alone will be able to induce protective immunity against malaria parasites. An example of vaccine-induced variation in the merozoite surface antigen of monkey malaria has been reported by Klotz et al. (1987). Antigenic diversity is the probable mechanism for the parasite's ability to evade the host's immune response. This agrees with the observation that in nature, an individual develops protective immunity after exposure over several years to repeated infections involving multiple strains of the malaria parasite.

Thus, it is evident that the antigenic diversity of malaria vaccine antigens pose a major obstacle to the realisation of an effective malaria vaccine. Overcoming this obstacle is a serious challenge facing researchers today.

6. *Genetic Differential Nonresponsiveness of Mice and Humans to Candidate Malaria Vaccine Antigens*

The present strategy for the development of subunit malaria vaccines assumes that the population in general will respond effectively to these vaccine antigens. However, several studies in mice and humans reveal that there is a widespread restricted immunogenicity to candidate *P. falciparum* subunit vaccines. These studies were first conducted using synthetic and recombinant CS vaccines in congenic mice of different H-2 haplotypes. The results showed genetic restriction in the immune response to a limited number of H-2 haplotypes (Del Guidice et al. 1986; Good et al. 1986). Similar results have been reported for a recombinant *P. vivax* CS antigen (Nardin et al. 1988), for synthetic and recombinant RESA antigens (Lew et al. 1989), and for native sexual stage antigens (Good et al. 1988b). In contrast to the latter study, Chang et al. (1989) reported a generalised immunological response to a native gp195 merozoite vaccine antigen by several strains of congenic mice with differing H-2 haplotypes. However, a series of studies conducted in the human population in Gambia (Gabra et al. 1986; Quakyi et al. 1989), Tanzania (Del Guidice et al. 1987), Gabon (Chizzolini et al. 1988), and Papua New Guinea (Carter et al. 1989; Graves et al. 1988) showed widespread restricted immunological response to subunit sporozoite, merozoite and gamete vaccines. These results are not surprising considering the human population constitutes individuals of different genetic background. These results along with those observed in mice suggest that genetic factors partly explain the differential immunological responses (Quakyi et al. 1989).

An elegant study has been reported recently (Good et al. 1988c) showing that human recombinant IL-2 when used as an adjuvant during immunisation of congenic mice can overcome genetic nonresponsiveness to malaria sporozoite peptide as determined by antibody response. However, low CD4 cellular response was not affected, suggesting the inability of IL-2 to circumvent the genetic restriction at T cell–mediated immunity – an essential effector mechanism for inducing protective immunity in malaria. Thus, the genetic restriction of the immunological response to malaria antigens may seriously compromise the effectiveness of subunit malaria vaccines now being developed.

7. *Immunotherapy and Possible Vaccines Against Malaria Pathology*

Despite medical management, the mortality rate due to cerebral malaria is reported to be 20 to 50% in endemic malarious areas (McPherson et al. 1985; Warrell et al. 1982). It is believed that sequestration of erythrocytes infected with *P. falciparum* is responsible for cerebral malaria. Using hyperimmune pool sera in an *in vitro* melanoma cell system, reversal and inhibition of cytoadherence of parasitised cells has been demonstrated (Singh et al. 1988). Based on these results, further investigation of the possible use of immunotherapy in the management of cerebral malaria, especially in high risk groups, has been suggested (Hommel & Semoff 1988). A protein, Pf EMPI, responsible for the cytoadherence has been reported (Marsh & Howard 1986), which may lead to the development of a vaccine against sequestration of parasi-

tised erythrocytes, thereby preventing cerebral malaria.

A very different approach has been suggested for developing a vaccine against cerebral malaria and other severe pathological lesions caused by *P. falciparum* malaria. In response to parasite exoantigen liberated during the asexual schizogony of malaria parasites, excessive amounts of cytokines, such as tumour necrosis factor (TNF), are produced. It has been postulated that these cytokines might be responsible for severe pathological complications like cerebral malaria (Clark et al. 1989; Grau et al. 1987). This has led to a novel suggestion that, if the action of parasite exoantigen can be blocked by antibody, this might be the basis for the development of an 'anti-disease' malaria vaccine (Playfair et al. 1990). This line of investigation merits serious consideration.

8. Conclusions

The prospect for the development of effective malaria vaccines is reasonably good. But the realisation of this goal will require some time. Parasite antigenic diversity and genetic restrictions of immunological responses to these antigens in the human population pose great problems to the effectiveness of subunit malaria vaccines now being developed. The necessity for safe and effective adjuvants and for incorporation of T cell epitopes in the vaccine molecule are additional major constraints towards subunit malaria vaccine development. Some of these hurdles may be circumvented by designing a synthetic and/or recombinant polyvalent vaccine composed of a mixture of sporozoite, merozoite and gamete defined antigens. Furthermore, each of the antigens must contain conserved antigenic structures which could elicit both antibodies and T cell responses. Alternatively, development of safe recombinant virus vectors capable of carrying genes for such multiple antigens may overcome these hurdles.

To find solutions to these challenging problems requires the persistent efforts of malaria immunologists, molecular biologists and parasitologists as well as the steady financial support from national and international research sponsoring agencies. We must be able to convince the governments and research sponsoring agencies that the use of vaccines is the most cost-effective method to prevent and control infectious diseases like malaria. Finally, it should be pointed out that the malaria vaccine, when available, will serve as a supplement to existing control measures. Hence, research towards the development of novel antimalarial drugs and insecticides should also be encouraged.

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