REVIEW

TOWARDS A MALARIA VACCINE: RIDING THE ROLLER-COASTER BETWEEN UNREALISTIC OPTIMISM AND LETHAL PESSIMISM

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Abstract. Activity in many laboratories over the past decade has resulted in many vaccine candidates nearing clinical trials. These include several antigens from the sporozoite stage; merozoite surface antigens MSA1 and MSA2, RESA, the rhoptry proteins RAP-1 and RAP-2 from the asexual blood stage; the pfs25, pfg45 and pfg230 from the ookinete and gamete stages. This progress in the identification of potential vaccine candidates now highlights a series of scientific, developmental, economic and operational problems the solutions to which will be as critical to the development of a vaccine as the cloning and expression of parasite genes.

INTRODUCTION

Malaria is one of the major infectious diseases facing mankind today. Estimates of the number of deaths due to malaria are difficult to verify, but a recent figure of 800,000 children per year in Africa is probably a good guide. Global estimates are of 120 million clinical cases per year with 300 million people carrying malaria parasites. Perhaps a better figure is the proportion of the world's population at risk from malaria. This can be estimated with more confidence: current WHO estimates put it at 40% of the world's population (WHO, 1992).

Of course, not all of these people are equally at risk. Much of the world's attention is focused on countries such as Nigeria, The Gambia, Kenya, Papua New Guinea which have a high profile since they have very high endemicities and a great deal of valuable research has been done in them. Existing control programs are unable to prevent a great deal of illness and death, and the development of a malaria vaccine for such countries is seen as being of major importance.

In other countries such as India, China, Indonesia, The Philippines, Malaysia, Thailand malaria, although prevalent, is not quite such a critical problem because these countries continue to spend considerable sums of money on malaria control programs. Even in such countries, which between them contain a large proportion of the world's population at risk from malaria, the disease continues to have a major indirect impact through the economic strains on finite health budgets. For these countries, a vaccine which made the control measures more cost effective would be welcome.

In the 1970s a number of developments led to the prospect that a malaria vaccine may be feasible. These included the development of an *in vitro* system for the cultivation of malaria (Trager and Jensen, 1976) and advances in the cloning and expression of genes coding for malaria protein antigens. The prospect of a vaccine was greeted with enthusiasm. From a scientific point of view, a malaria vaccine represented a great intellectual challenge. No recombinant vaccine had been made to a complex parasite infecting any host, let alone humans. A subunit malaria vaccine represented the medical equivalent of putting a man on the moon, requiring major intellectual advances in the fields of parasite biology, molecular biology, biochemistry and immunology. Some of these advances were attractive to biotechnology companies, since commercial funding for a malaria vaccine could be justified not only on the basis of potential profits from a vaccine but also on the intellectual property acquired along the way.

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From the operational point of view, a malaria vaccine offered the prospects of a long term solution to the increasingly serious problems facing malaria control programs: the widespread appearance of drug resistance in the parasite (Wernsdorfer, 1991), insecticide resistance in the vector, increasing lack of compliance with control measures in populations living in endemic areas, and decreasing resources, both fiscal and motivational amongst malaria control services.

This early enthusiasm was important. It was a crucial factor in obtaining major funding for the malaria vaccine program but it had and continues to have a number of undesirable effects. There has been an unreasonable expectation that the "vaccine is just around the corner". Even in the early 1980s this led to singificant concern that existing control programs were being relaxed in the expectation of a vaccine.

There have been two very different philosophies pertaining to the production of a malaria vaccine. One approach is to develop a vaccine which mimics "natural immunity" and protects a person against the disease but does not prevent parasite growth and transmission. The second approach is to develop an anti-parasite vaccine which is aimed at giving sterile immunity or blocking parasite transmission. These two approaches are considered in more detail below.

ANTI-DISEASE VACCINE

Justification: The rationale for developing an anti-disease vaccine which has no significant effect on parasite prevalence is based on the following propositions:

1. A vaccine directed against pre-erythrocytic or erythrocytic stage which gives long lasting sterile immunity is unlikely.

2. In some highly endemic areas of the world, the available resources make it unlikely that an eradication program will be possible in the conceivable future. In these areas, computer models suggest that anti-parasite, transmission blocking vaccines used alone would need to have unrealistically high levels of both efficacy and coverage to have an impact on the incidence of malaria (Koella, 1991). There is concern that in these areas, an anti-parasite vaccine may be detrimental since it could lower the population's tolerance to the parasite, resulting in most of the population being at risk of morbidity and even mortality rather than just the young and immunologically compromised (*eg* pregnant women). Since this philosophy requires that transmission is not interrupted, then it follows that such a vaccine can make full use of natural boosting to maintain immunity.

Two approaches are being explored:

1. Attenuation of the parasite growth rates to ensure that parasite density stays below the level required to trigger disease. Effectively this is an extension of the control programs already used in most highly endemic areas where chemotherapy is used to attenuate parasite infections to prevent deaths and disease but is not used to significantly reduce the parasite prevalence.

2. Attenuation of the host's response to prevent the immunopathology associated with disease (Playfair *et al,* 1990).

Problems to be solved: Three areas need to be faced in the development of such a vaccine: conceptual, developmental and operational.

a) **Conceptual:** The difficulties lie in trying to develop vaccines which give the required degree of attenuation of either the immune response or of parasite growth. Work towards this type of vaccine has highlighted how little is known about the processes which lead to disease and death. Attempts to find antibody specificities which may correlate with protection have so far failed to lead to prototype vaccines (Perrin *et al,* 1982; Brown *et al,* 1982). This line of research has emphasised the complexity of the immune response to the pathogen. Although passive transfer of antibody led to parasite clearance (Cohen *et al,* 1961), the antibody specificities responsible have not been identified. There is evidence that much of the specific immune response may be at best irrelevant and at worst counterproductive (Anders, 1986).

In the target population, ie people with little or no prior exposure to malaria, recent results and the observations of over 60 years of induced malaria in neurosyphilic patients, human volunteers and clinical cases suggests that much of the morbidity associated with malaria is related to immunopathology related to the human's protective response to the infection (Clark *et al,* 1991).

The initial rapid parasite growth rate is abruptly halted at the time symptoms appear (Kitchen, 1949). Symptoms are associated with the production of lymphokines such as tumor necrosis factor (TNF) (Clark *et al,* 1989) and the symptoms are similar to those seen in cancer patients undergoing lymphokine therapy. It is not yer clear what triggers this production, although lipoproteins have been implicated (Bate *et al,* 1992). Since the symptoms can be induced in naive volunteer just hours after infusion of a sufficient number of parasitized cells, activation of specific immune responses appear unlikely. Consideration of the parasite biology suggests that this lymphokine response is critical to survival of both the host and the parasite. Without it, initial parasite growth rates are sufficient to destroy all of the host's red cells and thereby kill the host, before any efficient transmission of gametes to a mosquito could occur.

The details of how this response controls the growth of parasites are not clear, although the induction of pyrexia seems to be sufficient to slow parasite growth (Kwaitkowski, 1989). There is a slow development of tolerance with multiple attacks and it is not infrequent to find older children in endemic areas with high parasitemia although they are asymptomatic (Marsh, 1992). Following infection, it takes several weeks before a person will become asymptomatic (Kitchen, 1949; Powell *et al,* 1972). This tolerance is relatively short lived, and although there are few detailed studies available, there is a large body of anecdotal evidence suggesting that people who have moved out of an endemic area for periods as short as a year are at high risk of considerable morbidity on reinfection. If the maintenance of this threshold at relatively high parasitemias is a result of desensitization to lymphokines then an anti-disease vaccine other than a sterilizing immunity will be essentially impossible. The lower parasitemia caused by a vaccine will simply result in a lower disease threshold.

One proposal is that an anti-parasite vaccine may slow parasite growth sufficiently that following infection, a variety of effector mechanisms will have time to be induced to hold the parasites below the disease threshold. Again, in the target group, people with little or no immunity, this will be difficult. Left untreated, these people remain symptomatic for several weeks with a parasitemia at or above the disease threshold and so the induction of these putative mechanisms could not occur more quickly than a few weeks. This threshold is normally passed only a few days after parasites emerge from the liver (*ie* after about 2 blood stage replication cycles). The asexual erythrocytic stage probably has an intial multiplication rate between 4 and 10 fold per generation. From these figures, it is possible to calculate the efficacy a preerythrocytic or erythrocytic stage vaccine would require to slow the growth rate sufficiently to allow the expression of induced effector mechanisms which would control parasite growth before the disease threshold is past.

At a multiplication rate of 4 fold per generation, to give a delay of 2 weeks in the time it takes the parasites to reach the disease threshold (*ie* a total of about 9 generations, counting the 2 which normally occur), a pre-erythrocytic stage vaccine would need to reduce the number of liver stage parasites being released by a factor of $9^4/2^4$, or by 410. At a multiplication rate of 10 fold per generation, the same vaccine would need to reduce the release of liver stages by a factor of 34 million. Since even the lower limit of a 410 fold reduction is greater than most estimates of the number of sporozoites inoculated (Rosenberg *et al,* 1990), this calculation suggests that it is essentially impossible to produce an anti-sporozoite vaccine which will reduce the inoculum sufficiently to delay the onset of symptoms by two weeks without killing all parasites.

For a vaccine aimed at slowing the growth rate of erythrocytic stages, a similar calculation shows that it would have to reduce the multiplication rate from the lower limit of 4 fold per generation to 1.36 or by a factor of 3 to give an increase of 2 weeks in the prepatent period. This shows that the efficacy of the vaccine would have to fall in a very narrow range. Less than a three fold reduction will result in the person being ill in less than 2 weeks; more than a 4 fold reduction gives sterile immunity. At the higher estimate of an initial 10 fold multiplication rate, the corresponding range in which this vaccine would be effective is a 6 to 10 fold reduction. It is difficult to see how such a vaccine could be made to operate within these limits in a significant proportion of the population, over an extended period of time and in the face of considerable parasite diversity. It would appear that a vaccine is more likely to have

one of two outcomes: no effect or sterile immunity.

A vaccine which protects against death may be conceptually easier to produce since it is possible that a vaccine which is not able to prevent the parasitemia from rising to a disease threshold, may still result in reduced symptoms. A useful model for a vaccine which may prevent death but not prevent morbidity comes from the use of mala ria as a therapeutic agent for neurosyphilis. These patients often required subcurative doses of anti-malarial drugs to prevent life threatening complications. Such treatment appears to have had little effect on the duration of symptoms. An anti-erythrocytic stage vaccine which slowed parasite growth rates could be expected to have a similar effect. Howerver, as in the case examined above for a vaccine which slows infections, it is difficult to see how a vaccine could be produced which would have a sufficient effect to prevent death but which would not exert its major effect as an anti--parasite vaccine. Again there would be a fine line between sufficiently attenuating parasite growth and giving sterile immunity.

b) **Developmental:** A major difficulty in developing an anti-disease vaccine lies in the unavailability of suitable non-human models. *P. falciparum* and *P. vivax* infections is monkeys such as *Saimiri* and *Aotus* do not result in the typical immunopathology associated with human infections. Similarly, the rodent malarias in mice and rats do not show the typical pathology associated with human disease. As a result, anti-disease vaccines will need to be taken all the way through to field testing before it is likely that efficacy can be detected. This will make testing a very expensive and slow process since it is likely that many different vaccine constructs will need to be assessed and that each of these will require the complete process of toxicology testing and production of moderate quantities of clinical grade material. The toxicology and phase I testing will need to be particularly rigorous since the test vaccine be given to substantial numbers of infants in endemic areas as the primary trial of efficacy.

Testing such a vaccine for anti-mortality and anti-morbidity effects will probably need to be conducted separately. Testing for prevention of death is likely to be straight forward, but requires large numbers. For example, in The Gambia, the infant mortality is approximately 140 per thousand. It is believed that about 4% of these deaths are due primarily to malaria (Greenwood *et al,* 1987). If crude death rate is the end point and a vaccine was capable of preventing 50% of the malaria related deaths, group sizes of 280,000 each would be needed in the test and control groups to give a 90% power of detecting an effect of the vaccine at the 0.05 confidence level. The malaria death rate in older children (1 to 4 years old) is higher at about 10 per 1,000 per year and the overall death rate lower at about 43 per 1,000 per year but would still need group sizes of 35,000 over a year for each of the control and vaccinated groups (Day, 1988). Group sizes this large pose logistic problems, not the least being that in the case of The Gambia the sample size is substantially bigger than the available population! These numbers can be reduced if more specific criteria of death due to malaria are introduced and considerable effort has gone into developing verbal autopsy techniques (Greenwood *et al,* 1987). However, even if these techniques are perfected, and all deaths can be attributed either to malaria or other causes, then group sizes of 10,000 and 6,000 respectively for the <1 and the 1 to 4 year olds would still be required for the control and vaccinated persons to be 90% confident of detecting a 50% reduction in malaria deaths. A major difficulty with this approach is the intensive surveillance required. This in turn raises ethical problems associated with using death as an end point. In this situation, is clearly unacceptable to allow sick infants to go untreated. Effectively, the test is to see if the vaccine combined with the use of antimalarial drugs is more effective in preventing death than the use of antimalaria drugs alone. Under these conditions it is likely that the malaria related mortality in the control group would be substantially less than that quoted above, requiring a compensatory increase in group sizes.

Testing an anti-morbidity vaccine in one sense will be easier since many more people get sick with malaria than die, so in principle, group sizes will be smaller. Again taking The Gambian data as an example, weekly surveys show that children have about one febrile episode per year of which about half are likely to be to malaria. The size of the group repuired will depend on an expectation of what effect a vaccine will have on morbidity. If the vaccine has an all-or-none effect, then groups sizes can be quite small. For this example, a group size of 330 would be required for a 90% power of detecting a 50% reduction in malaria related

febrile episodes. However, if the vaccine attenuates the symptoms without preventing them altogether (as would be expected in a target group of relatively nonexposed subjects), then this will lead to major difficulties in testing. The group size needs to be substantially larger since the parameter being tested is now a continuous variable and there will need to be some way of assessing severity, possibly requiring an active follow up. The Gambian data show that this may need to be quite frequent since monthly reporting only detects about a quarter of the cases. This in turn leads to a major ethical dilemma. At what stage should a patient presenting with relatively mild symptoms be treated?

c) **Operational:** A number of constraints on the deployment of an anti-disease vaccine will need to be taken into account during its development. These include:

1. Corverage rates: An anti-disease vaccine will have no herd immunity effect, so only those people vaccinated will be protected. Since the target groups are infants and young children, the vaccine will require an efficient infrastructure to be useful. Clearly, one way this could be achieved would be to make the vaccination schedule compatable with the delivery of other vaccines through the WHO extended immunization program. Unfortunately, some of the areas where this type of vaccine is most needed are those areas in which the delivery of health services is most difficult.

2. Development of resistant serotypes of the parasite: An anti-disease vaccine, particularly one which works by reducing parasite growth rates but does not induce sterile immunity results in conditions where the selection of resistant serotypes of the parasite is maximized. Anti-disease vaccines will need to be designed to minimize this risk.

3. Implication for future control programs: Control programs which aim to reduce parasite prevalence leading to eventual eradication rely heavily on passive case detection. A major problem in these programs is to detect asymptomatic carriers. The use of a long lasting and effective anti-disease vaccine would be counterproductive in such situations. A limited effective life for an anti-disease vaccine may be a virtue rather than a drawback.

ANTI-PARASITE VACCINE

Justification: Arguments for developing an anti-parasite vaccine are as follows:

1. There is now substantial evidence from model systems that vaccines directed against sporozoites, asexual erythrocytic stages and mosquito stages can induce sterile immunity or block transmission.

2. Such a vaccine will make eradication programs possible, since no parasites guarantees no disease.

3. Anti-disease vaccines which have little impact on parasite growth are unlikely to ever be possible for reasons outlined above.

Problems to be solved: The problems to be solved fall into the same three areas as the anti-disease vaccines: conceptual, developmental and operational.

a) **Conceptual:** The major conceptual problem is how to make a vaccine which gives sterile immunity when even repeated infections with the parasite fail to do so.

One approach which has been successful at identifying potential vaccine candidates is to screen libraries of mouse or rat monoclonal antibodies raised against malaria parasites. By using an animal which is not the natural host of human malaria, it may be possible to overcome natural selection pressure which may make key antigens nonimmunogenic in humans. These antigens must then be presented in a form which is immunogenic in humans. Since the native antigen fails to elicit a sterile immunity in a natural infection, it does not follow that a near native recombinant protein will be the best form of an antigen. In fact, there are grounds to believe that many of the odd structures in some malaria proteins may exist as immunological smokescreens which divert immunological responses away from appropriate epitopes (Saul *et al,* 1984).

b) **Developmental:** The conceptual problems underlying the development of an anti-parasite vaccine requires a different philosophy towards vaccine development than more conventional anti-viral or anti-toxin vaccines. One model is the search for new drugs, where very large numbers of compounds are screened then systematically

modified and tested. This has been the approach taken by Patarroyo *et al* (1987) where large numbers of peptides were screened for signs of activity in a monkey infection model.

Reduced to its bare essentials, such an approach makes no assumptions about the mechanism of protection or even the target of the protective response. One of the outstanding antigenic characteristics of many malarial proteins is the propensity for frequent cross reactivity. In at least one well documented case this arises from sequences which are common in malaria proteins but relatively rare in humans. This particular epitope is recognized by a mouse antibody M26-32 which inhibits the growth of parasites *in vitro* (Cheng *et al,* 1991). In cases such as this epitope and other antigens under consideration such as RESA, we may never know the target of the protective response elicited by immune mechanisms which also recognize these antigens.

In practice, the choice of antigens to be tested can be prioritized by location and likely function of the antigen, and by its availability in a form suitable for trials. There are 10 to 20 leading antigen under consideration; there are multiple alleles of several of these; there is a large number of different forms of each which could be tested ranging from short peptides to full length recombinant proteins; even with a restricted set of adjuvants available, there is still a substantial number of combinations of adjuvant, dose and route of administration. Many candidate antigens are now moving into clinical trials and there is a pressing need to develop testing procedures which will allow for the orderly improvement of prototype vaccines. Since the production of each new clinical grade antigen may take 1 to 2 years to make, purify, carry out toxicity tests, and formulate at a cost of $100,000 to $500,000, even as a global community we don't have the resources to randomly test all possible combinations.

One of the problems in the test systems for anti-parasite activity in volunteer trials is the essentially all-or-none end point. As detailed below for individual antigens, there have been relatively few trials either in man or in animal models, where there has been a clear correlation between some measurable immune response and protection. Rational development of vaccines, or even our ability to put priorities on development will be greatly enhanced by an increasing understanding of the mechanisms underlying immunity.

The developmental status of several leading candidates is reviewed below and summarized in Table 1.

Anti-sporozoite vaccines

Attention has focused on the major circumsporozoite protein of *P.falciparum.* There have been 7 published phase I or phase II trials in humans (Ballou *et al,* 1987; Herrington *et al,* 1987; Etlinger *et al,* 1988; Guiduemde *et al,* 1990; Sherwood *et al,* 1991; Vreden *et al,* 1991; Fries *et al,* 1992). All of these have involved constructs of the repetitive region of protein, either in the form of synthetic peptide coupled to a carrier protein (Herrington *et al,* 1987; Etlinger *et al,* 1988; Guiduemde *et al,* 1990), or as a recombinant peptide expressed as a fusion protein (Ballou *et al,* 1987; Sherwood *et al,* 1991: R32tet32; Vreden *et al,* 1991: R16HBsAg; Fries *et al,* 1992: $R32NS1_{18}$). Hoffman *et al* (1991) have summarized work towards improved responses. This work has progressed further with another 3 trials reported at the 1991 meeting of the American Society for Tropical Medicine and Hygiene with $R32NS1_{18}$ using a monophosphoryl A, mycobacterial cell wall skeleton, squalene adjuvant and with R32 conjugated to *Pseudomonas aeruginosa* toxin A. There has been a steady improvement in immunogenicity as measured by antibody titers or inhibition of sporozoite invasion *in vitro* (Fries *et al,* 1992) but there is not yet any indication of substantial protection. It is not yet clear if this is due to insufficient quantity of antigen or an inappropriate specificity. In model systems both are important. While it is difficult to make direct comparisons of antibody levels from different laboratories, using different antisera, it would appear that the levels of specific antibodies so far achieved are below the levels of monoclonal antibody required to protect mice in passive transfer experiments.

There have been 3 trials of recombinant *P. vivax* CS proteins in *Saimiri* monkeys (Colling, 1990; Collins *et al,* 1990), involving 5 constructs of the CS protein covering different parts of the molecule. One of these, $V20NS1_{81}$ consists of 20 copies of the repeat fused to an 81 amino acid portion of the influenza protein, NS1 and expressed in *E.coli.* The other proteins were expressed in yeast. Some limited protection was seen on challenge. However, no correlation between antibody levels

Table 1

Antigen	Published trials		Status
	Monkey	Human	
Sporozoite Stage			
Circumsporozoite protein *(P. falciparum)*	-	7	Further trials underway, consistant protection not yet achieved.
Circumsporozoite protein *(P. vivax)*	3	2	Low immunigenicity in human
Sporozoite surface protein 2 (SSP2)	-	-	Protection in rodent studies
Asexual Erythrocytic Stage			
Merozoite suface antigen 1 (MSA1)	Many	-	Native protein protects some peptides protect recombinant protein, yet to give consistent result.
Merozoite surface antigen 2 (MSA2)	-	-	Trials in humans underway.
Rhoptry proteins 1 & 2 (RAP-1, RAP-2)	3	-	Native proteins protect.
Apical membrane antigen 1 (AMA1)	1	-	Protected in monkey. Rat monoclonal antibodies gives marked *in vitro* inhibition.
RESA/Pf155	3 (one pos)	-	*In vitro* inhibition data. Monkey data indicates some protective.
Serine rich protein (SERA)	3 (2 with other proteins)	-	Inhibition in monkeys.
SP166 (mixture of synthetic peptides)	Several	In field trials	Inhibition in monkeys and humans.
Mosquito Stages			
Pfs25 Zygote surface antigen	1	-	Monkeys developed transmission blocking immunity. Considerable *in vitro* evidence of efficacy.

and protection was observed. Analysis of the specificity of antibody generated from one experiment shows that the polyclonal antibody lacks the fine specificity exhibited by a monoclonal antibody against the repeat region which is able to protect on passive transfer (Charoenvit *et al,* 1991).

There have been 2 phase I studies of *P. vivax* CS constructs reported in man. One involved the $V20NS1_{81}$ *E.coli* recombinant protein (Gordon *et al,* 1990), the other a construct expressed in yeast which included about 70% of the molecule. Neither gave high antibody responses (Herrigton *et al,* 1991). In the first trial, 5/5 failed to develop detectable antibody at the lowest dose (10μg): 4/4 at the middle dose (100μg) developed antibodies, but only 1/4 at the highest dose (1,000ug), suggesting that there may have been an immunosuppressive epitope in the repetitive region. In the other trial, the groups with the highest doses (200 and 400μg) developed antibodies, but the titers were

low.

Recently another protein was identified on the surface of *P. yoelii* sporozoites, sporozoite surface protein 2 (SSP2) (Hedstrom *et al,* 1990). Immunisation studies in mice showed that a mixture of a fragment of SSP2 with the *P. yoelii* CS protein showed a marked improvement of protection compared with immunization with the CS protein alone (Khusmith *et al,* 1991). The gene coding for this protein has now been entirely cloned and sequenced (Rogers *et al,* 1992). It has a similar structure to the CS protein with a central region of short repeated peptide sequences. The protein contains motifs common to a number of proteins involved in cell adhesion. Both the N and C terminal regions also have a high homology to a *P. falciparum* protein, TRAP (Robson *et al,* 1988), suggesting that it may be the *P. falciparum* homolog. If this is the case, then the way may be open to a combination vaccine against *P. falciparum* sporozoites with a significant improvement in efficacy.

Asexual blood stage vaccines

There are a number of antigens undergoing development. This review will concentrate on the most advanced.

Merozoite surface antigen 1 (MSAI) (Holder) *et al,* 1985): This antigen is synthesized as a large (about 195 kDa) protein anchored to the merozoite surface membrane through a glycolipid tail. It is highly polymorphic. The known sequences fall roughly into two antigenic families (Peterson *et al,* 1988), but with individual sequence variation within each family and recombination between the families. MSA1 is extensively processed by the time of merozoite release (Cooper *et al,* 1992).

MSAI was one of the first antigens identified as a potential vaccine on the bassis of its location and the ability of the *P. yoelii* form to protect mice (Holder and Freeman, 1981). Two monoclonal antibodies have been reported which are able to passively protect mice against challenge (Majarian *et al,* 1984; Lew *et al,* 1989). *In vitro* inhibition data shows that MAbs can inhibit the growth of *P. falciparum in vitro* (Pirson and Perkins, 1985; Blackman *et al,* 1990; Cooper *et al,* 1992). Several vaccine studies have been done in monkeys using MSAI purified from parasites or recombinant antigen. Siddiqui *et al* (1987) were able to achieve impressive protection in *Aotus* with the native purified form, with a homologous challenge. Less impressive results have been obtained in *Saimiri* (Hall *et al,* 1984) or *Aotus* (Siddiqui *et al,* 1978) when challenged with an heterologous strain although Etlinger *et al* (1991) obtained protection with a native protein from the K1 strain in *Saimiri* monkeys challeged with the Palo Alto strain of *P.falciparum.* Studies with the recombinant protein constructs or synthetic peptides have shown some efficacy (Patarroyo *et al* 1987; Herrera *et al,* 1990a,b; 1992). Patarroyo *et al* (1987) screened 15 peptides derived from the N terminal half of the protein as BSA conjugates for their ability to protect *Aotus* monkeys. Several showed partial protection of which one (peptide 83.1) has been used in human vaccine trials (see below). There have been 4 reported trials of recombinant MSA1 in monkeys. Herrera *et al* (1990 a,b) immunized monkeys with a hybird recombinant protein, 190N, consisting of amino acids 146-312 fused to amino acids 1059 to 1196. These portions of the MSA1 were chosen since they contain relatively conserved regions. 2/5 test monkeys cured their infection whereas all control monkeys needed drug treatment. Etlinger *et al* (1991) obtained similar results with *Saimiri* monkeys immunized with a construct p190-3 similar to the 190N protein and another construct p190-1 (147-321) similar to the N terminal portion of 190N. 1/4 monkeys immunized with p190-3 cured their infections; 2/4 immunized with p190-1 did so. In the first experiments, there was no marked decrease in the initial rates of parasite growth as would have been expected if the induced antibody was playing a role in protection and in the second experiment, there was no correlation between protection and antibody levels. Herrera *et al* (1992) repeated a monkey trial in *Aotus* using the N terminal fragment, 190L (146-312) of the 190N construct used earlier, and a hybrid protein 190L-CS.T3 containing a T helper epitope from the *P. falciparum* CS protein. Poor protection was observed with the 190L. However, the 190L-CS.T3 gave markedly improved protection, with 3/4 monkeys curing their infections. There was no correlation between protection with antibody levels, or with the ability of the monkey sera to inhibit *in vitro* parasite growth. However there was a marked correlation with sera levels of

gamma interferon. Knapp *et al* (1992) used a portion of MSA1 in a monkey trial as a hybrid protein consising of part of MSA1 with a serine rich protein SERA (Knapp *et al,* 1989) and a histidine rich protein (HRPII) (Wellems *et al,* 1986). Monkeys were protected, although as a similar degree of protection was seen in animals receiving a hybrid protein containing only the SERA and HPRII, it is not clear if the MSA1 played a role.

Merozoite surface antigen 2: A second merozoite surface antigen MSA2, has been described (Epping *et al,* 1987) with an apparent size of about 45kDa. The gene coding for the antigen has been cloned from a number of isolates (Smythe *et al,* 1988); like MSA1 it shows considerable sequence diversity (Smythe *et al,* 1990; Fenton *et al.* 1991) and is anchored in the merozoite surface with a glycolipid tail. The protein has short N and C terminal domains which are conserved. There have been no protection studies involving the native or recombinant protein in monkeys or man, but *in vitro* inhibition data using monoclonal antibodies suggest that MSA2 is a potential target of inhibitory antibodies (Epping *et al,* 1987; Miettinen-Baumann *et al,* 1988; Clarke *et al,* 1989).A phase I trial in man of a full length recombinant protein derived from the 3D7 clone is underway. Available evidence suggests that the central antigenically diverse portion of the protein is immunodominant. For example, no anti-MSA2 monoclonal antibodies have been found which react with the constant regions. However, a strong anti-constant region response can be obtained using peptides from these regions. The antibodies raised not only react with all isolates tested of *P. falciparum* MSA2 but cross react with a similar protein from *P. chabaudi,* and are able give 100% protection in mice from an otherwise lethal challenge with *P. chabaudi.* As expected from an antibody mediated effect, the protected mice show a marked inhibition of the intial growth rates of the parasites with a high correlation between decreased growth rates and antibody titers. Mice with the highest titer have sterile immunity (Saul *et al,* 1992). By contrast, mice immunized with the recombinant MSA2 protein, although they made antibody which cross reacted with the *P. chabaudi* homolog, had only a small decrease in initial parasite growth rates, and most were not protected. The peptide data are encouraging and emphasise that problems of antigenic diversity may be overcome by careful choice of the antigenic form.

Rhoptry proteins RAP-1 and RAP-2: Antigens in the rhoptry organelles were amongst the first components identified as potential vaccine candidates. Holder and Freeman (1981) showed that a monoclonal antibody against a rhoptry protein of *P. yoelii* was able to confer protection on passive transfer and showed that the purified target of this antibody was able to induce active protection on immunisation. Many rhoptry proteins have now been identified from *P.falciparum* (reviewed in Saul *et al,* 1992), but the leading candidates are an 80kDa and a 42kDa protein which form a complex in the parasite. These antigens have been cloned (Ridley *et al,* 1990a; Saul *et al,* 1992) and have been called RAP-1 and RAP-2 for rhoptry associated proteins 1 and 2. Monoclonal antibodies directed against this complex inhibit growth *in vitro* (Schofield *et al,* 1986). Mixtures of the affinity and electrophoretically purified RAP-1 and RAP-2 have been tested in monkey trials. Ridley *et al* (1990b) obtained impressive protection in *Saimiri* monkeys. Earlier studies by Perrin *et al* (1985) are not so easy to interpret as these studies used a mixture of proteins, which apparently included the parasite's aldolase. However, their data also suggest that the combination of electrophoretically purified RAP-1 and RAP-2 gave marked protection, and that the RAP-2 alone may have been effective.

Apical membrane antigen 1: AMA-1 is a 62kDa protein found in an apical structure in merozoites prior to release but on the surface of free merozoites (Peterson *et al,* 1989). There is a similar protein found on the merozoites of other species and the genes coding for the *P. chabaudi* and *P. fragili* homologs have been cloned (Peterson *et al,* 1990). Rat antibodies directed against the *P. knowlesi* form gave substantial inhibition of growth *in vitro* (Deans *et al,* 1984) and monkeys immunized with the purified antigen showed evidence of protection when challenged (Deans *et al,* 1988).

RESA/Pf155: RESA is a 155kDa parasite protein which is associated with the cytoplasmic face of the infected red blood cell membrane originally identified by antibodies affinity purified on glutaraldehyde fixed, ring infected erythrocytes (Perlmann *et al,* 1984). RESA is synthesised in mature stage parasites and is stored as small electron dense granules (Culvenor *et al,* 1991) which dis-

charge directly into the newly formed parasitophorous vacuole. In spite of the apparent inaccessibility of RESA, naturally occurring antibodies (Wahlin *et al,* 1984) mouse monoclonal antibodies (Ruangjirachuporn *et al,* 1988), human monoclonal antibodies (Berzins *et al,* 1985), and rabbit polyclonal antibodies (Sjolander *et al,* 1990; Aslund *et al,* 1986) all show inhibition of parasite growth *in vitro. Aotus* monkeys immunised with recombinant RESA showed modest protection against challenge with *P. falciparum* (Collins *et al,* 1986 a, b) which correlated with antibody titers. Much of this protection may be due to the extensive network of antigenic crossreactivity which is seen between RESA and several other malaria antigens (Mattei *et al,* 1989; Udomsangpetch *et al,* 1989), especially as anti-RESA antibodies are able to inhibit the growth of a parasite line which totally lacks RESA (Wahlin *et al,* 1992).

Serine rich protein: This 140kDa protein (SERA) is a soluble protein secreted into the parasitophorous vacuole and released on schizont rupture. The gene has been cloned (Bzik *et al,* 1988) and major sequence diversity has not been observed (Knapp *et al,* 1989). This antigen is found in microscopic immune complexes formed when polyclonal immune sera react with and trap emerging merozoites (Lyon *et al,* 1989). Electrophoretically purified protein, of which SERA was a major component, protected *Saimiri* monkeys (Perrin *et al,* 1984) and an N terminal recombinant fragment protected *Aotus* monkeys (Inselburg *et al,* 1991). Hybrid proteins containing SERA and HRPII, or SERA, HPRII and MSAI gave impressive protection in an Aotus trial with a marked inhibition of the initial parasite growth rates (Knapp *et al,* 1992).

SPf66: The SPf66 *P. falciparum* vaccine is a combination of peptides (35.1, 55.1 and 83.1) from 3 merozoite proteins, 35kDa and a 55kDa proteins which are poorly characterised, and from the 83kDa fragment of MSA1. The construct also contains fragments of the CS protein. Initial studies in *Aotus* monkeys identified these peptides as eliciting partial protective immunity (Patarroyo *et al,* 1987) and a subsequent vaccine trial in humans gave further encouraging results (Patarroyo *et al,* 1988). Other workers have found it difficult to reproduce the immunogenicity in monkeys. In contrast to the results obtained by Patarroyo *et al* (1992), Ruebush *et al* (1990) were unable to obtain protection in *Aotus* monkeys with either a mixture of the three peptides or SPf66 but did elicit antibody responses. Herrera reported at the 1991 meeting of the American Society for Tropical Medicine and Hygiene that his group were unable to elicit any response to the vaccine following 6 immunization trials in *Aotus* monkeys and the animals were not protected. The reasons for this lack of consistency is not known, but may relate to both the peptides and the adjuvant. In one sense, these results with monkeys may no longer be relevant since the vaccine is undergoing extensive field testing in South America. Vaccine is also being produced in the USA under GMP conditions which will enable further independent testing in humans. Data from South America shows that the vaccine is generally well tolerated and immunogenic in most of the population (Patarroyo *et al.* 1992), generating antibodies which react with the parent proteins. However a minority of people fail to develop high antibody titers (Patarroyo *et al,* 1991). High response was associated with a specific V beta rearrangement of the T cell receptor involving the V beta-8 gene whereas low response was associated with V beta-10 (Murillo *et al,* 1992).

Vaccines against mosquito stages

A series of stage specific antigen is expressed on the surface of gametes, zygotes and ookinetes. These are attractive targets for transmission block ing vaccines since it has been shown that polyclonal (Carter and Chen, 1976) and monoclonal antibodies (Renner *et al,* 1983) directed against these that are taken up in the mosquito blood meal can block fertilization or subsequent development. The data to 1988 have been extensively reviewed by Carter *et al* (1988) and will not be considered in detail here. Briefly, the main antigens under consideration which have been shown to be the target of transmission blocking immunity are: a 230 kDa (Pfs230) (Vermeulen *et al,* 1986); a 48/45kDa doublet (Pfs48/45) (Carter *et al, 1990);* a 40kDa very acidic protein (Pfs40) (Rawlings and Kaslow, 1992) which are all on the surface of gametes and early zygotes, and Pfs25, a 25kDa protein found on the surface of zygotes and ookinetes. The genes coding Pfs40 (Rawlings and Kaslow, 1992) and Pfs25 (Kaslow *et al,* 1988) have been cloned. Several groups have been working on the genes

coding for Pfs230 and Pfs48/45 and it is likely that the full sequences of these will be available soon.

So far Pfs25 is the only mosquito stage antigen to have moved into the vaccine development phase. Pfs25 is a very cysteine rich protein and like many of the other mosquito stage antigens, antibodies which target this antigen and block parasite development recognise reduction sensitive epitopes (Fries *et al,* 1989). This may constrain the form of antigen used in a vaccine, since synthetic peptides or proteins expressed in E. coli may not have the correct conformation to elicit an appropriate response. Two expression systems have been studied. Mice inoculated with a recombinant vaccinia virus which expressed Pfs25 produced anti-Pfs25 after several inoculations and this was able to block transmission to mosquitos (Kaslow *et al,* 1991). A modified form of Pfs25 has been produced in a yeast expression system. The resulting recombinant protein elicits transmission blocking antibodies in experimental in both mice and monkeys (Barr *et al.* 1991).

In a different approach van Amerongen *et al* (1989) identified peptides derived from Pfs25 which contain the epitopes recognized by transmission blocking monoclonal antibodies, raising the possibility of a peptide based vaccine.

c) **Operational:** From kinetic considerations, vaccines which rely on antibody as the effector mechanism are likely to need relatively high concentrations of antibody to kill parasites (Saul, 1987), and in the absence of boosting are therefore likely to have a short effective life. while such vaccines are seen as satisfactory for the traveller market, there has been a feeling that these will not be suitable in endemic areas.

Several recent models of the effect of transmission blocking by vaccines (reviewed by Koella 1991; Halloran and Struchiner, 1992) suggest that in most areas of the world, the level of coverage required before transmission would be blocked by a vaccination program is unachievable. This conclusion has led to widespread pessimism that vaccines which kill parasites will not be of use in endemic countries and has emphasised the need for anti-disease vaccines.

However, these models are essentially equilibrium models that examine the effect of a transmission blocking vaccine on the long term level of malaria. Furthermore, they make the assumption that the vaccination program is the only measure introduced. A recent model (Saul, submitted) looks at the dynamic effect vaccination programs may have on the rate at which parasites spread during an outbreak, through a seasonal transmission period and following active intervention as part of a control program. An important finding is that even a short lived vaccine at quite achievable coverage rates (*eg* less than 70% coverage), can have a major effect on the rate at which malaria will spread. Vaccines directed against all stages of the parasite: preerythrocytic, erythrocytic or sexual all had similar transmission blocking effects. These data suggest that the future for such vaccines may be much more optimistic that generally realised, since the use of even short lived anti-parasite vaccines may make a major difference in the cost effectiveness of eradication *versus* indefinite control programs based on an antidisease approach.

THE FUTURE OF THE VACCINE PROGRAM

Six years ago there was widespread optimism that a vaccine would be readily achieved. Today, the view is much more pessimistic. Malaria vaccine research is no longer so fashionable; granting bodies are becoming impatient with the development time and many of the companies which were involved in the global vaccine program have either withdrawn or substantially limited the resources they provide. From the viewpoint of workers in this field this is frustrating since the pessimistic outlook on funding contrasts greatly with the optimistic view on the availability of good vaccine candidates and the progress which is being made towards a vaccine. There is a real difference between the situation now and that prevailing 6 years ago in our ability to make development plans in a realistic manner. Now that the task has been better defined, it is clear the view 6 years ago was unrealistically optimistic with respect to the rate of progress.

I believe that optimist's view is still the only path. For if the optimist is wrong and a malaria vaccine one day proves to be impossible, then the world will have at least gained a great deal of knowledge ranging form better understanding of basic immunology, molecular biology and parasi-

tology through to more practical aspects of malaria biology. This knowledge will undoubtably assist in improving control programs. If the pessimists carry the day but are wrong then millions will die needlessly.

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