Review Article

MALARIA VACCINE: MYTH OR REALITY?

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Malaria currently remains the highest killer disease nationwide despite existing control measures. Malaria vaccine would provide a more efficient means of control and prevention of this disease. The objective of this review is to present the current trends in the production of malaria vaccine thereby supporting the view that malaria vaccine would be a reality. The suggestions towards achieving this inevitable goal are also included.

INTRODUCTION

Malaria has remained the highest cause of morbidity and mortality nationwide coming before measles and diarrhoeal diseases (CDC Final Report, 1995). The under five-mortality rate is about 160/1000 in Nigeria with malaria accounting for approximately 15% of the mortality figure (CDC Final Report, 1995).

The discovery almost a century ago that malaria was transmitted by mosquitoes led to the belief that the disease could be eradicated by elimination of mosquitoes from areas of human habitation. Twice more in the last century, new tools - the residual insecticides and efficient chemo-prophylactic drugs led to renewed optimism that malaria could be eliminated or at least controlled. However, because of changes in vector behaviour, drug resistance, manpower constraints for public enlightenment, control measures seem to have failed. In most rural areas for instance, the only activity remotely associated with malaria control is the monthly environmental cleaning activity. This itself is sporadic and not well organised. A major question that is asked of malaria research today is therefore whether it will be possible to develop vaccines to control the disease.

Consequently, the rate of progress in studies relating to vaccine studies as well as international meetings focusing on malaria vaccine have taken gigantic leaps with significant successes. The European action for development of malaria vaccines (VINCOMAL) has been regularly bringing together a group of scientists from Europe and Africa to discuss limitations and potentials of malaria vaccine. That organisation also has a close collaboration with the Private Vaccine Evaluation Network (PVEN) and the African Malaria Vaccine Testing Network (AMVTN).

Similarly, the European Union programmes for Research and Development (RTD) now has the possibility of offering contracts to identify and optimally exploit existing facilities in the field of malaria vaccine research (Mons et. al., 1997).

Unfortunately, the level of optimism towards the production of this vaccine appears to remain low in Nigeria. This may perhaps be due to limited information on the rate of progress towards this goal in Nigerian journals. This review would therefore be targeted towards reporting current and past accomplishments in candidate malaria antigen vaccine research with more emphasis on the erythrocyte stage antigens.

VACCINE DEVELOPMENT

The skill involved in development of vaccines has always been the use of a whole organism or a fraction of it. Non-pathogenic organisms (attenuated strains) could be chosen provided they are able to induce the correct immune response that protects the host against the virulent

forms. Pathogenic organisms could also be chosen and subsequently inactivated by radiation or chemicals. Subsequently, these are used to produce sub-component vaccines.

The initial selection of the candidate vaccine antigen could be either rational or empirical. Rationally, the antigens are chosen based on the criteria that they have predetermined properties and induce the correct immune responses. However, empirically, the only criterion for antigen selection would be that an experimental animal is protected in vaccine trials.

Unfortunately, the conventional and historically accepted methods of vaccine development described cannot be used for malaria development. Presently, *in-vitro* culture facilities are very expensive, cumbersome and low scale. Similarly, growing the parasites in monkeys is expensive and might pose a health risk due to contamination by simian viruses (Pasloske and Howard, 1994). In addition to these problems are the difficulties in developing a well-defined, easily grown attenuated strains with a low reversion frequency to the original pathogenic form.

The limitations posed by those problems therefore made the application of molecular biology techniques necessary. Thus, specific malaria antigens and peptides that encode sequences of the antigens could thus be produced. The first malaria vaccine (Spf 66) became possible with the application of these novel techniques. However, workers in this area are still trying to assess and correctly select the appropriate malaria vaccine candidates before finally embarking on both animal and human vaccine trials singly, in recombinant or cocktail forms.

VACCINE CANDIDATES:
Pre-Erythrocytic Stage Antigens

The malaria pre-erythrocytic stage (MPES) network, multicellular, multidisciplinary approaches have been making concerted efforts to understand extra-erythrocytic immunity (Mons *et. al.,* 1997). This is targeted towards empirically selecting pre-erythrocytic antigens as potential vaccine candidates. Of the 20 antigens that are the focus of MPES, the Liver Stage Antigen-1 (LSA-1), LSA-3, Sporozoite and Liver Stage Antigen (SALSA), Sprozoite Threonine and

Asparagine Rich protein (STARP) are most advanced. The LSA-3 is however being tipped as a strong candidate for inclusion as a vaccine based on rodent, primate and human field studies. This antigen showed a clear B - and T- cell activation. The *Plasmodium falciparum* LSA-3 protected mice against P. Yoelli challenge.

However, the circumsporozoite protein (CSP) that serves as ligands for sporozite receptors on hepatocytes (Egan *et al.*, 1993) seemed to have recorded the highest interest possibly because it was among the first parasite antigens to be cloned. Protective immunity against this protein had been demonstrated in both experimental models (Nussenzweig and Nussenzweig, 1985) and following natural infections (Qari *et. al.,* 1994). These responses had both humoral and cellular components (Egan *et. al.,* 1993; Brown *et. al.,* 1992). Similarly, Nwagwu and colleagues had recorded a high number of responders to the CS protein in SouthWestern Nigeria (Unpublished Data) as a vital corollary for future vaccine trials in a Nigerian population. Interestingly, Pattarayo *et. al.,* in 1988 had produced the first synthetic vaccine (Spf 66) made up in part by the CSP - 1. Human vaccine trials are also reported to have been successful (Amador *et. al.,* 1992).

Erythrocyte Stage Antigens SPF 66

This is the first synthetically produced malaria vaccine. It is made up of a combination of three peptides (35.1, 55.1 and 83.1) whose sequences are derived from merozoite and proteins together with a peptide sequence comprising part of the Circumsporozoite Protein-1 (CSP-1). The initial human trials were done in Columbia (Patarroyo *et. al.,* 1988). Three of the volunteers immunized with the synthetic vaccine had only mild infections and totally recovered by day 21; whereas the four members of the control group all needed drug treatments to cure the increasing parasitemia. Current trials are going on in Gambia, Tanzania and Thailand (University of Liverpool Annual Report, 1997). In Nigeria, field trials are about to commence in the southwestern regions (Nwagwu, Oral communication) and the eastern regions (Oguariri, Oral communication). The fact that the vaccine was safe and highly immunogenic in children less than 1 year

old with antibodies that did not vary with age makes it highly recommended since the bulk of risk of malaria are children.

Merozoite Surface Antigen - 1 (MSA-1)

This is a 195-KD protein found on the surface of merozoites and processed proteolytically prior to invasion of the erythrocyte (Cooper, 1992). Although this protein is polymorphic, it still contains regions that are conserved in different isolates (Peterson et. al., 1988). Protective responses to MSP-1 have been well reported in humans (Patarroyo et. al., 1988, Amador et. al., 1992). Recently, Nwagwu and colleagues have been able to establish the humoral responses of individuals living in a malaria endemic community of western Nigeria (Unpublished Data).

Vaccine studies in Aotus monkeys demonstrated a remarkable protective immunity when homologously challenged by the native purified protein (Siddigui et. al., 1987). Peptides containing sequences derived from MSA-1 when conjugated to bovine serum albumin (BSA) also demonstrated some protection in Aotus monkeys (Patarroyo et. al., 1987). Presently, on of these peptides (Peptide 83.1) is a component of a peptide vaccine that has undergone vaccine trials (Patarroyo et. al., 1988). A fusing of MSA-1 to a T-cell epitope was able to induce a protective response in about 75% of monkeys challenged in comparison with a non-impressive 50% induced by the unfused antigen (Herrana et. al., 1992).

Merozoite Surface Antigen-2

The immunogenicity of the 45KD (Epping et. al., 1988) protein is compromised by the large diversity of the central repeat sequence although the W- and C- termini are conserved. There are however human trials using the full length recombinant protein thus circumventing the polymorphism that occurs at the central repeat regions. Immunization of mice with peptide encoded by the conserved sequences showed potentials as a vaccine candidate. One hundred percent of the mice immunized were protected against challenge by plasmodium Chabondi (Saul, et. al. 1992).

Analysis of human isotype specific responses against the recombinant antigen is currently in progress. (Mons et. al.,

1997). Thus, cytophilic antibody responses can be documented at the end of the study. However, previous preliminary report indicates that the antibodies produced are primarily of the IgG3 subclass (Ferrante and Rzepazyk, 1997). The cytophilic nature of the induced antibodies further makes this antigen a likely candidate, as there are evidences of IgG3-neutrophil/monocyte collaboration as the major effector against blood stage parasites (Bonhawnn - Tayoun and Druilhe, 1992).

Merozoite Surface Protein-3 (MSP-3)

The relevance of this novel antigen has been confirmed by Antibody Dependent Cellular Inhibition (ADCI) assays (Ferrate and Rzepczyk, 1997). Further epidemiological studies showed a clear correlation between the acquired protection and the titres of cytophilic (IgG1 and IgG3) classes of ant-MSP-3 antibodies (Mons et. al., 1997).

Development of severe combined immuno-deficiency mice - human system had enabled Druile and group (Unpublished Data) establish a parasitemia between 0.1% and 3% for periods up to two months. Preliminary results also showed that only a combination of the antibodies and the monocytes caused a drop in parasitemia thus suggesting that the antigens have cellular components.

Apical Membrane Antigen-1 (AMA-1).

This antigen with varying molecular weights in different species of Plasmodium (83-KD in P. Falciparum, 66KD in P. Knowlesi) first localizes at the apical end of the merozoite and subsequently spreads accross the entire merozoite prior to erythrocyte invasion (Peterson et. al., 1989). Sequence comparison of AMA-1 in different strains of Plasmodium falciparum indicates relatively high sequence conservation (Pasloake and Howard, 1994). Immunization of monkeys with live AMA-1 of P. Fragili resulted in four of five monkeys controlling parasitemia without treatment as compared with all the control animals that required treatment (Collins, 1986). Monoclonal antibodies to P. Kunoleski also prevented P. Kunoleski merozoites from invading rhesus monkey erythrocytes. The purified antibodies still protected four of six

rhesus monkeys from live challenge (Deans et. al., 1988).

What makes all these results interesting is that both the natural and recombinant antigens do not need adjuvants. However, due to the conformational nature of the protective AMA-1 epitopes, an expression system became essential. The Yeast *Pichia pastoris* processing have therefore been used successfully to express folded homogenous P. Vivax AMA-1 ectodomam at very high secretion levels. This highly immunogenic product during experimental vaccine trials induced high antibody levels in rabbits and primates (Kocken et. al., Unpublished data).

Glutamate Rich Protein (GLURP)

The fact that GLURP is expressed in both the pre-erythrocytic and erythrocytic stages of the parasite life cycle in the human host raises significantly the prospect of the potential as a future component of the malaria vaccine. Antibodies to at least two specific regions of the molecule have been identified which are preliminarily associated with protection (Mons et. al., 1997). The three reactive types: RO specific, R11 specific and cross-reactive (RO + R11) antibodies had a direct inhibitory effect in combination with monocytes *in-vitro*. This therefore suggests that the antigen possesses both humoral and cellular components. Nwagwu and colleagues at the University of Ibadan, Nigeria are about to commence a rational assessment of the correlation between the responders with exposure, age and disease with a view to future vaccine trials.

Ring-Infected Erythrocyte Surface Antigen (RESA)

This 155-KD protein is produced during the mature stages of the erythrocytic phase and concentrated in electron dense granules. These are released into newly formed parasitophorous vacuoles upon invasion of the erythrocyte (Pasloske and Howard, 1994). The fact that RESA is inaccessible to antibody but have the ant - RESA inhibiting the parasite *in-vitro* growth have generated a lot of controversy (Collins et. al., 1986). Weak protection was also observed in an Aotus monkey trial with the recombinant antigen of *Plasmodium falciparum* thus leading to the suggestion

that there could be cross reactivity between the RESA antigen and other blood stage antigens.

The properties of these antigens that have been summarised in this review make them eligible to be tested in human vaccine trials. There are however presently over 2000 different proteins synthesized by the sexual stage parasite (Pasloske and Howard, 1994). Some of the antigens are undergoing various preliminary stages of assessment although not all with the intent of vaccine development.

Mosquito Derived Antigens

Assessing the feasibility of immunizing vertebrate host with mosquito-derived antigens in order to shorten the life span of mosquitoes after feeding on such immunised individuals are going on (Mons et. al., 1997). Some of the mosquitoes mid gut fractions induced responses that have a significant effect on the life span of the mosquitoes in a laboratory setting (Oguarri, Oral Communication). Billingsley et. al., (Unpublished Data) recorded varying antibody titres to sub-cellular fractions of midguts from sugar fed A. Stephsi that were used as antigens. However, the effects on the longevity of the mosquito were complicated by the high variability in the assay system.

Conclusion / Suggestion

From the data that has been reviewed, it is obvious that more antigens have to be assessed rationally considering the approximately 2000 antigens synthesized by the malaria parasite. More parameters should also be used to assess the antigens to effectively increase the chances of the antigen as a vaccine candidate. Previously, the rational selection of a vaccine candidate was based on the ability of the induced antibody to react with sporozoites or merozoites as well as prevention of parasitized erythrocytes to bind to endothelial cells. It was only very recently that the cytophilic nature of the induced antibodies was put into consideration. Fortunately, VINCOMAL, after one year of consultation has submitted a report on development of a rational assessment of malaria vaccine candidate antigens, adjuvant and delivery system.

More widespread and science driven approaches should be continued and proposals strongly encouraged from third

world scientists where malaria is very prevalent. Steps should also be taken to empower the scientists in terms of assess to current information as presently being done by the E-mail system at the University College Hospital, Ibadan, Nigeria.

Some of the antigens might also have to be altered to produce an optimal protection as was achieved for some candidate antigens like AMA-1 where an expression system was used and MSP-2, CSP-1 where recombinant antigens of the conserved sequences were used. It can therefore be rightly assumed that the adjuvant or amount of antigens used can affect the protective status of the antigen.

Although animal host models are different from human hosts, it could be assumed that candidate vaccines showing a high efficacy in animals should be tested in humans, as the likelihood of success is high. It might also be better not to waive an antigen aside because it failed in one trial as experimenting with the antigen in a cocktail or recombinant from instead of singly might produce better results as was recorded in SPF 66 human trials. Despite the fact that a large amount of fund is currently being channeled into this goal, it is important to note that this is a huge developmental plan. Enthusiasm by different agencies funding this research therefore should not be dampened if immediate results are not achieved. After all, we can all appreciate the complexity and ingenuity of the parasite we are dealing with. We can however confidently conclude that more than ever, the path towards the production of this vaccine is shorter based on present and past accomplishments recorded in this review.

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