

The hope but challenge for developing a vaccine that might control malaria

Michael F. Good

The Queensland Institute of Medical Research, Brisbane, Australia

As World Malaria Day approaches again it is timely, as an immunologically focussed community, to consider the consequence of not yet having an efficacious vaccine but also the progress that is being made and the challenges that we face in developing one. Key issues that need to be addressed for the leading vaccine candidates include antigenic polymorphism and loss of immunological memory.

Key words: Immunological memory · Malaria vaccines

Introduction

According to WHO there are about 250 million cases of malaria and 900 000 deaths *per* year, with most loss of life and disease occurring in African children (www.who.int/healthinfo). The malaria parasite alone is responsible for over 3% of all the years of life lost throughout the world and 10% of years of life lost in Africa. Drug resistance continues to worsen and combination drug therapy is now recommended to prevent the development of resistant strains. The effect on poor economies is also devastating with malaria reducing GDP by up to 1.3%. However, in terms of prevention for endemic countries, bed nets and indoor spraying of insecticides are the main tools; vaccines are not yet ready.

To consider the strategies for vaccines that are currently under development it is necessary to first briefly review the life cycle of *Plasmodium* (Fig. 1). Infection commences with the bite of an infected *Anopheles* mosquito that injects sporozoites into the lymph and circulation. Those that travel to and invade liver hepatocytes can develop over a week into thousands of merozoites that rupture from the liver and invade red blood cells. One merozoite can develop inside an erythrocyte over a period of 2 days (for *Plasmodium falciparum* and *Plasmodium Vivax* – the commonest of the human malaria parasites) into 16 merozoites that rupture the red cell and further invade. The parasite burden thus rises rapidly and severe pathology can ensue. Sexual forms

develop within erythrocytes and these can be taken up by mosquitoes during a blood meal to continue the life cycle. Vaccine strategies aim to block the life cycle at these different stages. The most developed strategies are to block the sporozoite and/or destroy the infected hepatocytes (sporozoite/exo-erythrocytic vaccines) or to block invasion of merozoites into erythrocytes or to destroy infected erythrocytes (blood-stage vaccines).

The subunit road long traveled

In 1983, the subunit era for malaria vaccine research commenced with the cloning of blood-stage and sporozoite-stage antigens [1, 2]. Shortly afterwards, the gene for the sporozoite coat protein, the circumsporozoite protein (CSP) of *P. falciparum*, was cloned [3, 4], quickly leading to the first human subunit vaccine trial with a recombinant protein containing the central immunodominant NANP repeat sequence of the CSP genetically fused to a nonsense protein, and referred to as R32tet32 [5]. A similar study using (NANP)₃ conjugated to tetanus toxoid was also tested [6]. In these studies of malaria-naïve adults, one of six and one of three volunteers, respectively, were protected from subsequent malaria following the bite of infected mosquitoes. In those two protected individuals the vaccines had successfully induced an anti-CSP antibody response that was capable of neutralizing all sporozoites prior to their invasion into hepatocytes. In individuals not protected, the antibodies were not sufficient to prevent all sporozoites invading hepatocytes. This is one of the challenges for

Correspondence: Professor Michael F. Good
e-mail: Michael.Good@qimr.edu.au

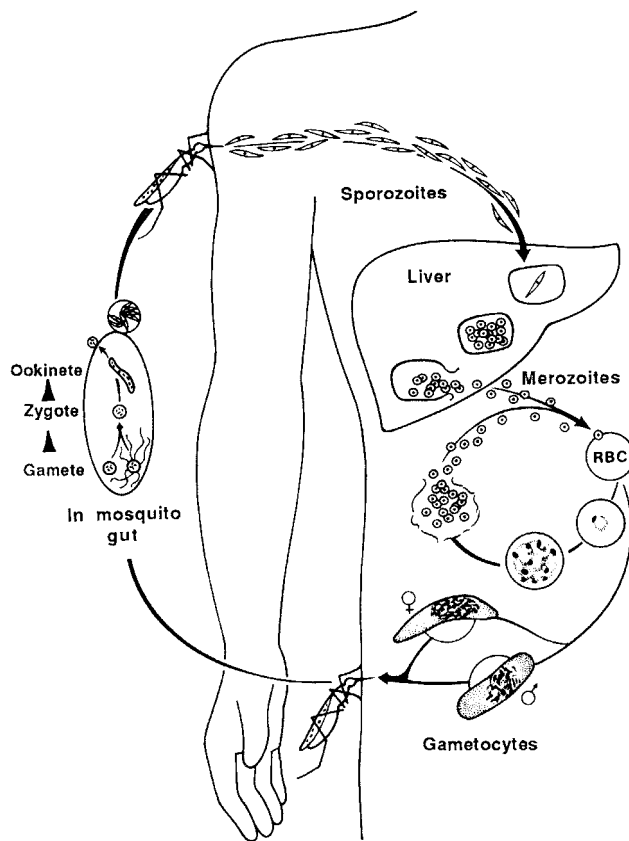


Figure 1. The life cycle of the malaria parasite provides a strategy for vaccine intervention and control (reprinted with permission from [41].)

exoerythrocytic vaccines – they must be 100% effective in either neutralizing sporozoites prior to invasion (*via* antibodies) or in killing infected hepatocytes (*via* T cells).

Since that time there has been an enormous amount of research into pre-clinical sporozoite vaccine research and a number of clinical studies involving recombinant, synthetic and DNA-based CSP candidate vaccines. However, in a Cochrane review, although Graves and Gelband [7] identified four subunit sporozoite vaccine preparations that had been tested in randomized control efficacy trials, only one preparation, “RTS,S”, [8] has been shown to reduce infections and clinical episodes of malaria. RTS,S consists of the central B-cell repeat epitope (NANP)_n of the CSP (“R”), with the T-cell epitope region of the CSP (“T”) linked to hepatitis B surface antigen (“S”) and mixed with free hepatitis B surface antigen (“S”), and formulated with an adjuvant.

RTS,S has undergone a number of trials with the most recent data coming from trials in Tanzania involving infants and children [9, 10]. The most encouraging data, which was for infants, showed that in the 6 months after vaccination the crude efficacy of protection from infection was 65% and for protection against febrile malaria it was 42%. In this study there was a correlation between the anti-CSP antibody titer and protection from infection. However, there was no evidence that those with high titers were more protected from clinical disease compared

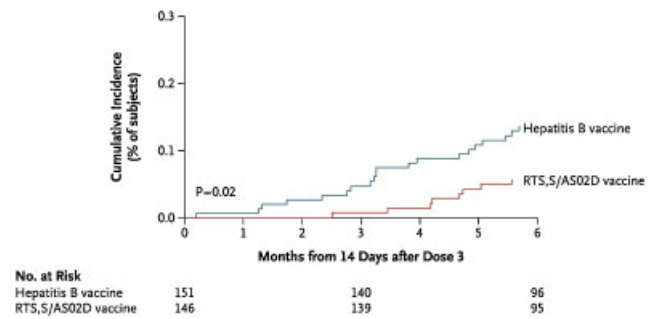


Figure 2. The cumulative incidence of at least one episode of malaria infection, following vaccination with either RTS,S or placebo (reprinted with permission from [10]).

with those with low titers. Overall, these results are encouraging but as immunologists they challenge us, with much more research needing to be done before an RTS,S vaccine could be considered an effective tool to reduce the global burden of suffering from malaria.

The main issues with RTS,S relate to the variable level of observed protection (ranging from 29 to 71% in different studies, although these figures depend on when during the study the efficacy is measured) and the apparent lack of immunological memory. As shown in Fig. 2, for the most recent study, 3 months after vaccination vaccinees were acquiring malaria at a rate similar to those who received a Rabies (control) vaccine. This phenomenon has been reported in most of the RTS,S studies. For example in a study involving Gambian adult men, protection was estimated by the authors to be 71% in the first 9 wk post vaccination, but after that time it reduced to 0% [11]. In another study (of African children) although it was claimed that the vaccine induced protection against clinical disease for at least 18 months [12] the efficacy in the last 3 months of the 18 month study was about half that during the first 3 months. Thus, 15.8% of control subjects developed malaria in the first 3 months after vaccination, compared with 10.6% of vaccinated subjects. Thus 33% of vaccinated subjects who could have been expected to develop malaria did not. In the last 3 months of the study 30.1% of control subjects who had not acquired malaria by 15 months did develop clinical malaria in the following 3 months, compared with 24.5% of vaccinated subjects who acquired malaria for the first time after 15 months – approximately 18.6% less than expected. However, this apparent but limited efficacy still masks the fact that all vaccinees are likely to eventually contract malaria within a relatively short period of time. The loss of efficacy reported for all these studies would be consistent with the drop of anti-CSP titers over time. For example, in the most recent study, antibody levels of 540 U/mL 1 month after vaccination had fallen to 72 U/mL when measured between 4.5 and 10.5 months post vaccination [9]. The reason for this, which underlines a major challenge for this vaccine, is likely to be that the exposure of the population to sporozoites post vaccination may not be sufficiently high to boost antibody levels, a situation compounded by the fact that the helper T-cell epitopes on the CSP and which are included in the vaccine are known to be polymorphic [13], with such polymorphisms having been shown to

be non-cross-reactive [14]. The rate of fall of anti-CSP titers over time is not inconsistent with the rate of fall of IgG passively administered to human volunteers, where the $T_{1/2}$, although quite variable, is between 22 and 96 days. Put differently, a passive immunization of human volunteers with anti-CSP antibodies (a strategy that has been shown to protect mice [15] and monkeys [16] from malaria) could be expected to have a similar outcome in terms of the extent and duration of protection to that observed in the RTS,S studies.

In an accompanying editorial it is argued that the vaccine studies were done in an area where the incidence of malaria was low and that the real challenge for the vaccine will be in Phase III trials in areas of high transmission [17]. This may be the case although it is possible that a higher rate of inoculation with sporozoites will lead to improved boosting and maintenance of titers, providing the sporozoites to which the vaccinees are exposed are immunologically cross-reactive with the T-cell epitopes present in the vaccine. These concerns are reminiscent of the issues raised in a paper in 1987 where it was demonstrated in a study in Kenya that adults with life-long exposure to malaria sporozoites did not have acquired protective immunity to sporozoites [18] – often daily sporozoite exposure was insufficient to induce and/or maintain a level of antibody sufficient for protection.

Vaccine trials for the blood stages of malaria are at a less advanced stage, although there has been more pre-clinical research. In the 1970s there was considerable enthusiasm for developing blood-stage malaria vaccines using *P. falciparum* parasites that could then for the first time be grown in culture in human red cells [19]. Analysis of malaria parasite density in syphilitic patients treated on multiple occasions with “malaria therapy” between 1940 and 1963 shows that infection leads to a significant drop in peak parasite densities during sequential infections [20]. Data such as these have lent support to a vaccine strategy using whole parasites. For example, it has been shown that monkeys could be nearly completely protected from infection with *P. falciparum* following exposure to 10^8 irradiated infected erythrocytes [21]. Similarly, experiments in monkeys immunized with infected blood emulsified with complete Freund’s adjuvant showed that they were also strongly protected [22]. However, the major problem seemed to be that large dose of infected red cells were apparently needed to induce efficacy. How would it be possible to culture such numbers of parasites when human blood was required for their production? The dawn of the subunit era effectively buried this research as there was then incredible optimism for developing a vaccine based on recombinant forms of merozoite surface proteins (MSP, such as MSP1 and MSP2, or apical membrane antigen 1 (AMA1); reviewed in [23]). Recombinant forms of MSP1 and AMA1 have been shown to be highly successful in inducing protection in animal models. However, as with the CSP, antigenic polymorphism is proving to be a significant hurdle with blood-stage vaccines [24, 25] and very high titers of antibodies may be required [26]. Furthermore, animal studies have also highlighted that infection can lead to apoptosis of memory B cells and long-lived plasma cells specific for MSP1 [27]. MSP1 and AMA1 now

have entered clinical trials but there have been no published efficacy trial results at this stage. In one study, MSP2, as part of a trivalent vaccine formulation, was suggested to be partially effective in controlling parasites containing the vaccine allele [25]. It thus remains to be seen whether the issues around antigenic polymorphism, immunological memory and the need for high titers will translate into significant clinical hurdles.

To address the issues of antigenic polymorphism there were a series of trials in the late 1980s and 1990s involving a synthetic peptide preparation incorporating sequences thought to be from three different blood-stage proteins and the repeats of the CSP. This candidate vaccine, referred to as SPf66 [28], was tested in ten clinical trials in South America, Africa and South-East Asia [7]. While there was evidence of efficacy in the South American studies (68% in one study), protection (31% efficacy) was observed in only one of two African studies and not in the Asian study [29]. In these studies, although the vaccine was shown to be immunogenic, there was either no correlation between the immune response and protection (where it was measured) or correlations were not tested. The authors of a Cochrane review concluded that there was no justification for further trials of SPf66 in its current form [7].

Back to the future

With the ongoing challenges of subunit vaccines for both sporozoite and blood-stage vaccines, there has been in recent years a re-appraisal of the whole parasite vaccine strategy. Irradiated sporozoites inoculated by mosquito bite have been shown to induce high levels of protection in naïve adults in the USA [30] and there are now intense efforts under way to prepare and formulate attenuated sporozoites that could be cryopreserved and then inoculated by syringe [31]. The first clinical trials will be done with radiation-attenuated *P. falciparum* sporozoites but future studies may be done with genetically attenuated sporozoites [32, 33]. After 5 years of research and development on the attenuated sporozoite vaccine approach, the challenges would seem to be more operational than scientific, although it is worth noting that maintenance of immunological memory to this vaccine might be an issue. Although an analysis of small numbers of volunteers immunized by irradiated infectious mosquitoes showed that four of five who received a secondary immunization were protected for between 23 and 42 wk, [30] natural exposure to sporozoites has been shown to not induce and/or boost immunity [18]. It is possible though that immunity once induced by an irradiated sporozoite vaccine could be boosted and maintained by natural exposure. Although this does not appear to occur following vaccination with RTS,S, immunity induced by irradiated sporozoites is likely to involve different immune mechanisms [34] and target multiple antigens thus reducing the impact of antigenic polymorphisms.

Efforts are also under way to re-examine whole blood-stage parasite vaccines. Since the exciting work in the 1970s where monkeys were protected following vaccination with large

numbers of irradiated or killed infected red blood cells, we now know that a subpatent infection of naïve human volunteers (with few as 32 live infected red cells, terminated by drugs after 8 days) can induce a robust cellular immunity (characterized by parasite-reactive CD4⁺ and CD8⁺ T cells) [35] and that ultra-low infections [36] and immunization of mice with as few as 100 dead parasites with CpG and alum can induce protection against both heterologous and homologous parasites associated with cell-mediated immune responses ([37] and unpublished data). The challenges for translation to humans include the choice of a human-compatible adjuvant capable of inducing potent cell-mediated immunity and concerns regarding immunopathology.

Concluding remarks

The major challenges in the development of a malaria vaccine remain the same as they have since the subunit strategy commenced and include poor immunogenicity and antigenic polymorphism with the realization now that the parasite might also modulate the immune response induced by a vaccine in order to enhance its survival. The successes to date of RTS,S relate largely to its ability to induce a moderate antibody response presumably as a result of the structure of the vaccine and the choice of adjuvant. The inability of the vaccine to induce enduring protection likely relates to failure of infection to boost the immune response or to abrogation of immunological memory. A similar failure to maintain antibody levels was observed after vaccination with the SPf66 vaccine in a study in South America and where this drop was attributed by the authors to an absence of immunological boosting by natural infection [38]. These challenges highlight the need to further understand immune regulation, adjuvant biology and the need to pursue polyvalent strategies whether they are based on synthetic, DNA-based, recombinant approaches or the ultimate polyvalent approach, a whole organism vaccine. Genome-wide studies involving immunomics may prove to be an invaluable way to identify multiple candidate epitopes for inclusion in a vaccine [39].

There certainly is hope for a malaria vaccine but the challenges are still formidable. In a 1991 book entitled, "Malaria. Waiting for the Vaccine", Miller wrote in the postscript that researchers and funders should stop reading every news release [40]. He further said that until we can grow parasites outside erythrocytes, vaccines will need to be subunits. This tacit endorsement of whole organismal vaccines may have been prescient but it is sobering that as World Malaria Day approaches, fully 18 years have passed. Yes, we are closer, but we are still waiting and resolve is still essential.

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Abbreviations: AMA1: apical membrane antigen 1 · CSP: circumsporozoite protein · MSP: merozoite surface protein

Full correspondence: Professor Michael F. Good, The Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Brisbane, Qld, 4029, Australia
Fax: +61-7-33620110
e-mail: Michael.Good@qimr.edu.au

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