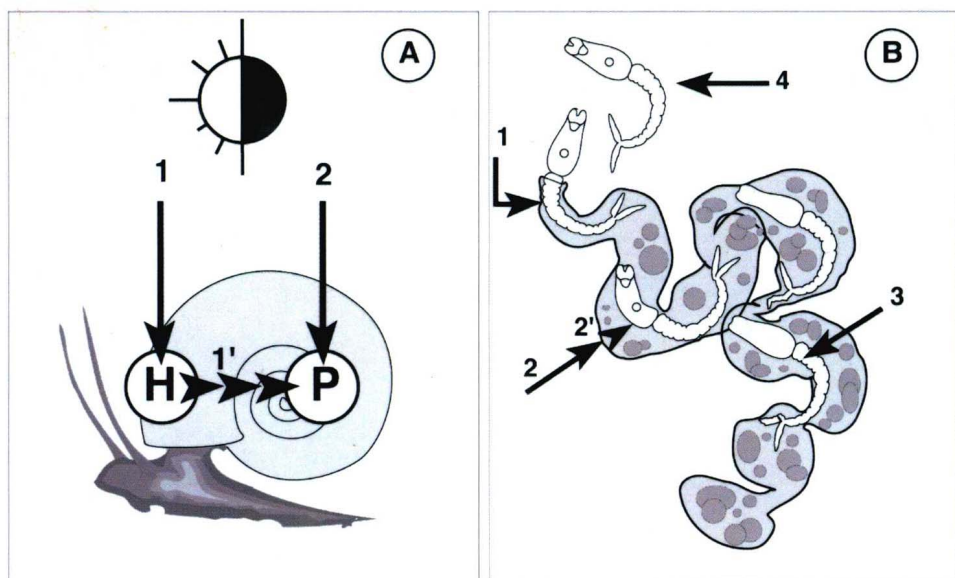


ADVANCES IN PARASITOLOGY



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D. ROLLINSON AND J.R. STOTHARD



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ADVANCES IN PARASITOLOGY

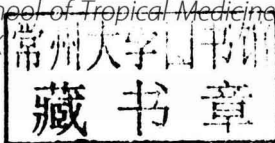
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Recent Developments in Malaria Vaccinology

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Abstract

The development of a highly effective malaria vaccine remains a key goal to aid in the control and eventual eradication of this devastating parasitic disease. The field has made huge strides in recent years, with the first-generation vaccine RTS,S showing modest efficacy in a Phase III clinical trial. The updated 2030 Malaria Vaccine Technology Roadmap calls for a second generation vaccine to achieve 75% efficacy over two years for both *Plasmodium falciparum* and *Plasmodium vivax*, and for a vaccine that can prevent malaria transmission. Whole-parasite immunisation approaches and combinations of pre-erythrocytic subunit vaccines are now reporting high-level efficacy, whilst exciting new approaches to the development of blood-stage and transmission-blocking vaccine subunit components are entering clinical development. The development of a highly effective multi-component multi-stage subunit vaccine now appears to be a realistic ambition. This review will cover these recent developments in malaria vaccinology.



1. INTRODUCTION

Malaria is one of the most important and life-threatening diseases worldwide. According to the Global Burden of Disease Study, malaria caused about 855,000 deaths in 2013 (Murray et al., 2014). African children under the age of five in sub-Saharan Africa were of highest risk. Furthermore, malaria caused more than 200 million clinical episodes in a population of approximately 3.4 billion people living in regions at risk of infection. In light of growing antimalarial drug resistance, there is an unquestioned need for better strategies to control malaria, such as improved treatment options, better mosquito control, or the development of a highly effective vaccine. In 2006, the WHO published a Malaria Vaccine Technology Roadmap which suggested a landmark goal to ‘develop and license a first-generation *Plasmodium falciparum* malaria vaccine that has a protective efficacy of more than 50% against severe malaria and death and lasts longer than one year by 2015’. Considerable progress has been made in recent years and the RTS,S vaccine candidate, the first ever malaria vaccine to enter Phase III clinical trials, has brought the field to within sight of this goal (The RTS,S Clinical Trials Partnership, 2014). The updated roadmap to 2030 has now set the bar even higher, aiming to increase the level and duration of efficacy, develop vaccines that prevent transmission, and also recognise *Plasmodium vivax* (Moorthy et al., 2013b). Here, we review recent and innovative developments in whole sporozoite vaccination (WSV), as well as subunit vaccination approaches, that seek to meet these ambitious, but arguably achievable, goals.



2. WHOLE SPOROZOITE VACCINATION

Many vaccines that are licensed and deployed today contain whole pathogens that are either inactivated or live-attenuated. Consistent with this, WSV approaches against malaria have also been widely considered. As heat-killed sporozoites were not found to protect mice (Alger and Harant, 1976), it became clear that viable, liver-invading parasites would be necessary to induce protective immunity. Over many years, numerous strategies for sporozoite attenuation approaches have been considered:

- Radiation-attenuated sporozoites (RAS)
- Genetically attenuated parasites (GAP)
- Infection-treatment vaccination (ITN)/Chemical prophylaxis sporozoite vaccination (CPS)

2.1 Radiation-attenuated sporozoites

During the 1960s, Ruth Nussenzweig and her colleagues demonstrated the first successful vaccination using whole malaria parasites in mice. After delivering radiation-attenuated *Plasmodium berghei* sporozoites intravenously (IV) or by mosquito bites, sterile protection against infectious sporozoite challenge was achieved (Nussenzweig et al., 1967). Subsequent clinical trials then showed that it is also possible to sterilely protect human volunteers by vaccination with bites of mosquitoes that had been infected with *P. falciparum* and irradiated with 150–200 Gy (Clyde et al., 1973; Rieckmann et al., 1979).

Noteworthy, more than 1000 immunising mosquito bites were necessary to achieve protective immunity, and each bite was estimated to inoculate about a hundred sporozoites (Medica and Sinnis, 2005) in $<0.5 \mu\text{L}$ of saliva (Rossignol et al., 1984). It was later established that with this vaccination strategy, long-lasting protective immunity, for at least 42 weeks, could be achieved (Hoffman et al., 2002). Even though it was generally not believed that WSV was deployable as a mass vaccination regime, these first clinical trials marked a milestone in malaria vaccine development, as they proved that sterile protection against pre-erythrocytic stages of the parasite was possible to achieve. Ever since that, the RAS approach has been commonly seen as the gold standard for malaria vaccine development.

In 2002, a biotechnology company (Sanaria Inc.) was founded to develop a way to manufacture: (1) aseptic, radiation-attenuated, purified and cryopreserved *P. falciparum* sporozoites (PfSPZ); under (2) current good manufacturing practice (cGMP) standards in adequate quantities to administer a sporozoite containing vaccine; by (3) a route that is standardisable and clinically realisable (Hoffman et al., 2010). After many production obstacles were overcome (Hoffman et al., 2010), Epstein et al. ran an open-label, dose-escalation study in which they immunised 80 healthy and malaria-naïve volunteers by administering purified and irradiated PfSPZ intradermally (ID) in the forearm or subcutaneously (SC) in the upper arm (Epstein et al., 2011). Those routes of administration were chosen as it had been shown before that during mosquito bites a substantial proportion of sporozoites remain in the dermis as well as SC tissues, and are not inoculated directly into the bloodstream of mice (Jin et al., 2007). However, the results of the human trial were rather disappointing: Even though the vaccine was reported to be safe and well tolerated, immunogenicity was suboptimal and only two volunteers were sterilely protected after being challenged

with malaria by exposure to bites of five mosquitoes infected with non-attenuated chloroquine-sensitive PfSPZ (Epstein et al., 2011).

This challenge model, also described as controlled human malaria infection (CHMI), is well established and often used to assess efficacy of new vaccine candidates. After infection, all volunteers are monitored by physical examination and thick blood smears, which are microscopically analysed. Unprotected volunteers are treated with antimalarial drugs, such as chloroquine, artemether plus lumefantrine or atovaquone plus proguanil, immediately after detection of blood-stage parasites (Sauerwein et al., 2011). Usually, the latter can be detected after 7–14 days (mean 11 days) (Epstein et al., 2007), although occasionally parasitaemia is also detected at later time points (Ockenhouse et al., 1998).

Epstein et al. postulated that the poor immunogenicity and efficacy found in their human clinical trial was due to their chosen route of administration (SC and ID). Thus, they proceeded to compare immunogenicity of RAS administered SC or IV in animal models. They found that IV, but not SC administration, induced a high frequency of sporozoite-specific CD8⁺ IFN- γ ⁺ T cells in peripheral blood and, most remarkably, in the liver of rhesus macaques and mice. In comparison to the IV route, 7–10 times more radiation-attenuated, purified and cryopreserved *Plasmodium yoelii* sporozoites were required to confer protection in mice if the SC or ID route were chosen. This could not be confirmed in rhesus macaques immunised with PfSPZ, as it is not possible to challenge these animals with *P. falciparum* to assess protection. Nevertheless, results demonstrated that changing the administration route from SC/ID to IV dramatically improved immunogenicity in the liver, which is the central site of immune protection against malaria (Hoffman and Doolan, 2000). This gave a clear rationale to start a new clinical trial in which the PfSPZ vaccine was administered IV (Epstein et al., 2011).

In 2013, Seder et al. reported a Phase I/IIa clinical trial in 40 adults to determine safety, immunogenicity, and protective efficacy against CHMI of the radiation-attenuated, aseptically purified and cryopreserved PfSPZ vaccine, which was administered IV. The vaccine was found to be safe and well tolerated. Most strikingly, six of six volunteers who received five doses of 1.35×10^5 PfSPZ were sterilely protected against CHMI about three weeks after the last immunisation. Six of nine subjects were protected after receiving four doses of 1.35×10^5 PfSPZ. In contrast, five of six unvaccinated control volunteers did develop malaria after CHMI. The sporozoite dose needed to achieve full protection in human volunteers was consistent

with earlier studies, which had shown that more than 1000 mosquito bites are necessary to achieve protection. Similar to observed efficacy, T cell and antibody responses against PfSPZ were also dose-dependent (Seder et al., 2013). Overall, this clinical trial confirmed that radiation-attenuated, aseptic, purified and cryopreserved PfSPZ can be highly efficacious if administered IV. Therefore, it prepared the ground for future clinical trials to: (1) investigate the duration of protection; (2) investigate the protective efficacy against heterologous parasite strains; and (3) to establish immune readouts that associate with protective efficacy (Seder et al., 2013). The results of these studies are pending and will be of great interest to the field.

Undoubtedly, the ability to produce attenuated cryopreserved PfSPZ under cGMP standards in high enough quantities, as well as the demonstration of their protective efficacy in humans, are striking achievements. Nonetheless, for mass deployment, substantial obstacles still have to be overcome. For instance, given IV administration is crucial for protection, the PfSPZ vaccine is currently not suited for deployment in infants or young children — target age groups for a malaria vaccine in sub-Saharan Africa. Furthermore, distributing the vaccine to rural areas will be difficult to achieve, as PfSPZ has to be shipped and stored in liquid nitrogen vapour phase. It remains likely in the shorter term, however, assuming that the PfSPZ vaccine induces sufficient breadth and duration of protection, that this current formulation could provide an effective vaccine for use by the military, travellers or for possible local malaria elimination campaigns in small island-based populations.

Cryopreserved PfSPZ is also proving to be of immense value for malaria vaccine research. Recently, investigators in the Netherlands, UK, Kenya and Tanzania have assessed the safety and infectivity of ID and IM administered non-attenuated, aseptic, purified, cryopreserved PfSPZ in adult volunteers. This 'PfSPZ Challenge' was safe, well-tolerated and infectious (Roestenberg et al., 2013; Sheehy et al., 2013b; Shekalaghe et al., 2014). Until today, sporozoite CHMI (delivered by mosquito bite) was only carried out in a small number of American and European research institutes, as secure entomology facilities are needed to produce *P. falciparum*-infected *Anopheles* mosquitoes (Shekalaghe et al., 2014). In contrast, PfSPZ Challenge can be conducted all over the world if suitable clinical trial facilities and adequately trained teams are present. This will significantly speed up malaria vaccine research — enabling CHMI studies to occur in more centres and in malaria-endemic target populations. Future CHMI studies in malaria-exposed individuals will also greatly facilitate studies of natural immunity (Hodgson

et al., 2014b) and facilitate the development of diagnostics for malaria or new antimalarial interventions, as CHMI has been proven to be a very powerful tool to investigate the underlying mechanisms of innate or acquired immunity against malaria. The development of new challenging parasite strains (like the Nijmegen *P. falciparum* strain NF135.C10), which are genetically distinct to the predominantly used NF54/3D7 clone challenge strain, will complement and expand the existing small repertoire of CHMI parasite strains (Teirlinck et al., 2013). Adding new heterologous parasite strains to future WSV might also be beneficial to achieve cross-strain protection (Teirlinck et al., 2013).

2.2 Genetically attenuated parasites

Another approach to attenuate parasites is to knockout genes that are crucial for liver-stage development. In comparison to RAS, this strategy overcomes the problem of fine-tuning irradiation of live parasites as part of a cGMP process, with the advantage that the parasite-based vaccine is also genetically defined and homogenous. However, absolute attenuation of parasites by gene deletion(s) is essential (Doll and Harty, 2014). After the first parasite genes that are only expressed in pre-erythrocytic stages were identified, the first knockout parasites that cause early liver-stage arrest were described in 2005. UIS3 (upregulated in infective sporozoites gene 3)-deficient (Mueller et al., 2005) and P36p-deficient *P. berghei* (van Dijk et al., 2005) parasites were unable to progress to blood-stages and conferred complete protection against sporozoite challenge with wild-type parasites in rodent models.

In the following years, many more parasite genes were identified that, if deleted, cause either full or partial arrest of parasites at distinct time points during liver-stage development. Identified genes include those expressed in the parasitophorous vacuole membrane or the apicoplast, or those involved in fatty acid synthesis or hepatocyte egress (reviewed in Khan et al., 2012).

In 2011, Butler et al. directly compared immunogenicity and ability to induce protective immunity of RAS, early-arresting *sap1*-deficient, and late-arresting *fabb/f*-deficient *P. yoelii* parasites (Butler et al., 2011). Strikingly, parasites that are capable of developing into later liver stages, and which undergo extensive intrahepatic schizogony but then fail to progress to blood stages, induced a superior effector and memory CD8⁺ T cell response. This also conferred higher levels of protective immunity in inbred and outbred mice than RAS and the early liver-stage-arresting parasites.

T cell specificity analysis and adoptive transfer experiments revealed that the larger CD8⁺ T cell response induced by fabb/f-deficient GAP was directed against parasite antigens that were expressed by late arresting GAP only. Furthermore, vaccination with late-arresting fabb/f-deficient sporozoites also conferred cross-stage protection against IV challenge with 100 *P. yoelii* parasite-infected erythrocytes (so-called blood-stage challenge) (Butler et al., 2011). This was not completely unexpected, as *Plasmodium* transcriptome and proteome analyses had predicted that late liver-stage parasites and blood-stage parasites share a number of antigens (Tarun et al., 2008). In contrast to these discoveries, earlier publications could not find cross-stage protection after vaccination with early liver stage-arresting RAS (Nussenzweig et al., 1967) or GAP (Butler et al., 2011).

In another study, a *P. yoelii* GAP that featured two simultaneous gene deletions (P52 and P36) showed complete liver-stage arrest and conferred sterile immunity against infectious mosquito bites or IV sporozoite challenge in mice (Labaied et al., 2007). Based on those findings, a *P. falciparum* GAP with corresponding P52 and P36 deletions was considered for human administration. After confirming complete liver-stage arrest in an in vitro hepatocyte infection model, as well as a humanised mouse model (VanBuskirk et al., 2009), the *P. falciparum* p52⁻/p36⁻ GAP of the NF54 strain was investigated in a human dose-escalation study with six volunteers (Finney et al., 2014; Spring et al., 2013). Sporozoites were delivered by bites of five infected mosquitoes followed by a further 200 bites one month later. After five bites, no volunteer was found to be blood-stage positive, indicating that GAP liver-stage arrest was complete. However, 12 days after exposure to 200 infected mosquitoes, one volunteer developed parasitaemia (confirmed by a positive blood smear) and developed malaria symptoms. Genotyping of the volunteer's blood-stage parasites discovered that the parasite retained both of their deletions (P52 and P36). Therefore, when administered in high dose, the *P. falciparum* p52⁻/p36⁻ GAP appears to be able to complete liver-stage development. Previous testing in the hepatocyte cell line model and liver-humanised mouse model did not disclose rare breakthrough infections. Alternative or improved humanised mouse models (Kaushansky et al., 2014; Vaughan et al., 2012a), such as humanised DRAG mice (Wijayalath et al., 2014), may be useful in the future to identify those rare events. Interestingly, studies in mice showed that *P. berghei* p52⁻/36⁻ GAP can develop within hepatocytes without formation of the parasitophorous vacuole membrane (Ploemen et al., 2012). This could explain why some of the double knockout parasites progressed into blood stage.

Mikolajczak et al. went on to develop a new *P. falciparum* GAP that is deficient in P52, P36, and also SAP1. These gene deletions did not influence gametocytogenesis, mosquito infectivity, sporozoite production or altered viability or infection. Whereas P52 and P36 are secreted proteins that are involved in the formation of the parasitophorous vacuole membrane, SAP1 is a cytoplasmic protein that regulates RNA stability. By knocking out two distinct biological processes, it is very unlikely that the parasite is still capable of progressing into blood stages. To preclinically investigate the risk of breakthrough blood-stage infections, the parasite was tested in an improved humanised mouse model transplanted with human hepatocytes and human erythrocytes and found to be completely attenuated. The authors argue that clinical testing of this new triple gene deletion *P. falciparum* GAP is now warranted (Mikolajczak et al., 2014). Provided liver-stage arrest is complete in humans, it would be interesting to see if protection after CHMI can be achieved. In the future, late liver-stage arresting *P. falciparum* GAP should also be assessed, as parasites progressing to later liver stages have been shown to induce higher levels of immunogenicity and efficacy in mice.

2.3 Infection-treatment vaccination/chemical prophylaxis sporozoite vaccination

In recent years it has also been established that administration of wild-type sporozoites under simultaneous chemoprophylaxis is another highly effective means to induce protective immunity.

In 2004, Belnoue et al. analysed the immunogenicity and efficacy of the administration of wild-type *P. yoelii* sporozoites under chloroquine treatment in BALB/c mice (Belnoue et al., 2004). Chloroquine is an antimalarial drug, which eliminates blood-stage trophozoites, but does not interfere with liver stages. The vaccination regime induced higher levels of protection against subsequent sporozoite challenge than those reported for RAS. When mice were immunised with wild-type sporozoites under chloroquine and additional primaquine treatment, which clears intra-hepatic parasites, protection against subsequent sporozoite challenge was abrogated. This indicates that the presence of liver-stage parasites is essential for protection. In line with this finding and similar to other WSV approaches, elimination of parasites was mainly mediated by $CD4^+$ and $CD8^+$ T cells directed against liver-stage parasites (Belnoue et al., 2004). More detailed analysis revealed that CPS (as well as RAS) induces expansion of hepatic $CD8^+ CD44^{hi} CD62L^-$ effector memory T cells up to 9 months post immunisation in mice.