Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Whole organism blood stage vaccines against malaria

Danielle I. Stanisic*, Michael F. Good**

Institute for Glycomics, Griffith University, Gold Coast Campus, Parklands Drive, Southport, Queensland 4222, Australia

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Plasmodium
Malaria
Vaccine
Whole parasite
Blood stage

ABSTRACT

Despite a century of research focused on the development and implementation of effective control strategies, infection with the malaria parasite continues to result in significant morbidity and mortality worldwide. An effective malaria vaccine is considered by many to be the definitive solution. Yet, after decades of research, we are still without a vaccine that is capable of inducing robust, long lasting protection in naturally exposed individuals. Extensive sub-unit vaccine development focused on the blood stage of the malaria parasite has thus far yielded disappointing results. There is now a renewed focus on whole parasite vaccine strategies, particularly as they may overcome some of the inherent weaknesses deemed to be associated with the sub-unit approach. This review discusses the whole parasite vaccine strategy focusing on the blood stage of the malaria parasite, with an emphasis on recent advances and challenges in the development of killed and live attenuated vaccines.

1. Introduction

Malaria infection is caused by parasites of the genus *Plasmodium*, with *Plasmodium falciparum* and *P. vivax* being the most prevalent of the 6 species that are able to infect humans. In 2012 there were over 3.4 billion people at risk of contracting malaria, over 200 million cases and 627,000 attributable deaths [1].

There is a long history of research into the development and implementation of different strategies which aim to both prevent and treat malaria infection. These include vector control (eg use of insecticide treated bed nets to reduce human-vector contact), chemoprevention (*e.g.* Intermittent Preventive Treatment (IPT) for vulnerable populations such as pregnant women and infants) and the prompt diagnosis and treatment of confirmed cases with appropriate anti-malaria drugs. While these have undoubtedly contributed to the progress in reducing case incidence and mortality rates [1], a vaccine is still seen as the definitive tool to prevent morbidity and mortality.

Despite decades of research, an effective malaria vaccine remains elusive. This is due to a number of factors including: the complex nature and life-cycle of the malaria parasite; its ability to rapidly evolve and evade the host immune system; and the lack of understanding of what precisely mediates immunity. Numerous sub-unit and whole parasite vaccine approaches targeting different parasite life-cycle stages are being examined in the pursuit of an effective vaccine. Disappointing results following the efficacy testing of sub-unit vaccines in clinical trials [2–4], including the most advanced vaccine candidate, RTS,S [5], have highlighted some of the intrinsic limitations of sub-unit vaccines that need to be addressed. Conversely, recent promising results from the clinical testing of radiation attenuated sporozoites [6] have re-ignited interest in the whole parasite approach. A radiation attenuated sporozoite vaccine must be 100% effective as even one mosquito-injected sporozoite developing through to blood stage could result in a fulminant blood-stage infection. For a whole parasite blood stage vaccine, even a partially effective vaccine could result in lower parasitemias and absence of symptomatic disease in recipients. This review will focus on recent advances in strategies to develop a whole parasite blood stage vaccine. It will also discuss some of the major challenges.

2. Evidence for a whole parasite blood stage vaccine approach

Historically, vaccine production has involved the isolation, inactivation/attenuation and injection of the whole infectious agent. This was facilitated by the development of methodology to cultivate the organisms to enable large-scale production of the killed or live attenuated vaccines and has been successfully employed for a number of organisms such as those causing the following diseases: tuberculosis, polio, measles, mumps, rubella and varicella (reviewed in [7]).

* Corresponding author. Tel.: +61 7 555 29433.
** Corresponding author. Tel.: +61 7 555 29435.
E-mail addresses: d.stanisic@griffith.edu.au (D.I. Stanisic), Michael.Good@griffith.edu.au (M.F. Good).

http://dx.doi.org/10.1016/j.vaccine.2015.09.057
0264-410X/

Whole organism blood stage malaria vaccine approaches were first investigated using animal models in the 1940s. Both ducks and monkeys were protected following immunisation with killed and adjuvanted *Plasmodium lophurae* and *Plasmodium knowlesi* infected red blood cells respectively [8,9]. Protection was also observed using the human malaria parasite, *P. falciparum*, in *Aotus* monkeys [10]. These and other non-human primate studies (reviewed in [11]) involved the use of Complete Freund's Adjuvant to enhance the immunogenicity of the parasite. Although this adjuvant is incompatible with human use, killed parasites in combination with alternative adjuvants were unable to successfully immunise monkeys [9]. The requirement for a human-compatible adjuvant to enhance the immunogenicity of the parasite and the inability at that time to generate sufficient quantities of human malaria parasites to enable clinical efficacy studies, were seen as substantial obstacles to furthering this vaccine approach. Although the discovery of how to culture *P. falciparum* [12] gave some impetus to the field, the perceived difficulties of this approach together with the ability to clone and express *P. falciparum* recombinant proteins [13,14], resulted in the sub-unit vaccine approach becoming the focus of malaria vaccine development. Recently, however, the challenges and disappointments of sub-unit vaccines led to a renewed interest in the whole parasite approach.

3. Advances in whole parasite blood stage vaccine strategies

A major advantage of the whole parasite vaccine approach is the broad array of antigens that such a vaccine presents to the immune system. Many are highly conserved between different parasite strains. This helps overcome two of the issues which have impacted on the efficacy of sub-unit vaccine candidates – immunological non-responsiveness and antigenic polymorphism. However, one of the key questions for the development of a malaria vaccine, is what type of immune response do we need to induce to result in robust protection. Immunity induced by natural exposure to the whole parasite during malaria infection is sub-optimal and takes several years to develop; therefore a whole parasite vaccine must improve on naturally acquired immunity by inducing either a different type or magnitude of immune response.

In the early 1900s, malariatherapy, which involved a protracted blood stage malaria infection, was a recognised treatment for neurosyphilis. A retrospective examination of these records suggests the induction of clinical and anti-parasite immunity during a second blood stage infection, manifesting as a decrease in parasitemia and febrile episodes [15]. More recently, multiple low doses of *P. falciparum* parasitised red blood cells (pRBC) were administered to malaria naïve individuals, with each infection terminated prior to patency with anti-malaria treatment [16]. Protection in 3 out of 4 volunteers was evident and was associated with the induction of vigorous parasite-specific cellular responses in the absence of detectable parasite-specific antibody, although the possibility that residual drug contributed to this protection could not be ruled out [17]. These immune responses differed from those that follow a natural (patent) infection where antibody responses are evident. Subsequent rodent studies also suggested that multiple subpatent malaria infections terminated by anti-malaria treatment could induce robust homologous and heterologous infection with induction of a similar antibody-independent protective immune response [18]. The results from a recent clinical study suggest that this protective immunity may be reliant on prolonged exposure to the malaria parasite during multiple asexual cycles. Multiple immunisations of sporozoites via infected mosquito bite while taking chloroquine prophylaxis induced immune responses to the *P. falciparum* blood stage, but these responses were not protective [19]. It was suggested that the prophylactic levels of chloroquine present in the volunteers' blood resulted in a short-lived parasitemia, and this exposure to the blood stage was not sufficient to generate protective immune responses [19]. These results collectively suggest that persistence of the blood stage parasite at low levels is associated with induction of protective immunity. Although the infection/drug treatment model is not a feasible malaria vaccine approach, it does inform vaccine development.

A number of approaches are currently being pursued to further this strategy and develop either a killed or live, attenuated blood stage malaria vaccine (Table 1). Each approach has its own advantages and disadvantages, which have implications for vaccine design.

Table 1
Characteristics of whole organism vaccine strategies.

Type of vaccine	Characteristics
Killed vaccines	Advantages: • Unable to replicate in the host. • Cannot revert to virulence and result in infection • Cannot be transmitted to susceptible individuals Disadvantages: • Most require the use of adjuvants to enhance immunogenicity • May require multiple doses to induce protective immunity
Live attenuated vaccines	Advantages: • Able to replicate in recipient so a lower number of parasites/fewer doses may be required. • Generally associated with longer lasting protection. Disadvantage: • Failure/instability of attenuation and reversion to virulence

4. Immunisation with killed blood stage parasites

As mentioned above, early work with rodents and monkeys demonstrated that vaccination with killed parasites/parasite lysate (including merozoites) can induce protective immunity (reviewed in [11]), when co-administered with Complete Freund's Adjuvant. A more recent study investigated the protective efficacy of low doses (100 and 1000) of frozen/thawed pRBC in the adjuvant CpG-ODN [20]. Homologous and heterologous protection was observed following immunisation, although low grade parasitemia was observed in vaccinated mice. Protection was dependent on $CD4^+$ T cells, IFN-γ and nitric oxide. This low dose parasite approach was chosen based on immunological considerations with low doses of parasites shown to induce robust T cell responses [18] without the deletion of effector cells that has been observed with higher parasite doses [21]. The induction of robust, protective T cell responses using a low dose of killed, adjuvanted parasites is very encouraging as the feasibility of producing large numbers of parasites during scale-up for human vaccine production is considered a limitation of the whole parasite approach.

Further development of this strategy requires the identification and testing of adjuvants appropriate for use in humans (reviewed in [22]). Issues encountered with the selection and utilisation of these compounds includes: limited access to novel or existing proprietary adjuvants; unacceptable safety profile of the final formulation; and a lack of understanding of the type of immune response that is required.

Please cite this article in press as: Stanisic DI, Good MF. Whole organism blood stage vaccines against malaria. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.09.057