Journal of Controlled Release 162 (2012) 242–254

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

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

Review

Vaccination Strategies against Malaria: novel carrier(s) more than a tour de force

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ARTICLE INFO

Article history:
Received 29 February 2012
Accepted 23 April 2012
Available online 28 April 2012

Keywords:
Hemagglutinin
Immunopotentiating reconstituted influenza virosomes
pH-sensitive liposome
Cationic lipid
TAP (Transcription activator protein)
Transfection
Immunogenicity
Subunit vaccines
Adjuvant
Microsphere
Virosomes

ABSTRACT

The introduction of vaccine technology has facilitated an unprecedented multi-antigen approach to develop an effective vaccine against complex systemic inflammatory pathogens such as *Plasmodium* spp. that cause severe malaria. The capacity of multi subunit DNA vaccine encoding different stage *Plasmodium* antigens to induce $CD8^+$ cytotoxic T lymphocytes and interferon-γ responses in mice, monkeys and humans has been observed. Moreover, genetic vaccination may be capable of eliciting both cell mediated and humoral immune responses. The cytotoxic T cell responses are categorically needed against intracellular hepatic stage and humoral response with antibodies targeted against antigens from all stages of malaria parasite life cycle. Therefore, the key to success for any DNA based vaccine is to design a vector able to serve as a safe and efficient delivery system. This has encouraged the development of non-viral DNA-mediated gene transfer techniques such as liposome, virosomes, microsphere and nanoparticles. Efficient and relatively safe DNA transfection using lipoplexes makes them an appealing alternative to be explored for gene delivery. Also, liposome-entrapped DNA has been shown to enhance the potency of DNA vaccines, possibly by facilitating uptake of the plasmid by antigen-presenting cells (APC). Another recent technology using cationic lipids has been deployed and has generated substantial interest in this approach to gene transfer. In this review we discussed various aspects that could be decisive in the formulation of efficient and stable carrier system(s) for the development of malaria vaccine.

Published by Elsevier B.V.

Contents

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0168-3659/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jconrel.2012.04.037

1. Background: The Burden of Malaria

Malaria continues to present a major health challenge in many of the poor countries in the world, with 225 million cases leading to an estimated 781,000 deaths in 2009 [1]. Numerous efforts towards control and eradication of this disease are directed at different areas including insect vector control, vaccine development, and the discovery of new therapeutic drugs. Although, battle to control malaria has been fought on several grounds including improved methodologies of diagnosis and chemoprophylaxis as well as integrated vector control through various physical methods such as treatment with insecticide and house spraying [2], prevalence and resurgence of malaria continues to persist because of drug resistant parasites and insecticide resistant vector [3,4]. Therefore, due to this bleak situation, the need to develop additional control measures such as malaria vaccine is both attractive and urgent. The malaria vaccine is still elusive despite of enormous and continued efforts on to develop an effective vaccine [5]

There are number of existing approaches to malaria vaccine based on attenuated sporozoite, synthetic and recombinant immunogenic peptides. These strategies have proved significant in terms of safety, duration of immunity and specificity [6–10]. As vaccines based on live, attenuated malaria parasites, are economically and technically not feasible, malaria research focuses on recombinant or synthetic subunit vaccines. The optimal vaccine should have the ability to elicit protective immunity blocking infection, prevents pathology and blocks transmission of parasite. Therefore, combination vaccine consisting subunits from different stage of the parasite would meet all these requirements. The progress in developing a malaria vaccine is not going up to that pace as it was expected after the complete genome sequencing of *P. falciparum* [11], perhaps, in part because of the larger genetic diversity of *plasmodium* parasite. Thus identification, expression and degree of variability of candidate vaccine antigens render it more complex to understand various biological processes of parasite. The complex life cycle of malaria parasite and antigenic diversity are the barriers associated with vaccine development [12].

2. Biology of malaria parasite: approaches for effective immune interventions

The causative agent of malaria parasite has a complex multi-stage life cycle involving both primary (mosquito) and secondary (human) hosts in different cellular environments (intra and extracellular) in which the parasite develops. The disease in humans is caused by one or a combination of four species of Plasmodia: *P. vivax, P. falciparum, P. malariae,* and *P. ovale.* Also, in geographically limited zones of South-East Asia, the Malaysian island of Borneo in particular, infections by *P. knowlesi* as a zoonose have been known to occur [13–15]. While it remains a possibility, there does not appear to be any evidence to indicate that infections of this "fifth human malaria parasite" can be transmitted from humans to other human hosts [16] and hence they are not considered to be important in terms of public health outside these zones. Malaria parasite has a large genome of 14 chromosomes comprising 26–30 mega bases encoding around 5000–6000 proteins [17,18]. Most of the *Plasmodium* strains have a complex life cycle that begins when a female mosquito injects sporozoites into the skin of an individual at the time of blood meal. After differentiation and passing through various forms, parasite produces thousands of merozoites that are released from the hepatocytes and rapidly invade circulating erythrocytes. Although the large numbers of parasites have developed during hepatic phase, infected hepatocytes are unable to produce liver or systemic symptoms in human host. The rupture of infected erythrocytes in the blood circulation release pigments initiating malaria related symptoms (Fig. 1).

3. Existing malaria vaccines

Although the complexity and genome variability of the parasite hampers the development of a universal, effective and long lasting vaccine, the feasibility of malaria vaccine is supported by the several line of evidence. The repeated exposure of malaria develops natural immunity against unwanted clinical manifestations of infection [19]. In addition, passive transfer of antibodies and immune effectors from an immunized animal to the susceptible host induces protection against malaria infection [20].

Immunization of naive humans and laboratory animals with radiation-attenuated sporozoites has shown up to 90% protection in the form of sterile immunity against infection [6,9,21,22]. Furthermore, sera of the immune animals and humans have demonstrated to block malaria transmission in mosquitoes [23,24]. Thus lack of immune correlates of protection, lack of reliable and predictive efficient laboratory animal model and genetic variability of parasite are the obstacles in vaccine development. Besides, lack of clinically acceptable adjuvants able to invoke cell-mediated immunity as well as epitope or antigen-presenting systems are the hurdles in vaccination program. Further, identification of antigens or epitopes for the construction of recombinant, subunit or synthetic malaria vaccines has been challenge for developing efficacious vaccine against malaria [18]. The attempts have been made to develop malaria vaccine by targeting one of the different stages of parasite development such as the pre-erythrocytic, the asexual (intra-erythrocytic) or the sexual stage by applying conventional approaches.

Pre-erythrocytic vaccine strategies aim to generate an antibody response able to neutralize sporozoites and prevent them from invading the hepatocyte, as well as to elicit a cell-mediated immune response able to interfere with the intra-hepatic multiplication cycle of the parasites. Asexual blood-stage (*erythrocytic stage*) vaccine strategies are directed to elicit antibodies that will inactivate merozoites and/or target malaria antigens expressed on RBC surface through

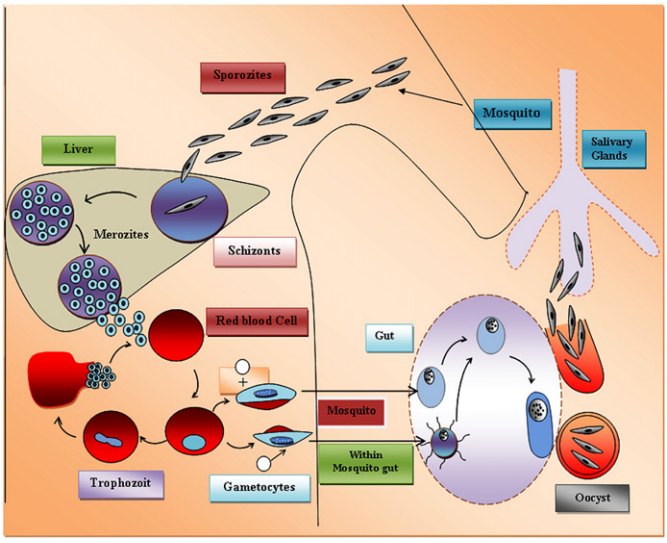

Fig. 1. Life cycle of malaria parasite.