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A semi-synthetic whole parasite vaccine designed to protect against blood stage malaria

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ABSTRACT

Although attenuated malaria parasitized red blood cells (pRBCs) are promising vaccine candidates, their application in humans may be restricted for ethical and regulatory reasons. Therefore, we developed an organic microparticle-based delivery platform as a whole parasite malaria-antigen carrier to mimic pRBCs. Killed blood stage parasites were encapsulated within liposomes that are targeted to antigen presenting cells (APCs). Mannosylated lipid core peptides (MLCPs) were used as targeting ligands for the liposome-encapsulated parasite antigens. MLCP-liposomes, but not unmannosylated liposomes, were taken-up efficiently by APCs which then significantly upregulated expression of MHC-II and costimulatory molecules, CD80 and CD86. Two such vaccines using rodent model systems were constructed – one with *Plasmodium chabaudi* and the other with *P. yoelii*. MLCP-liposome vaccines were able to control the parasite burden and extended the survival of mice. Thus, we have demonstrated an alternative delivery system to attenuated pRBCs with similar vaccine efficacy and added clinical advantages. Such liposomes are promising candidates for a human malaria vaccine.

Statement of Significance

Attenuated whole parasite-based vaccines, by incorporating all parasite antigens, are very promising candidates, but issues relating to production, storage and safety concerns are significantly slowing their development. We therefore developed a semi-synthetic whole parasite malaria vaccine that is easily manufactured and stored. Two such prototype vaccines (a *P. chabaudi* and a *P. yoelii* vaccine) have been constructed. They are non-infectious, highly immunogenic and give good protection profiles. This semi-synthetic delivery platform is an exciting strategy to accelerate the development of a licensed malaria vaccine. Moreover, this strategy can be potentially applied to a wide range of pathogens.

1. Introduction

In spite of many efforts to develop a malaria vaccine, a licensed product is not yet available. Sub-unit vaccine candidates that targeted a specific surface antigen of the parasite showed either no efficacy [1] or an effect of limited duration [2,3] due to poor immunogenicity and a failure of natural exposure to boost the vaccine-induced immune responses as a result of antigenic polymorphism [4]. Therefore, many vaccine development strategies are being re-directed to a whole parasite approach. The most advanced approaches are with sporozoites where irradiation [5], genetic modification [6] and chemical attenuation [7] render the parasite non-infectious, but still immunogenic. Whole parasite blood stage vaccines have generated less interest, in large part due to the need to culture the parasite in human red cells. Nevertheless, killed blood stage parasites administered with potent but toxic adjuvants or chemically attenuated intact parasitized red cells induce broad strain- and species-transcending immunity in mice [8,9]. While the molecular basis for the efficacy of this

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approach is not fully understood, whole parasite blood stage vaccines to date induced a cellular immune response with an absent or limited antibody component. Protective T cells respond to conserved antigens [10], thus offering a plausible explanation for their broad efficacy [11].

Whereas RBCs may be an efficient delivery system for a whole parasite vaccine, their use raises ethical and regulatory issues related to the presence of red cells and the possible induction of antibodies to red cell membranes. Furthermore, the use of chemicals to attenuate parasites necessitates the use of careful procedures to limit any attenuating agent being present in the vaccine. We explored delivery and targeting systems for whole parasite vaccines that do not rely on red cell membranes.

Pathogens are often presented to the immune system as particles with sizes ranging from a few nanometers (e.g. viruses) to several micrometers (e.g. bacteria and parasites). Translating this knowledge, many vaccine candidates are designed to mimic this particulate nature of pathogens, for efficacy [12–14]. In blood stage malaria, parasites spend most of their life inside the RBCs that are 6–8 μm in size. Therefore, to mimic the natural blood stage infection of malaria, delivery of whole parasite antigen in micron-sized particles would be an attractive strategy for the induction of protective immune responses.

Liposomes form large lipid-bilayered membrane vesicles that can deliver antigenic material and therefore mimic RBCs. However, unlike RBCs, they are non-immunogenic. They can be prepared with various lipid compositions in different particle sizes and charges [15–18], allowing tailored design of liposomal delivery systems that are optimal for the delivery of specific internal antigens and are ideal for the induction of specific T cell responses. Their other major advantage is their ability to be customized to target specific tissues or cells [13]. Efficient antigen delivery to APCs is crucial for induction of a potent cell-mediated immune response. C-type lectin receptors present on APCs can recognize specific pathogen-associated carbohydrate structures. Thus, carbohydrates have been vastly exploited for targeted delivery of vaccine candidates [19,20]. Among them, mannose is often used as a ligand for lectin receptors present on dendritic cells (DCs) and macrophages [21–23] and is an ideal molecule to target liposomes to antigen presenting cells (APCs). It is widely reported that targeting macrophages would enhance the immune response via activating T-cells [24,25].

We asked whether targeted liposomes that contained killed parasite antigen would be able to induce immunity to the blood stages of malaria. To enable us to directly compare the efficacy of liposome-targeted vaccines with attenuated vaccines, we constructed vaccines from the same stock of parasites and show that for *P. chabaudi* and *P. yoelii*, whole parasite vaccines using the liposome delivery system are as effective as attenuated parasites in inducing both cellular immune responses and protection.

2. Materials and methods

2.1. Animals

Four- to six-week-old female BALB/c mice (H-2^d), C57BL/6 (H-2^b), SCID mice (H-2^d), and A/J mice (H-2^a) were purchased from the Animal Resource Centre (Willeton, Western Australia). Mice were housed in a pathogen-free environment at the Griffith University Animal Facility, and allowed to acclimatize to the new environment for at least one to two weeks prior to the start of experimental.

2.2. Parasites

Cloned lines of *P. chabaudi AS*, and *P. yoelii 17X* (originally provided by Richard Carter (University of Edinburgh, United Kingdom)) from The Queensland Institute of Medical Research stock were used.

2.3. Attenuation of pRBCs (TFA vaccine) and extraction of parasites from pRBCs

To 9 mL of warm serum-free RPMI, 100 μL of infected blood (parasitemia was between 30% and 40%) and 1 mL of 20 μM TH-III-149 (tafuramycin A (TFA)) [26] were added and incubated at 37 °C in a 5% CO_2 incubator for 40 min. 40 mL RPMI was added and centrifuged at 1500 rpm for 5 min. Supernatant was discarded and the cell pellet was resuspended in 50 mL RPMI and incubated at 37 °C in a 5% CO_2 incubator for 20 min. The cells were washed thrice with PBS, counted and used for the immunization of positive control mice. Parasites were extracted from attenuated pRBCs by slightly modifying the method described in "Experimental asexual blood stage malaria immunity, support protocol 3" [27]. Briefly, the attenuated pRBC pellet was suspended in 1 mL of distilled water and incubated for 4 min at room temperature with frequent shaking, centrifuged for 10 min at 290*g*, 4 °C and the supernatant was then discarded. The above step was then repeated. The pellet was resuspended in 1 mL PBS, centrifuged for 10 min at 16,000*g*, 4 °C. This pellet was resuspended in 1 mL cold PBS and subjected to four freeze-thaw cycles. The resulting pellet was aliquoted at the desired concentration based on the equivalent pRBCs/mL concentration.

2.4. Formulation of liposomes encapsulating parasite extract

In a small round bottom flask, lipids (5 mg of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 2 mg of dimethyldioctadecylammonium bromide (DDAB), 1 mg of cholesterol, and 100 μg of desired MLCP) were dissolved in a chloroform/methanol solvent mixture (9:1, v/v). Then the solvent was removed by rota-vapour and connected to a vacuum overnight. A thin lipid layer at the base of the flask was formed. While maintaining the temperature of flask above 50 °C, the lipid film was hydrated with 2 mL of PBS containing the attenuated parasite extract. Hydration was performed for 20 min with vortexing at regular intervals. Independent batches of the vesicle systems were prepared either with or without MLCP or parasite extract.

2.5. In vitro uptake and APC maturation studies

Mouse spleen was transferred on to a 70 μm cell strainer and gently mashed using a 5 mL syringe plunger. The cell strainer was washed with cold PBS, and centrifuged at 400 g for 10 min. The supernatant was discarded and the pellet was suspended in 1 mL of erythrocyte lysis buffer to lyse RBCs and, then, incubated on ice for seven minutes, quenched with 30 mL PBS, and then centrifuged in the same manner as above. A second round of washing with 30 mL of PBS was completed. The pellet was resuspended in 20 mL phenol free IMDM Glutamax medium (supplemented with 10% fetal bovine serum (FBS), 50 μM 2-mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin) and the cells were counted. In a 12-well plate, cells were seeded at a density of 2×10^6 cells/well and incubated for two hours at 37 °C in a 5% CO_2 incubator. In the case of uptake studies using human blood, from a healthy human volunteer who had given written informed consent, blood was collected in heparin tubes. RBCs were lysed using erythrocyte lysis buffer and pellet was resuspended in 35 ml complete media. Cells were seeded in a 96-well round bottom plate at a density of 2×10^5 cells/well and incubated at 37 °C for at least two hours. Dextran or DiI-labelled liposome formulations were added and incubated overnight at 37 °C in a 5% CO_2 incubator. Dextran was used to show that APCs are viable