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Liposomes containing monophosphoryl lipid A and QS-21 serve as an effective adjuvant for soluble circumsporozoite protein malaria vaccine FMP013

Christopher J. Genito [a], Zoltan Beck [b], Timothy W. Phares [a], Fanta Kalle [a], Keith J. Limbach [c,d], Maureen E. Stefaniak [c,d], Noelle B. Patterson [c,d], Elke S. Bergmann-Leitner [e], Norman C. Waters [f], Gary R. Matyas [b], Carl R. Alving [b], Sheetij Dutta [a,*]

[a] Laboratory of Structural Vaccinology, Malaria Vaccine Branch, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA
[b] Laboratory of Adjuvant and Antigen Research, US Military HIV Research Program, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA
[c] Malaria Department, Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA
[d] Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., 6720A Rockledge Drive, Suite 100, Bethesda, MD 20817, USA
[e] Flow Cytometry Center, Malaria Vaccine Branch, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA
[f] Malaria Vaccine Branch, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA

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ABSTRACT

Malaria caused by *Plasmodium falciparum* continues to threaten millions of people living in the tropical parts of the world. A vaccine that confers sterile and life-long protection remains elusive despite more than 30 years of effort and resources invested in solving this problem. Antibodies to a malaria vaccine candidate circumsporozoite protein (CSP) can block invasion and can protect humans against malaria. We have manufactured the Falciparum Malaria Protein-013 (FMP013) vaccine based on the nearly full-length *P. falciparum* CSP 3D7 strain sequence. We report here immunogenicity and challenge data on FMP013 antigen in C57BL/6 mice formulated with two novel adjuvants of the Army Liposome Formulation (ALF) series and a commercially available adjuvant Montanide ISA 720 (Montanide) as a control. ALF is a liposomal adjuvant containing a synthetic monophosphoryl lipid A (3D-PHAD®). In our study, FMP013 was adjuvanted with ALF alone, ALF containing aluminum hydroxide (ALFA) or ALF containing QS-21 (ALFQ). Adjuvants ALF and ALFA induced similar antibody titers and protection against transgenic parasite challenge that were comparable to Montanide. ALFQ was superior to the other three adjuvants as it induced higher antibody titers with improved boosting after the third immunization, higher serum IgG2c titers, and enhanced protection. FMP013 + ALFQ also augmented the numbers of splenic germinal center-derived activated B-cells and antibody secreting cells compared to Montanide. Further, FMP013 + ALFQ induced antigen-specific IFN-γ ELISPOT activity, $CD4^+$ T-cells and a T_H1-biased cytokine profile. These results demonstrate that soluble CSP can induce a potent and sterile protective immune response when formulated with the QS-21 containing adjuvant ALFQ. Comparative mouse immunogenicity data presented here were used as the progression criteria for an ongoing non-human primate study and a regulatory toxicology study in preparation for a controlled human malaria infection (CHMI) trial.

1. Introduction

The sporozoite stage of *P. falciparum* is coated with the circumsporozoite protein (CSP), which is critical for hepatocyte invasion [1,2]. Antibodies to the CSP central repeat region, $(NANP)_n$, can effectively block invasion [3] and are believed to associate with protection against Controlled Human Malaria Infection (CHMI) [4]. Several early CHMI trials showed low level and inconsistent protection was conferred by aluminum hydroxide-adjuvanted CSP vaccines [5–7]. The first highly protective recombinant CSP vaccine was a hepatitis B particle fusion protein, RTS,S, formulated in an oil-in-water adjuvant (AS02) containing bacterial membrane monophosphoryl lipid A (MPLA) and QS-21, a triterpene glucoside compound derived from the *Quillaja saponaria* tree [8]. Subsequently, RTS,S formulated with a liposomal adjuvant containing

* Corresponding author.
E-mail address: sheetij.dutta.civ@mail.mil (S. Dutta).

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MPLA and QS-21 (AS01), showed further improvement in protection against CHMI [4]. Several Phase 2 and Phase 3 field trials have since shown AS01 to be safe in adults and children and that optimization of the vaccine schedule and dose may further augment RTS,S protection [9,10].

Despite the success against CHMI, the RTS,S + AS01 vaccine-induced protection in endemic areas has remained low (30–50% range) [9,11–13]. Protection wanes over time and one report suggests negative efficacy in RTS,S vaccinees after a long-term follow-up [14]. Antigenic escape by non-vaccine strain parasites has been cited as a possible cause of low RTS,S efficacy [15]. Measures attempting to counter the limited field efficacy of RTS,S could be to include blood stage antigens [16], transmission blocking antigens [17] and prime-boost approaches that improve the CD4+ and CD8+ T-cell responses [18,19]. A vaccine that ultimately eliminates malaria may be a combination of multiple delivery platforms and contain antigens of both *P. falciparum* and *P. vivax* [20]. However, to build upon the partial success of CSP-based vaccines, researchers need unfettered access to RTS,S or another CSP vaccine that can reproducibly protect humans against CHMI. As a step in that direction, WRAIR Malaria Vaccine Branch has developed the Falciparum Malaria Vaccine-013 (FMP013), a soluble, *E. coli*-derived, nearly full-length CSP which contains 19 NANP and 3 NVDP repeats, as well as the C-terminal and the N-terminal regions. The N-terminal region of CSP is not included in RTS,S, although several B and T cell epitopes of CSP have been mapped to this region along with a functional protease cleavage site [1,2,21–23]. FMP013 antigen has met all of the purity and stability criteria for advancing to CHMI studies [24], and we are in the process of down-selecting a suitable human-use adjuvant to be combined with FMP013.

Molecular adjuvants are designed to directly target innate and adaptive immune pathways [25]. MPLA is a ligand for Toll-like-receptor 4 (TLR4) and can activate a signaling cascade terminating at transcription factors NF-kB and IRF-7 (MyD88 pathway) as well as IRF-3 (TRIF pathway) [26]. QS-21 can activate the NOD-like receptor P3 (NLRP3) inflammasome complex present within the APC cytosol [27]. Among other pathways, aluminum adsorption of the antigen can activate the NLRP3 inflammasome complex [28], but unlike QS-21, aluminum salts induce primarily a T_H2 response [29]. The WRAIR Antigen and Adjuvant Research Laboratory has developed the Army Liposome Formulation (ALF) series of adjuvants. ALF is based on small unilamellar liposomes, 50–100 nm diameter, containing a synthetic MPLA derivative, 3D-PHAD® (Avanti Polar Lipids). ALF formulations with a recombinant HIV-1 envelope protein have been characterized in mice [30]. Here, FMP013 was tested with ALF, ALF containing QS-21 (ALFQ) or ALF containing aluminum hydroxide (ALFA). The data presented here provide the rationale for continued evaluation of FMP013 with ALFQ in Rhesus macaques and in a future human vaccine trial.

2. Materials and methods

2.1. Ethics statement

Research was conducted under an IACUC-approved animal use protocol in an AAALACi accredited facility in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition.

2.2. Preparation of liposomes

Dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), cholesterol and synthetic monophosphoryl lipid A (MPLA) derivative 3-deacyl monophosphoryl lipid A (3D-PHAD®) were obtained from Avanti Polar Lipids (Alabaster, AL). ALF contained DMPC:DMPG phospholipids (9:1 M ratio), cholesterol (43 mol%) and 3D-PHAD® (0.26 mM). For ALFQ liposomes, the cholesterol concentration was adjusted to 55 mol%. Briefly, multilamellar liposomes were formed using the lipid deposition method by combining DMPC and cholesterol (both in chloroform), DMPG and 3D-PHAD® (in chloroform:methanol; 9:1 v/v) [31]. Multilamellar liposomes were then microfluidized (LV1 instrument, Microfluidics, Westwood, MA) to yield small unilamellar liposomes, which were sterile filtered and stored in lyophilized form at +4 °C. Final cholesterol concentration was quantified by colorimetric assay [32].

2.3. Preparation of vaccine formulations

FMP013 was cGMP-grade nearly full-length recombinant 3D7 strain *P. falciparum* CSP expressed and purified from *E. coli* [24]. A total of 2.5 μg of FMP013 was present in each vaccine dose. Liposomal formulation compositions are summarized in Table 1. For ALF formulation, lyophilized FMP013 was reconstituted and added to dried liposomes. For ALFA formulation, reconstituted FMP013 was mixed with Alhydrogel (Brenntag Biosector, Frederikssund, Denmark) before adding to dried liposomes. For ALFQ, QS-21 (Desert King International, San Diego, CA) was mixed with small unilamellar liposomes before adding FMP013. Montanide formulations containing 70% Montanide ISA 720 VG (SEPPIC Inc., Fairfield, NJ) and 30% antigen (v/v) were vigorously vortexed for 25 min and emulsification was confirmed by a water surface dispersion test.

2.4. Protein and liposome analysis

Particle size dispersion was measured on a Zetasizer Nano S (Malvern, Worcestershire, United Kingdom). Thermal stability was assessed by incubating the formulations at +37 °C (kinetic stability) or room temperature and analyzing the samples at different time-points by SDS-PAGE followed by silver staining (Pierce Silver Stain Kit, Thermo Fisher Scientific, Waltham, MA). Western blot was performed to stain CSP specific degradation products using polyclonal mouse anti-CSP (1:2500) essentially as described previously [33].

2.5. Immunization of mice and challenge

Female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA) were immunized intramuscularly (IM) with 50 μl of the vaccines by injection in alternate rear thighs at 0, 3, and 6 weeks. The animals were bled three weeks after the first and second immunizations and two weeks after the third immunization. Protective

Table 1
Amount (μg) of each component in 50 μL dose of ALF, ALFA, and ALFQ formulated vaccines administered to mice. The contents listed for ALFQ apply to both ALFQ-B and ALFQ-L.

Group	CSP (μg)	DMPC (μg)	DMPG (μg)	Cholesterol (μg)	MPLA (μg)	Aluminum Hydroxide (μg)	QS21 (μg)
ALF	2.5	70	7.9	33.4	20	–	–
ALFA	2.5	70	7.9	33.4	20	30	–
ALFQ	2.5	700	79	541	20	–	10

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