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Development of a self-assembling protein nanoparticle vaccine targeting *Plasmodium falciparum* Circumsporozoite Protein delivered in three Army Liposome Formulation adjuvants

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ABSTRACT

We have developed FMP014, a vaccine candidate against *Plasmodium falciparum* malaria, which is comprised of 60 identical monomer protein chains that form an icosahedral shaped self-assembling protein nanoparticle (SAPN). Each monomer contains selected *P. falciparum* Circumsporozoite Protein (P*f*CSP) CD4+ and CD8+ epitopes, universal T_H epitopes, portions of the α-TSR domain, and 6 repeats of the NANP motifs of the P*f*CSP. Here we describe the conditions that are required for successful scale-up and cGMP manufacturing of FMP014 with a yield of ≈1.5 g of drug substance per 100 g of wet bacterial paste. When adjuvanted with an Army Liposomal Formulation (ALF) based adjuvant, the nanoparticle vaccine is highly immunogenic and prevents infection of mice by an otherwise lethal dose of transgenic *P. berghei* sporozoites expressing the full-length P*f*CSP.

1. Introduction

Malaria is a life-threatening disease that is transmitted to humans by the injection of sporozoite stage parasites during blood feeding of the infected female *Anopheles* mosquito. Only a handful of vaccine candidates, most targeting the abundant sporozoite protein *Pf*CSP, have shown promise in combating this major public health threat but have yet to achieve a fully protective, long-lasting immune response. One of the most successful candidate is the RTS,S vaccine which was developed initially a in collaboration between GlaxoSmithKline (GSK) and the Walter Reed Army Institute of Research (WRAIR) and further refined at GSK and brought to numerous clinical trials [1]. While RTS,S, formulated with the adjuvant AS01 is the most effective recombinant malaria vaccine to date, it is only ≈40% effective in children ages 5–17 months of age when administered under a traditional dosing regimen. In addition protective efficacy waned significantly in the first year after immunization [2]. RTS,S contains 16 NANP repeats and a large portion of the flanking C-terminus region from the P*f*CSP fused to the Hepatitis B Surface Antigen to form a small VLP [3]. Within the C-terminal region of P*f*CSP, numerous CD4+, CD8+ and T-helper epitopes have been identified [3]. We theorized that critical epitopes may only require a different display and formulation in a new adjuvant to induce stronger immunogenic responses ultimately leading to higher and longer-lived levels of protection [4].

The WRAIR Laboratory of Adjuvant and Antigen Research have developed an Army Liposome Formulation (ALF) adjuvant system to increase the immune responses of various vaccines. For decades,

Abbreviations: 3D-PHAD, 3-deacyl monophosphoryl lipid A; ALF, Army Liposomal Formulation; CSP, Circumsporozoite protein; DPMC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPMG, 1,2-dimyristoyl-sn-glycero-3-phospho-(1rac-glycerol); MPLA, monophosphoryl lipid A; *Pf*CSP, *Plasmodium falciparum* CSP; SAPN, Self-assembling protein nanoparticles; VLP, Virus-like particle.

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liposomes containing monophospholipid A (MPLA) have demonstrated effectiveness in inducing high titer antibodies and cytotoxic T lymphocytes against a variety of cancer antigens along with infectious pathogens such as malaria, HIV and *Neisseria meningitides* [5,6]. The addition of components such as QS-21 and Alhydrogel®, has increased the effectiveness of formulations containing MPLA [7]. The core of the ALF system is a liposomal membrane made of all synthetic compounds DMPC, DMPG, cholesterol and the MPLA 3D-PHAD® (Avanti Polar Lipids, Alabaster, AL). The mol% cholesterol relative to the phospholipids content was adjusted in some ALF formulations that incorporate QS-21 (Desert King International, San Diego, CA) to allow for maximum binding of the QS-21to the membrane bound cholesterol, reducing potentially toxic effects of free QS-21 [7]. Here we provide the experimental methods for cGMP manufacturing of FMP014 and show its immunogenicity and protective efficacy when formulated with three ALF adjuvant variations ALFA, ALFQ or ALFQA.

2. Method and materials

2.1. FMP014 construct design and gene synthesis

The monomer polypeptide for FMP014 was designed based on the immunogenic domains of the *P. falciparum* 3D7 strain CSP. Using computer modeling, amino acids with specific charge and hydrophobicity were selected and aligned to form the core of linked pentameric and trimeric coiled-coil sequences. Antigenic epitopes and structural domains of P*f*CSP were placed on either the N-terminal or C-terminal ends of the core. The α-TSR domain of P*f*CSP was attached to the N-terminus of the protein chain while the $(NANP)_6$ repeat region was placed at the C-terminal end. These two B-cell epitopes will be displayed on the surface of the nanoparticle. The malaria-specific CD8+ epitopes YLNKIQNSL and KPKDELDY are an integral part of the α-TSR domain while the GLIMVLSFL epitope, also located in C-terminal region of the P*f*CSP, was engineered into the trimeric coiled coil of the SAPN [8]. Furthermore, two CD4+ epitopes LIDYNKAALSKFKED and IRHENRMVL from the glycoprotein of lymphocytic choriomeningitis virus and from the matrix protein 1 of influenza A virus, respectively, were engineered into the scaffold of the SAPN, to render it more immunogenic, as they are predicted to bind to the MHC II molecules of the human HLA-DR phenotype [8]. The core and the antigenic epitopes together are termed the FMP014 monomer (Fig. 1a–c).

2.2. FMP014 monomer cloning and expression

A synthetic gene encoding the FMP014 monomer was constructed, using proprietary technology, by DNA2.0, Inc. (Menlo Park, CA) with codon usage predicted to yield high protein expression in *E. coli*. The gene for the FMP014 monomer was cloned by restriction site based ligation into the pJ411 expression plasmid. This plasmid contains a T7 promoter, a pUC origin of replication, a *lacI* gene for constitutive expression of protein and a kanamycin antibiotic selection marker. Lyophilized plasmid was reconstituted in sterile water and transformed into One Shot BL21(DE3) *E. coli* cells (Life Technologies, Grand Island, NY) by heat shock. Cells were incubated at 37 °C for ≈16 h on kanamycin antibiotic-selection agar plates. Five isolated colonies of transformants were selected and grown for plasmid analysis by SimpleSeq Sanger Sequencing (Eurofins Genomics, Huntsville, AL).

2.3. Research cell bank (RCB)

After small research-scale expression studies, one clone was selected for process development and passaged three times in Select APS Super Broth (SB) (DifcoTM Select APSTM, Becton Dickinson, Sparks, MD) liquid culture, a plant-derived medium suitable for growth of human-use products to prepare glycerol stocks that were designated "RCB".

2.4. Master and Production cell banks (MCB or PCB)

The MCB was prepared by inoculating SB media containing 50 μg/mL kanamycin and 1% dextrose with an inoculum from the

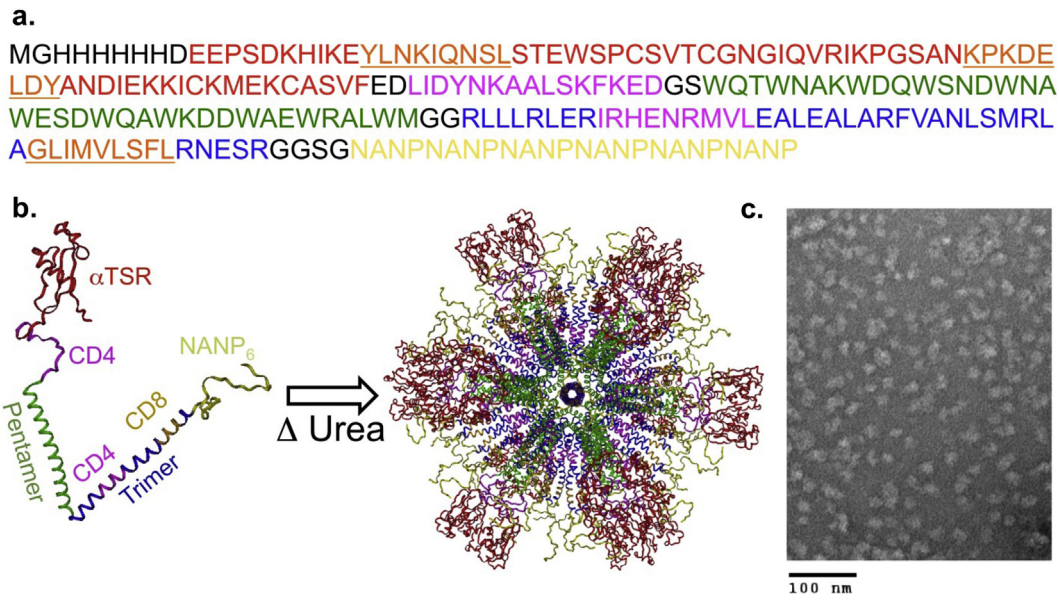

Fig. 1. Sequences, formation and structural analysis of FMP014. a: The color coding of the amino acid sequence of the FMP014 monomer identifies unique regions of the protein. The pentameric (green) and trimeric (blue) oligomeric domains form 5-stranded and 3-stranded coiled-coils, respectively, serve as the scaffold of the nanoparticle displaying the B cell epitopes α-TSR (red) and NANP-repeat (yellow). Two CD4 epitopes (pink) and three CD8 epitopes (orange, underscored) are built into the scaffold of the particle. b: SAPN is formed by the oligomerization of 3- and 5-stranded coiled-coiled domains within a single polypeptide monomer. c: Individual particles as visualized using electron microscopy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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