

## MALARIA VACCINE OF SELF-ASSEMBLING POLYPEPTIDE NANOPARTICLES

### REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/076,963 filed Jun. 30, 2008. The entirety of each of these documents is specifically and entirely incorporated by reference.

### RIGHTS IN THE INVENTION

[0002] This invention was made with support from the United States Government and, specifically, the Walter Reed Army Institute of Research and, accordingly, the United States government has certain rights in this invention.

### BACKGROUND

[0003] 1. Technical Field

[0004] This invention is directed to self-assembling polypeptide nanoparticles for the diagnosis and treatment of malaria and, in particular, nanoparticles containing specific epitope constructions of antigens derived from malarial proteins.

[0005] 2. Background

[0006] Malaria is caused by protozoan parasites of the genus *Plasmodium*. At least four types of the *plasmodium* parasite infect humans, although the most serious forms of the disease are caused by *Plasmodium falciparum* and *Plasmodium vivax*. Related species include *Plasmodium ovale* and *Plasmodium malariae*, which also infect humans. The group of human-pathogenic *Plasmodium* species is usually referred to as malaria parasites.

[0007] The organism itself is transmitted by the bite of an infected *Anopheles* mosquito. When an infected mosquito bites a human, sporozoites enter the human circulation. These travel to and penetrate liver cells where they asexually reproduce, via the process of schizogony. The intracellular, asexually dividing form of the parasite is referred to as a schizont, and because this schizont is in liver cells and not red blood cells (RBCs), it is referred to as the exoerythrocytic schizont stage. In *Plasmodium vivax* and *Plasmodium ovale*, the development of the schizont is retarded, and a resting stage of the parasite, called the Hypnozoite, is formed; however, this is not the case in *Plasmodium falciparum*.

[0008] When the hepatocytes burst, exoerythrocytic schizonts release merozoites into the blood, which are capable of infecting erythrocytes. Inside the erythrocytes, the merozoites develop into ring-like trophozoites, which then form the erythrocytic schizonts. Mature erythrocytic schizonts form merozoites again by breaking apart inside the erythrocytes. These merozoites are a transient intracellular form, either rapidly infecting new red blood cells to complete the erythrocytic cycle, or dying. In addition, when infection of new blood cells occurs, instead of forming trophozoites the parasites may grow into the immature gametocytes. These can be taken up in the blood meal of another feeding mosquito. The male gametocyte undergoes rapid nuclear division and produces a flagellated microgamete, which fertilizes the female gametocyte forming a zygote. The zygote develops into an ookinete, which then sticks to the gut wall of the mosquito, moves to the outermost layer of the stomach to form an oocyst. When the oocyst breaks, it releases sporozoites, which migrate to the salivary glands of the mosquito to restart the parasite's life cycle.

[0009] The disease malaria afflicts 500 million people worldwide and annually kills about 3 million people, most of whom are children. The Walter Reed Army Institute of Research (WRAIR) and Glaxo Smith Kline (GSK) developed the most successful vaccine to date. That vaccine, referred to as "RTS,S", is based on the circumsporozoite protein (CSP), the most abundant surface protein on the sporozoite, the parasite stage that mosquitoes inject into humans that starts the infection. The RTS,S Virus-Like Particle (VLP) vaccine is comprised of a C-terminal fragment of CSP fused to the Hepatitis B Surface Protein and is synthesized by *S. cerevisiae*. It requires formulation with the adjuvant AS02A to achieve protective immunogenicity. At best, this vaccine provides only about 40% protective efficacy in human clinical and field challenge studies. Many other malaria vaccines based on the CSP and other malaria proteins have proven unsuccessful [3]. Also, many experimental adjuvants have been tested and shown to produce either insufficient immunogenicity or unacceptable reactogenicity. Furthermore, a variety of antigen presentations, including single recombinant proteins, multi-antigen combinations, malaria fusion protein fragments, or single or multiple peptide epitopes arrays have produced little success in preventing disease.

[0010] The development of a vaccine for *P. falciparum* malaria has been extremely difficult for at least two reasons. The first is that the *P. falciparum* parasites do not reliably infect animals, although a few non-human primate models are available for blood stage vaccine work, thus making the testing of vaccine designs difficult. For sporozoite vaccine work, therefore, rodent malaria models based on *P. berghei* or *P. yoelii* (or *P. chabaudi* or *P. vinckei*) are used for preliminary vaccine studies. Because the blood stage of the parasite can be cultured in human erythrocytes, antibodies against blood stage proteins can be tested for their capacity to prevent invasion of erythrocytes by merozoites, but this event has yet to be definitively identified as a correlate of protective immunity. The second is that most malaria epitopes are not very immunogenic in man. It is believed this is the result of thousands of years of evolution of the malaria parasite living in man and evolving epitopes on its functionally important proteins that are not recognized by the human immune system. Therefore, the advances in malaria vaccinology have had to rely on adjuvants to increase the immune response to many malaria proteins in vaccines developed for human use. Most adjuvants used in animal studies have adverse side effects that make them unsafe to use in humans, and while there are several new ones in clinical trials, only alum is currently approved for human use, and alum has proved to be a poor adjuvant for malaria vaccines.

[0011] Peptide based vaccines against malaria have been made before but all relied on strong adjuvants for protective efficacy. Mouse studies with the murine malaria parasite *P. berghei* have shown that vaccines based on immunodominant CSP B- or T cell epitopes can induce a protective immune response if given with strong adjuvants. Analysis of murine immune responses to vaccination with the *P. berghei* CSP (PbCSP) have shown its dominant B cell epitope to be (DPP-PNPND)<sub>2</sub> (SEQ ID NO 1) [4, 5], which, like the PFCSP epitope, is located in the central repeat portion of the protein. A cytotoxic T cell epitope, NDDSYIPSAEKI (SEQ ID NO 2), has also been identified [6]. A synthetic peptide vaccine containing a tandemly repeating domain (DPPPPNPN)<sub>2</sub> (SEQ ID NO 3) has been produced using a "multiple antigenic peptide system" (MAPS), in which the synthetic pep-