

**RECOMBINANTLY EXPRESSED  
PLASMODIUM CELTOS ANTIGEN AND  
METHODS OF USE THEREOF**

REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Application No. 61/117,863 filed Nov. 25, 2008. The entirety of which is specifically incorporated by reference herein.

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.

FIELD AND BACKGROUND OF THE  
INVENTION

**[0003]** The present invention relates generally to fields of medicine and biotechnology. More particularly, the invention relates to the manufacture and use of a vaccine incorporating recombinantly expressed *Plasmodium* cell-traversal protein for ookinetes and sporozoites (CeLTOS) to induce pre-erythrocytic immunity against malaria.

**[0004]** Malaria currently represents one of the most prevalent infections in tropical and subtropical areas throughout the world. Per year, malaria infections lead to severe illnesses in hundreds of million individuals worldwide, while it kills 1 to 3 million people, primarily in developing and emerging countries every year. The widespread occurrence and elevated incidence of malaria are a consequence of the increasing numbers of drug-resistant parasites and insecticide-resistant parasite vectors. Other factors include environmental and climatic changes, civil disturbances, and increased mobility of populations.

**[0005]** Malaria is caused by the mosquito-borne hematozoan parasites belonging to the genus *Plasmodium*. Four species of *Plasmodium* protozoa (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*) are responsible for the disease in humans; many others cause disease in animals, such as *P. yoelii* and *P. berghei* in mice. *P. falciparum* accounts for the majority of infections and is the most lethal type ("tropical malaria"). Malaria parasites have a life cycle consisting of several stages. Each stage is able to induce specific immune responses directed against the corresponding occurring stage-specific antigens.

**[0006]** Malaria parasites are transmitted to man by several species of female *Anopheles* mosquitoes. Infected mosquitoes inject the "sporozoite" form of the malaria parasite into the mammalian bloodstream. Sporozoites remain for a few minutes in the circulation before invading hepatocytes. At this stage, the parasite is located in the extra-cellular environment and is exposed to antibody attack, mainly directed to the "circumsporozoite" (CS) protein (CSP), a major component of the sporozoite surface. Once in the liver, the parasites replicate and develop into so-called "schizonts." These schizonts occur in a ratio of up to 20,000 per infected cell. During this intra-cellular stage of the parasite, main players of the host immune response are T-lymphocytes, especially CD8+ T-lymphocytes (Bruna-Romero, Gonzalez-Aseguinolaza et al. 2001). After about one week of liver infection, thousands of so-called "merozoites" are released into the bloodstream and enter red blood cells, becoming targets of antibody-me-

diated immune response and T-cell secreted cytokines. After invading erythrocytes, the merozoites undergo several stages of replication and transform into so-called "trophozoites" and into schizonts and merozoites, which can infect new red blood cells. This stage is associated with overt clinical disease. A limited amount of trophozoites may evolve into "gametocytes," which is the parasite's sexual stage. When susceptible mosquitoes ingest erythrocytes, gametocytes are released from the erythrocytes, resulting in several male gametocytes and one female gametocyte. The fertilization of these gametes leads to zygote formation and subsequent transformation into ookinetes, then into oocysts, and finally into salivary gland sporozoites.

**[0007]** The malarial protein designated CeLTOS, for cell-traversal protein for ookinetes and sporozoites, from *Plasmodium berghei* has previously been shown to mediate malarial invasion of both vertebrate and mosquito host cells and is required for establishing their successful infections (Kariu, Ishino et al. 2006). In the vertebrate host, *Plasmodium* sporozoites traverse to hepatocytes via a complex passage initiating at the dermis and traversing through cellular barriers in the skin and the liver sinusoid. Therefore, the induction of immunity targeted to molecules involved in sporozoite motility and migration into hepatocytes may lead to nonproductive and/or reduced hepatocytic infection.

**[0008]** Malaria-naïve individuals do not possess partial immunity developed over life-long exposures. The current malaria vaccines being tested are insufficient to induce appropriate long lived memory responses. Therefore the need exists for an efficacious, long-term, pre-erythrocytic stage malaria vaccine to be used alone or in combination with alternate treatment strategies.

SUMMARY OF THE INVENTION

**[0009]** Immunization with a CeLTOS vaccine could mimic the development of immunity from natural malaria exposures. The development of an efficacious pre-erythrocytic stage malaria vaccine from the *Plasmodium* protein CeLTOS, (cell traversal protein for ookinetes and sporozoites) has the potential to protect human populations in malaria endemic regions. Vaccination with a pre-erythrocytic stage vaccine reduces or eliminates the traversal of infective sporozoites through cells required for infection of liver cells and thus protect against infection and/or reduce the severity of the Malarial disease

**[0010]** The mechanism of protection induced by a pre-erythrocytic stage malaria vaccine would be mediated by the development of specific protective antibodies to proteins on the parasite surface and block the traversal of the sporozoite through cells leading to productive infection. The putative mode of action of these antibodies is to bind the surface of the sporozoites and block their ability to associate with and invade cells involved in hepatocyte infection. The effect of blocking this process would be to reduce the potential amplification of parasites in the liver and thus reduce parasitic load.

**[0011]** Recombinant proteins expressed in *E. coli* can yield high levels of properly folded soluble protein. Development of a recombinant protein PfCeLTOS vaccine can be used in combination with other candidate malaria vaccines, from either pre-erythrocytic stages or blood stages. These stage and combination antigen vaccine approaches may lead to the induction of a broader immune response.

**[0012]** One embodiment of the invention utilizes CeLTOS as target antigen for a pre-erythrocytic vaccine. An alternate