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**CR-2 BINDING PEPTIDE P28 AS  
MOLECULAR ADJUVANT FOR DNA  
VACCINES**

CROSS-REFERENCE TO RELATED  
APPLICATIONS

This application claims priority to U.S. Provisional Application 60/998,460, filed on Oct. 3, 2007, the entirety of which being incorporated herein by this reference.

FIELD AND BACKGROUND OF THE  
INVENTION

The invention relates to the field of medicine and biotechnology. More particularly, the invention relates to the use of a recombinant model antigen DNA vaccine incorporating tandem copies (as, for example linear concatamers) of the synthetic peptides derived from the CR2 binding motif of the C3d domain of the third component (C3) of the human complement system. Furthermore, this inventions relates to a method and kit for the implementation of said method of such a construct as a vaccine against malaria infections and as a treatment of the same.

Background on the Malarial Disease

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.

Malaria is caused by the mosquito-borne hematoprotozoan 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.

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 intracellular stage of the parasite, main players of the host immune response are T-lymphocytes, especially CD8+ T-lymphocytes (Bruna-Romero O., 2001A). 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-mediated 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

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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.

Malaria Vaccines Incorporating CSP Antigens

Current strategies for developing effective malaria vaccines are to elicit 1) humoral responses against sporozoite surface antigen (Ag) to reduce infection of the liver, 2) cellular responses against hepatic stages to kill infected liver cells and reduce the subsequent blood stage infection, 3) humoral responses against erythrocytic stage Ag to eliminate residual infection and disease, and 4) humoral responses against sexual stage Ag to reduce transmission. (Wolfgang W. Leitner \*. M., 1997). One candidate for inclusion is the circumsporozoite protein (CSP) which is present in three developmental stages, including sporozoites and infected hepatocytes (Nussenzweig. V., 1989) and exoerythrocytic merozoites, but not erythrocytic merozoites (Atkinson, 1989).

The CS protein is the only *P. falciparum* antigen demonstrated to consistently prevent malaria when used as the basis of active immunization in humans against mosquito-borne infection, albeit at levels that are often insufficient. Theoretical analysis has indicated that the vaccine coverage, as well as the vaccine efficiency, should be above 85% or, otherwise, mutants that are more virulent may escape (Clyde D. F., 1973).

The sporozoite stage of malaria parasites carries CSP on its outer surface (Aikawa, 1981) which expresses a unique immunodominant epitope recognized by immunized or repeatedly infected hosts (Zavala F. A., 1983). Sera from mice immunized with *Plasmodium berghei* sporozoites immunoprecipitate a single 44,000Mr protein, the circumsporozoite (CS) protein (CSP), from extracts of surface-labeled sporozoites (Zavala F. A., 1983). Immunoprecipitation of extracts of metabolically labeled sporozoites with a monoclonal antibody (3D11) directed to the CSP demonstrated that the 44,000Mr membrane form is derived from a 54,000-Mr intracellular precursor (Zavala F. A., 1983). The CSP from monkey and human malaria parasites contains amino and carboxy-terminal regions of relatively low immunogenicity which flank a central region of highly immunogenic, tandemly repeated amino acid units, the sequences of which differ from species to species (Damem, 1984). Monoclonal antibodies to the repeated amino acid units neutralize parasite infectivity (Nussenzweig, 1969), suggesting that CSP might be useful as sporozoite-stage vaccines.

The central region of the CSP contains tandem repetitive peptide sequences that appear to be the dominant targets for Ab responses during infection (Nussenzweig. V., 1989). The N and C-terminal regions flanking the central CSP repeats contain several immunologically and structurally important features, such as MHC class I and II epitopes (Romero, 1990), and peptide structures that appear to be important for sporozoite invasion of hepatocytes (Aley, 1986). CSP-specific immune responses have been induced with various synthetic peptides and fragments or full-length recombinant proteins (Egan, 1987). The responses were B cell (Egan, 1987) or T cell dependent and in the case of T cell responses, were either CD4 or CD8 dependent (Migliorini, 1993). However, only a few of the immunogens induced responses that reduced the infection rate upon challenge (Egan, 1987). While some CSP repeat region-specific mAbs reduce infection rates (Potocn-