

**EXPRESSION, PURIFICATION AND USES OF
A PLASMODIUM FALCIPARUM LIVER
STAGE ANTIGEN 1 POLYPEPTIDE**

This application is a divisional of U.S. application Ser. No. 10/706,435 filed on Nov. 12, 2003 now U.S. Pat. No. 7,550,275, which claims priority from U.S. Provisional Application Ser. No. 60/425,719, filed Nov. 12, 2002, all of which are incorporated by reference in their entirety.

INTRODUCTION

Plasmodium falciparum is the leading cause of malaria morbidity and mortality. The World Health Organization estimates that approximately 200 million cases of malaria are reported yearly, with 3 million deaths (World Health Organization, 1997, *Wkly. Epidemiol. Rec.* 72:269-276). Although, in the past, efforts have been made to develop effective controls against the mosquito vector using aggressive applications of pesticides, these efforts ultimately led to the development of pesticide resistance. Similarly, efforts at treatment of the disease through anti-parasitic drugs led to parasite drug-resistance. As the anti-vector and anti-parasite approaches failed, efforts became focused on malaria vaccine development as an effective and inexpensive alternative approach.

However, the complex parasitic life cycle has further confounded the efforts to develop efficacious vaccines for malaria. The parasite's life cycle is divided between the mosquito-insect host and the human host. While in the human host, it passes through several developmental stages in different organellar environments, i.e. the liver stage and the red blood stage. Although conceptually simple, in reality the problems that must be considered when designing subunit vaccines for malaria are great. Antigen diversity is a characteristic that must be taken into account and includes a high degree of developmental stage specificity, antigenic variation and antigen polymorphism.

The observation that sterile immunity to malaria can be induced by immunization with irradiated sporozoites (Clyde et al., 1973, *J. Med. Sci.* 266: 169-277; Krzych et al. 1995, *J. Immunol.* 155, 4072-4077; Hoffman et al., 2002 *JID* 185, 1155-1164) has focused attention on the sporozoite and liver stages of the parasite life-cycle as potential targets of an effective vaccine. Liver stage antigen 1 (LSA-1) is a prime candidate for development as a vaccine as it is expressed during the hepatic stage of infection. It is known that peptides or recombinant fragments of the LSA-1 protein elicit specific humoral, cellular and cytokine immune responses from cultured peripheral blood mononuclear cells (PBMC) taken from malaria exposed individuals. These immune responses are correlated with a reduction or absence of parasitemia and *falciparum* malaria disease in subsequent transmission seasons (Kurtis et al. 2001, *Trends in Parasitology.* 17, 219-223). The *P. falciparum* LSA-1 protein is found within the parasitophorous vacuole (PV), a space defined as that region between the inner plasmalemma and the outer parasitophorous vacuole membrane (PVM). The (PV) forms a distinct ring separating the parasite cytoplasm from the host hepatocyte (Fidock et al., 1994, *J. Immunol.* 153, 190-204). The PVM is of host origin and is formed by invagination of the host cellular membrane when the parasite invaded the host cell. The LSA-1 protein is approximately 230 kDa in mass. Its expression begins shortly after sporozoite invasion of the liver and increases with liver stage development. It is described as a flocculent material within the parasitophorous vacuole and may also adhere to the surface of merozoites, suggesting a

crucial role in liver schizogony perhaps protecting the merozoite surface (Hollingdale et al., 1990, *Immunol. Lett.* 25, 71-76). When the hepatocyte ruptures, it releases the merozoites encased in LSA-1 protein into the liver sinusoid and into the blood stream (Terzakis et al., 1979, *J. Protozool.* 26, 385-389).

The LSA-1 protein is characterized by a large central repeat region consisting of about eighty-six 17 amino acid tandem repeats flanked by short non-repetitive N-terminal and C-terminal regions which are highly conserved across strains. Studies have revealed the protein is a target of B-cells (Guerin-Marchand et al. 1987, *Nature* 329, 164-167; Fidock, et al., 1994, supra; Luty et al., 1998, *Eur. Cytokine Netw.* 9, 639-646), helper T-cells (Doolan and Hoffman, 2000, *J. Immunol.* 165, 1453-1462; Fidock et al. 1994, supra; Connelly et al., 1997, *Infect. Immun.* 65, 5082-5087; Luty et al., 1998, supra; Kurtis et al., 1999, supra) and MHC-restricted CD8+ CTLs (Hill et al., 1991, *Nature* 532, 595-600; Aidoo et al., 1995; Doolan and Hoffman, 2000, *J. Immunol.* 165, 1453-1462). T-cell epitopes have been defined amongst the amino acid residues in the N-terminal and C-terminal flanking regions and in the central repeat region (Doolan and Hoffman, 2000, *J. Immunol.* 165, 1453-1462, 2000; Krzych et al., 1995, *J. Immunol.* 155, 4072-4077; Hill et al., 1991, supra; Fidock et al., 1994, supra). Even though LSA-1 is one of the most immuno-epidemiologically studied *P. falciparum* malaria antigens a vaccine using the protein has not yet been developed.

The *P. falciparum* *lsa-1* gene sequences have been used for over a decade in an attempt to make a DNA vaccine against *P. falciparum*. NYVAC-Pf7, a multivalent poxvirus vector made by WRAIR and Virogenetics, contained an *lsa-1* gene that encoded a repeatless protein. The NMRC MuStDO5 (a mixture of DNA plasmids constructs encoding part or all of five *P. falciparum* genes: CS, SSP2, LSA-1, EBA-175 and MSP-1), and more recently CSLAM (a mixture of DNA plasmid vaccine constructs encoding all or parts of five *P. falciparum* malaria genes: CS, SSP2, LSA-1, AMA-1 and MSP-1) contain LSA-1 gene sequences in their vaccines. The Oxford University scientists have modified vaccinia Ankara (MVA) and cowpox constructs containing DNA sequences that code for several LSA-1 T-cell epitopes. NMRC is currently constructing alpha-virus (VEE replicons) and adenovirus constructs that contain *lsa-1* genes.

All these potential vaccines use LSA-1 gene constructs designed as injectable DNA sequences that will be transcribed and translated in the human host, for example in a DNA plasmid, poxvirus, adenovirus, etc. The researchers have used LSA-1 DNA sequences that express the protein in the immunized host rather than injection of isolated LSA-1 protein s because the LSA-1 protein has proven to be impossible to obtain. It has been very difficult or impossible to express in bacteria, yeast or baculovirus. Therefore recombinant expression and isolation of the protein at a scale that is commercially viable for vaccine development and use has never been achieved.

We have overcome this problem and now can express the protein in bacteria. The expressed product can be isolated and purified to high homogeneity for use as an immunogen or a vaccine.

SUMMARY OF THE INVENTION

The aim of the present invention is to develop a *P. falciparum* liver-stage directed vaccine that will result in lower parasite burden in the human host. To that aim, large-scale expression, purification and characterization of a *P. falciparum* LSA-1 (PflSA1) immunogenic peptide is necessary.