

PLASMODIUM LIVER STAGE ANTIGENS**CROSS-REFERENCES TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No. 60/722,254, filed Sep. 30, 2005, and U.S. Provisional Application No. 60/748,382, filed Dec. 8, 2005, both of which are incorporated herein by reference.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

This invention was made by employees of the United States Army Medical Research And Materiel Command, which is an agency of the United States Government. The Government has certain rights in this invention.

FIELD OF THE INVENTION

This invention relates to proteins that are specifically expressed by liver stage *Plasmodium* parasites, and their use in the prevention, diagnosis, and treatment of malaria.

BACKGROUND OF THE INVENTION

Malaria has a tremendous impact on human health, killing millions annually, and the disease is a major impediment for social and economic development of nations in malaria-endemic areas, particularly in sub-Saharan Africa (Sachs & Malaney (2002) *Nature* 415:680-85). Malaria infection begins when the Anopheline female injects infective sporozoites into the mammalian host. Sporozoites travel through different cells before settling into their final host hepatocyte. The sporozoite moves into a parasitophorous vacuole created by invagination of the hepatocyte plasma membrane. Inside this compartment the sporozoite transforms into a liver stage. The liver stage grows rapidly and undergoes multiple rounds of nuclear division. The mature liver stage releases thousands of merozoites that will establish red blood cell infection. Liver stages are predicted to express many different proteins, some possibly unique to this stage, but only few of those unique molecules have been identified so far. Identification of liver stage-specific molecules is important because the infected hepatocyte has been established as the primary target of the sterile protective immune response in the radiation attenuated sporozoite vaccine model and recently in the genetically attenuated sporozoite vaccine model (reviewed in Matuschewski (2006) *Curr. Op. Immunol.* 18:1-9). In addition, liver stage molecules that can be detected in human diagnostic sample may be useful for diagnosing early stage malaria.

There is a need in the art for vaccines that protect against malaria infection and disease. There is also a need in the art for diagnostic markers for malaria. The present invention addresses these needs and others.

SUMMARY OF THE INVENTION

One aspect of the invention provides isolated liver stage *Plasmodium* polypeptides. In some embodiments, the isolated liver stage *Plasmodium* polypeptides comprise an amino acid sequence selected from the group consisting of SEQ ID NOs:1-48. In some embodiments, the liver stage *Plasmodium* proteins are preferentially targeted by immune responses associated with protection from *Plasmodium* infection. The isolated liver stage *Plasmodium* polypeptides of the

invention may be recombinant or synthetic polypeptides. In some embodiments, the polypeptides of the invention are immunogenic derivatives of polypeptides comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1-48. Such immunogenic derivatives include, but are not limited to, peptides comprising an amino acid sequence selected from SEQ ID NOs:49-52.

Another aspect of the invention provides isolated nucleic acid molecules encoding the liver stage *Plasmodium* polypeptides of the invention. Thus, some embodiments provide an isolated nucleic acid molecule encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1-48 and immunogenic derivatives thereof.

A further aspect of the invention provides compositions comprising one or more liver stage *Plasmodium* polypeptides of the invention and a pharmaceutically acceptable carrier. Thus, some embodiments provide an immunogenic composition comprising a liver stage *Plasmodium* polypeptide and a pharmaceutically acceptable carrier, wherein the liver stage *Plasmodium* polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1-48 and immunogenic derivatives thereof. In some embodiments, the compositions of the invention are immunogenic compositions for inducing immune responses, such as vaccine compositions.

In another aspect, the invention provides methods for inducing an immune response against *Plasmodium* parasites, comprising administering an immunogenic composition comprising an effective amount of one or more liver stage *Plasmodium* polypeptides of the invention. Thus, in some embodiments the invention provides a method for inducing an immune response in a mammalian subject against *Plasmodium falciparum*, comprising administering to a mammalian subject a composition comprising an effective amount of at least one liver stage *Plasmodium* polypeptide selected from the group consisting of SEQ ID NOs:1-48 and immunogenic derivatives thereof.

Yet another aspect of the invention provides methods for treating a mammalian subject in need thereof, comprising administering to a mammalian subject in need thereof an immunogenic composition comprising an effective amount of one or more liver stage *Plasmodium* polypeptides of the invention. Thus, in some embodiments the invention provides a method for treating a human subject in need thereof, comprising administering to a human subject an immunogenic composition comprising at least one isolated polypeptide selected from the group consisting of SEQ ID NOs:1-48 and immunogenic derivatives thereof.

In addition, the invention provides genetically attenuated sporozoites from which at least one gene coding for a liver stage polypeptides of the invention has been eliminated. Thus, in some embodiments, the invention provides genetically attenuated *Plasmodium* sporozoites lacking a gene coding for a liver stage polypeptide selected from the group consisting of SEQ ID NOs:1-48.

The invention also provides expression vectors encoding the liver stage *Plasmodium* polypeptides of the invention, host cells comprising such expression vectors, antibodies that bind specifically to the liver stage *Plasmodium* polypeptides of the invention or immunogenic derivatives thereof, and