PLASMODIUM VIVAX HYBRID CIRCUMSPOROZOITE PROTEIN AND VACCINE

This application is a divisional of U.S. application Ser. No. 11/334,161 filed on Jan. 18, 2006, now U.S. Pat. No. 7,790,186, which claims the benefit for priority under 35 U.S.C. 119(e) from U.S. Application No. 60/644,712 filed on Jan. 18, 2005, now expired, all of which are incorporated by reference in their entirety.

INTRODUCTION

Plasmodium vivax is the most abundant of all human malarias. In addition to being present in tropical and subtropical regions, the ability of the parasite to complete its mosquito cycle at temperatures as low as 15 degrees Celsius has also allowed it to be spread in temperate climates. It accounts for approximately 50% of all malaria cases worldwide. However, due to the fact that the disease caused by *P. vivax* is rarely lethal, the efforts to control *P. vivax* malaria (through vaccine development) are lagging far behind vaccine development against *P. falciparum*. Although *P. vivax* does not usually kill the patient, the sheer number of clinical cases, and the fact that it causes severe morbidity, contributes to serious economic impact in developing countries. In addition, there have been increasing numbers of reported cases of severe disease, resulting in anemia and death, caused by this parasite. A unique feature of *P. vivax* is that some 'strains' are capable of causing delayed infection by remaining latent in the liver before emerging into the peripheral circulation to manifest clinical symptoms. Thus, individuals that get infected in an endemic region may not present with symptoms for several months. When they return to areas that are not endemic for the disease, but do have the appropriate vector population, they can potentially cause the spread of disease in hitherto clean areas. Thus, it is necessary to focus efforts towards developing vaccines to control the global spread of *P. vivax* infections.

P. vivax malaria infection remains latent within the liver while the parasite is undergoing pre-erythrocytic shizogony. If the parasite is controlled at any stage before it escapes the liver there are no clinical symptoms of disease. Thus, the pre-erythrocytic stages of the malaria parasite are ideal targets for designing vaccines to prevent the symptomatic stage of the disease by killing parasites before they enter peripheral circulation.

The sporozoite has long been shown to induce protection in animal and human models against various malarias. Immunization with irradiated sporozoites leads to complete protection from a homologous challenge. However, using sporozoites to vaccinate large populations presents logistical problems.

The circumsporozoite (CS) protein present on the sporozoites of all plasmodia is the most abundant protein. It is involved in the motility and invasion of the sporozoite during its passage from the site of inoculation into circulation, from where it migrates to the liver and enters the hepatocyte (Mota, M M and Rodrigues, 2004, Cell Microbiol: 6, 1113-1118). As a consequence, the CS protein is a very appealing target for a vaccine. Studies in animal models and humans have shown promising results. The CS antigen has been shown to induce protection in rodent (Py and Pb) models and is a part of RTS,s, the most advanced malaria vaccine developed so far (Heppner et al. 2005, Vaccine 23, 2243-50) which is based on the CS protein of *P. falciparum*.

A recombinant *P. vivax* CS protein was expressed and tested as a vaccine in the early 80s to 90s with limited success (Collins et al., 1989, Am. J. Trop. Med. Hyg. 40, 455-64) and was not pursued further. Subsequently, due to the limitations of producing large amounts of pure protein, synthetic peptide-based vaccines have been developed. Recently, a Phase I study was carried out with the N-terminal, C-terminal and repeat region of PvCS and shown to induce antibody and IFN-gamma responses in vaccinated individuals (Herrera et al. 2005, Am. J. Trop. Med. Hyg. 73, 3-9). The vaccine was comprised of three long synthetic peptides corresponding to the N-terminal, Repeat region and C-terminal portions which ranged in size from 48 amino acids to 76 amino acids and were based on the sequence of the *P. vivax* Sal I parasite. The vaccine was based on linear peptides that represent three different parts of the CS molecule. They did not represent the CS protein in its entirety. Additionally these peptides did not take into account the variations found in the repeat region and were derived from a single strain of *P. vivax* (Sal-1) which is not representative of global *P. vivax* CS protein sequences. Other vaccines based on Multiple Antigen Peptides (MAP) were developed and tested in monkeys. MAPS are based on one or more epitopes that are cross-linked on a lysine backbone (Nardelli and Tam, 1995, Pharm. Biotechnol. 6, 803-19).

Synthetic vaccines present several drawbacks. Due to technical limitations in the length of synthetic peptides that can be made, these vaccines can not represent the entire protein, but only fragments of a protein. Additionally, these vaccines are limited in the sequences that are incorporated in them, and therefore would not recognize most global constructs. We therefore decided to explore the feasibility of a recombinant protein-based vaccine utilizing new advances in the field of biotechnology. Despite being studied for several years, the structure, and exact significance of the various parts of the CS molecule are not clearly known. There are several studies alluding to the significance of several regions of the CS molecule. CS sequence from all plasmodia show dramatic differences, with no general sequence conservation. There are, however, two motifs, a 5 amino acid sequence at the N-terminal immediately preceding the repeat region, known as Region I (KLKQP, SEQ ID NO:1), that shows complete sequence conservation in all the plasmodia sequenced so far. The second motif, located at the C-terminal end of the molecule, has strong sequence and motif conservation among all plasmodia. This region is known as Region II plus (CSVTCG, SEQ ID NO:2). Both Region I and Region II plus have been shown to be involved in binding to hepatocytes. Generating an immune response against these motifs could prevent a receptor-ligand interaction, a feature important in preventing the establishment of infection.

The bulk of the CS molecule of all Plasmodia is constituted by a central repeat region. The repeat regions vary for each *Plasmodium* species. The central repeat region of *P. falciparum* comprises of NANP/NVDP repeats. All sequenced strains of *P. falciparum* have a common and highly conserved repeat sequence. *P. vivax* has two distinct forms of the CS protein designated VK210, or Type 1, and VK247, or Type 2. These two forms are almost identical at the N and C terminal, but differ in the central repeat region. The repeat regions were initially identified when antibodies against what are now known as the VK210 parasite failed to recognize certain sporozoites. Thus, antibodies directed against the repeat region of the two types do not cross-react with each other. While a majority of the field infections are attributed to VK210 sporozoites, a significant number of VK247 infec-