

RECOMBINANT *P. FALCIPARUM* MEROZOITE PROTEIN-1₄₂ VACCINE

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, 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. Vaccine candidates have been identified from each of the parasite's developmental stages. The major merozoite surface protein-1, MSP-1, is among the leading erythrocytic stage vaccine candidates (Diggs, et al, 1993, Parasitol. Today 9: 300-302). The objective of erythrocytic stage vaccines is to diminish the level of parasitemia in the bloodstream and thus reduce the severity of disease.

Although the MSP-1 molecule has been studied extensively, its function is not fully understood. There is evidence that MSP-1 binds to erythrocytes and may have a role in erythrocyte invasion (Perkins and Rocco, 1988, J. Immunol. 141, 3190-3196; Holder, A. A., 1994, Parasitology 108 (Suppl.) S5-18).

MSP-1 is secreted as a membrane-anchored (Haldar et al., 1985, J. Biol. Chem. 260, 4969-4974) 195 kDa precursor that is proteolytically processed to products with nominal molecular masses of 83, 28-30, 38-45, and 42 kDa during merozoite development (Holder and Freeman, 1984, Phils Trans R. Soc. Lond B. Bio. Sci. 307, 171-177; Lyon et al., 1987, J. Immunol, 138, 895-901; Holder et al., 1987, Parasitology 94, 199-208). These protein fragments form a non-covalent complex on the surface of merozoites (McBride and Heidrich, 1987, Parasitology 23, 71-84; Lyon, et al., 1987, supra) that remain attached to the merozoite surface through the C-terminal 42 kDa fragment (MSP-1₄₂). At the time of erythrocyte invasion MSP-1₄₂ is processed further to a 33 kDa fragment and a 19 kDa C-terminal fragment (MSP-1₁₉) (Blackman, et al., 1991, Mol. Biochem. Parasitol. 49, 35-44) which is bound to the merozoite surface through an N-glycosylphosphatidyl inositol anchor (GPI) (Haldar, et al., 1985, supra) This second proteolytic cleavage event results in the shedding of the non-covalent associated protein complex from the merozoite surface during invasion. During the invasion process, MSP-1₁₉ is present on ring forms in the newly invaded erythrocyte (Blackman, et al., 1990, J. Exp. Med. 172, 379-382). The apparent structure of

MSP-1₁₉ is complex, containing 12 cysteines within a span of 100 amino acid residues, and is arranged as two tandem domains that are homologous with epidermal growth factor (EGF) (Blackman, et al., 1991, supra; Morgan et al., 2000, J. Biomol. NMR 17, 337-347). Each putative EGF-domain contains six cysteine residues that would form three disulfide bridges per domain, which force the assembly of several well-defined discontinuous epitopes (Farley and Long, 1995, Exp. Parasitol. 80, 328-332; McBride and Heidrich, 1987, supra; Uthaipibull et al, 2001, J. Mol. Biol. 307, 1381-1394).

Because age-dependent development of immunity to malaria is due, at least in part, to antibody against erythrocytic stage parasites (Cohen, S. et al., 1964, Nature 192, 733-737), a malaria vaccine should induce effective antibodies against this developmental stage. Evidence supporting the use of MSP-1₄₂ and MSP-1₁₉ in a malaria vaccine is extensive. MSP-1₁₉-specific mAbs inhibit *P. falciparum* growth in vitro (Blackman et al., 1990, supra) and passively protect mice against infection with *P. yoelii* (Majarian et al., 1984, J. Immunol. 132, 3131-3137; Ling et al., 1994, Parasite Immunol. 16, 63-67). Immunization of *Aotus* monkeys with native *P. falciparum* MSP-1 (Siddiqui, et al., 1987, Proc. Natl. Acad. Sci. USA 84, 3014-3018), or *S. cerevisiae* recombinant MSP-1₁₉ (Kumar et al., 1995, Mol. Med. 1, 325-332; Egan et al., 2000, Infect. Immun. 68, 1418-1427; Stowers et al. 2001, Trends Parasitol. 17, 415-419), protect against a homologous challenge. *E. coli*-expressed *P. yoelii* MSP-1₁₉ (Burns et al., 1989, J. Immunol. 143, 2670-2676) protects against a homologous challenge in rodent models. Antibodies raised against MSP-1₁₉ grown in yeast weakly inhibit *Plasmodium* growth in vitro (Gozalo et al., 1998, Am. J. Trop. Med. Hyg. 59, 991-997) however this antigen lacks correct structure and induces a strong allergic response (Keitel, W. A., 1999, Vaccine 18, 531-539). MSP-1₁₉ may not be an optimal vaccine because it does not induce strong T-helper cell responses (Quin et al., 2001, Eur. J. Immunol. 31, 72-81). Poor MSP-1₁₉ T-cell immunogenicity may be a consequence of its structural stability, which allows it to resist proteolysis, and therefore to resist processing and presentation to the immune system.

Thus, MSP-1₄₂ may be a better choice as a vaccine candidate (Quin and Langhorne, 2001, Infect. Immun. 69, 2245-2251). Immunization of *Aotus* monkeys with baculovirus-expressed recombinant MSP-1₄₂, protects against a homologous challenge and the anti-sera raised inhibit *P. falciparum* growth in vitro, but product yield is low and it is not yet available in clinical grade (Chang et al., 1996, Infect. Immun. 64, 253-261; Chang et al., 1992, J. Immunol. 149, 548-555). The monoclonal antibodies that inhibit *P. falciparum* growth in vitro also inhibit the secondary processing of MSP-1₄₂ to MSP-1₁₃ and MSP-1₁₉ and react with disulfide dependent conformational epitopes that are conserved among all known strains of *P. falciparum*, (Chappel and Holder, 1993, Mol. Biochem. Parasitol. 60, 103-111). MSP-1₁₉ as well as EGF Domain 2 affinity purified antibodies from immune human sera also prevent parasite invasion in vitro (Egan, et al, 1999, Parasite Immunol. 21, 133-139). Rabbit anti-sera raised against recombinant MSP-1₄₂ (Chang et al., 1992, supra) inhibit *P. falciparum* growth in vitro.

Vaccination of *Aotus nancymai* monkeys with molecules derived from either native full length MSP-1 (Siddiqui, 1977, Science, 197, 388-389) or C-terminal fragments of MSP-1 has shown protection against infection from a virulent FVO strain of *P. falciparum* (Chang et al., 1996, supra; Kumar et al., 2000, supra; Stowers, et al, 2001, Infect.