Development of Pre-erythrocytic Malaria Vaccines

One of the most significant tools available to malaria vaccine developers is the ability to challenge individuals with malaria parasites in a controlled, reproducible manner. In 1985, a system was established for using membrane-fed anopheline mosquitoes carrying *P. falciparum* to infect volunteers (135). Seed lots of cloned parasites with known sensitivities to antimalarial compounds could be made and a repeatable infectivity model for evaluating vaccine or drug efficacy could be established. A retrospective analysis of the first 118 volunteers participating in studies using the 3D7 clone of a *P. falciparum* isolate conducted between 1985 and 1992 showed the method to be a reliable, safe and well-tolerated experimental model (136). An analysis of more recent data has confirmed the safety and reliability of the challenge model (132). To date, over one thousand three hundred people have been challenged by this method and no recrudescence of parasitemia has occurred after treatment, eliminating the risk of delayed clinical illness or secondary transmission after the trial. The more recent introduction of quantitative real-time PCR permits two- to four-day-earlier detection of parasitemia (shortening the pre-patent period) and allows the estimation of critical parameters in the parasite life cycle using a statistical model, including back-calculating the number of infected liver cells and estimating the rate of parasite multiplication of blood stages (137,138).

Pre-erythrocytic Malarial Vaccines in Clinical Development

Four pre-erythrocytic proteins, which have reached clinical testing, will be discussed: CSP and thrombospondin-related adhesive protein (TRAP or SSP2) from the sporozoite stage, and liver-stage antigen-1 (LSA1) and liver-stage antigen-3 (LSA3) from the liver stage.

Circumsporozoite protein. CSP is a leading candidate for pre-erythrocytic-stage vaccines. The protein is encoded by a single-copy gene and covers the surface of sporozoites (139). CSP from *Plasmodium* species display common structural features, including an N-terminal signal peptide, a C-terminal glycosylphosphatidylinositol (GPI) anchor that links the protein to the sporozoite surface, and a central domain composed mostly of amino acid repeats. The repeat region is immunodominant and is a target for neutralizing antibodies against sporozoites (140). Flanking the central repeats, all CSPs contain highly conserved domains designated as Region I, Region II-plus, and Region III. Region I contains a pentapeptide, KLKQP, and is involved in attachment of sporozoites to mosquito salivary gland and liver tissue (141,142). Region II-plus consists of a pair of cysteines and a six amino acid consensus sequence and is embedded at the proximal end of the thrombospondin domain. The domain contains $CD4^+$ and $CD8^+$ T-cell epitopes, and is involved in adhesion to and invasion of mosquito salivary gland and human liver tissues (143). Region III is predicted to form an amphipathic α-helix and may provide a proper framework for the neighboring Region II-plus adhesion motif (144).

While there have been many CSP-based vaccines tested, only the RTS,S recombinant protein/adjuvant combination has proven to protect people against sporozoite challenge in clinical settings and the field. RTS,S includes only a portion of the CSP molecule, and >80% of the antigenic component of the vaccine is actually HBsAg, which promotes spontaneously assembly into VLPs. To achieve protection, RTS,S is formulated by GSK-Bio in the adjuvant systems AS02A, AS01B, or AS02D, and consistently protects 40% to 50% of immunized volunteers against experimental sporozoite challenge with the homologous parasite strain (114,145). Results of meta-analyses by Graves and Gelband (146) of nine safety and efficacy trials, and two safety trials, with over 3000 participants of semi-immune children, of the RTS,S vaccine, showed that it reduced clinical episodes of malaria by 26% and severe malaria by 58% for up to 18 months. Prevalence of parasitemia was also reduced by 26% at six months after immunization. RTS,S also reduced clinical malaria episodes by 63% in semi-immune adult men in the second year of follow-up after a booster dose. Most recently, in a study not included in the meta-analyses, RTS,S formulated in AS02D reduced the rate of new onset parasitemia in infants over a six-month observation period by 66% (95% CI, 43–80%) (147). At the same time, other CSP-based vaccines tested in the field have shown no evidence for a protective effect, including the CS-NANP vaccine [307 participants, three trials (148–150)] and the CS102 peptide vaccine [14 participants, one trial (151)]. Likewise, trials of vaccines containing a second sporozoite antigen, the thrombospondin-related adhesive protein (TRAP or SSP2), also failed to protect in field trials [the ME-TRAP vaccine, 777 participants, two trials (152,153)]. The analysis by Graves and Gelband concluded that the RTS,S vaccine was effective in preventing a significant number of clinical malaria episodes, including good protection against severe malaria in children for 18 months with no severe adverse events attributable to the vaccine (146). While the report recommended progression of the RTS,S vaccine toward licensing, it stressed the need to increase its efficacy. The report did not identify evidence for supporting additional development of other vaccines included in the review, and recommended further research on other CSP-based vaccines.

In an early attempt to increase the efficacy of the RTS,S vaccine, GSK and WRAIR conducted a combination trial administering both RTS,S and TRAP proteins simultaneously. Though the number of volunteers was small, there were no protected individuals and it appeared that the TRAP protein interfered with the RTS,S vaccine's ability to induce a protective immune response (113). The prime-boost combination of RTS,S with other vaccine platforms, such as adenovirus vectored vaccines, may be profitable (154).

Thrombospondin-related adhesive protein. TRAP is a *P. falciparum* 90-kilodalton (kDa) protein expressed in both the sporozoite and asexual erythrocytic stages. TRAP is localized to the microneme and cell surface of mature sporozoites and has been considered to play a critical role in gliding motility and in the recognition and/or invasion of hepatocytes. Cytotoxic $CD8^+$ T cells recognizing TRAP have been identified in humans immunized with irradiated *P. falciparum* sporozoites and protected against experimental sporozoite challenge (155,156). Furthermore, antibodies against TRAP have been shown to block the sporozoite invasion into hepatocytes in vitro (157). Naturally acquired antibodies against TRAP in combination with high antibody titers to CSP and LSA1 correlate inversely with the malaria parasite densities among children in a hyper-endemic area (66,158).

Murine studies support the role of TRAP as a protective pre-erythrocytic antigen. *P. yoelii* SSP2 (the murine parasite equivalent of TRAP) has been shown to be the target of protective $CD8^+$ CTL that eliminate *P. yoelii*–infected hepatocytes in mice (159), and immunization with a synthetic branched-chain peptide including four copies of a PySSP2 sequence, NPNEPS, formulated in adjuvant, protected A/J, but not BALB/c or C57BL/6 mice (160). In the first study,