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INDUCTION OF *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING ANTIBODIES BY RECOMBINANT Pfs25

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The origins of modern malariology can be traced back to a crucial observation first made by Laveran one hundred and twelve years ago. His observation of a male gametocyte exflagellating was the first evidence that a parasite caused malaria (C.L.A. Laveran, 1880, *Bull. Acad. Med., 44:* 1268). It was also the beginning of our current understanding of sexual stage development. During the sexual stage of the *Plasmodium falciparum* life cycle, several surface proteins are expressed that are target antigens of transmission blocking antibodies (see review R. Carter et al., 1988, *Prog. Allergy, 41:* 193-214). These target antigens appear to fall into two categories (see review D.C. Kaslow, 1990, *Immun. Letters, 25:* 83-86): Group I antigens, consisting of target antigens expressed predominately in gametocytes and gametes, have limited immunogenicity in congenic mice and in humans; in contrast, Group II antigens, consisting of target antigens expressed predominately in zygotes and ookinetes, are widely immunogenic in mice, but elicits no naturally occurring antibodies in humans from malaria endemic areas. Most of the known target antigens fall into Group I, including Pfs230, Pfs48/45, and if they turn out to be target antigens, Pfs40 and Pfs10 (Kaslow & Rawlings, unpublished observations). Pfs25, a cysteine-rich 25 kDa glycolipoprotein expressed predominately in zygotes and ookinetes, is one of a least two Group II antigens (Kaslow & Duffy, unpublished observations). Besides being highly immnogenic, Group II antigens may have other advantages over Group II antigens as transmission blocking vaccine candidates (D.C. Kaslow, 1990, *loc. cit.*), for instance a lack of significant antigenic diversity.

To develop a Group II subunit transmission blocking vaccine and to study its immunogenicity and efficacy, the gene encoding Pfs25 was cloned and sequenced (D.C. Kaslow, 1988, *Nature, 333:* 74-76), and more recently expressed in a number of recombinant protein expression system (D.C. Kaslow et al., 1991, *Science, 252:* 1310-1313; Barr et al., 1991, *J. Exp. Med., 174:* 1203-1208). Analysis of the deduced amino acid sequence of Pfs25 revealed a cysteine rich protein with a putative secretory signal sequence, a hydrophobic carboxy terminus suggestive that Pfs25 was transferred to a glycosyl-phosphatidylinositol (GPI) anchor, and four potential glycosylation sites. The most striking feature of the deduced amino acid sequence, however, was the similarity of cysteine spacing in Pfs25 to that of epidermal growth factor (EGF) and other EGF-like domains present in a number of eukaryotic extracellular proteins (D.C. Kaslow, 1988 *loc. cit.*). As evidence that these disulfide bonds are crucial for the conformational integrity of Pfs25, reduction of the cystines in Pfs25 destroys all epitopes recognized by transmission blocking monoclonal antibodies (H.C. Fries et al., 1989, *Parasite Immunol., 11:* 31-45). Therefore, formation of the appropriate disulfide bonds was predicted to be critical in eliciting transmission blocking immunity. Three expression systems, bacterial, vaccinia, and yeast, have been studied in detail and will be reviewed herein.

After overcoming toxicity problems by removing the amino-and carboxy-terminal hydrophobic sequences of Pfs25, abundant amounts of recombinant fusion protein in the form of inclusion bodies were produced in *Escherichia coli* (D.C. Kaslow, unpublished observation). However, the transmission blocking monoclonal

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antibodies to Pfs25 available at the time did not recognize the resulting fusion protein (D.C. Kaslow, unpublished observations), indicating that at least those important conformational, target epitopes were not recreated. To determine if a linear target epitope was present in Pfs25, mice and rabbits were immunized with this recombinant protein. Despite eliciting antibodies that recognized the native protein by immunoprecipitation, live indirect immunofluorescence, and Western blot analysis under nonreducing conditions, these antisera failed to block transmission (D.C. Kaslow, unpublished observations).

In contrast, recombinant Pfs25 (rPfs25) expressed in mammalian cells transfected with recombinant eucaryotic shuttle plasmid, pD1234, or infected with recombinant vaccinia virus, vSIDK, specifically bind a panel of transmission blocking MAbs, indicating that at least those target epitope(s) are recreated in rPfs25. Furthermore, rPfs25 is transported to the surface of mammalian cells (D.C. Kaslow et al., 1991, *loc. cit.*) and appears to be transferred to a GPI anchor (K. Williamson & D.C. Kaslow, unpublished observation). Surface expression of immunogens encoded in recombinant vaccinia viruses has been shown to improve B cell responsiveness (C.L. Langford et al., 1986, *Mol. Cell. Biol., 6:* 3191-3199). Although mice given a single inoculation with live vSIDK failed to develop transmission blocking immunity, after three inoculations with vSIDK, these mice developed complete transmission blocking activity. Furthermore, MHC-disparate congenic mouse strains immunized with vSIDK elicited antibodies that recognized Pfs25 and blocked transmission, demonstrating that Pfs25 is highly immunogenic and that the ability to develop TABbs is not genetically restricted in mice (D.C. Kaslow et al., 1991, *loc. cit.*). Most recently, we have found that *Aotus* monkeys immunized three times with vSIDK also developed complete transmission blocking activity (unpublished observations).

Although live virus vector vaccines are an attractive alternative to conventional subunit vaccines, especially for delivery and administration of immunogens in developing countries, they are not yet universally accepted as a safe means of vaccine delivery. So in an effort to rapidly develop Pfs25 into a transmission blocking vaccine, recombinant Pfs25 was secreted from yeast transformed with an autonomously replicating recombinant plasmid. By deleting the signal and anchor sequences, as well as the potential glycosylation sites, and fusing the synthetic Pfs25 gene to the a-factor secretory signal sequence, gram amounts of recombinant Pfs25 have been purified from yeast culture supernatant (Barr et al., *loc. cit.*). Two chromatographic steps have been employed to purify the resulting 18 kDa polypeptide, Pfs25-B, to greater than 95% purity. The structural integrity of Pfs25-B has been examined in a series of western blot experiments. These data strongly suggest that, unlike the bacterial produced recombinant Pfs25, the yeast product partially recreates the native Pfs25 conformation (Barr et al., *loc. cit.*).

Mice immunized three times with 50 μg of Pfs25-B, emulsified in either Freund's adjuvant or an muramyl tripeptide (MTP)-type adjuvant, developed complete transmission blocking activity. Furthermore, two *Aotus* monkeys have been immunized with Pfs25-B in the MTP adjuvant and two control monkeys with adjuvant alone. ELISA titers of Pfs25 were not detectable in control monkeys or before the second immunization in the test monkeys. Peak titers were observed on week 12 and rapidly drop thereafter. Both test monkeys had complete transmission blocking activity at 12 weeks, and despite the rapid drop in titer, the transmission blocking activity was still present in both monkeys at 22 weeks (Barr et al., *loc. cit.*).

Next we tested the ability of yeast recombinant Pfs25 to induce transmission blocking activity without any adjuvant or adsorbed to alum (unpublished observations). Alum adsorbed Pfs25-B gave ELISA titers exceeding those seen with Pfs25 emulsified in an MTP adjuvant after two immunizations, and approximately equivalent titers following three immunizations. Pfs25-B alone gave titers two to three orders of magnitude lower than the alum absorbed Pfs25-B. Transmission blocking activity was assayed after one and three immunizations. Alum adsorbed Pfs25-B elicited complete transmission blocking activity after three immunizations but not after a single immunization. Pfs25-B alone failed to block transmission even after three immunizations. Other adjuvants such as microencapsulated Pfs25-B, and Pfs25-B combined with DPT are currently under investigation.

In conclusion, Pfs25 expressed in yeast or mammalian cells elicits transmission blocking activity. Boosting of the primary immune re-

sponse is required to elicit transmission blocking activity for both the vaccinia virus, vSIDK, and the yeast produced recombinant protein, Pfs25-B. The recombinant protein secreted from yeast recreates enough of the conformational structure of Pfs25 to induce transmission blocking activity. As alum is a well proven suitable vehicle for use in humans, and because the murine studies with alum look encouraging, full scale preclinical safety and toxicity studies are now being planned.

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