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Comparison of Novel *Plasmodium yoelii* and *P. berghei* Ookinete Antigens with Known Homologues Reveals Two Conserved Regions

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Vaccination of humans to prevent transmission of the malaria parasite to the *Anopheles* mosquito vector is one of the control strategies currently being developed. Targets of transmission-blocking immunity are proteins expressed by the sexual/sporogonic stages of *Plasmodium* species and by the mosquito midgut. In the post-fertilization-(zygote and ookinete) stages of *Plasmodium falciparum*, the prime vaccine candidates are Pfs25 and Pfs28, surface antigens of 25- and 28-kDa, respectively. The hallmark of these antigens is the presence of four EGF-like domains anchored to the surface by glycosyl phosphatidylinositol. The family can be divided into P25 and P21/28 subfamilies based on deduced amino acid sequences of the predicted mature proteins. Members of the P25 subfamily have 22 cysteine residues and a complete fourth EGF-like domain; whereas P21/28 family members have 20 cysteine residues and an incomplete fourth domain. The analogous proteins, Pgs25 and Pgs28, from the avian malaria parasite, *P. gallinaceum*, and Pbs21, from the rodent malaria parasite, *P. berghei*, have been described previously. Unlike Pfs25 and Pgs25, Pfs28, Pgs28 and Pbs21 have four rather than the usual six cysteines in the fourth EGF-like domain. Until recently, just one ookinete surface protein, Pbs21, had been reported from a rodent malaria parasite *P. berghei*. We have newly identified, cloned and sequenced the P21/28 and P25 subfamily members from *P. yoelii* and *P. berghei*, Pys21, Pys25 and Pbs25.

Aligning the gene sequences of four known proteins —Pfs25, Pgs25, Pgs28 and Pbs21— we identified two areas of nucleotide similarity and synthesized the unique PCR oligonucleotides to Pbs21. We amplified a 600 bp fragment from cDNA library of *P. yoelii* zygotes and sequenced. Then, we identified the whole sequence of the cDNA (Pys21) by using the gene specific primers and the vector specific primers. Analysis of the amino acid sequence deduced from the 693 bp open reading frame of Pys revealed a presumptive secretory signal sequence, followed by four EGF-like domains and $(GTGS)_5$ repeats, and then a short hydrophobic region at the carboxy-terminus. The deduced amino acid sequence had 20 cysteines spaced in a manner typical of EGF-like domains, but have only four cysteines in the fourth EGF-like domain. Furthermore, aligning the gene sequences of five known proteins —Pfs25, Pgs25, Pgs28, Pbs21 and Pys21— we synthesized degenerate PCR oligonucleotides. By using these primers and the vector specific primers, we successfully amplified the second gene (Pys25) from cDNA library of *P. yoelii* zygotes. Analysis of the amino acid sequence deduced from the 663 bp open reading frame of Pys25 revealed a presumptive secretory signal sequence, followed by four EGF-like domains, and then a short hydrophobic region at the carboxy-terminus. The deduced amino acid sequence of Pys25 had 22 cysteines spaced in a manner typical of EGF-like domains. Therefore the structure of the presumptive Pys21 and Pys25 protein were similar to those of sexual stage proteins with EGF-like domains of malaria parasite. Recently, we also cloned a second gene present in *P. berghei* encoding Pbs25 which is the homologue of Pfs25, Pgs25 and Pys25 by using a set of Pys25 specific PCR primers.

The overall sequence similarities of Pys25 and Pys21 are compared to each other and to the other known family members. The *P.*

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yoelii P25 homologue, Pys25, is most similar to the *P. berghei* P25 homologue, Pbs25. Likewise, Pys21 is most similar to Pbs21. Interestingly, the least similarity for each homologue in a species tends to be with the other subfamily member from that same species. Of the six conserved regions in Pfs25 and Pgs25 identified previously, four (Regions I-IV) are conserved in the P25 subfamily and two (Regions I and II) are conserved in both the P25 and P21/28 family. Region I, which has the most striking similarity between all members of the P25 and P21/28 family, is in the major loop (between Cys-30 and Cys-46) of the first EGF-like domain. The major loop of EGF mediates the biological activity of EGF and the major loop of the third EGF-like domain of Pfs25 is the target of several transmission-blocking monoclonal antibodies. Whether Region I contributes an important structural function to these malaria transmission-blocking vaccine candidates and is an important region to focus the immune response to generate transmission-blocking immunity against all species of malaria parasites remains to be determined.