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Ph.D. Synopsis:

Molecular Interactions Involved in Erythrocyte Invasion by Malaria Parasite

Specific molecular interactions mediate *Plasmodium* merozoite invasion of erythrocytes (1). *Plasmodium vivax* and *P. knowlesi* merozoites are completely dependent on the Duffy Blood Group Antigen for invasion of human red blood cells (RBCs)(2). Duffy-negative individuals are resistant to invasion by these parasite species. However, *P. knowlesi* can also invade rhesus monkey erythrocytes by using alternative pathways (3). *P. falciparum* commonly uses sialic acid residues on glycophorin A in order to invade human erythrocytes although invasion using other receptors is also reported.

Parasite ligands that bind RBC receptors to mediate invasion belong to a family of erythrocyte-binding proteins (EBP), which includes the *P. vivax* and *P. knowlesi* Duffy binding proteins, the *P. knowlesi* β and γ proteins which mediate Duffy-independent invasion pathways, and *P. falciparum* sialic acid-binding protein (also known as EBA-175), which mediates invasion by sialic acid residues on glycophorin A. They all contain two cysteine-rich domains, regions II and VI, which contain conserved cysteines and hydrophobic amino acid residues. The functional binding domain of each EBP has been mapped to the N-terminal cysteine-rich region, region II, and is referred to as the Duffy-Binding Like (DBL) domain after the first functional domain to be identified from *P. vivax*.

In addition to their role in erythrocyte invasion, DBL domains are involved in cytoadherance, which refers to the binding of *P. falciparum* infected RBCs to receptors on host endothelium. Members of the *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) family have been shown to mediate binding to endothelial receptors. Sequence analysis of *var* genes, which encode PfEMP-1, has revealed that they contain multiple DBL domains. DBL domains of PfEMP-1 have been shown to mediate rosetting to uninfected RBCs and also binding to endothelial receptors such as intercellular adhesion molecule-1 (ICAM-1), and chondroitin sulphate A (CSA-1).

DBL domains are involved in two important pathogenic mechanisms of malaria, namely, RBC invasion and cytoadherence. It is therefore important to understand the structural basis of the interaction of DBL domains with host receptors. Here, molecular approaches will be used to probe the structure-function relationship of DBL domains and their interactions with host receptors.

We will map the residues of *P. vivax* region II responsible for binding to the Duffy antigen. The full length *P. vivax* region II will be expressed as a secreted protein in an eukaryotic expression system and tested for binding to RBCs. Homologue scanning will be used to identify residues within *P. vivax* region II for site-directed mutagenesis. Mutant *P. vivax* region II will be tested for binding to RBCs. Changes in binding affinity will identify binding residues.

P. vivax region II is a promising vaccine candidate. Antibodies to this domain can be expected to block binding and invasion. However the extent of diversity from Indian isolates of *P. vivax* is not known. We will study the extent of polymorphism in field isolates and express some of these variant domains in an eukaryotic expression system. We will determine if antibodies raised against P. vivax region II from the SalI strain, which is being used for vaccine development will cross-react with variant domains from field isolates and block the binding of these variant domains to RBCs.

Interaction with the Duffy antigen is a critical step for invasion of erythrocytes by *P. vivax*. Molecules that block this interaction may block erythrocyte invasion. This strategy may be helpful to develop new drugs against *P. vivax* malaria. A rapid binding assay will be developed to allow screening of inhibitors that block the interaction of *P. vivax* region II with the Duffy antigen.

REFERENCES:

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