2010 Research Grant Program Winning Abstract

Flow Cytometric Measurement of P falciparum Erythrocyte Membrane Protein-1 (PfEMP-1)

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Worldwide, malaria is a leading cause of death for children under age 5 years. Plasmodium falciparum malaria is the most lethal form of malaria. P. falciparum's lethality derives from the fact that human red cells, once infected, display on their surface a parasite-encoded protein named P. falciparum erythrocyte membrane protein-1 (PfEMP-1). PfEMP-1 binds to several host ligands including CD36 (platelet glycoprotein IV); ICAM, chondroitin sulfate, and ABO antigens. Binding of PfEMP-1 to host ligands results in red cell adhesion to endothelium and cell clumping (rosette formation), which in turn block microvascular blood flow resulting in ischemia and organ failure.

Expression of PfEMP-1 is under control of parasite genes and varies among different sub-strains of P falciparum malaria. Strains expressing greater degrees of PfEMP-1 are more adhesive in vitro and associated with greater lethality in vivo.

Despite the fundamental importance of PfEMP-1 in malaria, there is no standard assay to measure its expression on red cells. The lack of a commercially-available antibody to PfEMP-1 has been an obstacle to its study. A validated assay for PfEMP-1 would be of enormous benefit to a wide range of researchers studying malaria pathogenesis, vaccine development, malarial immunity and new drug discovery.

Specific Aim: We propose to develop a standardized flow cytometric assay for expression of PfEMP-1 on the red cell surface. The assay takes advantage of two novel fusion proteins available commercially. One fusion protein, termed rhCD36-Fc, consists of recombinant human CD36 bound to a portion of the Fc domain of human IgG1. The second fusion protein (rhICAM-Fc) consists of recombinant human ICAM bound to a portion of the Fc domain of human IgG1.

Assay: Red blood cells obtained from peripheral blood venipuncture and collected in EDTA will be used. To a dilute suspension of washed red cells, rhCD36-Fc or rhICAM-Fc is added in separate tubes. After incubation and washing, the mixture is stained with a fluorescent-conjugated anti-IgG1-Fc and with Ethidium bromide – used to identify DNA-positive (infected) red cells. The final suspension is washed, resuspended and acquired by the cytometer. Red cells are easily discriminated by FSC-SSC light scatter properties. Analysis focuses on a comparison of staining between infected and uninfected red cells.

Preliminary data: In January 2010, the grant applicant did initial pilot work on this project at the flow cytometry laboratory of the Joint Clinical Research Center (JCRC) in Kampala, Uganda. Fifteen children with acute malaria were studied and the initial parameters of the assay developed. We demonstrated the feasibility of the above staining approach. The cytometric measurement of the proportion of RBCs infected with

malaria (percent parasitemia) matched that seen by an independent confirmatory laboratory using standard microscopy. A signal for PfEMP-1 expression was observed on infected cells and was not present on uninfected cells. We now seek funding to refine this assay. In particular, we wish to optimize the concentrations of reagents used and define a reproducible gating strategy for a generalizable assay.

Significance and practical value: A PfEMP-1 assay using standardized reagents would be of great interest worldwide to investigators working in malaria research. Agents designed to reduce PfEMP-1 expression on red cells, to block its role in cytoadhesion, or to elicit an immune response directed at PfEMP-1 would all be expected to hold great promise as novel approaches to combat malaria. Because the assay employs a fusion protein which is itself a natural pathologic ligand for PfEMP-1, binding characteristics are directly relevant to the disease. Because the assay successfully distinguishes infected red cells from uninfected cells, each patient serves as his own internal control for measurement of binding by the fusion proteins. Finally, because the staining technique distinguishes infected from uninfected red cells in the same specimen, the assay provides a highly quantitative measurement of “percent parasitemia” – a measure used clinically for disease risk assessment but currently done by manual microscopy.

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