

## SEQUESTRIN

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## INTRODUCTION

Alone among the malaria species which infect humanity, *Plasmodium falciparum* causes the erythrocytes which it invades to sequester in the deep vascular beds of various tissues. This sequestration phenomenon is observed in peripheral blood smears by the presence of immature (non-adherent) ring-stage parasitized erythrocytes and the absence of mature (adherent) trophozoite and schizont stage parasites, the latter having localized to postcapillary venules (Bignami and Bastianelli (1889) *Reforma Medica* 6: 1334–1335) (All documents cited herein supra and infra are hereby incorporated by reference). Sequestration allows the parasite to develop in a microenvironment of low oxygen tension and to evade splenic immune surveillance (Langreth and Peterson (1985) *Infect. Immun.* 47: 760–766).

*Faliparcum malaria* can have protean manifestations, ranging from asymptomatic infection, to mild disease (symptoms may include fever, arthralgias, abdominal pain, diarrhea, headache, nausea, fatigue and others in various combinations), to severe disease (recognized severe forms include cerebral malaria with coma, pulmonary edema with consequent respiratory failure, severe anemia with consequent hemodynamic/cardiopulmonary decompensation) often resulting in death. These symptoms of severe malaria resulting in vast mortality worldwide are believed to be imparted by sequestration (Warrell et al. (1990) *Trans. Soc. Trop. Med. Hyg.* 84:1–65). Owing to the devastating consequences of the disease, and the potential for therapeutic intervention, researchers have long sought to isolate the parasite protein(s) responsible for the cytoadherence of *P. falciparum* infected erythrocytes (IRBC) to postcapillary venular endothelium.

Cytoadherence appears to be a complex event, with multiple binding phenotypes displayed by both culture-adapted and wild-type IRBC isolates. In vitro models demonstrate that different cell lines, such as C32 amelanotic melanoma cells (Schmidt et al. (1982) *J. Clin. Invest.* 70: 379–386), human umbilical vein (Udeinya et al. (1981) *Nature* 303: 429–431) and human microvascular endothelial cells (Johnson et al. (1993) *J. Infect. Dis.* 167: 698–703) support adhesion of IRBC. More recently, with the availability of purified molecules, an array of endothelial ligands such as CD36 (Ockenhouse et al. (1989) *Science* 243: 1469–1471) ICAM-1 (Berendt et al. (1989) *Nature* 341: 57–59) VCAM-1 (Ockenhouse et al. (1992) *J. Exp. Med.* 176: 1183–1189), E-selectin (Ockenhouse et al., 1992), and the extracellular matrix molecules thrombospondin (Roberts et al., year, *Nature* 318: 64–66) and chondroitin sulfate A (Rogerson et al. (1995) *J. Exp. Med.* 182:15–70) have demonstrated the capacity to bind IRBC. While culture-adapted IRBC can be selected to bind each of these ligands, only CD36 is able to bind nearly all wild-type parasite strains isolated in the field (Ockenhouse et al (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88:3175–b 3179); Hansen et al (1990) *Blood* 76: 1845–1852). Furthermore, *P. falciparum* parasites which no longer cytoadhere in vitro to cells expressing CD36, are unable to establish a virulent infection in primates, suggesting the primary role of sequestration for parasite survival (Langreth and Peterson, 1985, supra).

Electron microscopy studies have demonstrated that IRBC adherence to endothelium occurs along electron-

dense protrusions, called “knobs”, on the erythrocyte surface (MacPherson et al. (1985) *Am. J. Pathol.* 119: 385–401). The IRBC surface ligands for both CD36 and thrombospondin receptors have been localized to these knobs (Nakamura et al. (1992) *J. Histochem. Cytochem.* 40:1419–1422). Although culture-adapted laboratory parasites may bind CD36 in the absence of surface knobs, (Udomsangpetch et al. (1989) *Nature* 338: 763–765) the prevailing view is that the CD36-binding protein(s) localizes at the surface of the knob of all wild-type parasites.

CD36, an 88 kD glycoprotein expressed on the surface of microvascular endothelium, platelets, and monocytes belongs to a family of related proteins containing extensive amino acid homology (Greenwalt et al. (1992) *Blood* 80:1105–1115; Calvo et al. (1995) *Genomics* 25: 100–106). Also known as platelet glycoprotein IV or IIIb, CD36 is expressed in a regulated fashion during cell development (Abumrad et al. (1993) *J. Biol. Chem.* 268: 17665–17668) and its expression is modulated by cytokines (Huh et al. (1995) *J. Biol. Chem.* 270: 6267–6271; Johnson et al. (1993) *J. Infect. Dis.* 167: 698–703). CD36 has binding sites for several molecules involved in hemostasis and atherogenesis, including collagen (Tandon et al. (1989) *J. Biol. Chem.* 264: 7570–7575), thrombospondin (Asch et al. (1992) *Biochem. Biophys. Res. Common.* 182: 1208–1217), oxidized low density lipoprotein (LDL) (Endemann et al. (1993) *J. Biol. Chem.* 268: 11811–11816), long chain fatty acids (Abumrad et al. (1993) supra), and anionic phospholipids (Rigotti et al. (1995) *J. Biol. Chem.* 270: 16221–16224).

Cytoadherence of IRBC in vitro can be inhibited or reversed by antibodies directed against either the surface of parasitized erythrocytes or against endothelial cell ligands. IRBC binding to CD36 is blocked by monoclonal antibodies OKM5 (Barnwell et al. (1985) *J. Immunol.* 135:3494–3497) and OKM8 (Ockenhouse et al., 1991) directed against discontinuous epitopes but not by other CD36 monoclonal antibodies which recognize linear continuous epitopes (Ockenhouse et al., unpublished observations) establishing the importance of conformationally-correct protein structure for IRBC binding to CD36. In monkeys, sequestration can be reversed by passive transfer of hyperimmune sera, leading to the clearance of IRBC in the spleen (David et al. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80: 5075–5079).

A number of parasite-derived and altered host proteins have been postulated to mediate IRBC cytoadherence. PfEMP1, a large molecular weight, size variable protein which is the product of the var gene family involved in malaria antigenic variation (Baruch et al., 1995; Su et al. (1995) *Cell* 82: 89–100; Smith et al., 1995), has characteristics which suggest its involvement in cytoadherence: PfEMP1 is expressed on the external erythrocyte surface as demonstrated by radioiodination and immunofluorescence; PfEMP1 is readily cleaved from the IRBC surface by proteolytic enzymes such as trypsin at concentrations known to abolish IRBC adhesion, and PfEMP1 varies its size in a manner which correlates with changes in both strain specificity and IRBC adhesion (Leech et al. (1984) *J. Exp. Med.* 159: 1567–1575; Baruch et al., 1995). Another candidate, band 3, is a surface protein of normal human erythrocytes which has been shown to be altered by malaria infection, exposing cryptic peptides in loops 3 and 7 (Crandall and Sherman (1994) *Parasitol.* 108:389–396). Monoclonal antibodies against altered band 3 inhibit cytoadherence in vitro (Crandall and Sherman, 1994) and synthetic peptides based on the cryptic epitopes of band 3 affect sequestration in vivo (Crandall et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:4703–4707).