Our infant-toddler studies (Projects I and II of this renewal) have demonstrated a preliminary relationship between erythrocyte phosphatidylserine (PS) and vaso-occlusive crisis (VOC). We have also demonstrated that PS on the RBC is a critical determinant of RBC-endothelial adhesion (Blood – In Press). These observations have led us to pursue the exact nature of the adhesive interaction between PS-positive RBCs and the microendothelium. Additional preliminary experiments have demonstrated that ionophore-treated PS-positive HbAA erythrocytes adhere to human retinal microendothelium (HRCECs) and that this adhesion is enhanced by agonists such as hypoxia and cytokines which also upregulate the endothelial PS receptor (PSR), a recently demonstrated antigen that recognizes cell surface PS. In addition, blocking studies suggest that while CD36 is involved in the adhesion of PS-positive RBCs to endothelium under basal conditions, PSR appears to be the major receptor involved in agonist-stimulated adhesion. In other work, anionic heparin and other GAGs inhibited the binding of PS-positive RBCs to immobilized thrombospondin (TSP), suggesting that this anionic phospholipid competes with GAGs for the heparin-binding site of TSP. In **Aim I**, we will evaluate endothelial PSR regulation and its interaction with PS-positive RBCs, initially using ionophore-scrambled HbAA controls red cells, such that red cell PS alone will presumably provide the adhesive interactions with the major EC counter-receptors. Confirmation of the role of PSR in adhesion will be demonstrated in a heterologous cell system transfected with an expression vector containing the cDNA for PSR, and sickle cell adhesion tested. Adhesion experiments using a recently described CD36 peptide will be used to evaluate the involvement of endothelial CD36 in endothelial-PS erythrocyte adhesion. Other studies in Aim I will evaluate the mechanism of PSR expression induced by agonists relevant to SCD pathophysiology such as hypoxia, cytokines and free radicals, (steady-state mRNA levels and gel shift assays will evaluate the level at which the agonist effect is mediated). Final studies in Aim I will test whether the cytoprotective mediators Nitric Oxide and prostacyclin inhibit agonist-induced PSR upregulation. **Aim II** will be devoted to a dissection of the specific domain(s) on the TSP molecule with which PS-positive RBCs interact. In view of our preliminary results, such work will be initiated with peptides corresponding to the heparin-binding domain of TSP. Our human studies will evaluate both endothelial PSR and endothelial PS. **Aim III** will assess for PSR (antigen, mRNA and functional studies) on circulating endothelial cells (CECs) both in steady-state and VOC. We hypothesize that during VOC quantitative and qualitative increases in PSR will occur (as assessed by histochemistry, and more quantitative microparticle capture assays) while levels of red cell PS will decrease. **Aim IV** will take a second look at a recently proposed hypothesis that the endothelium in SCD is in a state of anti-apoptotic tone by performing micro-capture quantitative assays for endothelial PS vesicles and an evaluation of CECs. An increase in PS endothelial positivity dissociated from apoptosis would suggest that the PS exposure is due to other non-apoptotic mechanisms of EC activation. A delineation of the EC receptors involved in erythrocyte PS binding will be a first step in the potential for anti-adhesion therapy based on the red cell PS-endothelial cell connection. In addition, the importance of these interactions is underscored by the clinical observations linking erythrocyte PS with pain, the protean manifestation of SCD.