The Tipping Point: How Little Adhesive Proteins Make a Big Difference

By Margaret Ragni, MD, MPH

Today's Scientific Committee on Thrombosis and Vascular Biology, chaired by Eric Grabowski, ScD, MD, Massachusetts General Hospital, will include three talks that focus on the mechanism of thrombosis in microangiopathy, highlighting recent studies in trophoblastic and decidual cells in pregnancy loss and glomerular endothelial cells in hemolytic uremic syndrome, a disorder in children.

Hartmut Weiler, PhD, Blood Research Institute, BloodCenter of Wisconsin, Milwaukee WI, will discuss the regulatory role of the trophoblast and hemostasis. A number of studies have shown the essential role of placental trophoblastic cells in the regulation of hemostasis during pregnancy. Not only do these cells express molecules with anticoagulant function, they also express procoagulant receptors (e.g. PAR1, 2, and 4). It is well established that in mice lacking intact thrombomodulin, organogenesis fails and pregnancy loss ensues. Thrombomodulin is a glycoprotein synthesized by vascular endothelial cells, which plays a key role in natural anticoagulation, specifically through activation of thrombin-induced protein C (the most potent natural anticoagulant). The mechanism of the organ dysfunction and pregnancy loss in this animal model appears to be mediated by three independent mechanisms: platelet activation, apoptosis, or disrupted PAR signaling: 1) trophoblasts in thrombomodulin knockout animals activate maternal platelets through the PAR4 thrombin receptor, resulting in fetal organ dysfunction; 2) trophoblasts may undergo apoptosis through mitochondrial activation, internalization of fibrin fragment E, and cell death by fibrinolysis and pregnancy loss; or 3) lack of thrombomodulin may interfere with thrombomodulin-dependent PAR signaling, leading to dysregulated trophoblastic signaling and, ultimately, pregnancy loss. These findings underscore the importance of thrombomodulin and protein C in placental health and successful pregnancy, and suggest novel targets for drug development to reduce pregnancy loss in women with thrombophilia.

In the second talk of this session, Michael Paidas, MD, Yale University School of Medicine, will consider the unique role of the decidual cell in thrombosis. Stromal endothelial cells in the endometrium, exposed to the increasing estrogen and progesterone of pregnancy, transform into decidual cells that have a central role in hemostasis during pregnancy. Decidual cells produce tissue factor, subsequent thrombin generation, and regulation of plasminogen activator inhibitor type 1, thereby regulating hemostasis in pregnancy. Specifically, decidual cells prevent bleeding as the growing trophoblast invades endometrial capillaries during implantation and with growth of the placenta. In pregnancy, if this mechanism is not regulated and excess thrombin is produced, pathologic outcomes of pregnancy may ensue (for example, premature rupture of membranes). Excess thrombin induces decidual cell production of tyrosine kinase receptor sFit-1, matrix metalloproteinases MMP-1 and MMP-3, and interleukin-8. sFit-1 may impede trophoblastic invasion, leading to preeclampsia; MMP-1 and MMP-3 may restrict fetal growth; and IL-8 may lead to neutrophil infiltration and elaboration of proteases that degrade the extracellular matrix and lead to premature membrane rupture. To inhibit these pathways and reverse adverse pregnancy outcomes, *in vitro* studies of heparin to prevent apoptosis of primary trophoblasts and of toll-like receptor ligands to reduce cytokine production by decidual cells are in progress.

In the third talk, Dr. Eric F. Grabowski will discuss models to study upregulation of adhesion molecules which mediate endothelial activation in hemolytic uremic syndrome. Studying glomerular endothelial cells in flowing blood in a chamber, conditions are varied to determine real-time videomicroscopy of platelet activation with adhesive molecules $\alpha_v\beta_3$, ICAM-1, and P-selectin, as well as Shiga toxin. In the latter setting, studies showed that the platelet aggregates and adherent platelet strings which occur may be eliminated by a monoclonal antibody against human tissue factor or by the experimental antithrombotic agent, site inactivated, irFVIIa. These findings may have potential application for intervention in hemolytic uremic syndrome.