

## Identification of the megakaryocyte receptor that mediates endocytosis of Factor V from plasma

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10 Platelet- and plasma-derived factor Va serve essential roles in thrombin generation catalyzed by prothrombinase, a 1:1, calcium-dependent complex of the cofactor factor Va and the protease factor Xa, assembled on the activated platelet surface. Factor Va plays a central role in this enzymatic complex and profoundly influences the amount of thrombin generated. Deficiencies in the procofactor factor V can lead to severe hemorrhage and possibly death.

Several clinical observations indicate that the platelet-derived cofactor is more important in maintaining normal hemostasis than its plasma counterpart. This notion is consistent with observations by our laboratory that platelet-derived factor Va, as compared to the plasma molecule, possesses physical and functional differences that give it an increased procoagulant potential and allow it to sustain coagulant events on the platelet surface. Recent studies have demonstrated unequivocally

that the entire platelet-derived factor V pool originates from plasma via clathrin-dependent, receptor-mediated endocytosis of plasma factor V by megakaryocytes.

We hypothesize that subsequent to its endocytosis, factor V is modified physically, processed proteolytically, and packaged into alpha-granules to yield the pool of the unique platelet-derived cofactor. Equilibrium binding analyses indicate that <sup>125</sup>I-labeled factor V binding to megakaryocytes is specific, saturable and reversible, and is defined by a dissociation constant of low affinity. Factor V consists of a heavy chain and a light chain, linked by the B domain, which is removed upon activation to factor Va. The binding of factor V to megakaryocytes appears to be mediated by the light chain.

The overall goal of this proposal is to identify and characterize the megakaryocyte membrane receptor that regulates the binding of factor V to the cell surface and its endocytosis from plasma. Three approaches will be used. Factor V ligand blotting and affinity chromatography will be used to detect putative factor V binding proteins. Isolated factor V light chain will be used to probe for factor V binding proteins in these experiments. Factor V binding proteins isolated by these techniques will be identified by N-terminal sequencing and MALDI-TOF mass spectrophotometry. The role of LDL receptor-related protein (LRP) or an LRP-like protein in mediating factor V endocytosis will also be assessed. Preliminary experiments using the LRP agonist, Receptor Associated

Protein, suggest that an LRP family member mediates factor V binding and endocytosis.

If the approaches described above prove to not be useful for receptor identification, we will screen a megakaryocyte cDNA library for a cell surface expressed factor V receptor using expression cloning techniques. As the cellular mechanisms imparting and regulating the phenotypic differences between the plasma- and platelet-derived cofactor molecules are unknown, these studies will increase our understanding of how megakaryocytes acquire, process, and package this critical coagulation factor.

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