

Plasma factor V (FV) is endocytosed by megakaryocytes, the platelet-precursor cells, and modified to form the structurally and functionally distinct platelet FV molecule essential for physiologically-relevant blood coagulation. Previous studies indicate that FV endocytosis is mediated by two receptors composed of an unknown, specific FV receptor and low density lipoprotein receptor-related protein-1 (LRP-1), a ubiquitous cell surface receptor involved in endocytic and cell signaling processes regulated by phosphorylation/dephosphorylation events. Recent observations are consistent with a role for the ganglioside GM3 in FV endocytosis. GM3 bind its ligands via sialic acid moieties and can function as a co-receptor by modulating receptor phosphorylation. Confocal microscopic analyses demonstrated that GM3 colocalizes with FV and LRP-1 in megakaryocytes. Thus, we hypothesize that FV binding to GM3 effects phosphorylation/dephosphorylation of LRP-1 resulting in endocytosis of FV. To test this hypothesis, a protocol was developed to immunoprecipitate LRP-1 from a megakaryocyte-like cell line. Western blotting using anti-phospho-amino acid antibodies indicated that LRP-1 is phosphorylated on tyrosine and threonine residues. Consistent with the presence of GM3 on the megakaryocyte cell surface, binding of the specific lectin, MAA, was inhibited following the treatment of cells with neuraminidase to remove sialic acid. In addition, treatment of FV with neuraminidase increased its electrophoretic mobility, and reduced its staining with Schiff periodate reagent consistent with removal of sialic acid. Preincubation of cells with MAA inhibited both the binding and endocytosis of FV. These combined observations suggest that sialic acid residues present on FV and/or the megakaryocyte cell are involved in FV binding and/or endocytosis. Future experiments will test the effect of neuraminidase treatment of FV and cells on FV binding/endocytosis. The effect of FV binding/endocytosis on basal LRP-1 phosphorylation will also be tested. Finally, a specific role for GM3 will be described.