CHANGES IN LRP-1 PHOSPHORYLATION MEDIATED BY FACTOR V BINDING TO ITS SPECIFIC RECEPTOR ON MEGAKARYOCYTES

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Plasma-derived factor V is endocytosed by megakaryocytes, platelet-precursor cells, and modified to form the structurally and functionally distinct platelet-derived factor V molecule essential for physiologically relevant blood coagulation. Previous studies indicate that factor V endocytosis is clathrin-dependent and mediated by a two receptor system composed of an unknown, specific factor V receptor and low density lipoprotein receptor-related protein-1 (LRP-1), a ubiquitous cell surface receptor involved in various endocytic and cell signaling processes. These functions of LRP-1 and related receptors appear to be regulated by phosphorylation-dependent interactions with numerous cytoplasmic adaptor proteins. As phosphorylation at tyrosine, serine and threonine residues appears to attenuate LRP-1's association with adaptor proteins involved in endocytosis, we hypothesize that factor V's interaction with the specific factor V receptor decreases phosphorylation of LRP-1 and induces factor V endocytosis. Whole cell lysates from a megakaryocyte-like cell line, CMK, were immunoprecipitated with an anti-LRP-1 antibody using protein G beads. Western blotting with a second anti-LRP-1 antibody confirmed immunoprecipitation of LRP-1 from CMK cells. In parallel experiments, specificity of the antibodies was established using cell lines positive or negative for LRP-1 expression. Subsequent western blotting with an anti-phosphotyrosine antibody demonstrated a basal level of phosphorylation on LRP-1 immunoprecipitated from CMK cells. In future experiments, western blots will also be probed with anti-phosphoserine and anti-phosphothreonine antibodies. The effect(s) of factor V binding and/or endocytosis on LRP-1 phosphorylation will be assessed in parallel reactions. Relative levels of LRP-1 phosphorylation under these different conditions will be compared using densitometry. We anticipate that binding/endocytosis of factor V will decrease phosphorylation of LRP-1; however, an increase in phosphorylation would be consistent with the notion that LRP-1-mediated endocytosis of factor V is controlled by phosphorylation/dephosphorylation events at the megakaryocyte cell surface.