Studies from our laboratory have shown that platelet-derived factor V originates via endocytosis of plasma FV by platelet precursors, megakaryocytes. The endocytosis is mediated by two receptors: an unidentified, specific FV receptor, and LDL receptor related protein-1 (LRP-1). According to the model for FV endocytosis, FV initially binds to the FV receptor and this binding interaction facilitates the binding of a second FV molecule to LRP-1, which then mediates endocytosis. FV is a blood coagulation protein, crucial for thrombin generation at sites of vascular injury, that consists of a heavy and a light chain component with a domain structure of A1-A2-B-A3-C1-C2. The protein exists in two whole blood pools, with ~80% found in plasma and ~20% stored in the alpha granules of platelets following its endocytosis and modification by megakaryocytes. The goal of this study is to determine what region of factor V (FV) mediates its endocytosis from plasma to form the physically and functionally unique, physiologically-relevant platelet-derived molecule. Using anti-FV monoclonal light chain antibodies and isolated FV components, the role of the light chain was determined using binding assays, displacement studies and protein competition assays performed in the absence of calcium to distinguish binding from endocytosis. Two antibodies, anti-FV #2 and E9, which do not inhibit FV coagulant activity, were utilized primarily in this study. Initial observations suggest that the light chain of FV is involved in binding and/or endocytosis. Both antibodies were observed to alter the time-dependent binding of FV to the surface of a megakaryocyte-like cell line (CMK). FV binding assays performed in the presence of anti-factor V #2 indicated that this antibody enhanced binding of FV to CMK cells, contrary to E9, which inhibited FV binding. Inhibition with E9 was also concentration-dependent and nearly complete inhibition was observed even at low concentrations of antibody. Moreover, E9 displaced binding of FV from the cell surface. Western blotting analyses indicated that while anti-FV #2 has a continuous

epitope, E9's epitope is discontinuous. Thus far, initial attempts at localizing E9's epitope have proven unsuccessful. These data suggest that the binding and/or endocytosis of FV by megakaryocytes is mediated by the FV light chain. Future experiments will focus on attempts to epitope map E9, as well as binding experiments performed in the presence of the LRP-1 ligand, RAP to elucidate which FV/receptor interaction is being affected.