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THROMBOSPONDIN: CELL BIOLOGY OF AN ADHESIVE GLYCOPROTEIN. R.L. Nachman, R.L. Silverstein, and A.S. Asch. Cornell University Medical College, New York, USA.

Thrombospondin (TSP), a multifunctional 450 KD glycoprotein is a secretory product of thrombin stimulated platelets. It is a major component of the platelets alpha granule constituting approximately 3% of total platelet protein. Thrombospondin does not circulate in appreciable concentrations ($\sim$ 0 100 ng/ml); however, the tissue distribution is broad. In addition to its expression on the membrane of activated platelets, the protein is synthesized by fibroblasts endothelial cells, glial cell smooth muscle cells alveolar pneumocytes mononuclear phagocytes and various tumor cells. TSP is a major constituent of the extracellular matrix and has been demonstrated in the vessel wall, basement membrane and glandular connective tissue. Fibroblasts, smooth muscle cells and endothelial cells in tissue culture incorporate TSP into the extracellular matrix. Matrix TSP is under cell-cycle regulatory control. Mesenchymal cells in the proliferative phase synthesize greater amounts of TSP than non growing cells. Platelet derived growth factor induces smooth muscle cell and glial cell synthesis of TSP. Atheromatous lesions contain increased amounts of TSP compared to normal vessels emphasizing the potential role of TSP in the interaction of proliferating cells with the matrix. TSP binds specifically, saturably, and reversibly to mouse peritoneal macrophages and to cells of the monocyte-like human cell line U937. Binding was time dependent and was optimal in the presence of both Ca^{++} and Mg^{++}. PMA stimulated U937 cells and activated macrophages bound TSP to an equivalent extent as resting cells. The TSP binding site on the surface of U937 cells and peripheral blood monocytes mediates the adhesive interaction between these cells and thrombin-stimulated platelets. Using a sensitive rosetting assay we found that monocytes were not rosetted by resting platelets while >90% were rosetted by thrombin-stimulated platelets. Monoclonal and polyclonal anti-TSP antibodies markedly inhibited rosetting as did TSP itself. Antifibronectin or non-immune control antibodies did not inhibit rosetting, nor did fibronectin, fibrinogen, the fibronectin adhesion tetrapeptide arg-gly-asp-ser (RGDS), or heparin. The TSP membrane receptor, an 88 KD glycoprotein, formely known as GPIV has been identified in platelets, endothelial cells, monocytes and a variety of tumor cells. TSP may thus serve as a molecular bridge linking activated platelets with monocytes at sites of early vascular injury. Such interactions involving the TSP receptor complex may be of critical importance in the regulation of thrombosis and the initiation of atherosclerosis.

REVERSIBLE DEFICIENCY OF INTACT THROMBOSPONDIN AND MEMBRANE GLYCOPROTEIN Ia IN PLATELETS OF A PATIENT WITH A BLEEDING DISORDER Friday

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REVERSIBLE DEFICIENCY OF INTACT THROMBOSPONDIN AND MEMBRANE GLYCOPROTEIN Ia IN PLATELETS OF A PATIENT WITH A BLEEDING DISORDER. B. Kehrel (1), L. Balleisen (2), R. Kokott (1), W. Stenzinger (2), K.J. Clemetson (3) and J. van de Loo (2). Inst. for Arteriosclerosis Research, Univ. of Muenster, FRG (1), Dept. of Internal Medicine, Univ. of Muenster, FRG (2) and Theodor Kocher Institute, Berne, Switzerland (3).

A 52 year old female patient with a severe bleeding tendency since the age of two was studied. Her history revealed recurrent petechial bleedings, two severe postoperative haemorrhagic episodes and intensive menstrual bleedings which required blood transfusions. Coagulation studies ruled out any coagulation disorder including von Willebrand's disease. Platelet count and morphology (using light and electron microscopy) were normal. The patient had prolonged bleeding times (up to 15 min). Her platelets aggregated normally in response to ADP, arachidonic acid, thrombin, ionophore A 23187, epinephrine and ristocetin. In contrast, platelet aggregation in the presence of collagen and wheat germ agglutinin could only be achieved with very high doses of these agonists. Repeated analyses of the patient's platelet proteins by two-dimensional O'Farrel gel electrophoresis followed by silver staining or blotting onto nitrocellulose and staining with a mixture of peroxidase-coupled lectins showed that glycoprotein Ia and intact thrombospondin were absent. An immunoblot for thrombospondin showed several proteins with lower molecular weight than thrombospondin. Preincubation of the patient's platelets with thrombospondin normalized collagen-induced aggregation.

At a recent follow-up examination the patient had no petechial bleedings. Platelet protein analysis revealed that intact thrombospondin and glycoprotein Ia were present. These results suggest that both glycoprotein Ia and thrombospondin have essential roles in collagen-induced platelet aggregation.