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ROLE OF PLATELET MEMBRANE GLYCOPROTEIN IV IN PLATELET-COLLAGEN INTERACTION: A MICROTITER ASSAY TO STUDY PLATELET ADHERENCE. N. N.Tandon and G.A. Jamieson. American Red Cross Laboratories, Rockville, MD 20855, U.S.A.

The role of platelet glycoprotein IV (GPIV) in platelet function has not been elucidated. We have now isolated GPIV (Mr 88,000) from platelet membranes in homogeneous form by a series of steps involving (i) phase partitioning in Triton X-114, (ii) ion exchange chromatography on DEAE-cellulose, (iii) lectin affinity chromatography on WGA-Sepharose, and (iv) size exclusion chromatography on Ultrogel AcA44. Purified GPIV inhibited platelet shape change and aggregation induced by collagen (2 ug/ml; 7 nM as tropocollagen) in a dose-dependent fashion (ID_{50} ~1 ug/ml; 10 nM) but did not affect aggregation induced by thrombin, ADP, epinephrine, arachidonate or ionophore A23187. To study the role of GPIV in platelet interaction with collagen we have developed a microtiter assay involving (i) coating acid soluble or fibrillar Type I collagen onto microtiter plates, (ii) incubation of coated collagen with ^{51}Cr-labeled platelets and (iii) quantitation of platelet adherence by analysing the radioactivity of the SDS lysate of the adhered platelets. In this assay system, Fab fragments of anti-GPIV antibody inhibited platelet adherence by 75% to both acid soluble and fibrillar Type I collagen while nonimmune serum was without effect. Fab fragments also inhibited collagen-induced aggregation and secretion (ID_{50} ~10 ug/ml; 200 nM) and, slightly less effectively, aggregation by ADP and epinephrine (ID_{50} ~300 nM), but did not affect platelet activation by thrombin, arachidonate or ionophore. Fab fragments also inhibited platelet attachment to collagen-Sepharose columns by 80%. These results suggest a role for GPIV in the interaction of platelets with collagen, probably at the level of primary platelet adherence.

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PURIFICATION AND PROPERTIES OF HUMAN PLATELET MEMBRANE GLYCOPROTEIN V (GP-V). Rasheeda Zafar and Daniel A. Walz. Wayne State University, Department of Physiology, Detroit, MI, U.S.A.

Platelet membrane glycoproteins function as specific ligand receptors or substrates for selected platelet agonists and antagonists. GP-V is the only such membrane glycoprotein known to be a thrombin substrate. We have purified GP-V to homogeneity in order to better characterize the nature and specificity of this thrombin proteolysis. GP-V was extracted from fresh human platelets and purified through a combination of gel filtration, hydroxylapatite, DEAE and mono S chromatographies. The resulting protein had a molecular mass of 80 kDa by both non-reduced and reduced SDS electrophoresis. Amino-terminal analysis of GP-V failed to yield any detectable residues, suggesting a blocked N-terminus. Incubation of purified GP-V with 1 nM human thrombin for 20 minutes resulted in the generation of a stable (thrombin resistant) fragment of 63 kDa; smaller GP-V peptides have not been identified. The amino-terminus of the 63 kDa GP-V fragment is also blocked, indicating that thrombin proteolysis is occurring at the carboxyl-terminal region of the protein. Purified GP-V is also a substrate for human γ-thrombin, although its rate of proteolysis is much slower than that observed using α-thrombin; the α- and γ-thrombin generated GP-V fragments are electrophoretically similar. Polyclonal antibodies raised against purified GP-V react equally with the GP-V fragment. Such antibodies have been used in immunoblotting procedures to screen bovine platelets, human endothelial cells and a transformed erythroid cell line known to express other platelet proteins; all these cells failed to give a positive reaction, suggesting the absence of GP-V in these cell membranes or its presence in an amount significantly lower than that observed for human platelets.

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PADGEM PROTEIN EXPRESSION IN HUMAN ERYTHROLEUKEMIA (HEL) CELLS. **E. Yeo, B.C. Furie, and B. Furie. New England Medical Center and Tufts University School of Medicine, Boston MA, U.S.A.**

PADGEM (Platelet Activation-Dependent Granule ==> External Membrane) glycoprotein, a platelet alpha granule integral membrane protein with a molecular weight of 140,000, is translocated to the plasma membrane during granule secretion. PADGEM protein is expressed solely on activated platelets, but is not on the surface of resting platelets. Because HEL cells contain platelet alpha granule-like organelles and proteins (e.g. platelet factor 4, von Willebrands factor, β-thromboglobulin) and express certain platelet membrane proteins (e.g. GP IIb/IIIa, GPIb), we evaluated induced and uninduced HEL cells for the synthesis and expression of PADGEM protein. HEL cells were induced with 1.25% DMSO for 3-4 days, then grown in the absence of DMSO for 1-3 weeks. After eight cycles of DMSO exposure, the induced HEL cells were found to increase the expression of PADGEM, in contrast to the uninduced cells. Intact fixed and unfixed induced HEL cells were observed by immunofluorescence, utilizing KC4, a monoclonal anti-PADGEM antibody, to express PADGEM while non-induced HEL cells expressed low levels of PADGEM. Both induced and uninduced HEL cells bound A2A9, an anti-GP IIb/IIIa monoclonal antibody. Quantitative analysis by fluorescence activated cell sorting demonstrated a 2.5-fold increase in mean surface expression of PADGEM and 3.3-fold mean increase in GP IIb/IIIa surface expression compared to uninduced cells. By fluoresence microscopy, 70% of induced HEL cells expressed PADGEM protein versus 20% of the uninduced cells. GP-IIb/IIIa expression increased from 40% in noninduced cells to 90% in induced cells. The induced HEL cells contained PADGEM with a molecular weight identical to that of platelets, as demonstrated by Western blotting using the KC4 antibody. Direct binding experiments with ^{125}I-KC4 antibody demonstrated that surface binding was specific, saturable, and time-dependent. Surface expression of PADGEM protein was not increased with platelet agonists (thrombin, epinephrine, ADP) nor cytokines (IL-1, IL-2, tissue necrosis factor). The surface density of PADGEM protein on induced HEL cells and activated platelets appears similar. HEL cells should provide a useful model to assist in the elucidation of the structure, function and biology of PADGEM protein.

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PLATELETS EXPRESS A MEMBRANE PROTEIN COMPLEX IMMUNOLOGICALLY RELATED TO THE FIBROBLAST FIBRONECTIN RECEPTOR. F.G. Giancotti (1), L.R. Languino (2), A. Zanetti (2), G. Grignani (3), G. Tarone (1) and E. Dejana (2). University of Torino, Torino (1), Ist. Mario Negri, Milano (2), Policlinico S. Matteo, Pavia (3), Italy.

The heterodimer complex GpIIb-IIIa on human platelets can specifically bind fibronectin (FN) only when platelets are activated by thrombin. However unstimulated platelets can adhere and spread on a FN substratum. This suggests the existence of a second binding site for FN on the platelet surface that does not require activation for its expression. We have previously identified and characterized a membrane glycoprotein complex (Gp 150/135) that functions as fibronectin receptor (FN-R) in mouse fibroblast adhesion. To investigate whether this molecule was also present in platelets we have produced an affinity purified polyclonal antibodies monospecific for the lower subunit of the fibroblast FN-R. These antibodies specifically stained human and rat platelet surface as determined by fluorescence flow cytometric analysis and reacted with a component of 138 Kd m w in Western blot of platelet membranes. Experiments of differential extraction revealed that the 138 Kd component is an integral membrane protein. Moreover the antibodies precipitated the 138 Kd component together with a 160 Kd protein suggesting that the two molecules are associated in a supramolecular complex. A comparative analysis indicated that this protein complex is clearly distinct from the GpIIb-IIIa. In addition platelets from a thrombastenic patient reacted normally with 138 Kd but not with GpIIb-IIIa antibodies by Western blot analysis. These data indicate that normal human platelets express both GpIIb-IIIa and FN-R on their membrane and that these receptors are composed of structurally and antigenically distinct proteins.