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A NEW FIBRIN-SPECIFIC ANTIBODY DISCRIMINATING BETWEEN FIBRIN AND FIBRINOGEN IS DIRECTED AGAINST THE SYNTHETIC PEPTIDE LEU-ILE-ASP-GLY-LYS-MET. U. Scheefers-Borchel and G. Müller-Berghaus. Clinical Research Unit for Blood Coagulation and Thrombosis of the Max-Planck-Gesellschaft, D-6300 Giessen, FRG.

To determine soluble fibrin in blood of patients with coagulation disorders we produced monoclonal antibodies which distinct fibrin from fibrinogen and other blood constituents. Fibrin-specific monoclonal antibodies were obtained by immunizing mice with the synthetic hexapeptide Leu-Ile-Asp-Gly-Lys-Met which was covalently linked to KLH via its C-terminus. Several of the monoclonal antibodies which reacted with the hexapeptide also reacted with batroxobin-induced desAA-fibrin and thrombin-induced desAABB-fibrin, but not with fibrinogen. No reaction was observed with plasmin-induced fibrinogenolytic and fibrinolytic degradation products, respectively. The epitope recognized by these fibrin-specific antibodies is located on the α-chain of fibrin and is not accessible for an antibody in native fibrinogen. One monoclonal antibody (B/H_{11}) was used to quantify the amount of soluble fibrin in plasma of patients with a variety of coagulation disorders. This antibody could also be used to develop an ELISA based on two different fibrin-specific monoclonal antibodies. For this assay anti-fbn 17 (Scheefers- Borchel et al., Proc. Natl. Acad. Sci. USA 82: 7091, 1985) was coated onto ELISA plates. After adding plasma which contained soluble fibrin, the fibrin bound was detected by the second fibrin-specific antibody B/H_{11} to which biotin was covalently linked. The second antibody was probed by the addition of peroxydase conjugated streptavidin and the substrate ABTS for peroxydase. This test can be used to detect fibrin at concentrations as low as 70 ng/ml. With this assay system, it is possible to measure the amount of soluble fibrin present in plasma samples without the interference of fibrinogen which is associated with soluble fibrin.

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FIBRIN-STIMULATED RELEASE OF VON WILLEBRAND FACTOR FROM ENDOTHELIAL CELLS IS LINKED TO FIBRINOPEPTIDE B CLEAVAGE. **J.A. Ribes, D.D. Wagner and C.W. Francis. Hematology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA.**

von Willebrand factor (vWf) is synthesized in endothelial cells and stored in specialized organelles, the Weibel-Palade bodies. We have examined the role of fibrin as a potential physiological secretagogue of vWf from Weibel-Palade bodies using indirect immunofluorescence staining of endothelial cells to detect release. Addition of fibrinogen to endothelial cell cultures resulted in the formation of a clot, and this was temporally associated with vWf release. Addition to endothelial cells of preformed fibrin prepared by clotting fibrinogen with thrombin also stimulated release of vWf within 10 minutes. Hirudin inhibition or heat denaturation of clot-bound thrombin abolished most of the thrombin activity but did not diminish release. The role of fibrinopeptide A and B (FPA, FPB) cleavage in stimulating release was examined using reptilase or the venom from A. contortrix to selectively remove FPA or FPB. Release was stimulated by fibrin from which FPB had been cleaved by either thrombin or A. contortrix, while desAA fibrin prepared with Reptilase was an ineffective stimulus. The formation of a stimulatory fibrin clot with the contortrix enzyme, which does not cause release by itself, demonstrates that fibrin stimulation was completely independent of thrombin activity. The capacity to stimulate release was found to be independent of factor $XIII_a$ crosslinking with both crosslinked and noncrosslinked FPB cleaved fibrins demonstrating stimulation. We conclude that fibrin stimulates rapid release of vWf from endothelial cells independent of thrombin activity and may function as a physiologic secretagogue. Furthermore, the stimulating capacity is dependant on cleavage of FPB suggesting that release is mediated by an active site near the N-terminal of the β chain or is dependent on a fibrin structure resulting from FPB cleavage.

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LOCALIZATION OF THE DOMAINS OF FIBRIN INVOLVED IN BINDING TO PLATELETS. Hantgan, R. R. Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC, USA.

The molecular basis of platelet-fibrin interactions has been investigated by using synthetic peptides as potential inhibitors of binding fibrin protofibrils and fibrinogen to ADP-stimulated platelets, adhesion of fibrin fibers to the platelet surface, and platelet-mediated clot retraction. Synthetic peptides RGDS and HHLGGAKQAGDV, corresponding to regions of the fibrinogen α and γ chains previously identified as platelet recognition sites, inhibited the binding of radiolabelled soluble fibrin oligomers to ADP-stimulated platelets with IC_{50} values of 12 and 40 μM, respectively. The IC_{50} values obtained with fibrinogen as the ligand were 3-fold higher. Synthetic GPRP and GHRP, corresponding to the N-terminal sequences of the fibrin α and β chains, were minimally effective in blocking soluble fibrin oligomer binding to ADP-stimulated platelets. The extent of fibrin:platelet adhesion was determined with a microfluorimetric technique which measures the quantity of fluorescein-labelled fibrin attached to the surface of platelets. The signal obtained from the brightly fluorescent platelet:fibrin adducts was time- and concentration-dependent, and was fully inhibited by a monoclonal antibody directed against the glycoprotein II:IIIa complex (HP1-1D, kindly provided by Dr. W. Nichols). Inhibition of fibrin:platelet adhesion by RGDS, HHGGAKQAGDV, and GHRP all exhibited a similar, linear dependence on the peptide concentration, reaching 1/2 maximum at about 200 μM, suggesting nonspecific effects. GPRP inhibited fibrin assembly but did not appear to have specific effects on fibrin:platelet adhesion. The time course of clot retraction was followed by right angle light scattering intensity measurements. Only RGDS affected clot retraction, causing a 4-fold decrease in rate at 230 μM. These results indicate that fibrinogen and fibrin protofibrils, which are obligatory intermediates in the fibrin assembly pathway, share a set of common platelet recognition sites located at specific regions of the α and γ chains of the multinodular fibrin(ogen) molecules. The RGDS site is also involved in mediating interactions between the three dimensional fibrin network and ADP-stimulated platelets.

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THE N-DSK γ-CHAIN BINDS TO IMMUNOPRECIPITATED GP IIb-IIIa. L.I.Thorsen (1), B.Hessel (2), F.Brosstad (1), G.Gogstad (1) and N.O.Solum (1). Research Institute for Internal Medicine, Rikshospitalet, Oslo, Norway (1) and Karolinska Institutet, Stockholm, Sweden (2).

We have previously demonstrated binding of the CNBr-split N-terminal disulphide knot of the fibrinogen molecule (N-DSK) to blood platelets and to their immunoprecipitated fibrinogen receptor, the glycoprotein IIb-IIIa complex. To further investigate which part of the N-DSK molecule that is responsible for its binding to GP IIb-IIIa, this fragment was split into its separate Aα- (1-51), Bβ- (1-118) and γ- (1-78) chains and carboxymethylated. GP IIb-IIIa was immunoprecipitated by crossed immunoelectrophoresis (CIE) of Triton X-100 extracts of platelets against rabbit antibodies to whole platelet proteins. The CIE plates were incubated with 125-I-radiolabelled N-DSK chains, and investigated for binding by autoradiography. The γ-chain but not the Aα- or Bβ-chains demonstrated binding to the GP IIb-IIIa. These results demonstrate that the fibrinogen molecule contains a third sequence of amino acids which is capable of binding to the fibrinogen receptor of the blood platelets, in addition to the two previously reported sequences C-terminally in the Aα- and γ-chains. Fragment E derived from fibrinogen (E fg) does not interact with the fibrinogen receptor. Thus, the γ-chain interaction site can not be present in the E fg-γ-chain (1-53). However, fragment E derived from fibrin (E1) with the γ-chain of residues 1-62, does react. The residues of N-DSK and E1 which are involved in binding to GP IIb-IIIa therefore appear to be present in the γ-chain sequences 54-62.