

INHIBITION OF COLLAGEN AND ADP-INDUCED PLATELET AGGREGATION BY PLASMA FIBRONECTIN. D.G. Moon and J.E. Kaplan, Department of Physiology, Albany Medical College, Albany, NY 12208.

Platelets contain fibronectin a glycoprotein with an established affinity for collagen. This observation has led other investigators to postulate that fibronectin is the platelet collagen receptor. The much greater concentration of fibronectin in the plasma surrounding platelets, however, has led us to suggest that plasma fibronectin may bind to collagen and competitively inhibit the platelet-collagen interaction. Rat platelets were isolated by Stractan density gradient centrifugation and aggregated with acid-solubilized rat tail tendon collagen (Type I) in a Payton 300B Aggregometer. Fibronectin was twice purified by affinity chromatography with gelatin linked to CNBr-activated Sepharose 4B. Simultaneous addition of 50 µg fibronectin and 25 µg collagen to platelets suspended in Tyrodes solution at 37°C resulted in a 2-fold increase in lag time and a 30% decrease in aggregation rate as compared to control values. When collagen was preincubated in Tyrodes solution for 12 minutes at 26°C without platelets to allow for prior fibrillogenesis, the addition of 50 µg fibronectin with the platelets resulted in <20% increase in lag time and a 20-30% decrease in aggregation rate. In a separate series of experiments, fibronectin was also found to inhibit ADP-induced aggregation. In this case, the initial rate of aggregation was comparable with and without fibronectin, but this maximal rate was maintained for a shorter period in the presence of fibronectin. Thus, fibronectin reduced the *in vitro* aggregation response to two different physiological stimuli. Our data supports previous studies which indicate that fibronectin reduces the reactivity of platelets with collagen and provides evidence of a role for fibronectin in modulating platelet responses in the absence of collagen.

FURTHER EVIDENCE FOR THE ROLE OF PLATELET SURFACE-ASSOCIATED FIBRONECTIN IN THE STIMULATION BY COLLAGEN: H.B. Bensusan, D. Holderbaum, K.G. Khor, and M. Orlando. Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio

We have previously published evidence showing that 1) fibronectin (FN) can be extracted from intact platelets, 2) collagen fibers preincubated with plasma FN do not stimulate platelets, and 3) antibodies to human plasma FN stimulate platelets. We now present evidence that platelet stimulation by anti-human plasma FN antibodies depends upon antigen-antibody compatibility in platelets from individual donors. Antibodies to human plasma FN produced in rabbit and goat were purified on affinity columns of FN. Platelets from some donors were stimulated by antibodies from both species, some by one but not the other, and some by neither. These results suggest that there is a genetic variability of human platelet associated FN, presumably residing in the carbohydrate side chains. For this reason, donor-antibody compatibility must be considered in studies of platelet-membrane FN using antibodies. F(ab')₂ fragments of goat antibody caused stimulation of platelets from compatible donors. These data show that the stimulation of platelets is not due to any form of immune complex, which requires the Fc portion of the antibody. The Fab' fragment caused no stimulation of platelets. However, when varying amounts of these fragments were preincubated with platelets, a dose-dependent inhibition of stimulation by collagen was seen. These data clearly indicate a role of platelet-membrane associated FN in the stimulation of the platelet by collagen. Three lines of monospecific antibodies were derived from hybridoma-induced mouse tumor ascites. Two of the antibodies from these lines stimulated the platelets from all donors while the other stimulated none. Furthermore, the plasma FN from all donors was shown to be antigenic to all antibodies of the goat whether or not the antibody stimulated their platelets. This fact, together with the observation that one monoclonal antibody caused no response, suggests that a portion of the platelet-membrane associated FN molecule is somehow masked or buried.

THROMBASTHENIC PLATELETS DO NOT BIND PLASMA FIBRONECTIN. Mark Ginsberg, Juan Chediak, Alton Lightsey and Edward F. Plow. Scripps Clinic, La Jolla, CA, Michael Reese Hospital, Chicago, IL, and Naval Regional Medical Center, San Diego, CA

Plasma fibronectin (fn) binds to thrombin-stimulated normal platelets and may thus contribute to platelet participation in hemostasis. Glanzmann's thrombasthenia is an inherited bleeding disorder in which the affected platelets do not aggregate in response to thrombin or other stimuli and have diminished ability to retract clots. To evaluate the ability of thrombasthenic platelets to bind fn, suspensions of gel filtered platelets and 2nM ¹²⁵I fn were incubated at 37°C in the presence or absence of 2 units/ml purified human thrombin. At various times, cells were centrifuged through 20% sucrose in microfuge tubes and bound ¹²⁵I fn measured in amputated tube tip. In the case of normal cells, in the presence of thrombin, there was time dependent uptake, plateauing by 20 min incubation. At equilibrium, greater than 95% of binding was saturable, and Scatchard plots indicated a single class of binding sites with K_d = 3x10⁻⁷ M and a maximum of 120,000 molecules/platelet. No binding was observed in the absence of thrombin. In contrast, platelets from 7 thrombasthenic individuals from 4 unrelated kindreds showed little or no detectable uptake of fn in the presence or absence of thrombin even after a 60 min incubation. Nevertheless, thrombin did induce serotonin secretion from these cells, showing that stimulation was occurring. Thrombasthenic platelets are defective in their ability to bind fibrinogen in the presence of ADP. These data indicate a defect in platelet interaction with another plasma protein in this disease. They also suggest the possibility that deficient fn-platelet interaction contributes to the thrombasthenic bleeding diathesis.

FIBRONECTIN-BINDING GLYCOPROTEIN FROM HUMAN PLATELET MEMBRANES.

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Fibronectin (cold insoluble globulin) has been suggested as a possible mediator of platelet adhesion. A fibronectin-binding protein was partially purified from washed solubilized human platelet membranes by affinity chromatography on fibronectin-Sepharose. The isolated protein migrated as a single band in SDS-polyacrylamide gel electrophoresis with a molecular weight of approximately 125,000 under reducing conditions. In non-reduced gels the protein migrated as a dimer. The purified protein did not react with immunoglobulins against fibrinogen or fibronectin when tested in crossed immunoelectrophoresis or electroimmuno assay. The protein and purified fibronectin formed a complex which had a significantly faster mobility in crossed immunoelectrophoresis than did native fibronectin. The presence of heparin in the binding protein-fibronectin mixture resulted in an even faster mobility of the complex, while the mobility of native fibronectin was unaffected. Crossed affinoimmunoelectrophoresis of the complex using different lectins suggested that the binding protein is a glycoprotein containing N-acetyl-glucosamine residues. The complex, but not purified fibronectin, bound to phenyl-Sepharose on crossed hydrophobic interaction immunoelectrophoresis. The results strongly suggest the presence of a fibronectin binding glycoprotein in the platelet membrane.