

HUMAN BLOOD PLATELET FACTOR VIII-RELATED ANTIGEN: A GLYCOCALICIN-LIKE PROTEIN. B.N. Bouma, S. de Graaf and T.S. Zimmerman. Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.

The location of Factor VIII-Related Antigen (FVIII R:AG) in human blood platelets was evaluated by treating intact platelets with chymotrypsin and observing the effect on the relative distribution of FVIII R:AG in different platelet fractions. Platelets were fractionated using the glycerol gradient technique. In untreated platelets from 12 normal individuals 95.5% of total platelet FVIII R:AG was found in the soluble fraction, 4.5% in the granules and less than 0.01% in the membrane fraction. Treatment of washed platelets with chymotrypsin resulted in the appearance of FVIII R:AG outside the platelets, and the amount of FVIII R:AG in the soluble fraction decreased in parallel. This suggests that at least part of platelet FVIII R:AG is exposed on the surface of the platelets, and that this FVIII R:AG is released from the membranes during homogenization. FVIII R:AG present in the soluble fraction was analyzed on SDS-polyacrylamide gels in the presence of reducing agents. A PAS-positive band with an apparent mol wt similar to plasma FVIII R:AG was observed. This band was identified as FVIII R:AG by specific removal with insolubilized anti-factor VIII. Granule FVIII R:AG showed crossed immunoelectrophoretic and ultracentrifugational characteristics similar to plasma FVIII R:AG. In contrast studies of FVIII R:AG in the soluble fraction using the same techniques showed a smaller average size than plasma FVIII R:AG. Our results indicate that platelet FVIII R:AG, isolated in the soluble platelet fraction, originates in part from the surface of the platelet and is therefore a glyocalicin-like protein.

THE BINDING OF BOVINE FACTOR VIII TO HUMAN PLATELETS, Edward P. Kirby and Sha May Tang, Dept. of Biochemistry and the Thrombosis Research Center, Temple Univ. Health Sciences Center Phila., Pa.

Highly purified bovine Factor VIII (FVIII) was iodinated by the lactoperoxidase method. Subsequent chromatography on agarose and extensive dialysis against sodium iodide solutions was required to remove non-covalently bound I-125 which adhered tightly to FVIII. Iodination destroyed the procoagulant activity (Antihemophilic Factor-AHF) of FVIII, but did not affect its Platelet Aggregating Factor (PAF) activity. Binding to human platelets was determined by incubating radiolabelled FVIII with formalin-treated platelets, centrifuging, and measuring both bound and free radioactivity. Results obtained by this method were much more precise than those obtained by measuring disappearance of unlabelled AHF, PAF, or FVIII-related antigen from the supernatant, although the estimates of total binding obtained were comparable. Binding of FVIII to formalin-treated platelets was approximately the same as to unfixed platelets, and the binding could be saturated by adding an excess of unlabelled FVIII. Maximal binding occurred within 1-2 minutes at 37° and binding could be blocked by dextran sulfate, Evans Blue or other inhibitors of FVIII-induced platelet agglutination. Treatment of platelets with trypsin inhibited binding of labelled F-VIII. Binding was not affected by the presence of plasma, or high levels of purified human fibrinogen or FVIII.

SUBCELLULAR LOCALIZATION AND SECRETION OF FACTOR V BY HUMAN PLATELETS. D.D. Pifer, R.W. Colman, and C.McI. Chesney. University of Tennessee Center for the Health Sciences, Memphis, Tennessee, U.S.A. and University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.

Previous studies have suggested that factor V in platelets is derived from plasma. In order to test this hypothesis, we studied the subcellular localization, release by aggregating agents, molecular size, and stability of factor V in human platelets. When platelet homogenates were fractionated on sucrose density gradient the factor V activity distributed primarily into the granules (0.076 units factor V activity/mg protein) with less amounts in the membrane fraction (0.014 units/mg protein) and virtually none in the dense granules or cytosol. Collagen released 79.3% of total platelet factor V clotting activity while ADP and epinephrine failed to liberate factor V activity, further supporting significant localization in the lysosomal granules rather than the dense granules. Platelet factor V can be solubilized by 0.1% Triton and has an apparent molecular weight of 470,000 as determined by 4% agarose gel filtration. In contrast, plasma factor V in the presence or absence of Triton has a molecular weight of 300,000. Factor V activity from the platelet homogenate exhibited biphasic decay at 37°C, suggesting more than one form, whereas plasma factor V shows a first order decay curve. These data suggest that platelet factor V is predominantly localized in the granules and is an intrinsic constituent of the platelet with different properties from plasma V.