LOCALIZATION OF FACTOR VIII ANTIGEN IN PLATELETS AND MEGAKARYOCYTES OF NORMAL AND VON WILLEBRAND PATIENTS. (ELECTRON MICROSCOPY STUDIES WITH PEROXIDASE LABELLED ANTIBODIES). C. Jeanneau, Y. Sultan, E. Savariau and J. Caen. Laboratoire d'Hémostase et de Thrombose Expérimentale, Hôpital Saint-Louis, Paris, France.

Using peroxidase coupled antibodies to human factor VIII, factor VIII related antigen (F VIIIRA) was localized in platelets and megakaryocytes of normal and Von Willebrand's patients.

Megakaryocytes and washed platelets were fixed either in glutaraldehyde alone or in a mixture of tannic acid, paraformaldehyde and glutaraldehyde and then incubated with the peroxidase coupled antibodies. In normal platelets, the peroxidase was found in two locations, on the plasma membrane and surface connecting system (S.C.S.) and in the cytoplasm associated with the membrane complex and dense tubular system.

The plasma membrane of the megakaryocyte and the demarcating membrane system were not labelled. Antigen was localized in the perinuclear space, in smooth reticulum and clear vesicles, known to be the future platelet S.C.S.

In homozygotes with severe forms of Von Willebrand's disease (VWd) no F VIIIRA was detected around or in the platelets either before of after transfusion of normal cryo or after incubation of their washed platelets in normal plasma. In less severe forms, with detectable F VIIIRA in the plasma there is no such clear correlation between plasma and platelet F VIIIRA. Some have antigen in all platelets with a low plasma level. Others have two populations of platelets some with and others totally lacking antigen. In genetic variants of the disease all the platelets are normally labelled.

URINARY EXCRETION OF FACTOR VIII RELATED ANTIGEN AFTER RENAL TRANSPLANTATION. Z. M. Ruggeri, R. Coppola, Y. B. Gordon, N. Ardaillou, C. Ponticelli and P. M. Mannucci. Hemophilia & Thrombosis Centre, Milano, Italy; St. Bartholomew's Hospital, London; Institut de Pathologie Cellulaire, Paris, France

Urinary excretion of factor VIII related antigen (VIIIR:AG) and fibrin(ogen) fragments (Fg) D and E was measured by radio-immunological techniques in unconcentrated urines obtained from 20 normal individuals, 20 patients (pts) followed serially after renal transplantation (tx) and 48 pts with constantly good renal function after tx. Pts serially followed showed a transitory excretion of VIIIR:AG, Fg D and E immediately after tx; subsequently, excretion was found only in connection with acute rejection episodes. Out of 20 episodes, VIII:AG was present in 16, Fg E in 13 and Fg D in 8, either accompanying or preceding of 1-2 days the increase of serum creatinine. In the group with good renal function the three proteins were occasionally present in urines but to a much lower extent than in patients with rejection episodes. Statistical analysis with the corrected X^2 test showed a significant difference ($P<0.001$) between the two groups for all the three measurements. The false positive rate (high excretion in patients with no rejection) was 6% for VIIIR:AG, 8% for Fg E and 10% for Fg D. Urinary excretion of VIIIR:AG proved to be more sensitive than Fg D and E as a parameter of kidney rejection, with a lower incidence of false positive results. This confirms that immunologically related endothelial damage and platelet aggregation precede intra-renal fibrin deposition and appearance of fibrin (ogen) degradation products in patients with acute rejection episodes.

ISOLATION OF AN APPARENTLY ABNORMAL VON WILLEBRAND FACTOR: COMPARISON WITH PLASMA AND SERUM VON WILLEBRAND FACTOR. R. F. Baugh, C. Burnand and C. Hougie. Department of Pathology, University of California, San Diego, School of Medicine, La Jolla, California, U.S.A.

A patient with an extremely high level of von Willebrand factor (8X normal) failed to show any response to ristocetin with her platelet-rich plasma. The patient also had a long bleeding time and a large amount of a paraprotein. Using a washed platelet assay system and dilutions of her platelet-poor plasma, ristocetin-induced platelet aggregation activity could be recovered to 40% of normal. Purification of the von Willebrand factor from the patient's plasma resulted in an increase in ristocetin-induced platelet aggregating activity associated with the von Willebrand factor. Electrophoretic comparison by SDS disc gel electrophoresis and crossed immunoelectrophoresis showed no differences between this patient's von Willebrand factor and either purified normal plasma or serum von Willebrand factor. The patient's plasma, following removal of the von Willebrand factor, inhibited ristocetin-induced platelet aggregation in normal platelet-rich plasma and in a washed platelet system using purified normal von Willebrand factor. These observations indicate the presence of an abnormal plasma protein which binds ristocetin and thereby inhibits platelet aggregation. Furthermore, the abnormal protein must interfere with the in vivo functions of the von Willebrand factor as witnessed by the increased bleeding time presented by the patient. This behavior is explained by a model system in which ristocetin mimics a structural component found in the lining of the vessel walls which is exposed by tissue injury. The abnormal plasma protein competes with the von Willebrand factor and interferes with von Willebrand dependent platelet adhesion.

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