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INVITED SYMPOSIUM I

Molecular Biology of Factor VIII.

STRUCTURE-FUNCTION STUDIES OF FACTOR VIII/VON WILLEBRAND PROTEIN(S). Patrick A. McKee. Duke University Medical Center, Durham NC, U.S.A.

Factor VIII (FVIII) procoagulant activity was initially thought to be a glycoprotein with a molecular weight (MW) >1 million and composed of disulfide-linked ~200,000 MW subunits. A protein with similar properties, except lacking procoagulant activity, is in hemophilic plasma; it was identical to normal FVIII by SDS-gel analyses, isoelectric focusing, and PAS staining. Subsequently it was shown that the FVIII glycoprotein also has von Willebrand factor (vWF) activity, suggesting that both FVIII and vWF activities might be properties of the same molecule. When the FVIII/vWF protein(s) is rechromatographed on 4% agarose and 0.25 M CaCl₂, virtually all the protein and vWF activity elute in the void volume, but most of the FVIII procoagulant activity elutes much later. The extent of separation of the two activities depends on the amount of protein applied to the column. Also, exposure of the FVIII/vWF to thrombin before gel filtration strikingly accentuates separation of the two activities. The reduced SDS-gel pattern of the void volume protein peak showed the 200,000 MW subunit while that of the procoagulant peak contained several subunit bands which ranged from ~30,000-100,000 MW. Removal of sialic acid from FVIII/vWF is associated with reduced ristocetin induced platelet aggregation and causes a 50-fold increase in the rate of clearance of protein from the circulation by the hepatocyte. Currently, our data suggest that FVIII procoagulant and vWF activities are properties of a single molecule composed of disulfide-bound identical subunits. Cleavage by thrombin then results in FVIII procoagulant activity. Additional cleavages, to which the molecule appears very sensitive, results in FVIII inactivation. The vWF activity is very stable—even to proteolysis—and it appears to be a function of the carbohydrate side chains of the molecule.

IMMUNOLOGIC APPROACH TO THE STUDY OF FACTOR VIII. Leon W. Hoyer. University of Connecticut School of Medicine, Farmington, Connecticut, U.S.A.

In contrast to direct biochemical analyses, immunologic studies of factor VIII are less limited by that factor's lability and low protein concentration. Both human and heterologous anti-factor VIII have been used in these studies. Human antibodies inactivate factor VIII procoagulant activity but they do not fix complement or form immunoprecipitates. Their interaction appears to involve the formation of an immune complex with a univalent component distinct from that which has been designated factor VIII-related protein. This component is synthesized—but remains nonfunctional—in hemophilia, and at least two different defects have been distinguished. Very different reactions can be identified using heterologous antibodies. Although many of these antibodies do not react directly with the procoagulant site, they do fix complement, precipitate with factor VIII-related protein, and inactivate ristocetin cofactor activity. These antibodies are useful for classification of factor VIII-deficiency diseases according to the relative levels of factor VIII procoagulant activity and factor VIII-related antigen; identification of the hemophilic carrier state; characterization of heterogeneity in factor VIII structure; and purification of factor VIII procoagulant activity by immunoadsorbent chromatography. In general, the information obtained by immunologic studies has complemented that from direct biochemical analyses. Both approaches contribute to our evolving understanding of factor VIII structure and function.