Tuesday, July 14, 1981

Symposium VIII

Factor VIII/ von Willebrand Factor

15:30–17:30 h

Grand Ballroom East

0476

MOLECULAR BIOLOGY OF FACTOR VIII COAGULANT PROTEIN.

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Several laboratories report factor VIII coagulant (VIII:C) from human, bovine and porcine sources interact with Willebrand factor (WF). Bovine coagulant may be S-S bonded to WF; the human and porcine VIII:C may reversibly bind to WF. This interaction has been used by various laboratories to help purify the VIII:C. The VIII:C from all 3 species are activated by IIa and inactivated in EDTA. Our work has involved the porcine proteins bound by WF and anti-VIII:C antibodies. Human antibodies to human VIII:C bind porcine plasma proteins which on SDS-PAGE band at apparent MW 160K, 130K and 80K. These data are consistent with the human VIII:C (Switzer and McKee (JCI 60:819, 1977)). Their human material appears to contain S-S linked chains, porcine chains separate in SDS alone. Thrombin treatment results in loss of high molecular weight proteins and an appearance of activation/deactivation products at MW = 80K and 73K and lower. Two murine monoclonal anti-porcine VIII:C antibodies partially inactivate VIII:C while a third doesn't. This latter antibody, when immobilized, removes activity from solutions of VIII:C dissociated from WF but not from solutions of undissociated VIII:C. The VIII:C immobilized through this antibody is still active, can be removed by organic solvent in active form, and contains the same protein chains as those bound to the polyclonal human anti-VIII:C. The monoclonal antibody VIII:C complex is inactivated and releases the 130K + 160K chains in EDTA. The 80K MW chain can be removed separately. Based on relative staining intensity in SDS gels, the porcine VIII:C at this stage of purity may be heterogeneous, (130K + 80K and 160K + 80K), with the chains associated through Ca++. The 80K MW chain, may bind the VIII:C to porcine WF. The site of this interaction does not seem to be critical to the measurement of VIII:C activity in vitro.

0477

MONOCLONAL ANTIBODIES AS PROBES OF FACTOR VIII/VON WILLEBRAND FACTOR.

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Monoclonal antibodies are essential for the isolation and specific assay of the various components of Factor VIII/von Willebrand Factor (F.VIII/vWF) and for the immunochemical analysis of the surface epitopes of this protein. In this study, we have produced and characterized a panel of thirty-eight antibody synthesizing hybridomas. Mice were hyperimmunized with human F.VIII/vWF and three days after a 10-20 µg booster dose, spleen cells were harvested and fused with P3X63Ag8 or SP2/O myeloma cells. All antibodies reacted by solid phase ELISA with purified F.VIII/vWF and normal cryoprecipitate and did not bind to cryoprecipitate from severe vWD, fibrinogen, or fibronectin. Antibody titers (20 µl) varied from 10^2 to 10^6. Seven antibody producing hybridomas derived from P3X63Ag8 myeloma cells were cloned, recloned, and were stable in ascites growth. Two of these partially blocked VIIIR:RCo while the mixture of the seven exhibited significant inhibition, suggesting a cooperative effect in steric hindrance or induction of allosteric effects on the VIIIR:RCo function of the molecule. Three out of thirty-one hybridomas derived from SP2/O myeloma cells blocked Factor VIII activity. A spatial map of the epitopes at the surface of F.VIII/vWF was derived from competitive displacement studies of the monoclonal antibodies on immobilized F.VIII/vWF. Three types of results were observed : no displacement, indicating independence of two epitopes; partial displacement, indicating steric proximity; and complete displacement, indicating epitope identity or extremely close proximity.

0478

VON WILLEBRAND FACTOR IN PLATELET INTERACTION WITH SUBENDOTHELIUM.

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Studies using citrated blood. A defect in adhesion of platelets from VWD patients to rabbit aorta subendothelium was first demonstrated in 1974 using our annular perfusion chamber. Later on we showed that the magnitude of the defect increased with the blood shear rate. Addition of void-volume fractions of cryoprecipitate chromatographed on Bio-gel A-15m to blood of patients corrected the adhesion defect. By treatment of normal human blood with homologous and heterologous antibodies to human FVIII/VWF a shear rate dependent adhesion defect was experimentally induced. Studies with reconstituted human blood by Sixma's group confirmed the involvement of FVIII/VWF in platelet adhesion to subendothelium. Treatment of rabbit aorta subendothelium with antibodies to rabbit FVIII complex gave different results: an antiserum raised in goat inhibited adhesion, whereas an antiserum raised in guinea pig had no effect. The latter antiserum did, however, inhibit adhesion when added to rabbit blood. It is conceivable that the two antisera are predominantly directed towards different epitopes on FVIII/VWF.

Studies avoiding anticoagulation. In these studies blood is directly drawn from a vein through the annular chamber at a controlled flow rate. In addition to a shear rate dependent inhibition of platelet adhesion to subendothelium a significant inhibition of maximum thrombus heights, thrombus growth and/or stability was observed at shear rates which were not associated with an adhesion defect. It is not yet clear whether this additional defect is due to FVIII/VWF and/or VIII:C deficiency.

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