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DIVERGENT FATES OF VON WILLEBRAND FACTOR AND ITS PROPOLYPEPTIDE (VON WILLEBRAND ANTIGEN II) AFTER SECRETION FROM ENDOTHELIAL CELLS. D.D. Wagner, P.J. Fay, L.A. Sporn, S. Sinha, S.O. Lawrence and V.J. Marder. Hematology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

The intracellular site of cleavage of pro-von Willebrand factor subunit and the subsequent fate of the propolypeptide (von Willebrand antigen II) and of the mature von Willebrand factor (vWf) were investigated. Both the propolypeptide, which was found to be a homodimer of non-covalently linked subunits, and mature vWf were released from Weibel-Palade bodies of endothelial cells following stimulation with secretagogues. The stoichiometry of the two proteins in the releasate was essentially equimolar. This indicates that vWf and the propolypeptide were packaged into the Weibel-Palade bodies as one unit, pro-vWf, and that the proteolytic cleavage of pro-vWf is likely to be a post-Golgi event. The association of prosequences into dimers provides support for their hypothetical role in the multimerization process. After secretion, the two proteins were distributed differently, as based on the following observations. The propolypeptide did not associate with vWf in the culture medium, did not co-distribute with vWf in the extracellular "patches of release" on stimulated endothelial cells, and was not detected in the endothelial cell extracellular matrix, which did contain vWf. Additionally, in contrast to vWf, the propolypeptide did not bind to matrix of human foreskin fibroblasts. Since the propolypeptide does not associate with vWf and does not interact with extracellular matrices in vitro, it is highly unlikely that it would promote platelet adhesion to subendothelium in vivo.

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PREVENTION OF OCCLUSIVE CORONARY THROMBOSIS BY MONOCLONAL ANTIBODY TO VON WILLEBRAND FACTOR IN SWINE. T.C. Nichols, D.A. Bellinger, M.S. Read, R.L. Reddick, K.M Brinkhous, T.R. Griggs, and M.A. Lamb. University of North Carolina at Chapel Hill, Departments of Medicine and Pathology and the Center for Thrombosis and Hemostasis Research, Chapel Hill, NC, USA.

Von Willebrand's disease protects pigs from developing cyclic or permanent coronary thrombosis (C/P-Th) following experimentally induced stenosis and injury (S/I) in an open chest anesthetized pig (Nichols et al, Circ Res., 1986; 59:15-26). To confirm that this protection is related specifically to absence of von Willebrand factor (vWF), an IgG kappa monoclonal antibody (MAb) to purified porcine vWF was produced in mice. MAb inhibited platelet aggregation by ristocetin and botrocetin, but not ADP, thrombin, or collagen. MAb was infused into seven normal pigs. Bleeding times (BT) in all pigs were prolonged to >10 min, and platelet agglutinating factor levels as a measure of vWF activity were <2% for approximately 2 hours or longer. There was no significant change of hematocrit, platelet count or Factor VIII coagulant activity. Coronary stenosis was produced by placing a Goldblatt clamp (GC) around the left anterior descending coronary artery (LAD). A 20-MHz Doppler velocity crystal was placed distal to GC to measure LAD blood flow velocity. The LAD was injured at the GC site with spring loaded forceps. C/P-Th were detected by flow velocity changes and vessels were examined by light and scanning electron microscopy. Five of seven pigs were given MAb prior to S/I; after S/I, four of these five had no C/P-Th. The fifth pig developed C/P-Th after 2 hours when vWF activity had returned to 10% and BT had shortened to 2.5 min. The other two pigs were given MAb after S/I had produced C/P-Th. The first had total resolution and the second partial resolution of these cyclic thromboses. This S/I technique produced C/P-Th in 8 of 9 normal pigs. Thus, this MAb reduces vWF activity and prevents C/P-Th. This study confirms a direct role of vWF in supporting occlusive coronary thrombosis in this porcine model and suggests reduction of vWF activity could be a therapeutic approach to arterial thrombotic diseases. (Supported by Grant Nos. HL26309-06 and HL01648-40. MAb supplied by Bruce Evatt, M.D., C.D.C., Atlanta, Georgia).

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A FREQUENT TAQI RFLP AND A GENE LESION OR RARE TAQI RFLP IN THE VON WILLEBRAND FACTOR GENE

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A cDNA for vonWillebrand factor (vWf) has been used to investigate lesions and RFLPs in the vWf gene.

In hybridizations with the 3'cDNA portion (a 2 Kb SacI fragment) a frequent polymorphism has been found with TaqI restriction enzyme. The alleles are 3.3 Kb and 2.6 Kb ; the frequencies of 0.51 and 0.49 respectively enable to investigate an appreciable portion of vW disease (vWd) families.

In a patient with type III vWd an abnormal TaqI pattern has been observed. A 4.5 Kb band is absent and an additional band of 2.3 Kb is present. This pattern has been inherited from the consanguineous heterozygous parents and has been traced in several members of this large family. The presence of the abnormal gene pattern is related to total or partial vWf deficiency in the family and has not been found in several normal subjects. The BamHI and BglII restriction patterns are normal and suggest a small mutation originating a new TaqI site.

These findings are compatible with a gene lesion or a rare RFLP.

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VON WILLEBRAND FACTOR RELEASED FROM WEIBEL-PALADE BODIES BINDS MORE AVIDLY TO EXTRACELLULAR MATRIX THAN THAT SECRETED CONSTITUTIVELY. L.A. Sporn, V.J. Marder and D.D. Wagner. Hematology Unit, Department of Medicine, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA.

Large multimers of von Willebrand factor (vWf) are released from the Weibel-Palade bodies (WPB) of cultured endothelial cells following treatment with a secretagogue, whereas predominantly dimeric forms are secreted constitutively. These two pools of vWf were used to compare binding of the various multimeric forms of vWf to the extracellular matrix (ECM), the in vitro model of the basement membrane. The released multimers and an equal number of subunits of constitutively secreted vWf were placed, for 72 hours, on cultures of human foreskin fibroblasts (HFF) grown on glass coverslips, then fixed and stained by fluorescence using anti-vWf antiserum. Constitutively secreted vWf produced only a trace of matrix decoration, whereas the released large multimers bound more extensively. In order to determine if increased binding of released vWf was due to the presence of another component in the releasate, releasate from which vWf was adsorbed was combined with constitutively secreted vWf, and this mixture was overlaid onto HFF. The presence of the adsorbed releasate did not promote binding of constitutively secreted vWf. Therefore, it appears that the enhanced binding observed was due to the large multimeric size of vWf stored in the WPB. To further substantiate this, iodinated plasma vWf which was presumably constitutively secreted from endothelial cells was overlaid onto HFF for 72 hours, labeled vWf was removed, and cells were washed extensively and lysed. Samples of iodinated plasma vWf (starting material) and cell lysates were electrophoresed, non-reduced on an agarose gel. Densitometric scans of starting material and of bound vWf revealed that the large multimeric forms bound preferentially. It appears that multivalency is likely an important property in vWf interaction with the ECM, just as has been shown for vWf interaction with platelets. The pool of vWf contained within the WPBs, therefore, is not only especially suited for platelet interaction, but also for interaction with the ECM.