1296

PLATELET VON WILLEBRAND FACTOR-INDUCED SPONTANEOUS PLATELET AGGREGATION IN VON WILLEBRAND'S DISEASE. H.R. Gralnick, L.P. McKeown*, S. Williams*, J. van Mourik.
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We have previously described a form of von Willebrand's disease (vWd) with spontaneous aggregation induced by an abnormal plasma von Willebrand factor (vWf). We have studied the ability of these patient's platelet (P) lysate to induce P aggregation and compared the results with P lysates from other vWd patients. P were prepared from whole blood by centrifugation on an arabinogalactan gradient. The P yield varied from 86-93%. When ^{125}I purified plasma vWf added to whole blood, it was not detectable in the isolated P preparation. The P were lysed by Triton X-100 and after freezing were used in the assays. We found that as little as 25 ul of P lysate (1 x 10^9 platelets/ml) induced SPA in normal platelet-rich plasma (PRP). Normal or other vWd did not induce PA when (1) a monoclonal antibody directed the fibrinogen/vWf binding site on the GPIIb/IIIa was incubated with PRP; (2) when Glanzmann's thrombasthenic PRP was used or (3) when platelets were suspended in a fibrinogenemic plasma. The monoclonal antibodies, 10E5 and PLT-1 directed against the GPIIb/IIIa complex, totally inhibited the P-vWf induced platelet aggregation. SPA did not occur unless the plasma fibrinogen concentration was 40 mg/ml. The monoclonal antibody 6D1 (directed against the plasma vWf binding site on GPIb) only partically inhibited the P-vWf SPA. But an antibody directed against the plasma vWf-GPIb binding domain totally inhibited the SPA. We conclude that P-vWf has a domain similar to plasma vWf which binds to GPIb; however, the P-vWf binds to a site on the GPIb which is not identical to the plasma vWf binding site.

1297

IMPORTANCE OF LARGE VON WILLEBRAND FACTOR (vWF) MULTIMERS IN vWF INTERACTION WITH PLATELET GLYCOPROTEIN IIb/IIIa. M. Yamamoto, Y. Ando, K. Watanabe, H. Iri (1), Y. Araki, M. Murata, H. Murakami, K. Satoh (2) and Y. Ikeda (3). Department of Laboratory Medicine (1), Department of Hematology (2) and Blood Center (3), Keio Univ. Tokyo, Japan.

Recently it has been reported that, in addition to binding to glycoprotein (GP) Ib, vWF also interacts with GPIIb/IIIa, although the physiological relevance of this interaction is not completeley clear. In this paper, we have investigated the role of different size of vWF multimers in vWF-mediated platelet aggregation. Different size of vWF multimers were purified from human plasma through Sephacryl S-1000 column according to the method of Fowler et al. Fractions were analysed by SDS-agarose gel electrophoresis by the method of Ruggeri et al. When each fraction was examined for ristocetin cofactor activity (RCo), only larger multimers exhibited significant RCo. The maximum extent of ristocetin-induced platelet agglutination by larger multimers (10 µg/ml) was 80%, while that of intermediate and lower multimers at the same concentration was 20% and 0%, respectively. Each fraction was then added to washed platelet suspensions in the presence of 10 µM ADP and 0.3 mM $CaCl_2$. Only larger multimers induced platelet aggregation, while intermediate and lower multimers failed to induce platelet aggregation. The maximum extent of aggregation in the presence of larger multimers (10 µg/ml) was 70% of that in the presence of fibrinogen instead. Similar experiments were peformed using platelet-rich plasma from a patient with afibrinogenemia in stead of washed normal platelets. ADP caused significant aggregation only when purified vWF larger multimers or fibrinogen was added. This vWF-mediated aggregation was completely inhibited by monoclonal antibody to GPIIb/IIIa (1 µg/ml) and synthetic peptide, Arg-Gly-Asp-Ser, (1 mM).

Our results indicate that larger multimers of vWF play major roles in vWF interaction with GPIIb/IIIa.

1298

LOCALIZATION OF THE PLATELET-BINDING AND HEPARIN-BINDING DOMAINS OF BOVINE VON WILLEBRAND FACTOR. Edward P. Kirby, Mary Ann Mascelli, Carol Silverman, and Daniel W. Karl. Dept. of Biochem. and the Thrombosis Research Center, Temple Univ. School of Medicine, Philadelphia, PA 19140 U.S.A.

Bovine von Willebrand Factor (vWF) binds directly to human platelets and also to heparin-agarose. Cleavage of vWF with Protease I, a metalloenzyme isolated from the venom of the western diamondback rattlesnake, produces two major fragments with apparent Mr of 250 kD and 200 kD. The 200 kD fragment competes with native vWF for binding to the GPIb-associated vWF receptor on formalin-fixed human platelets and has weak platelet-agglutinating activity. It is composed of three polypeptide chains of apparent Mr of 97 kD, 61 kD, and 35 kD. Monoclonal antibodies #2 and H-9, which inhibit binding of vWF to a GPIb-associated receptor of platelets, recognize the 200 kD fragment.

Modification of vWF with ^{125}I-labeled Bolton-Hunter reagent (I*-BHR) causes inhibition of platelet-agglutinating activity at very low levels of incorporation. Modification of less than 2% of the amino groups in vWF causes 50% loss of platelet agglutinating activity and a decreased affinity of vWF for binding to platelets. Labeling with I*-BHR does not block binding to heparin-agarose, even when 5-10% of the amino groups are modified. Differential labeling at pH 7.0 and pH 8.5, followed by proteolytic fragmentation with Protease I, suggests that it is the modification of amino groups on the 200 kD fragment which is responsible for the decrease in platelet binding activity. Modification of the 97 kD peptide chain is best correlated with this loss of platelet binding activity.

Heparin inhibits the agglutination of human platelets by bovine vWF. The 200 kD fragment of vWF binds both to platelets and to heparin-agarose. These observations suggest that the heparin-binding and platelet-binding domains of vWF, although distinct from one another, reside in the same region of the vWF molecule. The platelet-binding domain contains a small number of very reactive amino groups which are required for vWF binding to human platelets.

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1299

ASIALO VON WILLEBRAND FACTOR ENHANCES PLATELET ADHESION TO VASCULAR SUBENDOTHELIUM. A.Ordinas(1), E.Bastida,(1) M.Garrido,(1) J.Monteagudo,(1) L.de Marco(2) and R.Castillo,(1). Hospital Clinic i Provincial. Facultad de Medicina. Universidad de Barcelona. Barcelona. Spain(1). Centro Immunotrasfusionale. Ospedale Civile. Pordenone. Italy(2).

Native Von Willebrand factor (NvWF) binds to platelets activated by thrombin,ADP or ristocetin,and also supports the adhesion of platelets to subendothelium at high shear rates.In contrast,asialo von Willebrand factor (AvWF) induces platelet aggregation in absence of platelet activators.We investigated the role of AvWF in supporting the adhesion of platelets to rabbit vessel subendothelium under flow conditions at a shear rate of 2000 sec-1 for 5 min using the Baumgartner perfusion system. We also studied the effects of blockage of platelet GPIb or GPIIb/IIIa on platelet adhesion using monoclonal antibodies (Mabs),and we measured the rate of binding of ^{111}I-labeled NvWF and AvWF to subendothelium. Perfusates consisted of washed platelts and red cells resuspended in a 4% human albumin solution to which increasing concentrations of NvWF or AvWF had been added.Platelets interacting with the perfused vessels were evaluated morphometrically using a computerized system.At a concentration of 1.2 µg/ml the percentage of total coverage surface was 21.3 $\pm$ 4.8% and 40.0$\pm$14.6%, for NvWF and AvWF,respectively ($p < 0.01$). Addition of either Mab against GPIb (LJ1b1) or against GPIIb/IIIa (CP8) to the perfusates, reduced platelet deposition ($p < 0.01$). The rates of binding of ^{111}I-labeled NvWF and AvWF to perfused vessel subendothelium were similar (0.83$\pm$0.1µg and 0.95$\pm$0.1 µg ,respectively).
Our results indicate that AvWF enhances the interaction of washed platelets with the vessel subendothelium under flow conditions.Furthermore, they suggest that this effect is related to the interaction of AvWF with platelets and not to an increased affinity of AvWF for subendothelium.