728

BINDING OF FIBRINOGEN TO PLATELET GLYCOPROTEIN (GP) IIb/IIIa IS CRUCIAL FOR SHEAR-INDUCED PLATELET AGGREGATION. Y.Ikeda, M.Murata, Y.Araki (1), M.Yamamoto, Y.Ando, K.Watanabe (2), M.Ichitani, K. Sakai (3), I.Itagaki and Y.Mori (4). Department of Hematology (1) Department of Laboratory Medicine (2), Keio Univ., Department of Engineering, Waseda Univ (3) and Basic Research Laboratories, Toray Industries, Inc. (4), Japan.

It is well known that human platelets can aggregate in vitro under certain shear stress without adding aggregating inducers. However, the mechanism of this shear-induced platelet aggregation has not been clarified yet. In this paper, we have investigated the role of fibrinogen and GP IIb/IIIa in shear-induced platelet aggregation. Citrated human platelet-rich plasma (PRP) was subjected to controlled shear stress levels in a polycarbonate cone and plate viscometer at 37°C for 2 minutes. After shearing the particle count was measured by an electronic particle counter. Particles with sizes from 3 to 20 μ cum were considered as single platelets. In unsheared PRP most of the particles were single platelets, but platelet doublets and platelet fragments larger than 3 μ cum were also counted. After exposure to shear rate of 3,600 - 9,000 sec^{-1}, the particle counts were decreased in a shear rate dependent manner, while LDH leakage from platelets was not significantly increased and ^{3}H-serotonin release was 2 - 7%. Scanning electronmicroscopy clearly showed the presence of large platelet aggregates when the particle counts were decreased. Platelets from two patients with thrombasthenia and one patient with afibrinogenemia, however, failed to aggregate at a shear rate of 9,000 sec^{-1}. Shear-induced aggregation was inhibited by monoclonal antibody to GPIIb/IIIa (1 μg/ml) and synthetic peptide, Arg-Gly-Asp-Ser, (1 mM). When fibrinogen was added to PRP from a patient with afibrinogenemia, shear-induced aggregation became evident as seen in normal platelets. Apyrase and hirudin showed no effect on shear-induced aggregation. Indomethacin (100 μM) and TXA_2 synthetase inhibitor, OKY-046 (100 μM) markedly inhibited aggregation, while TXA_2 competitive inhibitor, ONO-3708 (100 μM) exhibited only partial inhibition.

Our results indicate that binding of fibrinogen to GPIIb/IIIa is also crucial for shear-induced platelet aggregation and that the exposure of fibrinogen receptor on GPIIb/IIIa may partially depend upon TXA_2 synthesis in platelets.

729

IDENTIFICATION OF YUK(b) ALLOANTIGEN ON PLATELET GLYCOPROTEIN IIIa*. S. Santoso (1), Y. Shibata(2), V. Kiefel (1), and C. Mueller-Eckhardt (1). Institute of Clinical Immunology and Blood Transfusion, Justus-Liebig-University, Giessen, FRG (1) and Department Immunohematology, Toranomon Hospital, Tokyo, Japan (2).

Neonatal alloimmune thrombocytopenic purpura (NATP) is caused by IgG platelet alloantibodies (ab), produced by the mother and directed against antigens present on the platelets of the child. The specificity of the platelet-specific ab is anti-Pl(A1) in the majority of cases. Other specificities, i.e. anti-Pl(E2), anti-Bak(a), anti-Pen, and anti-Pl(A2) have also been found. Recently, Shibata et al (1986) have described a new platelet antigen system Yuk(a)/Yuk(b) involved in NATP. The Yuk(a) and Yuk(b) antigens are not expressed on thrombasthenic platelets indicating that these antigens do exist on glycoprotein (GP) IIb and/or IIIa. In order to investigate the molecular localization of these antigens, we studied the interaction of anti-Yuk(b) purified ab with membrane components of platelets using immunoblot procedure and compared their immunochemical behaviour with that of other platelet specific ab (anti-Pl(A1), -Lek(a), -Bak(a)). In the absence of disulfide reduction Yuk(b) ab reacted with an antigen of molecular weight (mol wt) 92 kDa with an electrophoretic mobility identical to GP IIIa. An identical result was obtained for Pl(A1) ab. In contrast, the Bak(a) ab as well as Lek(a) ab detected an antigen of mol wt 134 kDa which comigrated with GP IIb. After reduction with 2-mercaptoethanol binding of anti-Pl(A1) and anti-Yuk(b) was not observed. To further localize the Yuk(b) antigen on GP IIIa, immunoblotting experiments were performed with anti-Pl(A1) and anti-Yuk(b) of chymotrypsin treated platelets. While anti-Pl(A1) bound to GP IIIa and a 68 kDa component, anti-Yuk(b) bound only to GP IIIa when the platelets had been treated for 45 min with chymotrypsin. This discrepancy became even more pronounced by prolonged treatment of platelets (225 min) in that the reactivity of anti-Yuk(b) was entirely abolished, whereas binding of anti-Pl(A1) shifted completely from the 92 kDa to the 68 kDa component. Thus, unlike the Pl(A1) antigen, the Yuk(b) determinant either resides on the 30 kDa fragment of GP IIIa or it is destroyed by chymotrypsin treatment.

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730

PLATELET DYSFUNCTIONS DUE TO AN ANTI-GP IIIa AUTOANTIBODY IN A HEMORRHAGIC PATIENT WITH MALIGNANT LYMPHOMA. T. Kubota(1), K. Tanoue(2), H. Kitagawa(2), I. Muronashi(1), N. Aoki(1) and H. Yamazaki(2). Dept. of Internal Med., Tokyo Med. & Dental Univ.(1) and Dept. of Cardiovascular Res., Tokyo Metropolitan Inst. of Med. Sci.(2)., Tokyo 113 , Japan.

A 38-aged woman developed a moderate purpura and enanthema. Before 3 years when she had no bleeding tendency, she was diagnosed with retroperitoneal malignant lymphoma (follicular, large cell type) by a laparotomy and successfully treated with surgery and radiation. Laboratory tests at the time of the present admission revealed normal coagulation tests, platelet counts of 140,000/μl and prolonged bleeding time. Platelet aggregation by 1-10μM ADP showed a immediate lag time lasting 30-40 sec, then followed by an aggregation. Aggregation by epinephrine showed a loss of the first wave with a normal second wave. Collagen-induced aggregation showed an abnormally long lag time. Crossed immunoelectrophoresis (CIE) of 1% Triton X-100 solubilized platelets from the patient revealed a two-peaked immunoprecipitate of GP IIb/IIIa complex, of which the abnormally slow peak was thought to be the GP IIb/IIIa complex coated with an antibody. CIE of her platelets also showed abnormally decreased amounts of both albumin and fibrinogen in the platelets, suggesting intravascular release reaction from her platelets stimulated with the antibody. She gradually developed EDTA-induced pseudo-thrombocytopenia with true platelet counts of 200,000-250,000/μl. Then, the patient developed true thrombocytopenia ranging from 9,000 to 37,000/μl and was found to have the left tonsillar swelling, which was subsequently diagnosed as a relapse of the malignant lymphoma by a biopsy and treated successfully by radiation. After the disappearance of the tumor, the patient's platelet counts increased to 160,000/μl. At this time, aggregation patterns by ADP, epinephrine or collagen were completely normalized. CIE of her platelets showed a normal shape of GP IIb/IIIa complex as well as normal amounts of albumin and fibrinogen. The purified IgG from the patient in the thrombocytopenic stage inhibited dose-dependently ADP- or epinephrine-induced aggregation of normal PRP. Western-blot analysis showed that the patient's IgG reacted with a protein of approx. 100kDa, corresponding to GP IIIa, in unreduced SDS-PAGE of normal platelets.

731

PRODUCTS OF LIMITED PROTEOLYSIS OF GLYCOPROTEIN IIIa ON THE PLATELET MEMBRANES. S. Niewiarowski, A. Eckardt, H. Lukasiewicz, K. Norton, Tur-Fu Huang and E. Kornecki. Dept. of Physiol. and Thromb. Res. Ctr. Temple University Sch. of Med., Philadelphia, PA and Dept. of Psychiatry, University of Vermont, Burlington, VT.

Previous investigations from our laboratories have demonstrated the limited proteolysis by chymotrypsin of GPIIIa and the formation of a major component migrating on SDS polyacrylamide gel electrophoresis with an apparent molecular weight of 60 kDa in a nonreduced system and 66 kDa in a reduced system. The formation of this component occurred in parallel with the expression of fibrinogen receptors on the platelet surface. We raised in rabbits a monospecific polyclonal antibody against highly purified 60 kDa component. Using available rabbit polyclonal antisera (anti-GPIIIa and anti-66 kDa) and murine monoclonal anti GPIIIa antisera (AP3 and SSA6) a complete immunological crossreactivity between GPIIIa and 60 kDa was established. The limit of detection by immunoblotting assay was 20 ng of GPIIIa or 60 kDa in Triton X100 extract of platelets. Extracts of intact platelets, of ADP- or thrombin-stimulated platelets separated on SDS PAGE showed a strong band corresponding to GPIIIa, a light band migrating with an apparent molecular weight of 120 kDa and no detectable 60 kDa component. Incubation of platelets with chymotrypsin, pancreatic elastase or human granulocyte elastase resulted in the appearance of 60 kDa and in a progressive increase of 120 kDa component detectable by all polyclonal and monoclonal antisera. In contrast to 120 kDa, reduced GPIIIa (108 kDa) was not recognized by AP3 and SSA6. The 120 kDa did not dissociate in the presence of 6M urea and 5 mM EDTA. The component migrating with an apparent molecular weight of 120 kDa also appeared during prolonged storage of purified 60 kDa component. The partial purification of 60 kDa and 120 kDa components together with GPIIIa was accomplished by Con A sepharose chromatography. In conclusion 60 kDa and 120 kDa platelet membrane components represent products of proteolysis of GPIIIa.

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