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EXTRACELLULAR CALCIUM AND PLATELET GLYCOPROTEINS. I. Jabbal-Gill, G.I. (1) Johnston (2) and S. Heptinstall, (1). Department of Medicine, University of Nottingham, Nottingham, U.K. (1) and Department of Medicine, University of Texas, San Antonio, Texas, U.S.A. (2).

Platelet membrane glycoproteins IIb and IIIa form Ca^{++}-dependent heterodimer complexes that contain binding sites for fibrinogen and therefore are relevant to the ability of platelets to aggregate together. In this study we investigated the effects of extracellular Ca^{++} on the stability and expression of IIb-IIIa complexes using a IIb-IIIa complex-specific monoclonal antibody M148. Its specificity was examined using crossed immunoelectrophoresis: the antibody reacted only with intact IIb-IIIa complexes and not with either glycoprotein alone.

SDS-polyacrylamide gel electrophoresis of immunoprecipitates of soluble glycoproteins that interacted with M148 showed that IIb and IIIa were present as complexes in Ca^{++}-depleted media at 25°C, pH7.4. However, Ca^{++}-depletion at 37°C, pH7.4 or 37°C, pH8.7 or 25°C, pH8.7 caused dissociation of the complex.

The effect of extracellular Ca^{++} on the expression of IIb-IIIa complexes on the surface of intact platelets was studied by a technique which is based upon indirect binding of M148 using a fluorescent- labelled second antibody (FITC-RAM) and measuring the fluorescence per platelet using the FACS IV cytofluorometer. Intact platelets were incubated in Ca^{++}-depleted media at 25°C, pH7.4 or 37°C, pH7.4 either (i) prior to or (ii) after adding M148. At 25°C increased M148-binding was observed, compared to the value prior to Ca^{++}-depletion. This increased binding could be reversed by adding Ca^{++} back to the preparation. Under condition (i) at 37°C a marked decrease in M148 binding was observed, which could not be reversed by restoring Ca^{++}, while under condition (ii) at 37°C the results were the same as at 25°C.

Our studies demonstrate that (a) Ca^{++}-depletion at 37°C and/or alkaline pH causes dissociation of the IIb-IIIa complex (b) Ca^{++} depletion at 25°C possibly alters distribution of the complexes thereby increasing their availability to the antibody and (c) M148 prevents the dissociation of complexes in Ca^{++}-depleted media at 37°C, possibly by holding IIb and IIIa together.

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PLATELET MEMBRANE GLYCOPROTEINS ABNORMALITIES IN MYELO-PROLIFERATIVE DISORDERS. STRUCTURE/FUNCTION RELATIONSHIP. M. Mazzucato (1), M.G. Del Ben (1), A. Casonato (2), V. De Angelis (1), and De Marco L. (1). Centro Immuno-trasfusionale U.S.L. n. 11 Pordenone (1) and Patologia Speciale Medica II Universita' di Padova (2), Italy.

The platelet membrane glycoproteins (GP) Ib and GPIIb/IIIa were investigated in 10 patients with myeloproliferative disorders. 2 patients had essential thrombocytemia (ET), 2 had chronic myeloid leukemia (CML) and 6 policytemia vera (PV). The number of GP molecules were detected by radiolabelled monoclonal antibodies anti GPIb and anti GPIIb/IIIa complex (gift of dr. Z.M. Ruggeri) and their function was evaluated by using, in a binding assay, purified radiolabelled asialo von Willebrand factor (125I ASvWF) and purified radiolabelled fibrinogen (125I F). Binding isotherms were evaluated by Scatchard type analysis using the computer assisted program Ligand. The binding of 125I anti GPIb to the platelets of the ten patients showed 14,955 ± 4,636 molecules/platelet (M/Plt) compared to 19,798 ± 3,791 M/Plt of 11 normals with a p value < 0.01. The binding of 125IAsvWF to the GPIb of nonstimulated platelets in platelet rich plasma (PRP) was then measured and found to be decreased. The dissociation constants (Kds) were within normal values except in one patient. There was a good correlation ($r = 0.91$, $p < 0.01$) between the amount of 125I ASvWF bound and GPIb molecules. The binding of radiolabelled anti GPIIb/IIIa to the platelets of six patients (4 with PV and 2 with CML) was measured and found to be constantly decreased in all patients with a mean value of 25,349 ± 2,077 M/Plt compared to 43,192 ± 6,354 M/Plt found in normals ($p < 0.01$). 125I fibrinogen binding to the GPIIb/IIIa complex of ADP + adrenalin stimulated washed platelet was studied in 2 patients and we found 16,267 M/Plt and 14,752 M/Plt respectively, significantly diminished when compared to the mean value of 36,591 M/Plt found in 2 normal controls. The Kds were within normal values. Our studies demonstrate a significant decrease of GPIb and GPIIb/IIIa on the platelet membrane of patients with myeloproliferative disorders. Furthermore this decrease is accompanied by a diminished binding of both vWF and F to their platelets receptors. These findings may partly explain the hemorragic tendency often encountered in these patients.

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ANALYSIS OF THE FUNCTIONAL ROLE OF PLATELET MEMBRANE GLYCOPROTEINS WITH MONOCLONAL ANTIBODIES. H. Nagata, S. Nomura, K. Oda, T. Kokawa and K. Yasunaga. The First Department of Internal Medicine, Kansai Medical University, Osaka, Japan.

Eight monoclonal antibodies were obtained which recognized platelet surface antigens of these, 5 (NNKY1-32, NNKY2-5, NNKY2-6, NNKY2-11, NNKY2-18) recognized GP Ⅱb-Ⅲa complex, 2 (NNKY5-4, NNKY5-5) recognized GP Ⅰb and 1 (NNKY1-19) recognized CD 9 antigen. They were used to research the platelet membrane antigens.

Monoclonal antibodies that recognize CD 9 antigen, which exists on the surface of platelets, acute lymphoblastic leukemia cells, eosinophils and other tissue, are known to act as an aggregating agent to platelets and NNKY1-19 was fond to induce platelet aggregation accompanied by ATP release. NNKY5-4 had no effect on platelet functions. NNKY5-5 inhibited aggregation induced by ristocetin but had no effect on aggregation induced by ADP, collagen, thrombin, and NNKY1-19. NNKY1-32, 2-5, 2-6, 2-11, and 2-18 inhibited aggregation induced by ADP, collagen, thrombin, and NNKY1-19, although slight release of ATP was recognized when NNKY1-19-induced aggregation was completely inhibited by NNKY1-32. Mutual inhibition of binding to platelet membranes between the 3 groups of monoclonal antibodies was not recognized.

NNKY1-19-induced aggregation was associated with a lag time that was plolonged in inverse proportion to antibody concentration. Aspirin had almost no effect on NNKY1-19-induced aggregation. A TXA_2 receptor antagonist, a calcium-channel blocking drug and EDTA inhibited NNKY1-19-induced aggregation. These results indicate that GP Ⅰb, GP Ⅱb-Ⅲa complex and the cyclooxygenase pathway are not involved in NNKY1-19-induced platelet activation, that the target of NNKY1-19 on the platelet membrane is same as that of TXA_2, and that the mechanism of activation by NNKY1-19 is related to calcium flux.

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A GENETIC VARIANT OF PLATELET MEMBRANE GLYCOPROTEIN Ib ASSOCIATED WITH A MILD BLEEDING DISORDER. M. Meyer (1), I. Schellenberg (1), B. Hofmann (2) and G. Vogel (3). Department of Medical Genetics (1), Institute of Pathological Biochemistry (2) and Department of Internal Medicine (3), Medical Academy Erfurt, G.D.R.

Mild bleeding symptoms in a female patient were shown to be related to defective platelet function: Aggregation induced by ADP and PAF was decreased and platelet spreading was disturbed. Platelet membrane glycoproteins were analyzed by various electrophoretic procedures (SDS electrophoresis, nonreduced-reduced 2-dimensional and high resolution 2-dimensional electrophoresis). These studies revealed a decreased concentration of normal glycoprotein Ib (GP Ib) and the appearance of an additional glycopeptide with an apparent M_r of 160.000 under reducing conditions. This component was strongly labeled in intact cells by periodate-^{3}H-sodium borohydride. Staining characteristics of the additional glycopeptide were also similar to those of GP Ib. Immunoblotting using a monospecific anti-glycocalicin antiserum provided conclusive evidence for a structural variant of GP Ib molecularly characterized by an increase of about 20.000 in apparent M_r of the large subunit. The abnormal glycopeptide is cleaved by the endogenous calcium-dependent neutral protease and the resulting glycocalicin exhibits the same increase in M_r as intact variant GP Ib. The abnormal glycopeptide was also detected in 3 relatives of the proposita. All of the carriers are obviously heterozygous for the underlying mutant gene.