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REDISTRIBUTION OF PLATELET GLYCOPROTEINS INDUCED BY ALLO- AND AUTOANTIBODIES. S. Santoso, V. Kiefel, C. Mueller-Eckhardt
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It is now well established that two of the major membrane glycoproteins (GP) of human platelets, GP Ib and IIb/IIIa, are functionally prominent for adhesion, aggregation and carry the binding sites for all known types of human platelet specific antibodies (ab). Although a number of in vitro effects of ab on platelet function have been described, the role of the GP specificity of the various ab with regard to membrane mobility and redistribution phenomena is as yet unknown. In this work, we studied the effect on platelet membrane redistribution of allo-ab, auto-ab and a quinidine-dependent ab directed against various epitopes on GP Ib, IIb and IIIa using immunofluorescence and a quantitative radioimmunoassay. The platelet GP's carrying the corresponding epitopes were determined using immunoblot technique or radioimmuno-precipitation. When unfixed platelets were incubated with allo- or auto-ab against epitopes on GP IIb or GP IIIa cap formation and internalization of antigen-antibody complexes were visualized by fluorescence. In contrast, no changes of antigen distribution were seen with auto-ab or quinidine-dependent ab directed against GP Ib. To quantitate antigen-antibody complexes internalization a specially designed radioimmunoassay was employed. If unfixed platelets were treated with allo- or auto-ab against GP IIb or GP IIIa precipitous reduction of external radioactivity was found, whereas the total radioactivity remained essentially unchanged. This indicated that a portion of approximately 50-70% of GP IIb or GP IIIa had been removed from the platelet surface and had been internalized. Internalization could not be induced with auto-ab or quinidine dependent ab against GP Ib. We conclude that membrane redistribution of human platelets can be induced by various human ab with specificity for GP IIb and/or IIIa and is a function of the target GP rather than the source of the respective ab.

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AN EPITOPE OF A MONOCLONAL ANTIBODY (TM83) AGAINST GLYCOPROTEIN IIb/IIIa COMPLEX. N. Yamamoto, H. Kitagawa, K. Tanoue and H. Yamazaki. Department of Cardiovascular Research, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

GPIIb/IIIa is forming a heterodimer complex on the human platelet membrane. Many monoclonal antibodies against GPIIb/IIIa complex obtained elsewhere react with neither GPIIb nor GPIIIa separately when GPIIb/IIIa is blotted to filter after SDS-PAGE. Therefore the epitope of GPIIb/IIIa complex is not identified actually. Our attemption is to clarify the epitope recognized by a monoclonal antibody against GPIIb/IIIa designated TM83. TM83 (30 µg/ml) inhibited collagen-, ADP-, or thrombin-induced aggregation, but it did not inhibit ATP-secretion induced by 0.01 U/ml of thrombin. TM83 also inhibited fibrinogen-binding approximately to 50 % of total binding. The binding of ^{125}I-TM83 to platelets decreased to 40% of control when platelets were incubated in the presence of 1 mM EDTA at 37°C for 30 min. However the incubation at 25°C for 30 min did not change any binding capacity of ^{125}I-TM83 to platelets. Thus the binding of TM83 to platelets was dependent on both temperature and calcium concentration in surrouding medium, suggesting that TM83 bound to GPIIb/IIIa complex.

If the small amounts of epitope of GPIIb/IIIa complex is not injured during SDS-PAGE and blotting, we may identify clearly the epitope of GPIIb/IIIa complex. For this aim, GPIIb/IIIa complex was extracted carefully in the presence of 1 mM calcium by the phase separation using Triton X-114, and was run on SDS-PAGE in the presence of 100 µM calcium. Western-blot of the membrane preparation showed that ^{125}I-TM83 was incorporated into both GPIIb and GPIIIa on Durapore filter. Further radio-crossed immunoelectrophoresis showed that ^{125}I-TM83 was incorporated only into immunoprecipitin of GPIIb/IIIa complex in the presence of 1 mM calcium. While after addition of 25 mM EDTA to the membrane preparation containing 1 mM calcium, ^{125}I-TM83 was mainly incorporated into GPIIb/IIIa complex as well, however very faint radioactivity from the immunoprecipitin corresponding to GPIIIa was observed, but the radioactivity from GPIIb was not identified. While there is a discrepancy in our result, it must be further studied whether one monoclonal antibody can recognize or not two kinds of GPs, GPIIb and IIIa, which are formed in complex in physiological condition.

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DIFFERENT INTEGRITIES OF GP IIb/IIIa COMPLEX ARE REQUIRED FOR ADP- OR THROMBIN-INDUCED AGGREGATIONS. K. Tanoue and H. Yamazaki. Tokyo Metropolitan Inst. of Med. Sci., Bunkyo 113, Japan.

A relation between the integrity of platelet GP IIb/IIIa complex and aggregability by ADP or thrombin was studied on intact platelets with EDTA-induced irreversibly dissociated GP IIb/IIIa complex. Human platelets were washed once and suspended in Ca-, Mg-free HEPES-Tyrode's solution (pH 7.4). Aliquots of the suspensions were incubated with 2mM EDTA at 37°C for 2 to 60 min. Control platelets were incubated at 22°C. Then, 4mM $CaCl_2$ were added to the samples, which were incubated for another 30 min at 37°C. The platelets were washed twice with HEPES-Tyrode's solution (pH 6.7). For the measurement of amounts of GP IIb/IIIa complex, the platelets were solubilized with 1% Triton X-100 to 4 X 10^6/µl, five µl of which were subjected to crossed immunoelectrophoresis using constant volumes of anti-platelet antibody. The areas under the immunoprecipitates of GP IIb/IIIa complexes were measured as the amounts of GP IIb/IIIa complex. For the aggregation studies, the platelets were suspended in HEPES-Tyrode's solution (pH 7.4). ADP-aggregations were measured in the presence of added 1mg/ml fibrinogen and 2mM Ca. Platelets incubated with EDTA or $CaCl_2$ at 22°C showed the same amounts of GP IIb/IIIa complex. The relation between the amounts of GP IIb/IIIa complex and aggregability by ADP or thrombin were in Tab.;

Incubation Time (min)	0	2	5	15	30	60
GP IIb/IIIa Complex (%)	100		60.3	22.0	19.9	11.8
Aggregation (%)						
ADP(10µM)	100	50.9	36.0	0	0	0
ADP(100µM)	100	52.5	16.3	0	0	0
Thrombin (0.05U/ml)	100	97.1	71.1	60.1	50.7	45.4

ADP-aggregability declined more rapidly than the decrease in GP IIb/IIIa complex. In contrast, thrombin-aggregation were much better maintained than ADP-Aggregation during the incubation with 2mM EDTA. These results suggest either that the integrity of GP IIb/IIIa complex required for ADP-aggregation is more strict than for thrombin-aggregation, or that thrombin-aggreagtion can be caused by an alternative mechanism which does not require the integrity of GP IIb/IIIa complex.

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PLATELET CONTENTS OF VON WILLEBRAND FACTOR ANTIGEN (vWF:Ag) AND FIBRINOGEN (I) AND PLATELET FUNCTION (PF) IN MYELODYSPLASTIC SYNDROMS (MS). S. Meschengieser, A. Blanco, N. Maugeri, J. Fernández, M. de Tezanos Pinto, M. Lazzari. Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Buenos Aires, Argentina.

Bleeding in MS is usually associated with thrombocytopenia, but may occur in patients (P) with a normal platelet count (PC) and defects in the release (R) reaction and the arachidonic acid (AA) pathway. We investigated the PF and contents of vWF:Ag and I in 6 P with diagnosis of: Acquired Idiopathic Sideroblastic Anemia (2), Refractory Anemia (2) and Refractory Anemia with Excess of Blasts (2). Laboratory tests included: PC; bleeding time (BT)(Ivy); platelet retention to glass beads (PR); platelet aggregation (PA) and R induced by ADP(2.5µM), epinephrine (E)(0.1mM), collagen (C) (1-8ug/ml), AA(0.8-1mM) and A23187 (A)(19µM); spontaneous PA (SPA); generation (G) and response (Re) to thromboxane A_2 like material (TXA_2); vWF:Ag and I (electroimmunoassay)in platelet lysates. Results: PC were normal in 6/6, BT were prolonged in 3/6, PR were reduced in 4/6 , PA induced by E were absent in 4/6 and abnormal in the other 2, absence of secondary PA induced by ADP were observed in 4/6, PA induced by C and AA were abnormal in 5/6. No SPA were observed in 6/6. ATP R were absent with ADP and E in 4/6, with C and AA in 5/6 and abnormal with A in 2/6. The G of TXA_2 were absent or decreased and the Re to TXA_2 were normal in all the P. vWF:Ag ($\bar{X}$: 0.088U/10^9cells) and I ($\bar{X}$: 16.8µg/10^9cells) were decreased in 6/6 P and the mean values were lower ($p<0.001$) than controls ($\bar{X}$: 0.284U/10^9cells and $\bar{X}$: 61.2µg/10^9cells respectively). The PF abnormalities observed are similar to those described by other authors, but the decrease of vWF:Ag and I have not been previously reported. These low levels could be the result of platelet activation or megacaryocyte dysfunction.There was no evidence of platelet hyperactivity in our P. So, we assume that defective megacaryocytopoiesis with an alteration in the storage of the granules contents could be responsible for the depletion observed.