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ELECTRONMICROSCOPIC STUDIES ON PLATELETS AND MEGAKARYOCYTES IN GIANT PLATELET SYNDROME. Sozo Suzuki, Kazuo Mori, Koji Sugai, Yasuyuki Akutsu, Masaaki Ishikawa, Hideaki Sakai and Katsuhide Hiwatashi. Third Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan.

Giant platelet syndrome are characterized morphologically by many giant platelets associated with several functional abnormalities in the peripheral blood. However, the mechanism of large platelet production has not yet been clarified. In 1981, we reported acase with Bernard-Soulier syndrome(BSS) in whom giant platelets were considered to be formed by fusion of two or three platelets in the circulating blood. We examined the ultrastructure of platelets and megakaryocytes in another case with BSS (29 year-old female) and a case with May-Hegglin anomaly (31 year-old male). Whole blood and bone marrow specimens were fixed with glutaraldehyde-osmium solution. Thin sections were prepared and stained with uranyl acetate and lead cytrate. Membrane systems of platelets and megakaryocytes in a case with BSS was investigated by staining of surface coating with ruthenium red.

In a case with BSS, most platelets were very large and similar in morphology to those in formerly reported case. Giant platelets contained several-fold increased number of α-granules and mitochondria. Typical dense bodies were also observed. Contents of ATP/ADP, platelet factor-4(PF-4), ß-thromboglobulin(ß-TG) and platelet factor-3 availability(PF-3) were increased. Disorganization of microtubules was recognized. Some giant platelet contained membrane systems similar to demarcation membranes(DM) in megakaryocytes, characteristically. In mature megakaryocytes, areas divided by DM similar in size to those in normal megakaryocytes were observed. Several of these areas appeared to fuse together to form the giant platelets containing many granules and remnants of DM. In a case with May-Hegglin anomaly, typical Döhle's bodies were shown in neutrophilic granulocytes. Giant platelets in this case also contained large number of α-granules and some of them contained membrane systems similar to DM. Areas similar in morphology to these giant platelets were clearly noted in the cytoplasm of mature megakaryocytes.

In these cases, most giant platelets in the peripheral blood may be formed in the cytoplasm of megakaryocytes by fusion of several areas divided by DM, each of which may become normal sized platelets in normal megakaryocytes.

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BERNARD-SOULIER SYNDROME: WHOLE BLOOD DIAGNOSTIC ASSAYS OF PLATELETS. W.L. Nichols, S.E. Kaese, D.A. Gastineau, L.A. Otteman and E.J.W. Bowie. Mayo Clinic/Foundation, Rochester, MN, U.S.A.

Diagnosis of Bernard-Soulier syndrome (BSS) is complicated by the difficulty of separating the giant platelets from other blood cells to pursue analyses of platelet function and structure. We report on the utility of three whole blood assay techniques for diagnosis of a patient with BSS. To our knowledge, these three techniques have not been simultaneously applied or compared for efficacy in laboratory diagnosis of BSS. (1) Whole blood platelet aggregation responses, studied with an electrical impedence aggregometer, were equivalent to those more laboriously obtained using platelet-rich plasma prepared by unit gravity sedimentation, studied with an optical light transmittance aggregometer. Platelet aggregation responses were normal with ADP or collagen stimulation, and absent with Ristocetin or bovine plasma stimulation. (2) Whole blood radioimmunoassay of platelet glycoprotein (GP) expression was performed using iodinated murine monoclonal antibodies HP1-1D (anti-GP IIb/IIIa) and 6D1 (anti-GP1b, kindly supplied by Dr. Barry Coller, Stony Brook, NY). After incubation with citrated whole blood, centrifugation was used to separate cell-bound antibody which was quantitated with a gamma counter. The patient's whole blood had a normal level of cell-bound GP IIb/IIIa, but a markedly reduced level of cell-bound GP Ib (5% of normal mean; n = 20). (3) Whole blood smear immunocytochemical staining with the monoclonals (indirect immunoalkaline phosphatase technique), and qualitative analysis by light microscopy, revealed a marked reduction of GP Ib expression by the patient's giant platelets, whereas GP IIb/IIIa expression was normal. This latter technique might be especially valuable as a screening technique when the patient is not directly available for laboratory study. Together with the patient's life-long history of thrombocytopenia and moderate bleeding diathesis, and other laboratory observations including markedly prolonged bleeding times and reduced whole blood prothrombin consumption, these data established diagnosis of BSS. We conclude that these three relatively simple assays of platelets in whole blood should be of particular value in the laboratory differential diagnosis of patients with congenital thrombocytopenias and giant platelet syndromes.

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MEGAKARYOCYTES FROM THE MARROW OF A PATIENT WITH BERNARD-SOULIER SYNDROME LACKED GP Ib AND WERE DEFICIENT IN GP IX. P. Hourdillé (1), F. Belloc (1), E. Heilmann (1), M. Pico (2), A.T. Nurden (3). (1) Laboratoire d'Hémobiologie, Hôpital Cardiologique, Bordeaux, France, (2) Haemostasis Section, Hosp. Vall d'Hebron, Barcelona, Spain. (3) U-150 INSERM/UA 334 CNRS, Hôpital Lariboisière, Paris, France.

Although it is recognized that glycoprotein (GP) Ib/IX complexes are deficient in the platelets of patients with the Bernard-Soulier syndrome (BSS), the nature of the genetic defect remains unknown. We have looked for these GPs in permeabilized megakaryocytes (MK) of a BSS patient employing immunofluorescence (IF) or immunocytochemical procedures combined with electron microscopy. The study involved the use of monoclonal antibodies AP-1 (anti-GP Ib), AP-2 (anti-GP IIb-IIIa) and FMC 25 (anti-GP IX), gifts from Drs. T. Kunicki and M. Berndt respectively. Bound IgG were revealed by biotinylated anti-mouse IgG followed by Texas Red-streptavidin and MK identified in IF by a double-staining procedure using a polyclonal antibody to fibrinogen (Fg). Platelet morphology was typical of BSS with a high percentage of "giant" platelets. Flow cytometry confirmed that platelets of all sizes were negative for AP-1 and FMC 25 but normally bound AP-2. MK from a marrow aspirate obtained by sternal puncture were concentrated on a Percoll gradient. Electron microscopy showed the MK to be of normal size with a normal granule distribution. However, a striking feature was an irregular distribution of the demarcation membranes which often had a vacuolar appearance. Whereas all permeabilized MK from normal individuals were strongly fluorescent with AP-2, AP-1 and FMC 25, those from the BSS patient were negative for AP-1, weakly positive for FMC 25 and normal for AP-2. Incubation of intact cells with AP-1, with bound antibody located by anti-IgG bound to gold particles, confirmed the absence of GP Ib from the surface membranes of BSS MK. Our results show that the platelet membrane GP defect in BSS results from an abnormal synthesis and/or stability of both GP Ib and GP IX in the MK.

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ACQUIRED STORAGE POOL DISEASE IN DECOMPRESSION SICKNESS. H. Yamazaki (1), K. Tanoue (1), K. Kuroiwa (1), H. Suzuki (1), M. Shibayama (2) and Y. Mano (2). The Tokyo Metropolitan Institute of Medical Science (1) and Department of Public Health, Tokyo Medical and Dental University (2), Tokyo, Japan.

The presence of hemostatic abnormalities has been reported in decompression sickness. It is suggested that platelets recognize air bubbles in the blood stream as a foreign surface and adhere to them with ensuing platelet aggregation. It is important to determine if consumption of platelets occurs in vivo to understand a role of platelets in the genesis of decompression sickness. To analyse the problem, platelet behavior was studied in 34 rabbits with decompression sickness which was brought about by the exposure to 6 ATA (atmospheres absolute) for 40 min followed by rapid decompression. All rabbits died within one hr after the decompression due to apnea which always preceded the cardiac arrest. Platelet counts decreased significantly during the time course of decompression. A regression line can be drawn between the changes in platelet count (Y) and the time after decompression (X): $Y=100.2 - 0.8X$, $r= -0.876$, $p<0.001$. Platelet counts measured just before the apnea were 56 to 72% (65.7 ± 6.1%) of the precompression value. Kinetic studies with ^{111}In-oxine-labeled platelets revealed shortened survivals of the circulating platelets and autoradiograms indicated the accumulation of radioactivity in the lungs after the decompression. Although there was no change in the mode volume of platelets after the decompression, the transient appearance of smaller or fragmented platelets suggested a random over-destruction of platelets. Whole and releasable adenine nucleotide contents of platelets decreased significantly after the decompression. There were no significant changes in cytoplasmic adenine nucleotide contents. Therefore, in decompression sickness, the circulating platelets behaved similarly to those of acquired storage pool disease. Platelet thrombi were found in the pulmonary artery, compatible with the accumulation of the labeled platelets. These findings suggest that circulating air-bubbles interact with circulating platelets, causing the platelet release reaction, and these activated platelets participate in the formation of thrombi in decompression sickness.