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COMPARISON OF THE MAJOR MEMBRANE GLYCOPROTEINS OF HUMAN, RABBIT AND RAT PLATELETS. B. Toor, J.L. McGregor, K.J. Clemetson, L. McGregor, M. Dechavanne and E.F. Lüscher. INSERM Unité 63, Fac. de Med. Alexis Carrel, Lyon, France and Theodor Kocher Institute, University of Berne, Switzerland

Rabbit and rat platelets have been extensively investigated under in vitro or in vivo conditions to try to understand the pathology of thrombosis in man. Here, surface-labelling techniques have been used to find out if the platelet surface has a similar composition in these two animals and in man or not. Human, rabbit and rat platelets were isolated, washed and surface-labelled by techniques specific for protein or for sugars (sialic acid or penultimate galactose/N-acetyl galactosamine residues). Labelled platelets were solubilized in sodium dodecyl sulphate and separated under reducing conditions on 7.5 % Laemmli polyacrylamide gels. Dried gels were exposed to film by fluorography or indirect autoradiography. Terminal Gal/Gal NAc residues (no neuraminidase treatment) were strongly labelled with rat and rabbit platelets compared to human platelets which labelled very poorly. Terminal sialic acid labelling with rat and rabbit platelets showed a weak labelling of a glycoprotein (GP) with the same M.Wt. as GPIb which is the most intensely labelled GP in man. However two GP (with rabbits) and one GP (in rats) were intensely labelled at a M.Wt. similar to that of GPIa in man. These GP had a different M.Wt. with terminal Gal/Gal NAc labelling. Bands with a similar M.Wt. to GPIIb and IIIa in man were strongly iodinated with rabbit platelets but with rat platelets only a single band at the position of GPIIb was strongly iodinated. These results strongly indicate that there are considerable differences in surface composition between rabbit, rat and human platelets.

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LECTIN BINDING DEMONSTRATES DIFFERENT GLYCOPROTEIN ABNORMALITIES IN THROMBASTHENIC AND BERNARD-SOULIER PLATELETS. Philip A.Judson and David J.Anstee. Department of Immunochemistry, South Western Regional Blood Transfusion Centre, Bristol, U.K.

We have previously shown that the lectins from Maclura aurantiaca (Ma) and Arachis hypogaea (Ah) (peanut) bind selectively to GP-I in human platelet membranes. In contrast Concanavalin A lectin binds primarily to GP-III. GP-I is deficient or absent from the membranes of individuals with Bernard-Soulier Syndrome (B-S.S.) while GP-II and III are deficient or absent from Thrombasthenic (G.T.) platelets. We have investigated the binding of radioiodinated lectins to SDS polyacrylamide gels of whole platelets from patients with B-S.S. and G.T. The object of this study was to define further the nature of the glycoprotein abnormalities in these conditions.

The results clearly demonstrated a marked reduction in the binding of Ma and Ah lectins to the GP-I region of B-S platelets when compared to that of normal platelets. No new lectin binding components were observed. The binding of Con A lectins to B-S platelets did not appear to be significantly different from that to normal platelets. In contrast Ma and Ah binding to G.T. platelets appeared normal whereas the binding of Con A to GP-III was markedly reduced. Two faint Con A binding bands were apparent in the GP-III region of the G.T. platelets. It is not clear whether either of these represent residual GP-III or if they are minor components masked by GP-III in normal platelets. The carbohydrate of GP-I in normal platelets consists almost entirely of O-glycosidically linked oligosaccharides. Ah and Ma lectins bind selectively to galactosyl and N-acetylgalactosaminyl residues in this type of oligosaccharide. We conclude that B-S platelets have a gross deficiency of O-glycosidically linked oligosaccharides. Con A binds selectively to branched N-glycosidically linked oligosaccharides with a mannose-rich core. We conclude that such structures normally present on GP-III are grossly deficient in G.T. platelets.

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MEMBRANE GLYCOPROTEIN DEFECTS IN BERNARD-SOULIER SYNDROME PLATELETS. K.J. Clemetson, J.L. McGregor, E. James, M. Dechavanne and E.F. Lüscher. Theodor Kocher Institute, University of Berne, Switzerland and Faculté de Médecine, Alexis Carrel, Lyon, France.

It is well established that Bernard-Soulier syndrome (BSS) platelets are deficient in a major membrane glycoprotein (Ib). In order to investigate if this is the only defect in this disorder and to see if the β-subunit of glycoprotein Ib is also diminished, platelets from 3 BSS patients and from healthy donors were isolated, washed and surface labelled by lactoperoxidase-catalysed iodination, periodate/NaB^3H_4 or neuraminidase/galactose oxidase/NaB^3H_4. Labelled platelets were solubilized in sodium dodecyl sulphate and separated by 2-dimensional gel electrophoresis (isoelectric focusing, discontinuous polyacrylamide gel electrophoresis). Glycoprotein Ibα was virtually absent in 2 patients and strongly decreased in the third patient. The β-subunit was also absent in the 2 patients and present at about 40 % of normal in the third. Glycoprotein IIbβ was present normally in all patients. In addition, a further low molecular weight glycoprotein with a M.WT. of 17,000 and a pI of 6.8-7.5 was absent or present at levels paralleling glycoprotein Ibβ. The thrombin cleavable glycoprotein (GP IV or V) appeared greatly diminished with BSS platelets labelled by carbohydrate specific methods though no difference could be seen with iodination. This finding was confirmed in a fourth BSS patient using one dimensional gel electrophoresis.

The defects in BSS platelets are thus more complex than previously thought.

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FURTHER CHARACTERIZATION OF PLATELET MEMBRANE GLYCOPROTEINS. P. Clezardin, J.L. McGregor, K.J. Clemetson, M. Dechavanne and E.F. Lüscher. INSERM Unité 63, Fac. de Med., Alexis Carrel, Lyon, France and Theodor Kocher Institute, University of Berne, Switzerland.

The binding of ^{125}I-labelled lectins to major and minor platelet glycoproteins (GP) and their subunits has been investigated. Human platelets were isolated, washed, solubilized in sodium dodecyl sulphate (SDS) under non-reducing conditions and separated on 5, 7.5 and 10 % non-reduced/reduced 2-D polyacrylamide gels. The gels were incubated with ^{125}I-labelled lectins: Lens culinaris lectin (LCL), concanavalin A (ConA) wheat germ agglutinin (WGA) or Ricinus communis agglutinin (RCA-120), then washed extensively, dried and exposed to X-ray film by indirect autoradiography. Surface-labelled platelets were similarly separated. WGA and RCA bound predominantly to GPIbα but also to two minor bands above and below it which were affected by neuraminidase treatment. One of them bound two ^{125}I-lectins (LCL and ConA) while GPIbα did not. Additional GP bands were detected by lectin binding and by surface-labelling beneath GPIIIb (IV). With platelets labelled by the neuraminidase/galactose oxidase/NaB^3H_4 method a GP was detected between IIa and IIIa which was not found with periodate/NaB^3H_4 labelling (not affected by reduction). Two spots on the diagonal bound LCL and ConA. GP Ibβ bound LCL more strongly than IIbβ. GPIbβ also bound WGA and RCA. GPIcβ apparently bound only ConA. GPIbβ and IIbβ were labelled equally strongly by surface labelling techniques, Icβ was apparently not labelled. Further GP subunits were detected one below Ibβ and IIbβ and another which originated in the GPVII region. These techniques demonstrate that the platelet surface is even more complex than previously thought.