826

TOPOLOGY OF PLATELET GPIb INVESTIGATED BY LOCATION OF MONOCLONAL ANTIBODY EPITOPES. K.J. Clemetson (1), S. Weber (1) and J.L. McGregor (2). Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-312 Berne, Switzerland (1) and Fac. de Méd. Alecia Carrel-INSERM U63, Lyon, France (2).

A large number of monoclonal antibodies to platelet membrane glycoprotein Ib (GPIb) have been described but for most of these the position of the epitope is not known. Since many of these influence platelet function, a better understanding of structure-function relationships requires this knowledge. The position of the epitopes for the monoclonal antibodies AP1 (Dr. T.J. Kunicki), AN51 and SZ-2 (Dr. C-G. Ruan), WM23 (Dr. M.C. Berndt) and P1 were determined by analysis of proteolytic cleavage fragments of glycocalicin via affinity chromatography on the monoclonal antibodies coupled to Sepharose, elution with diethylamine solution, separation on SDS-gel electrophoresis and detection by silver-staining. First, intact glycocalicin was examined and was found to bind to all monoclonals with the exception of P1. All monoclonals bound intact GPIb. WM23 bound a 70 kDa glycopeptide from the highly-glycosylated 90 kDa tryptic fragment of glycocalicin. AP1, AN51 and SZ-2 all bound to 45 kDa and 40 kDa, poorly glycosylated tryptic fragments. The 40 kDa fragment is derived from the 45 kDa fragment and has been shown to be the N-terminal region of GPIb. All these monoclonals have been shown to inhibit von Willebrand factor induced platelet agglutination. Platelets were treated with either elastase or calcium activated protease and monoclonal binding checked by immunofluorescence. The immunofluorescence with AP1, AN51 and SZ-2 was minimal compared to control platelets whereas that of P1 remained as strong as the controls. This indicates that the epitope for P1 lies on GPIb in a region other than glycocalicin and its absence from glycocalicin is not simply due to conformational changes in that fragment. Since P1 inhibits platelet activation by thrombin and ADP it must act via conformational effects and not by blocking the thrombin receptor which lies on the 45 kDa region of glycocalicin. These results support a more complex role for GPIb in platelet activation.

827

THE DISTRIBUTION OF GP Ib AND THE STABILITY OF THE PLASMA MEMBRANE ARE DEPENDENT UPON AN INTACT MEMBRANE SKELETON. J.K. Boyles (1), J.E.B. Fox (1), M.C. Berndt (2). Gladstone Foundation Laboratories, Department of Pathology, University of California, San Francisco, CA, U.S.A. (1) and Department of Medicine, University of Sydney, Westmead Hospital, Sydney, New South Wales, Australia (2).

Platelets are know to have a cytoskeleton of actin filaments. We have presented evidence that they also have a membrane skeleton linked to the cytoskeletal filaments and that the membrane skeleton is linked to GP Ib-IX on the plasma membrane via actin-binding protein. In the current study, electron microscopy of thick (0.2 µm) epoxy sections was used to identify the distribution of GP Ib. After various treatments, platelets were fixed and incubated with affinity-purified GP Ib antibody and colloidal gold-labeled Protein-A. The entire cell surface was covered with a network of short intersecting chains of relatively evenly spaced gold particles. This was true of platelets in blood dripped directly from a vein into fixative, of washed discoid platelets, and of platelets activated by thrombin, ionophore, or cold under conditions in which aggregation did not occur. This pattern was not affected by the size of the gold label, the immunocytochemical protocol, or the fixative. The number of gold particles per cell was between 10,000 and 20,000, indicating a 1:1 ratio of label to GP Ib. The distribution of GP Ib was not affected by a level of cytochalasin B sufficient to disrupt the actin filaments of the platelet cytoskeleton. Proteolysis of actin-binding protein is known to be induced by treatment of platelets with dibucaine and by platelet activation (with either ionophore or thrombin) under conditions in which cell aggregation occurs. These same treatments caused GP Ib to cluster. They also produced platelets with unstable membranes that vesiculated when the cells were subjected to shear force during centrifugation or osmotically stressed during fixation. These studies show that both the distribution of GP Ib and membrane stability are dependent upon the integrity of actin-binding protein and the membrane skeleton. In the high-shear environment of the blood vessel, the membrane skeleton and its linkage to GP Ib-IX and the cytoskeleton may be essential for proper platelet function.

828

EXPRESSION OF A PLATELET GLYCOPROTEIN Ibα RELATED PROTEIN IN THE HUMAN HISTIOCYTIC CELL LINE U937. A.N. Wicki(1), N. Kieffer (2) and K.J. Clemetson (1). Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-3012 Berne, Switzerland (1) and Institut National de la Santé et de la Recherche Médicale U.91, Hopital Henri Mondor, 94010, Créteil, France.

It has previously been shown that platelet glycoprotein Ib is expressed in a minority of cells of the human leukemic cell line HEL (Tabilio et al., EMBO J. 3, 453-459). The present study was performed to determine wheter U937 cells also share membrane proteins with platelets. Indirect immunofluorescence on histiocytic U937 cells with F(ab)2 fragments of polyclonal anti GPIb antibodies and with different monoclonal antibodies revealed a uniform staining of the cell surface. A single polypeptide chain of apparent molecular mass 60,000 was precipitated under reducing and nonreducing conditions from nonanoyl-N-methyl glucamide solubilized, surface labelled and metabolically labelled U937 cells by the different antibodies. Competitive immunoprecipitation performed in the presence of an excess amount of unlabelled platelet glycocalicin, a large fragment of GPIbα, completely displaced the 60 kDa polypeptide. We were unable to demonstrate the presence of a polypeptide corresponding to the α-subunit of GPIb. Therefore, we conclude that U937 cells produce a 60,000 Da protein which is immunologically closely related to platelet GPIbα.

829

IDENTIFICATION OF GLYCOPROTEIN Ib IN "IN VITRO" TRANSLATES FROM ISOLATED HUMAN PLATELET mRNA. A.N. Wicki, A. Walz and K.J. Clemetson. Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-3012 Berne, Switzerland.

Platelet membrane glycoproteins play a crucial role in platelet adhesion and activation. To understand how they function it is of great importance to know their amino acid sequences and structures. It is rather difficult to purify membrane glycoproteins in amounts that are sufficient to determine their amino acid sequences by protein sequencing techniques. The easier way seems to be molecular cloning of the genes for these proteins.

Metabolically stable mRNA derived from nucleated megakaryocytes is known to be present in the anuclear human platelets. We have developed a purification method for the isolation of platelet mRNA. Starting with 100 units of blood platelets we isolated 0.5 mg of mRNA by guanidine chloride/lithium chloride/phenol extraction. Crude mRNA as well as oligo-dT purified, polyadenylated mRNA, was assayed for protein synthesis in a reticulocyte lysate translation system in the presence of different labelled amino acids. SDS-PAGE of nonreduced and reduced samples of the translation products showed molecular masses up to at least 200 kDa. Immunoprecipitation and affinity column chromatography with poly- and monoclonal anti platelet glycoprotein Ib antibodies showed that a protein with a molecular mass of 60 kDa cross-reacting immunologically with glycocalicin is specifically recognized by these antibodies. The 60 kDa protein seems to be the nonglycosylated form of GPIbα.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.