1103

VINCULIN ISOFORMS IN HUMAN BLOOD PLATELETS. G.M. Asijee (1), T. Bruin (1), A. Sturk (1), J.W. ten Cate (1), and L. Muszbek (2). Div. of Hemostasis and Thrombosis, Academic Medical Center, Amsterdam, The Netherlands (1), and Dept. of Clinical Chemistry, Medical School, University of Debrecen, Hungary (2).

In several cells vinculin has been implicated in the interaction between the cytoskeleton and the outer cell membrane. We have recently demonstrated that vinculin becomes associated with the Triton X-100 insoluble cytoskeleton of blood platelets upon thrombin-induced activation (Asijee et al, Exp Cell Res, 1987, in press). In the present study we demonstrate by SDS-PAGE and immunoblotting that vinculin is also present in the membrane skeleton of both non-activated and activated human blood platelets. The membrane skeleton was prepared by the method of Fox (J Clin Invest, 1985: 76, 1673), and platelets were stimulated 5 min at 37 °C with 0.1 U/ml thrombin. The association was specifically inhibited by DNase I-induced depolymerization of the actin filaments.

Protein analysis by the O'Farrell technique (first dimension IEF, second dimension SDS-PAGE) and subsequent immunoblotting demonstrated purified vinculin to consist of 4 major isoforms (pI 6.8 - 7.2). These isoforms differed in subcellular distribution. Upon thrombin-induced platelet activation, cytoskeletal vinculin consisted of the 2 most acidic isoforms, and cytoplasmic vinculin of 2 more basic isoforms. The membrane skeleton-associated vinculin contained all 4 isoforms.

We conclude that: 1. vinculin is a component of the membrane skeleton of both non-activated and activated human blood platelets, 2. similar to chicken gizzard smooth muscle cells, human blood platelet vinculin consists of several isoforms with differing subcellular distribution.

1104

A STUDY OF HUMAN PLATELET SUBPOPULATION HETEROGENEITY IN FRACTIONS SEPARATED BY CONTINUOUS FLOW ELECTROPHORESIS (CFE). M. Crook and N. Crawford. Department of Biochemistry, Royal College of Surgeons of England, London, U.K.

Earlier density gradient studies showed that the circulating platelet pool has substantial heterogeneity with respect to size, buoyant density and metabolic and functional properties. The interpretation of this heterogeneity is, however, controversial; some workers attributing it to in vivo ageing processes, whilst others consider it relates more closely to the megakaryocyte ploidy classes at the time of platelet production. Such considerations are important for further understanding of disease states where the heterogeneity profile may be distorted by the presence of megathrombocytes or other atypical platelet forms due to changes in the thrombopoeitic equilibrium (e.g. idiopathic or drug-induced thrombocytopaenias, splenomegaly and leukaemia etc). We describe a new procedure for investigating platelet heterogeneity using a continuous flow method to isolate subpopulations on the basis of differences in surface membrane electrokinetic properties. To maintain the cells quiescent during the isolation and CFE they have been pre-treated with taxol, a microtubule stabilising agent. Taxol maintains platelet discoidicity, yet the cells show full functional responses to conventional agonists. The heterogeneity profile emerging from the CFE chamber extends over 15-20 fractions and spans a range of electrophoretic mobility (measured with a Laser Doppler Analytical apparatus) between 0.83-0.93 μmetres/sec/V. cm. Pooled fractions from the profile show differences in total and surface neuraminidase-labile sialic acid which correlate well with their electrokinetic properties. Interestingly, DTNB-titratable -SH groups in surface membrane proteins show an inverse relationship to platelet electronegativity as do phosphate-labile phosphate groups. There are significant differences in platelet size (Coulter volume) with the larger platelets being more electronegative in terms of unit charge density than the smaller ones. This novel procedure for studying platelet subpopulations on the basis of surface electrokinetic properties may also have value in profiling platelets from patients with specific platelet defects, during extracorporeal circuitry, transplant rejection and in vessel wall diseases.

1105

INFLUENCE OF CALCIUM FLUX ON STABILITY OF PLATELET MICROTUBULE COILS. Gundu H. R. Rao, Ph.D. and James G. White, M.D., Departments of Laboratory Medicine and Pathology, Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455.

Resting human platelets have a characteristic discoid form supported by a circumferential microtubule (MT). Ultrastructural, immunocytochemical and immunofluorescence studies have shown that the circumferential MT is a stable structure which undergoes constriction following exposure of platelets to aggregating agents. However, some biochemical and morphological studies suggested that MT coils dissolved almost completely within seconds after exposure to aggregating agents, then reassembled 1-4 minutes later. One investigation suggested that disappearance of the MT coils was associated with calcium flux caused by agonist stimulation or exposure to the ionophore, A23187. The present study has examined the latter hypothesis directly. Fura 2, a calcium sensitive fluorophore, was loaded into washed platelets at a concentration of 1 μM, the cells washed once and resuspended in HEPES buffer. Fluorescence changes were monitored in a Fluorolog spectrofluorometer. Thrombin stimulation of Fura 2 loaded platelets resulted in an immediate eight fold increase in the level of cytoplasmic calcium. The calcium ionophore, ionomycin, stimulated a 15 fold rise in the cytoplasmic calcium of Fura 2 loaded cells. Samples of thrombin and ionomycin stimulated platelets were fixed in glutaraldehyde and osmic acid at the peak of calcium flux indicated by the rise in fluorescence. Examination of activated platelets in the electron microscope revealed shape change and internal transformation. MT coils were constricted, but did not disappear from platelets fixed during the maximum elevation of cytoplasmic calcium. The findings do not support the concept that the calcium rise produced in platelets following exposure to potent agonists causes disappearance of the circumferential MT.

1106

PLATELET EXTERNAL SURFACE MEMBRANE IS OSMOTICALLY DOUBLED IRRESPECTIVE OF SIZE OR SPECIES (HUMAN/BOVINE): DYNAMICS AND MEMBRANE SOURCES. Mony M. Frojmovic[1], Truman Wong[1], Jane Wylie[1] and J.G. White[2]. [1]Department of Physiology, McGill University, Montreal, Canada H3G 1Y6 and [2] Departs. of Lab Med. and Path., Pediatrics, Univ. of Minnesota, Minneapolis, Minnesota 55455.

Osmotic swelling can double the external plasma membrane surface area of human platelets independently of size, proposed to recruit the open surface-connected canalicular system ((SCCS) (Thrombos. Res. Suppl. VI: 119, 1986). As bovine (B) platelets have been reported to lack SCCS, we compared osmotic swelling for B and human (H) cells. Addition of water to platelet-rich-plasma (10-90% v/v) caused sequential shape change and osmotic spherocyte (OS) formation, analyzed for size and surface area changes from time-dependent phase-contrast videomicroscopic images. Selected samples were fixed and stained with tannic acid prior to osmic acid fixation for visualization of open SCCS by transmission electron microscopy. B platelets required 3-4x less water dilution of PRP than H platelets, with significant OS forming at 20% water addition. Continued water dilution converted 50% of platelets to OS, with maximally stable swelling and no significant lysis for bovine OS up to 60% dilution. Electron micrographs of unactivated discocytes (D) and of optimally-swollen OS showed open SCCS in human D not detectable in any of the swollen platelets, though granules, mitochondria and a small number of vesicles and vacuoles persisted; no evidence for any open SCCS was found for bovine D or OS, though the OS otherwise appeared similar to H-OS. Geometric measurements of D and nonlysed OS showed a stable, maximal 2.1±0.1 fold increase in external plasma membrane surface area with osmotic swelling, identical for different-sized H platelets (mean volume = 2.8-6.8 fl) or for B platelets (3.6 fl). B platelets show equal or greater sensitivity for ADP-induced activation as H platelets, with 2-fold slower maximal rates of recruitment in early aggregation. As osmotic swelling appears to primarily externalize SCCS in H platelets, the identical relative amounts of internal membrane externalized for B platelets is hypothesized to arise from an osmotically more labile, "closed", and structurally simpler SCCS or from a distinct membrane source than in H platelets.