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IN VITRO CHARACTERIZATION OF THE PROCOAGULANT ACTIVITY WITHIN THE BRONCHOALVEOLAR COMPARTMENT OF NORMAL HUMAN LUNG. D.S. Fair (1), H.A.Chapman (2), C.L. Allen (2) and R. Yee (2). Univ. Texas Health Center, Tyler, TX, U.S.A. (1) and Brigham and Women's Hospital, Boston, MA, U.S.A. (2)

The procoagulant activity (PCA) of the normal human bronchoalveolar (BA) lining layer was examined qualitatively and quantitatively. Unconcentrated, cell-free lavage freshly obtained from normal volunteers clotted whole plasma in 84 ± 20 sec. The PCA was initiated by factor VII (F VII) and tissue factor (TF) complexes as judged by differential activities in various plasmas genetically deficient in single clotting factors and by neutralization of the PCA with antibodies to either F VII or TF. The cell-free fluid contained about 8500 thromboplastin units/mg total protein. The fluid also contained F VII activity estimated by clotting activity to be 2 ng of plasma F VII/ml of lavage fluid or 22 ng/mg total protein compared to 7 ng/mg protein for normal human plasma. Amidolytic activity measurements suggested that the F VII concentration was 0.57 ng/ml of lavage fluid. Because of the increased amounts of F VII expressed in the lung relative to plasma, we investigated the activation state of the F VII by the ratio of clotting to amidolytic (VIIc/VIIam) activities. The ratio of unfractionated alveolar fluid was about 19, suggesting the presence of the more active two-chain F VIIa. However, immunoblots of concentrated lavage protein revealed only single-chain F VII and ^{125}I-F VII added to the fluid was not converted to ^{125}I-F VIIa. Additional studies showed that the enhanced PCA of lavage fluid F VII over that of plasma was due to a factor(s) in the sedimentable fraction of the lining layer which directly increased the prothrombinase activity of exogenous, purified factor Xa and V/Va and phospholipid (PL). Although normal alveolar macrophages express TF and F VII, we estimate that the cells contribute < 15% of the total PCA within the alveolar compartment. However, these cells are the only suitable PL surface for prothrombinase complex formation within the lavage material, suggesting that macrophages are a major site for thrombin formation within alveoli. Thus, it appears the enhanced rates of thrombin formation rather than an increase in the rate of factor Xa generation explain the accelerated clotting times and the high F VIIc/VIIam ratio, and that macrophages may play a significant role in the total PCA output observed in the lung.

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HEPARIN BINDING TO HUMAN MONOCYTES: MODULATION BY HISTIDINE-RICH GLYCOPROTEIN. L. Leung, K. Saigo and D. Grant. Stanford University School of Medicine, Division of Hematology, Stanford, CA, U.S.A.

Heparin and its related glycosaminoglycans interact with a variety of cell types and, irrespective of their anticoagulant activities, have a complex and biologically important influence in blood vessel wall biology. In this study, the binding of heparin to the human monocytoid cell line U937 was characterized. ^{35}S-Heparin bound to U937 cells in a specific, concentration-dependent, and saturable manner, while binding to human erythrocytes was minimal. The binding was rapid, reaching a steady state at 30 min at 4°C and was reversible. Excess unlabeled heparin, but not chondroitin sulfate or dermatan sulfate, inhibited heparin binding, demonstrating the specificity for heparin. Scatchard plot analysis revealed a single class of heparin binding sites, with an apparent Kd of 0.32 uM and approx. 2.0 x 10^6 sites/cell. When binding was performed at 37°C, significant amount of cell-bound heparin was not removed by cell surface trypsinization, indicating endocytosis of heparin by U937 cells. Taken together the binding characteristics suggested the presence of specific heparin receptors on U937 cell surface. Histidine-rich glycoprotein (HRGP), a potent heparin-binding protein found in human plasma and platelets, decreased the affinity of heparin for U937 cells without a significant change in maximal binding. This negative modulation of heparin binding was specific for HRGP since antithrombin III was much less effective. While EDTA abolished the HRGP-heparin interaction, it had no effect on the HRGP modulation of heparin cell binding, suggesting that HRGP interacted directly with the U937 cell and altered its affinity for heparin. Specific binding of labeled HRGP to U937 cells was demonstrated. Cell-surface bound heparin markedly accelerated the inactivation of thrombin by antithrombin III. Its anticoagulant potency was equivalent to heparin in the fluid phase. Human monocytes and macrophages provide an optimal cell surface for the assembly of prothrombinase and the generation of thrombin. The specific binding of anticoagulantly active heparin to monocytes and macrophages may modulate the procoagulant properties of these cells at sites of inflammation and thrombosis. Furthermore, since heparin fractions with high or low affinity for antithrombin III bound equally well to U937 cells, heparin may have a direct effect on monocyte cell biology. The current study also raises the possibility that the significant modulation of heparin cell binding by HRGP may represent a major function for this protein in vivo.

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STUDIES OF PLATELET AND LEUKOCYTE ATTACHMENT TO WHOLE HUMAN ARTERY SEGMENTS. Anna E. Schorer, Debra J. Johnson and James G. White. Minneapolis VA Medial Center and the University of Minnesota, Minneapolis, MN, U.S.A.

Activation of endothelial cells (EC) by inflammatory stimuli can result in heightened procoagulant properties towards coagulation proteins. These alterations can be readily studied in existing tissue culture models which lack a component of flow (shear). It is generally believed that thrombosis in the venous (low shear) regions of the vasculature are relatively dependent upon the coagulation proteins, whereas clots in the arterial system (high shear) are more dependent upon platelet plugs. Adhesion of the platelets to vessel wall elements under controlled conditions of flow have also been addressed in a number of models, such as the "Baumgartner chamber". In this model, blood or platelets are perfused over denuded, everted vessel segments, usually rabbit aortae. We have modified this model to study attachment of platelets to arterial segments of human origin which retain an intact layer of native EC. Arteries are aseptically removed from umbilical cords, trimmed, washed, and gently everted. The segments are placed in Medium 199 with 20% calf serum and standard growth supplements. After 4-10 days, these segments are placed on rods and perfused with whole citrated blood (800 sec^{-1}). Such segments permit attachment of single platelets, but not platelet aggregates. Identical segments from which EC are mechanically removed promote attachment of large (>5 cell) platelet thrombi. Endothelialized segments which are pre-treated (16 hours) with thrombin or phorbol myristate acetate show reduced attachment of platelets. In selected regions, clusters of leukocytes are attached to these segments, either upon or beneath the still- confluent layer of EC. Artery segments treated with bacterial endotoxin (1 mcg/ml) for 90 minutes, by contrast, show enhanced attachment of platelets, both as a monolayer of platelets and as small (2-5 cell) aggregates. This novel application of the Baumgartner assay demonstrates that platelet and leukocyte attachment to confluent EC may occur under conditions of moderate shear, and may be regulated in part by EC, which in turn may be modulated by exogenous mediators.

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CHARACTERIZATION OF THE FUNCTIONAL ADHESION PROPERTY OF THE MONOCYTE FIBRINOGEN RECEPTOR WITH A MONOCLONAL ANTIBODY (Mab) DIRECTED TO THE ACTIVATED STATE OF THE PLATELET GLYCOPROTEIN (GP) IIb/IIIa.

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We recently showed that human blood monocytes bind fibrinogen through a genuine surface antigen only in part similar to the platelet GP IIb/IIIa. Moreover, some anti-GP IIb/IIIa Mabs cross-react with monocytes. In this study we used the 7E3 Mab which preferentially binds to the activated conformation of the platelet GP IIb/IIIa to characterize the dynamic mechanism of "exposure" of the monocyte fibrinogen receptor. 7E3 Mab (25 μg/ml) completely suppressed the binding of ^{125}I-fibrinogen to ADP (10μM)-stimulated monocytes. However, differently from the platelet GP IIb/IIIa, ^{125}I-7E3 binding to unstimulated monocytes was a non-specific and non-saturable reaction. In contrast, after stimulation with ADP (10 μM), suspensions of human monocytes bound ^{125}I-7E3 with saturation of 25-30 μg/ml of added Mab. Scatchard plot analysis was a single-affinity straight line revealing 97,400 binding sites/monocyte with a dissociation constant of 5.2x10^{-8} M. The monocyte surface antigen uniquely expressed after ADP-activation recognized by 7E3 was visualized by immunoprecipitation studies. Surface iodinated platelet lysate subjected to immunoprecipitation with 7E3 revealed a single band with molecular weight (M_r) of 116,000 corresponding to the platelet GP IIb/IIIa. In contrast, monocytes showed a dimeric surface antigen precipitated by 7E3 in two subunits with M_r=155,000 and 95,000 respectively. These data indicate that the adhesion properties of the monocyte fibrinogen receptor defined by an anti-platelet GP IIb/IIIa cross-reacting Mab are structurally and functionally distinct from those of the platelet receptor.