

REGULATION OF VASCULAR PROSTACYCLIN (PGI<sub>2</sub>) SYNTHESIS. S. Coughlin, M. Moskowitz, H.N. Antoniades, L. Levine. M.I.T., Cambridge, MA; Harvard Medical School, Boston, MA; Center for Blood Research, Boston, MA; Brandeis University, Waltham, MA.

We have examined the possibility that substances released during platelet degranulation modify vascular PGI<sub>2</sub> synthesis. PGI<sub>2</sub> is a potent inhibitor of platelet function produced by vascular endothelial and smooth muscle cells. Regulation of PGI<sub>2</sub> synthesis by blood vessels is not well understood. We report that a platelet-dependent factor in serum dramatically stimulates PGI<sub>2</sub> synthesis by vascular endothelial and smooth muscle cells in culture. We further report that platelet-derived growth factor (PDGF), a releasable protein found in platelet alpha granules, stimulates PGI<sub>2</sub> synthesis by the above cell types by over 100 fold. The concentration of PDGF required to elicit this effect is below that reported in human serum. The above mentioned serum factor is relatively heat stable, non-dialyzable, and cationic; preliminary studies indicate that anti-PDGF antiserum is capable of blocking stimulation of PGI<sub>2</sub> synthesis by both PDGF and serum. These data suggest that the serum factor may indeed be PDGF. PDGF acts synergistically with other platelet granule constituents (serotonin, ATP) and with thrombin to stimulate PGI<sub>2</sub> synthesis by vascular cells in culture. We thus postulate that platelet-released PDGF, in concert with other substances generated during clotting, acts to increase vessel wall PGI<sub>2</sub> synthesis as part of a negative feedback mechanism controlling platelet aggregation. A defect in the ability of a blood vessel to increase PGI<sub>2</sub> production in response to platelet degranulation, as may occur in atherosclerotic vessels, could perhaps contribute to the genesis of thromboembolic events.

ALTERED FACTOR VIII ANTIGEN: A SENSITIVE INDICATOR OF ENDOTHELIAL DAMAGE. A.C.A. Carvalho, S. Bellman, J. Saullo, J. Lauer and W.M. Zapol. Roger Williams Gen Hospital, Providence, RI and Massachusetts Gen Hosp, Boston, MA, USA.

Acute respiratory distress syndrome (ARDS) is characterized by pulmonary vascular injury and altered endothelial permeability. Since Factor VIII antigen (VIII:Ag) is synthesized by endothelial cells, we studied FVIII:Ag and coagulant (VIII:C) activities in 100 patients with ARDS. Factor VIII:C was determined by one-stage assay, FVIII:Ag by quantitative immunoelectrophoresis and FVIII complexes by cross-immunoelectrophoresis (CIE). We studied 56 patients with mild acute lung injury (lobar infiltrate, mortality 30%), 30 with moderate ARDS (mortality 60%), and 14 with severe ARDS (mortality 90%) secondary to various etiologic agents. The results are expressed in % (Mean±SEM) as shown below:

Group	(n)	FVIII:C	FVIII:Ag	Ag/C
Normal	28	95±6	134±11	1.2±0.07
Mild ARDS	56	190±15	487±50	3.5±0.5
Mod. ARDS	30	190±21	590±60	3.8±0.5
Severe ARDS	14	171±25	567±80	4.0±0.5

In all ARDS groups, there was a marked increase in FVIII:Ag by 4- to 5-fold the normal average. By CIE, both slow- and fast-moving components of FVIII:Ag displayed abnormal patterns. In moderate and severe ARDS, there was an increase in the fast-moving component of FVIII:Ag, mimicking vonWillebrand's defect; this FVIII change may result in hemorrhage in the lung and elsewhere. In contrast, in mild ARDS there was an increase in FVIII:Ag slow-moving component which may account in part for the increase in pulmonary micro and/or macrothrombi formation. Recovery from ARDS resulted in return towards normal of FVIII:Ag patterns. Thus, FVIII:Ag appears to be a sensitive indicator of pulmonary endothelial injury and repair. In addition, our data strongly suggests that excessive concentrations and altered FVIII:Ag patterns may contribute to the thrombo-hemorrhagic complications commonly seen in acute lung injury.

THE EFFECTS OF ESTROGEN ON VON WILLEBRAND FACTOR PRODUCTION AND ENDOTHELIAL CELL NUMBER IN CULTURE. R.L. Harrison and P.A. McKee. Howard Hughes Med. Inst., Duke Univ. Med. Ctr., Durham NC, USA.

Subconfluent and confluent endothelial cell monolayers derived from human umbilical veins were treated with pharmacologic levels of estrogen and compared functionally to control monolayers. The levels of von Willebrand ristocetin cofactor (vWF) present in media from cell cultures after three days of incubation were assayed by ristocetin-induced platelet aggregation (PA) as well as by a radioreceptor platelet assay (RR) for vWF. DNA content of each monolayer was determined fluorometrically as a measure of cell number. The incorporation of <sup>3</sup>H-thymidine into 17β-estradiol (E2)-treated endothelial cell monolayers vs. control cultures was also quantitated. Whether measured by PA or RR, levels of vWF in media from E2-treated cells were reproducibly and significantly greater (1.2±0.33 μg vWF/ml/μg DNA at 2 ng/ml E2) than in media from control endothelial cells (0.69±0.084 μg vWF/ml/μg DNA, p<0.01) and showed a dose-response effect with respect to the E2 added to the culture media. Compared to control cultures, E2-treated monolayers consistently contained greater DNA (2.0±0.10 μg DNA) than control cultures (1.7±0.12 μg DNA, p<0.001) and incorporated more <sup>3</sup>H-thymidine into trichloroacetic acid insoluble material (30.2±6X10<sup>3</sup> CPM with E2, vs. 21.2±2X10<sup>3</sup> CPM control, p<0.05). However, differences in cell number between E2-treated and control monolayers did not account for the elevated vWF levels in the presence of exogenous E2. We conclude that estrogen has at least two effects on endothelial cell cultures: 1) estrogen directly stimulates the cells to increase their rate of production of vWF; and 2) estrogen causes an increased rate of endothelial cell replication.

PURIFICATION, CHARACTERIZATION AND RADIOIMMUNOASSAY OF A NEW ANTIHEPARIN PROTEIN SECRETED BY RABBIT PLATELETS. R. Muggli, B. Glatthaar, E. Mittelholzer and T.B. Tschopp. Pharma Research and Diagnostic Departments, F. Hoffmann-La Roche & Co. Ltd., 4002 Basel, Switzerland.

We isolated a platelet specific protein from rabbits for studying the usefulness of such proteins as marker for in vivo platelet activation in animals.

The protein was purified by chromatography on heparin-agarose and on Sephadex G75 from the soluble release products of thrombin aggregated washed rabbit platelets. On heparin-agarose the protein eluted in front of rabbit PF<sub>4</sub> at a salt concentration of about 0.9 M NaCl, and had a MW of about 20,000 dalton as judged by gel filtration. The purified protein migrated in SDS PAGE in the presence or absence of mercaptoethanol as a single band with a MW of <12,000 dalton. It had a heparin-neutralizing activity of 0.16 mg Polybrene/mg and a pI of >9.5.

A radioimmunoassay with second antibody precipitation was developed. Antisera were produced in goats. Plasma and serum levels were 26 ± 6 ng/ml (n = 16,  $\bar{x}$  ± SD) and 2900 ± 960 ng/ml (n = 8), respectively. Washed platelets contained 2.0 ng of the protein per 10<sup>6</sup> platelets. The protein was released in parallel to platelet aggregation induced by collagen. Plasma levels rose from 26 ± 8 ng/ml (n = 6) to 72 ± 29 ng/ml 30 s after i.v. injection of collagen into rabbits. After injection of the iodinated protein into rabbits (n = 4) the disappearance of radioactivity followed two-compartment kinetics with half-lives of 3.8 min and 137 min for the fast and slow component, respectively. No increase of plasma radioactivity was recorded after injection of 500 U heparin per rabbit after 3 hrs. At that time 51 ± 13 % of the injected radioactivity was recovered in the urine.

This new rabbit platelet secretory protein shows similar but not identical biochemical and in vivo behaviour as human BTG.