

Monday, July 13, 1981

Poster Presentations

White Blood Cells – I

11:00–12:30 h

Grand Ballroom Lobby Boards 225–230

0172

HEMORRHAGE AND EXUDATION IN THE MICROCIRCULATION INDUCED IN RABBITS WITH HUMAN NEUTROPHIL LYSOSOMAL ENZYMES. S. Wasi and H. Z. Movat. Division of Experimental Pathology, Department of Pathology, Medical Sciences Building, University of Toronto, Toronto, Canada.

The purpose of this study was to assess the nature of the lesions in the microcirculation induced in rabbits by lysosomal enzymes derived from human neutrophil leukocytes (PMNs). PMNs were isolated from ACD blood. Their lysosomal contents were specifically released upon incubation with immune precipitates of bovine serum albumin (BSA)-anti BSA. Several enzymes were purified by means of cation exchange chromatography (SP-Sephadex) and gel filtration (Sephadex G-200). Two parameters of microvascular injury were quantitated: hemorrhage with ^{59}Fe -labelled erythrocytes and vascular permeability with ^{125}I -labelled serum albumin. The crude lysosomal lysate induced both hemorrhage and exudation. Several enzymes were characterized with respect to their size, charge and inhibition patterns with specific proteolytic inhibitors. When crude lysosomal lysate was treated with diisopropylfluorophosphate, MeO-Suc-Ala-Ala-Pro-Val-NMec, or heated, both hemorrhage and exudation were inhibited. Hemorrhage was also partially inhibited by p-hydroxy mercury benzoate and leupeptin. Hemorrhage and exudation were produced by at least two different enzymes: one more basic, with an approx molecular weight (MW) of 23,000–27,000 daltons and one more acidic with an approx. MW of 69,000–76,000 daltons. Small doses of the enzymes induced only increase in exudation and large doses induced exudation, followed by hemorrhage. With the aid of ultrastructural studies it was demonstrated that the increase in vascular permeability was due to leaky vessels through which electron dense tracers escaped, whereas hemorrhage occurred through disruption of basement membranes and necrosis of endothelial cells. Proteases, when released from PMNs, can induce lesions of the microcirculation. Mild lesions are characterized by leaky vessels and severe lesions by vascular wall disruption.

0173

THE SOURCE OF THROMBOXANE FORMATION ASSOCIATED WITH COMPLEMENT-MEDIATED PULMONARY LEUKOSTASIS IN SHEEP. J.W.D. McDonald and M. Ali, University of Western Ontario, London, Ontario, Canada. J.D. Cooper and E.R. Townsend, University of Toronto, Toronto, Ontario, Canada.

The infusion of plasma containing Zymosan-activated complement (ZAC) into sheep produces leukopenia with pulmonary leukostasis and transient pulmonary arterial hypertension (PAH). Previous work has related PAH to elevations of plasma thromboxane B_2 (TXB_2) rather than to mechanical obstruction by sequestered leukocytes (WBC). We have investigated the source of the TXB_2 formation in this model. Incubation of platelet-poor WBC preparations with arachidonate resulted in negligible TXB_2 formation. WBC-poor platelet preparations on the other hand formed significant amounts of TXB_2 (approximately 6–18 ng/ 10^8 platelets). Incubation of whole sheep blood or plasma with ZAC failed to generate significant amounts of TXB_2 . Thus, WBC agglutination *in vitro* did not induce platelet TXB_2 formation.

Pretreatment of sheep with aspirin (ASA) (10 mg/kg IV) completely blocked TXB_2 formation and PAH in response to infusion of plasma containing ZAC. The infusion of 10–50% normal platelets into sheep 4 hours after ASA pretreatment failed to restore TXB_2 formation and pulmonary vascular response to subsequent challenge with ZAC. TXB_2 formation during blood clotting *ex vivo* was restored by the platelet infusions. These experiments make it appear unlikely that platelets are the source of the TXB_2 . It is possible that the transfused platelets respond to thrombin but are unable to interact with sequestered leukocytes. Sheep lung and pulmonary artery were incubated *in vitro* with arachidonate. Lung formed 630 ng TXB_2 and 39 ng 6-keto-PGF $_{1\alpha}$ /g of wet tissue. Pulmonary artery formed 9 ng TXB_2 and 180 ng 6-keto-PGF $_{1\alpha}$ /g of wet tissue. The relative proportions of TXB_2 and 6-keto-PGF $_{1\alpha}$ formed by lung parenchyma but not pulmonary artery resemble the proportions observed in previous *in vivo* experiments with ZAC. It appears that lung tissue is the most likely source of TXB_2 formation causing PAH in response to ZAC-mediated pulmonary leukostasis.

0174

COAGULATION DEFECTS DUE TO INACTIVATION OF PLASMA INHIBITORS BY GRANULOCYTIC ELASTASE. M. Jochum, St. Lander and H. Fritz. Department of Clinical Chemistry and Clinical Biochemistry, Surgical Clinic of the University of Munich, Munich, GFR.

Neutral proteinases such as elastase and cathepsin G are liberated from secretory granules of PMN leukocytes in the course of diseases like septicemia, acute myelocytic leukemia or emphysema. Despite the presence of a high antiproteinase potential (α_1 -antitrypsin, α_2 -macroglobulin, anti-thrombin III, α_2 -antiplasmin, α_1 -antichymotrypsin) in plasma these proteinases are able to degrade clotting and other plasma factors unspecifically thus contributing to severe coagulation disorders. Evidence is presented that the plasma inhibitors (antithrombin III, α_2 -antiplasmin and α_1 -antichymotrypsin) which are not able to inhibit granulocytic elastase, are rapidly inactivated by this proteinase and may thereby intensify consumption of clotting and other plasma proteins during inflammation.