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ERYTHROCYTES(RBC)AS SUICIDAL ENDOGENOUS SCAVENGERS IN IMMUNE TRIGGERED GRANULOCYTE(PMN)MEDIATED VASCULAR DAMAGE. M.A.Boogaerts, P.Zachée*,M.P.Emonds,W.Goossens,R.L.Verwilghen,R.L.Lins*. University Leuven and A.Z.Stuyvenberg*Antwerp,Belgium.

PMN produced toxic oxygen radicals(TOR)have been implicated in the generation of endothelial injury in a number of clinical conditions e.g. in apheresis,hemodialysis,ARDS and atherosclerosis. RBC have shown to inhibit TOR induced damage in a number of hyperoxic lung injury models.We surmised RBC may serve as endogenous TOR scavengers in those in vivo situations where PMN are immunologically triggered(e.g. complement-activation in hemodialysis)to produce endothelial damage.However,RBC in their role as scavengers may become more vulnerable to further oxydant stress and display a reduced life span.Confluent monolayers of ^{51}Cr-labeled human umbilical vein endothelial cells will release 7.5 ± 1.3% of their label upon incubation with complement triggered PMN.When these PMN (1)are premixed with RBC(10),the endothelial damage can be inhibited by 78.4 ± 3.2%.This inhibition can be reproduced by replacing intact RBC by their hemolysates,but not by red cell ghosts.In a ^{51}Cr-RBC cytotoxicity system,phorbolester stimulated PMN will lyse 52.6 ± 4.2% RBC.Addition of unlabeled RBC(1/5),inhibits cytotoxicity by 31.1%,their hemolysate by 100%.Pretreatment of added unlabeled RBC with the anion channel blocker DIDS,did not significantly block the scavenger effect.
RBC-targets from hemodialysis-patients(n=8),are more vulnerable to PMN mediated cytotoxicity than normal controls(+24.1%,$p<0.001$). This vulnerability is further increased(+16.3%,$p<0.05$)during the early stages of the hemodialysis-procedure,at the time when granulocyte counts are lowest and TOR-generation highest.The GSH of hemodialysis-RBC can be more rapidly depleted upon challenge by APH, while they also display a significant higher methemoglobin production upon sodium ascorbate challenge,both indicative of their increased oxidative sensitivity. High dose Vitamin C($10^{-3}M$)or desferioxamine($10^{-3}M$)inhibit all RBC cytotoxicity.
We conclude that RBC serve as endogenous scavengers of TOR,generated by PMN,triggered by complement activation during hemodialysis. However,by doing so,they become themselves more vulnerable to further oxidative stress,which may contribute to the chronic anemia of hemodialysis-patients.

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INHIBITION BY AD6 (8-MONOCHLORO -3-BETA-DIETHYLAMINOETHYL -4- METHYL -7- ETHOXY CARBONYL METHOXY COUMARIN) OF POLYMORPHONUCLEAR LEUKOCYTES ADHESION TO ENDOTHELIAL CELLS

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Increased leukocyte adhesion to the endothelial lining of blood vessels is an essential event in inflammation and the pathogenesis of certain vascular deseases. The monocyte product interleukin-1 (IL-1) has been shown to enhance the adherence of human periferal blood polymorphonuclear leukocytes (PMN) to human umbilical vein endothelial cells (EC). In this study AD6, a coumarin derivative which inhibits platelet aggregation and coronary thrombus formation in experimental animals, was found to inhibit PMN adhesion to control and IL-1-treated EC. Suspensions of washed ^{51}Cr labeled PMN were added to cultured human umbilical vein EC which have been treated either with buffer or IL-1 (10 units/ml for 4 hours). The amount of PMN adherent to 10^5 EC after 5 min incubation was about $2.1x10^5$ to control and $4x10^5$ to IL-1-treated EC. When AD6 was added to the medium during the adhesion experiment PMN adhesion was inhibited in a concentration dependent way. The minimal active concentration of the drug was 0.5μM(20-30% inhibition to both control and IL-1 treated EC) but inhibition was maximal at 500μM (80-90 % inhibition to control and 65-80 % to IL-1-treated EC). AD6 also blocked (90-95 % inhibition at 500μM) PMN adhesion to plastic wells. The drug effect on PMN required at least 5 min incubation to be apparent and declined after 30 min. AD6 pretreatment of either PMNs or ECs followed by washing reduced PMN adhesion to unstimulated or IL-1-treated EC.The effect was however maximal when both cell types were treated with AD6, thus indicating that the inhibitory activity of AD6 was directed at both ECs and PMNs and might be cumulative.

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CHEMOTAXIS OF POLYMORPHONUCLEAR LEUKOCYTES BY VASCULAR PERMEABILITY FACTOR FROM BOVINE PLATELETS. N. Hoshikawa, H. Niino, J. Imura, Y. Ashihara and K. Shirasawa. First Department of Pathology, Kyorin University School of Medicine, Mitaka-shi, Tokyo, JAPAN.

Bovine platelet α-granule acid extract (BPAE) contains vascular permeability factor (VPF) as human platelets. VPF plays as a mediator depending on polymorphonuclear (PMN) leukocytes and possesses a potent cell growth activity in cultured fibroblasts. This study was carried out to examine the chemotactic activity of VPF for PMN leukocytes of rabbits. For the study of chemotaxis, the PMN leukocytes were collected from the ascites after production by an injection of 0.1% oyster glycogen in 0.85% NaCl solution into the peritoneal cavity of rabbits. They were suspended in the Gey's balanced salt solution with supplementation of 2% human albumin. Partial purification of BPAE was performed by gel filteration using Sephadex G-50 so as to exclude the influence of PF-4 and β-TG. BPAE, as the attractant solution, was prepared at the several concentration in the Gey's solution. Human serum activated with zymosan was used as the positive control. Effects of protamine sulfate, a competitive inhibitor of platelet-derived growth factor (PDGF), on the chemotactic activity of VPF were also studied. Chemotaxis of the PMN leukocytes was measured in the modified Boyden chamber with a 3μm-pore filter. Five high power (x400) grids were counted per filter and cell migration was corrected by subtraction of blanks in which the lower compartment contained medium only. The most striking chemotactic response was obtained when BPAE was used at the concentration of 0.1μg/ml. However, the response was reduced at the concentration of more than 1μg/ml. The activity of BPAE (0.1μg/ml) was about 60% of the positive control level. The migration was reduced to 18% of the positive control by protamine sulfate (3μg/ml) at that concentration of BPAE.

It is concluded that VPF induces chemotaxis to PMN leukocytes at the concentration lesser than that enhancing the vascular permeability response. Furthermore, protamine sulfate inhibits the VPF-induced migration of PMN leukocytes. Those findings may indicate that VPF acts as PDGF in function. Therefore, it is likely that the factor attracts PMN leukocytes and promotes cell growth in the inflamed region.

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MEASUREMENT OF ELASTASE-INDUCED FIBRINOGEN-DERIVED PEPTIDES IN VITRO. Th. Eckhardt, S. Haas, B. Lange, H. Pfeiffer. Dep. of Med., JL-University, Giessen,FRG.

Leukocyte elastase (EL) cleaves peptides from the N-terminal fibrinogen (fbg) Aα and Bβ chains. The Aα peptide (Aα 1-21) and the as yet unidentified Bβ peptide contain thrombin (TB)cleavage sites. As we were interested in fbg proteolysis in sepsis and leukemia, we have tried to measure these peptides (generated by incubating a crude granulocyte extract with fbg) using available fibrinopeptide A (FPA) - und Bβ 15-42-antigen-RIA techniques. As the indirect measurement of Aα 1-21 in terms of FPA releasable by TB treatment in vitro (TB-inducible FPA, TIFPA) requires the use of a highly specific anti-FPA-antiserum to avoid cross reaction of Aα 1-21 we tested the specificity of several antisera. Only R_2, provided by Dr. J. Owen, proved suitable whereas two commercial antisera measured 6-15% of Aα 1-21 as apparent FPA. Simultaneous recovery of FPA and Aα 1-21 from plasma using R_2 was between 80-100% after precipitation of cross-reacting fbg by ethanol, whereas bentonite adsorbed Aα 1-21 and the EL induced Bβ peptide. TIFPA was fully recovered after incubation with plasma (37°C, 2 hr) whereas cross-reactivity of Aα 1-21 in the FPA-assay did not increase. EL-induced proteolysis in plasma in terms of TIFPA generation did not occur unless the normal granulocyte-plasma ratio was increased about 300-400 fold. The Bβ 15-42 antigen RIA allowed quantitative measurement of the as yet undefined EL-induced Bβ peptide, since the "Bβ 15-42" concentration measured was equal to the amount of FPB (measured as Bβ 1-13) releasable from the EL-induced Bβ-peptide by TB. The immunoreactivity of this peptide is stable in plasma and completely recovered after ethanol precipitation. This finding suggests that the EL-induced Bβ peptide is longer than Bβ 1-42 and not susceptible to C-terminal degradation of the Bβ41/42 region which is crucial for recognition of the peptide by the antiserum (supported by Deutsche Forschungsgemeinschaft).