7. Inflammation/Mediators – Free Papers

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HYPEROXIA INDUCED CYTOKINE PRODUCTION IN HUMANS BREATHING 100% O₂ FOR 60 MINUTES.

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Objectives: Critically ill patients often require mechanical ventilatory support and increased inspiratory O₂ concentration. Experimentally, continuous breathing of 100% O₂ in animals leads to progressive irreversible damage to the lung, ARDS and death. In critical care units 100% O₂ is often administered for several hours. The mechanism of hyperoxia induced lung injury is unclear. The objective of this study is to explore inflammatory mediator responses following breathing 100% O₂ in humans. Ten healthy subjects aged 32-46 breathed 100% O₂ for 60 minutes. Minute ventilation, arterial O₂ saturation, blood pressure, heart rate and EKG were continually monitored.

Methods: Interleukin-1 beta (IL-1 β) and Interleukin-1 receptor antagonist (IL-1ra) were determined by ELISA in the supernatant of whole blood incubated with Endotoxin by the method of Nerad and Dinarello, before (To), at the end of 60 minutes of 100% O₂ breathing (T₆₀) and at 90 (T₉₀) and 180 (T₁₈₀) minutes following resumption of air breathing.

Results:

pg/ml	O ₂ Breathing		Air Breathing	
	T ₀	T ₆₀	T ₉₀	T ₁₈₀
IL-1ra	22,611	29,488	28,993	26,456
IL-1 β	1,190	2,095	2,292	2,521

Conclusion: Sixty minutes of 100% O₂ breathing in healthy subjects leads to a significant *in vitro* increase in production of IL-1ra (To-T₉₀, p=0.007) and IL-1 β (To-T₁₈₀, p=0.046). It is postulated that hyperoxia induced lung injury is partly mediated through release of inflammatory cytokines, and initiated earlier than previously reported in the literature.

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EFFECT OF DOPEXAMIN ON THE INTERLEUKIN-1 β -STIMULATED SYNTHESIS OF NITRIC OXIDE IN THE RABBIT CAROTID ARTERY

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OBJECTIVES: Catecholamines are major therapeutic agents used to treat the loss of vascular responsiveness in septic shock, which is due, in part, to the cytokine-induced synthesis of nitric oxide (NO) in the blood vessel wall. The expression of inducible nitric oxide synthase in vascular smooth muscle cells is partly mediated by the synthesis of adenosine 3':5'-cyclic monophosphate (cAMP). As catecholamines through the stimulation of β -adrenergic receptors, can activate adenylyl cyclase in vascular smooth muscle cells, the effect of doxepamin (DPX) on the interleukin-1 β (IL-1 β)-induced NO synthesis was examined in rabbit carotid arteries.

METHODS: Rabbit carotid artery rings without endothelium were incubated with IL-1 β (60 U/ml) and/or with DPX (0.1 - 10 μ M) for 6 hours. Thereafter the segments were suspended in organ chambers containing Krebs-Henseleit solution and the cyclooxygenase inhibitor diclofenac (1 μ M). A cumulative concentration-response curve to phenylephrine (PE, 0.01 - 100 μ M) was constructed in the absence and presence of the NOS-inhibitor N^G-nitro-L-arginine (L-NNA, 100 μ M). In addition the tissue level of guanosine 3':5'-cyclic monophosphate (cGMP) (an index of NO-production) was determined in the presence of isobutyl-methyl-xanthine (0.1 mM, an inhibitor of the degradation of cGMP by phosphodiesterases), by radioimmunoassay.

RESULTS: Phenylephrine induced a concentration-dependent contraction of the carotid artery rings. The maximal contraction to PE (10 μ M, E_{max}: 3.7 $\pm$ 0.2 g) was significantly attenuated in the IL-1 β (100 U/ml)- or DPX (10 μ M)-treated rings (IL-1 β : E_{max}: 2.5 $\pm$ 0.2 g; DPX: E_{max}: 2.6 $\pm$ 0.3 g), and to a greater extent in IL-1 β - and DPX-treated rings (E_{max}: 2.2 $\pm$ 0.2 g). The IL-1 β - but not the DPX-mediated attenuation of the PE-evoked contraction was inhibited by L-NNA. In addition, DPX (10 μ M) did affect neither the basal nor the IL-1 β -stimulated production of cGMP.

CONCLUSION: These findings indicate, that both IL-1 β and DPX caused an attenuation of the phenylephrine-mediated vasoconstriction of rabbit carotid arteries. In contrast to IL-1 β , the hypocontraction to PE in DPX-treated carotid arteries was not abolished by L-NNA and was also not associated with the production of cGMP. Thus, the loss of responsiveness in the DPX-treated arteries is unlikely due to an induced synthesis of NO but may possibly involve an alteration of α -adrenergic receptors or the intracellular signal transduction pathways.

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Intratracheally administered histamine increased lavage neutrophil numbers but did not increase acute lung leaks in Tumor Necrosis Factor (TNF)- α treated rats

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Objectives: Histamine increases the rolling of neutrophils via increase of P-selectin expression on the surface of endothelial cells and is known to have some interrelationships with interleukin (IL)-1, IL-8 and TNF- α . We investigated the effect of the histamine on the acute lung injury of the rats induced by intratracheally treated TNF- α .

Design: Randomized prospective animal study

Subjects: Sprague-Dawley rats weighing 280-380 gm

Methods: We intratracheally instilled saline or TNF (R&D, 500ng) or histamine of various dosage (1.1, 11, and 55 μ g/kg) with and without TNF separately, then after 5 h measured the lung lavage neutrophil numbers, lung myeloperoxidase activity (MPO), and lung leak in rats.

Statistical analyses: Kruskal-Wallis nonparametric ANOVA test with Dunn's multiple comparison test

Results: We found that rats given TNF, histamine alone (55 μ g/kg), and TNF with histamine (1.1, 11, and 55 μ g/kg) intratracheally had increased (P<0.05) lung MPO activity compared to saline-treated control rats. TNF and TNF with histamine (1.1, 11, and 55 μ g/kg) intratracheally had increased (P<0.05) the lung leak compared to control rats. TNF with histamine (1.1, and 55 μ g/kg) only had increased (P<0.05) the lavage neutrophil numbers compared to saline or TNF treated rats (0.91 $\pm$ 0.3, 0.82 $\pm$ 0.407 $\times 10^6$ / mL vs 0.055 $\pm$ 0.04, 0.391 $\pm$ 0.179 10^6 / mL, values are mean $\pm$ SEM). But there were no additive effect of histamine with TNF compared to TNF alone in lung MPO activity and acute lung leak in rats.

Conclusion: Histamine administered intratracheally did not have synergistic effects on TNF-induced acute lung leak and lung MPO in spite of additive effect on increase in lavage neutrophil numbers in rats. These observations suggest that instilling histamine intratracheally would not play synergistic roles in neutrophil-mediated acute lung injury in rats.

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ASCORBIC ACID DECREASES THE PERMEABILITY OF MESOTHELIAL CELL MONOLAYER.

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OBJECTIVES: We have previously reported an antioxidant therapy such as ascorbic acid reduces requirement of intravenous fluid for initial management of patients with burn in clinical studies. Since the study implied that ascorbic acid may decrease leakage from intravascular lumen to interstitial space and body cavity, we examined changes in permeability of mesothelial cell monolayer in the presence of ascorbic acid *in vitro*.

DESIGN: The study was performed using an *in vitro* assay system and results were statistically analyzed by ANOVA and Student's *t*-test.

SUBJECTS: Rat mesothelial cells and polymorphonuclear leukocytes. **METHODS:** Rat peritoneal mesothelial cells were cultured on filter membranes of co-culture chambers to form a confluent monolayer.

Assay medium consist of 10⁵ cells/ml polymorphonuclear leukocytes, primed by Phorbol 12-myristate 13-acetate, 1 μ M FITC-labeled 70,000 dextran and 1 mM ascorbic acid was added to the upper chamber, and the concentrations of FITC-dextran that permeated to the lower chamber through the mesothelial cell monolayer were determined in 4 hours and expressed as a percentage of FITC-dextran that permeated through the filter membrane without cells. The drug diluents were used as controls.

RESULTS: One mM ascorbic acid decreased the permeability from 30.61 $\pm$ 0.84% to 18.05 $\pm$ 1.79% (p < 0.05). However, same concentration of ascorbic acid didn't alter the permeability of mesothelial cell monolayer without neutrophils.

CONCLUSION: Since ascorbic acid doesn't alter the permeability of mesothelial cell monolayer directly, it is suggested that ascorbic acid may decrease the permeability of mesothelial cell monolayer by modulating inflammatory process via neutrophils. Further studies will be required to elucidate the mechanism of ascorbic acid effect while a high dose of ascorbic acid might be considered as a part of immediate treatment to prevent third spacing in critically ill patients.

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PLASMA GM-CSF LEVELS AND HLA-DR VALUES ON MONOCYTES IN MODS *D Kammermeier¹, J Birkmann¹, L Blinzler², WM Gallmeier¹, D Heuser²*

Objectives: Low HLA-DR values were shown earlier to be an indicative parameter for the immunological state in septic patients. To minimize the risk of a possible therapeutical intervention with immune-stimulating drugs, additional parameters are useful to differentiate between hyper- and hypoinflammation.

Design: Pilot investigation to estimate a flow-cytometrically independent parameter for the immunological phase diagnosis in MODS.

Patients and methods: Flow-cytometric analysis of leukocytes, cell function assays (phagocytosis, oxidative burst), ELISA of 6 plasma cytokines of 40 patients suffering from either necrotizing pancreatitis or peritonitis derived MODS were monitored 4-12 times during their ICU stay and compared with the clinical courses.

Results: Low HLA-DR expression on monocytes in MODS corresponds with abnormal low plasma GM-CSF levels (0-40 pg/ml) compared to healthy volunteers (>150 pg/ml), often with monocytopenia, and altered monocytes (e.g. CD15 positive, reduced phagocytosis). All patients with persistent low GM-CSF below 40 pg/ml died. Improvement of the clinical course results in an increase of HLA-DR values and in normalization of plasma GM-CSF levels. Severe septic shock corresponds with decreasing HLA-DR values, and often with an increase of the endotoxin receptor (CD14) on granulocytes. High values for IL-6, TNF- α and IL-10 are usually found in these cases.

Conclusions: Evaluation by ELISA of plasma GM-CSF values in MODS provides an additional information of the immune response to flow cytometrical techniques which are not yet standardized.

A monocytic proliferation failure, followed by monocytopenia and maybe immature monocytes with low HLA-DR values and therefore reduced antigen presentation could be a possible result of low GM-CSF stimulation. Sufficiently monitored immune stimulation in the hypoinflammatory phases of the MODS with e.g. interferon- γ or GM-CSF might be useful.

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INTER- α -TRYPSIN INHIBITOR (ITI) IMPROVES HEMODYNAMICS AND OXYGENATION PARAMETERS IN PORCINE ENDOTOXIN SHOCK

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OBJECTIVES: Inter- α -trypsin inhibitor (ITI) is an inhibitor of proteases and cytokines. We investigated the post-treatment effects of ITI on gas exchange, oxygen transport, hemodynamics and endotoxin-induced disseminated intravascular coagulation (DIC) in a porcine model of endotoxin shock.

METHODS: 3 groups of pigs were studied: Control group (n=6), LPS group (n=5) receiving *E. Coli* endotoxin (5 μ g/kg/min over 30min) and LPS+ITI group (n=6) receiving endotoxin and just after 30mg/kg ITI over 30 min. LPS groups were given isotonic saline to maintain mean arterial pressure (MAP) $\geq$ 60mmHg. ITI levels, hemodynamic, oxygenation and coagulation parameters were measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 5 h. ITI urinary degradation products were measured at 5h.

RESULTS: Compared with the LPS group, we observed in the LPS+ITI group: 1) a significant decrease in ITI plasma levels with an increase in ITI urinary degradation products. 2) a normalization of MAP without changes in systemic vascular resistances. 3) a less pronounced decrease in cardiac index (CI). 4) significant differences in oxygen extraction ratio (OER), lactate, pulmonary arterial pressure and resistances. 5) significantly lower thrombin-antithrombin complexes and plasminogen activator inhibitor-1 levels at 5h.

CONCLUSION: In this hypodynamic model of endotoxin shock, ITI administration was followed by a marked improvement in hemodynamics, oxygenation and endotoxin-induced DIC.

Labs: ER 150 Inhibiteurs de la coagulation (F.Fourrier) - EA 1042 Inhibiteurs des sérine protéases (J. Mizon)- Université Lille II
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PROPHYLACTIC ANTI-INTERLEUKIN-1 β MONOCLONAL ANTIBODY THERAPY ATTENUATES THE HAEMODYNAMIC DERANGEMENTS OF OVINE GRAM-NEGATIVE SEPTIC SHOCK

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Objective: To examine the efficacy of anti-interleukin-1 β (IL-1 β) monoclonal antibody (mAb) immunotherapy in a reproducible model of ovine hyperdynamic gram-negative septic shock. **Subjects:** Eight mature, instrumented, awake, female sheep of mean[SD] weight 43[7] kg. **Methods:** At T0h, 4 sheep randomly received murine IgG, mAb against ovine IL-1 β (7 mg/kg over 1 hour) and 4 sheep received the equivalent amount of non-specific protein immediately prior (T0+1h) to an LD₁₀₀ intravenous infusion of live *Escherichia coli* (2.4[0.2] $\times$ 10⁹ organisms/kg over 1 hour). Invasive haemodynamics and plasma IL-1 β , TNF α and IL-8 levels were measured at T0h, T0+1h and at multiple time-points for 24 hours. Gentamicin 3 mg/kg intravenous (T0+3h and T0+15h) and 0.9% saline (4ml/kg/hour maintenance and 10 ml/kg boluses to maintain baseline cardiac filling pressures) were administered. Immediately following death an autopsy was performed and organ injury was graded histologically (score 0 - 3). **Results:** Anti-IL-1 β mAb therapy was associated with a significant and progressive linear increase in mean arterial pressure, cardiac index and systemic vascular resistance relative to control animals ($p < 0.01$, ANOVA). Fluid requirements in the mAb and control groups were 4.5[3.1] and 10.1[1.8] ml/kg/hr respectively ($p < 0.01$, t-test). Pulmonary artery occlusion pressure was unchanged over time and not significantly different between the two groups ($p > 0.01$, ANOVA). Peak IL-1 β levels (T0+6h) were 208[133] and 63[25]pg/ml for the mAb and control groups respectively ($p > 0.01$, t-test). IL-1 β remained elevated in the mAb group (184[124] pg/ml at T0+24h). Two mAb animals survived to 24 hours (survival time of non-survivors 11.5 and 18.5 hours). All control animals died (survival time, 11.5[3.6] hours). The pooled histological organ injury scores for the heart, lung, liver, spleen, kidney and small intestine are shown. The observed group difference achieved statistical significance ($p = 0.04$, χ^2).

Pooled histological injury score across organs (n = 6)	0	1	2	3
Count, mAb group (n = 4 sheep $\times$ 6 organs = 24)	4	5	13	2
Count, control group (n = 4 sheep $\times$ 6 organs = 24)	1	13	6	4

Conclusions: Anti-IL-1 β mAb therapy attenuates observed haemodynamic changes in ovine gram-negative septic shock. The increased organ injury trend in the mAb group may reflect time-dependent histological changes associated with an increased survival time.

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EXTRACELLULAR SUPEROXIDE DISMUTASE (EC-SOD) IN PATIENTS WITH SEPSIS

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OBJECTIVE: Superoxide dismutase (SOD) plays an important antioxidant defence against oxidative stress. Extracellular SOD is the major SOD isoenzyme in extracellular fluids and also occurs in tissues. Nutrient antioxidant concentrations are reduced in patients with sepsis associated with increased oxidant mediated damage and clinical disease severity. We measured EC-SOD in serum from patients with severe sepsis in relation to 28 day mortality. **METHODS:** 32 patients with septic shock were studied. Blood samples were taken within 12 h of the onset of shock. EC-SOD protein was determined by enzyme linked immunosorbent assay.

RESULTS: Serum EC-SOD was significantly elevated in patients who died ($p=0.018$ Mann Whitney U test) Figure.

CONCLUSION: EC-SOD concentrations are highest in the patients who die from septic shock and this may represent either enhanced shedding of enzyme from damaged endothelial cells or an adaptive response to excess concentrations of oxygen derived free radical species.

We are grateful to Bayer Plc for its support of this study and Dr S Marklund for help with the assays.

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PBMC CYTOKINE PRODUCTION AND THE ACUTE PHASE PROTEIN RESPONSE IN FULMINANT HEPATIC FAILURES.J. Wigmore¹, T.S. Walsh², P. Hopton², A. Lee², J.A. Ross¹

Aims: Patients with fulminant hepatic failure (FHF) have elevated serum concentrations of acute phase proteins (APP) such as CRP. Such increases in APP production correlate with serum IL-6 concentrations. This study investigates production of the APP-stimulating cytokines IL-6 and TNF by peripheral blood mononuclear cells (PBMC) and the ability of PBMC supernatants to stimulate CRP production by isolated human hepatocytes.

Methods: 12 patients with FHF (10-paracetamol toxicity, 2-NANB hepatitis) who did not have evidence of sepsis, were studied on admission to the intensive care unit. In 6 patients PBMC were isolated and cultured in the presence and absence of LPS. TNF and IL-6 in serum and supernatants were measured by ELISA. PBMC supernatants were then added to isolated human hepatocytes and concentrations of CRP were measured by ELISA.

Results: Serum IL-6 348 ± 172 pg/ml and TNF 118.5 ± 15.5 pg/ml were elevated compared with healthy controls (not detected) and these observations were matched by elevated serum CRP in patients with FHF 38.9 ± 7 mg/l. Both the production of IL-6 and TNF by PBMC isolated from patients with FHF and the potential of supernatants from these cells to stimulate CRP production by hepatocytes in vitro was significantly reduced compared with controls.

	Controls	FHF
PBMC IL-6 pg/ml(LPS)	7367 ± 720	$1652 \pm 381^{\dagger}$
PBMC TNF pg/ml(LPS)	1701 ± 296	$787 \pm 150^*$
Hepatocyte CRP ng/ml	149 ± 39	$83 \pm 7^*$

ANOVA * $p < 0.05$, $^{\dagger}p < 0.01$

Conclusions: PBMC from patients with FHF have reduced potential to produce cytokines and elicit an acute phase response by the time of patient admission to the intensive care unit. One explanation for this observation is activation of PBMC *in vivo*, prior to study. This may indicate that activation of the inflammatory response occurs early in the natural history of FHF and prior to the development of sepsis.

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CD14 DEPENDENT ACTIVATION OF MONOCYTES BY GRAM POSITIVE BACTERIAL CELL WALLS (G+)

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OBJECTIVES: LPS induces cytokine release from monocytes via the cell surface receptor CD14. Peptidoglycan (PG) of G+ also stimulate cytokine from monocytes, but the role of CD14 is equivocal.

DESIGN & METHODS: We investigated the role of CD14 by i) measuring IL-8 release from a non-differentiated monocyte cell line transfected or not with the human CD14 (CD14- and CD14+) gene stimulated with whole PG from *Streptococcus pneumoniae* (WCW), that solubilized by the autolytic enzyme, amidase (ADCW) or LPS; ii) measuring the ability of WCW, ADCW & LPS to inhibit binding of fluorescein-labeled LPS (FITC-LPS) to CD14+ cells.

RESULTS: i) transfection of CD14 resulted in an increase of IL-8 production over that measured in CD14- cells; ii) 1000-fold more G+ material was required to elicit a response compared to that required with LPS; iii) solubilization of G+ by amidase did not increase the specific activity; iv) CD14 blockade with anti-CD14 mAb of CD14+ cells was effective at low, but not at high concentrations of WCW, ADCW or LPS; v) LPS (10 μ g) inhibited binding of FITC-LPS to monocytes whereas 100 μ g of WCW and ADCW had no effect.

CONCLUSIONS: Thus, CD14 is a target for G+ mediated activation of monocytes. As with LPS, activation pathways other than CD14 are probably responsible at high G+ concentrations. The inability of WCW and ADCW to out-compete binding of FITC-LPS suggests that the bulk of the cell wall macrostructure binds to monocytes by a pathway distinct to that of LPS, which is LPS-binding protein and CD14 dependent. However, inhibition of IL-8 production in the presence of anti-CD14 mAb suggests that fractions within it do.

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DOWNREGULATION OF TNF PRODUCTION BY BLOOD MONOCYTES OF PATIENTS WITH SEPTIC SHOCK. COMPARISON WITH PATIENTS WITH CARDIOGENIC SHOCK AND PNEUMONIA

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OBJECTIVES: Downregulation of TNF production by monocytes from septic shock (SS) patients has been reported. To investigate whether this was due to shock or to infection, we compared patients with septic shock and patients with cardiogenic shock (CS) and bacterial pneumonia (BP).

DESIGN & METHODS: Whole blood was stimulated with LPS in 14 normal donors as controls, 6 SS patients at Day 0 and Day 1, 10 CS and 10 BP patients on the day of admission. TNF was measured.

RESULTS:

	Number of patients in which TNF release (ng/ml) was:			
	<0.05 ng/ml	0.05-0.3 ng/ml	0.3-1 ng/ml	>1 ng/ml
Controls	0	0	6	8
SS Day 0	6	0	0	0
SS Day 1	6	0	0	0
CS Day 0	1	5	2	2
BP Day 0	3	1	1	5

An important reduction of TNF release by monocytes of SS patients was observed compared to that of controls. Production of TNF was intermediate in patients with CS and BP. Whereas TNF reduction in SS was associated with a lower number of monocytes and a decreased expression of CD14, the number of monocytes and the expression of CD14 were similar in controls and in CS and BP patients.

CONCLUSIONS: If CD14 downregulation was associated with the lack of stimulation of SS monocytes, other mechanisms were certainly also involved, since downregulation also occurred in patients with CS and BP presenting who had a normal expression of CD14.

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INDUCTION OF IMMUNOINHIBITING CYTOKINES IN PATIENTS UNDERGOING OPEN HEART SURGERY

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OBJECTIVES: Exposure of blood to extracorporeal artificial surfaces during cardiopulmonary bypass (CPB) induces alterations in cell-mediated immune response, resulting in a state resembling whole body inflammation. This inflammatory response might be associated with morbidity caused by severe organ dysfunctions observed after CPB. A variety of inflammatory pathways are activated after CPB, leading to release of different cytokines. A large number of studies have reported different patterns of cytokine production in patients undergoing CPB, but there were no references about immunoinhibiting cytokines interleukin-10 (IL-10) and transforming growth factor- β .

DESIGN AND SUBJECTS: Our subjects were 30 adult patients, scheduled for elective coronary artery bypass grafting with extracorporeal circulation (ECC). The study protocol was approved by the Ethics Committee of our hospital and informed consent was obtained. Blood samples were obtained at 8 predetermined intervals before, during as well as 6, 24 and 48 hours after CPB.

METHODS: Blood was withdrawn from an indwelling arterial line into sterile plastic tubes and immediately cooled in ice. After centrifugation with 4000 rpm at 4°C for 20 min, the plasma was removed and stored at -80°C until investigation. The levels of plasma IL-10 and TGF- β were determined by enzyme-linked immunosorbent assays. The detection limits were 5 pg/ml for TGF- β and 0.1 pg/ml for IL-10.

RESULTS: Preoperative only very low plasma IL-10 levels (mean: 2.5 pg/ml) were detectable. The concentrations remained low during CPB and peaked significantly after weaning from CPB until skin closure. The mean peak value was 308 ± 180 pg/ml. 6 hours after skin closure IL-10 decreased to 19.8 ± 9.8 pg/ml ($p < 0.05$), remaining on this level until the second postoperative day. TGF- β levels significantly increased after weaning from CPB from 1150 ± 900 pg/ml to 1544 ± 716 pg/ml and showed a peak value at the end of operation with 1860 ± 1020 pg/ml. 6 hours after operation there was a decrease to 1250 ± 630 pg/ml ($p < 0.05$).

CONCLUSION: Our study presents for the first time alterations in plasma levels of IL-10 and TGF- β in patients undergoing coronary artery bypass grafting. The production of both cytokines is significantly increased at the end of CPB. This findings demonstrate that IL-10 and TGF- β are involved in dysregulation of immune response following CPB.

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PTCA INDUCES PROMPT NEUTROPHIL-DERIVED OXYGEN FREE RADICAL-ACTIVITY IN HUMANS

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OBJECTIVES: The inflammatory consequences of mechanical endothelial-trauma are believed to contribute restenosis after balloon angioplasty (PTCA). This inflammatory response might be initiated by neutrophil (PMN) derived oxygen free radical (OFR) activity.

DESIGN: We measured neutrophil-derived OFR-activity using chemiluminescence (CL) before and shortly after PTCA and correlated this activity with the presence of coronary ischemia (CI). The CL is commonly used as a method for indirekt measurement of intra- and extracellular activity.

SUBJECTS: Sixteen patients (pts) (mean age 59.2 years [range 37- 78] years) undergoing elective single vessel PTCA (left anterior descending (n=12), right coronary (n=2), left circumflex (n=2)) for symptomatic coronary artery disease were studied. Pts with prior PTCA, recent myocardial infarction, bypass surgery, heart failure, rheumatic disease, diabetes or ongoing inflammatory disease were excluded.

METHODS: Simultaneous blood samples were taken from the aorta (A) and coronary sinus (CS) before, and 2 and 10 minutes after PTCA. Leukocytes were separated using a density gradient method. Thereafter, luminol and lucigenin-enhanced CL were measured before and after stimulation with phorbol- myristate- acetate (7.5 µg/ml PMA). Cardiac lactate production ($\text{lactate}_{CS} > \text{lactate}_A$) or decreased lactate extraction ratio (LER: $(A-CS)/A \times 100 < 10\%$) were regarded diagnostic for CI.

RESULTS: In 15/16 pts luminol- and lucigenin-enhanced CL from CS blood increased by a mean of 98.2 % (range: 0.8-415.8) and 153.3 %, respectively (range: 13.8-1039.9), indicating an intracoronary activation of neutrophils within minutes after PTCA. Coronary ischemia was present in 15/16 pts, diagnosed by lactate production (n=12) or decreased LER (n=3).

CONCLUSION: Our data indicate a close relationship between mechanical or ischemic trauma, or both, and prompt intracoronary activation of neutrophils within a few minutes after PTCA. Neutrophil-derived OFR-activity may trigger the inflammatory cascade resulting in restenosis.

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LEUCOFILTRATION AS NOVEL THERAPY FOR SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS): PRELIMINARY FINDINGS.

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OBJECTIVES: To assess the clinical safety of a newly developed leucocyte depleting filter in the critically ill and to determine its *in vivo* efficacy in removing white blood cells (WBC) from patients with SIRS.

DESIGN: A preliminary safety study.

SUBJECTS: Fifteen patients fulfilling SIRS criteria, WBC > 12,000 cells/mm³, and with no contraindication to heparinisation.

METHODS: Subjects were filtered using a standard venovenous renal extracorporeal circuit for 30 minutes at 200 ml/minute. Heparin was used for anticoagulation. Filtration cycles were repeated every 12 hours until recovery, death, or failure to meet trial entry and safety criteria. Patients received an average of 2.6 cycles (range 1 to 8). Average APACHE II score for the group was 21 (range 9 - 33).

RESULTS: The filter removed an average of 3.6×10^{10} WBC (range 1.3 to 7.8×10^{10}) over 15 minutes; platelet depletion was insignificant and there were no adverse effects. Interestingly, peripheral WBC count was restored to pre-filtration levels within one hour. 10 of the 15 patients survived.

CONCLUSION: We have demonstrated that the filter safely removes WBC. We are currently investigating whether such removal is selective for a subpopulation with characteristics that have been implicated in endothelial disruption.

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ANTITHROMBIN III IN PATIENTS WITH SEVERE SEPSIS, A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND MULTICENTER TRIAL

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OBJECTIVES: Antithrombin III (ATIII) is an important physiological inhibitor of blood coagulation as it interferes with the clotting process at various levels. Recently, evidence has been accumulated that there is an additional anti-inflammatory potential of AT III as it reduces the release of oxygen radicals from activated neutrophils. Furthermore, it diminishes the release of TNF α from activated macrophages and reduces platelet aggregability. Aim of the placebo-controlled, double-blind study was to evaluate the safety and potential efficacy of AT III in reducing mortality in patients with severe sepsis.

SUBJECTS AND METHODS: We studied 42 patients with severe sepsis who received standard supportive care and antimicrobial therapy, in addition to the administration of AT III (Kyberlin P) or placebo. Patients received either an intravenous loading dose of 3,000 I.U. AT III followed by a maintenance dose of 1,500 I.U. every 12 hours for 5 days, or equivalent amounts of placebo.

RESULTS: The administration of AT III was safe and well-tolerated. It was followed by a 39 % reduction in 30 days all-cause mortality (n.s.). The reduced mortality rate was accompanied by a considerably shorter stay on ICU. Patients treated with AT III exhibited a better performance in overall severity of illness and organ failure scores (APACHE II, MOF, OSF) which already could be noticed early after initiation of treatment. Patients treated with AT III demonstrated a better resolution of preexisting organ failures and a lower incidence of new organ failures during the observation period.

CONCLUSION: A metaanalysis comprising this and two other double-blind, placebo-controlled trials with AT III with a total of 122 patients suffering from severe sepsis confirms the positive trend. The results of the metaanalysis demonstrate a 22.9 % reduction in 30 days all-cause mortality in patients treated with AT III. Although still too small to be confirmative, the metaanalysis clearly points to the fact that a sufficiently powered phase III trial is warranted to definitely prove whether there is a beneficial role of AT III in the treatment of severe sepsis.

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WHITE CELL RESPONSE TO BLOOD TRANSFUSION IN CRITICALLY ILL PATIENTS

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Objectives: To verify that red cell transfusion in critically ill patients is the cause of an acute white cell response and to attempt to determine its cause.

Methods: Forty eight consecutive patients who were receiving a blood transfusion on the Intensive Care Unit (ICU) were studied. Patients who were actively bleeding, were on steroid medication, who had evidence of malignancy, whose white blood cell count was below 4 or above $20 \times 10^9/l$ or who had received blood in the previous 48 hours were excluded. The following data was collected: age, sex, APACHE II score for previous 24 hours, presence of sepsis, ventilatory status, and number of units transfused. Blood was taken 12 hours before, immediately before and after blood was given, and at 12, 24 and 48 hours post transfusion and measured for haemoglobin concentration and differential white cell count with a Technion H3 analyser.

Results: There were 23 males. Mean age 55 (SD 15) years, mean APACHE II score of 18 (6). Mean number of packed cells transfused was 2.05 (0.3). The mean white cell count before transfusion was 10.95 (4.77). There was a significant rise in white cell count at 4 hours post transfusion ($p < 0.001$) which returned to baseline value by 24 hours. There was no correlation between APACHE II score, presence or absence of sepsis or mode of ventilation with white cell response. Rise in white cells was due to an increase in peripheral neutrophil count.

Conclusion: Transfusion of packed red cells to critically ill patients causes an acute neutrophilia. The cause is unknown.

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IS NITRIC OXIDE (NO) INVOLVED IN THE PATHOGENESIS OF INFLAMMATORY ANEMIA?

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OBJECTIVES: Anemia is a common finding in several inflammatory processes in the ICU. At present the precise mechanisms for the low hemoglobin levels during inflammation are unclear. One suggested pathogenetic mechanism is the suppression of erythropoietin (EPO) production by proinflammatory cytokines. Many cytokines, e.g. IL-1 and TNF- α stimulate nitric oxide synthase and release NO. Thus we hypothesize that nitric oxide (NO) mediates the inhibition of EPO production during inflammation.

MATERIAL AND METHODS: We selected the EPO producing cell line HepG2 (hepatocellular carcinoma). Cells were incubated under hypoxic conditions for 24h with following substances: sodiumnitroprusside (SNP, 0.1 mM), 3-morpholino-sydnominine (SIN-1, 1mM), S-nitroso-N-acetyl-D,L-penicillamine (SNAP, 1 mM), pyrrogaolol (300 μ M), hydrogen peroxide (H_2O_2 250 μ M) as well as 8-bromo-cGMP (10 μ M). Statistical analysis was performed using the Dunnett's test. Data are given as means $\pm$ SD of 4-8 separate cultures. Statistically significant differences versus untreated control cultures are indicated: * $P < 0.05$, ** $P < 0.01$.

RESULTS:

	EPO production (% of untreated control cultures)
Control	100 $\pm$ 18
SNP	45 $\pm$ 22 **
SIN-1	66 $\pm$ 16 **
SNAP	31 $\pm$ 12 **
Pyrrogaolol	71 $\pm$ 14 **
H_2O_2	76 $\pm$ 13 *
8-bromo-cGMP	102 $\pm$ 8

CONCLUSION: The NO-liberating agents SNP, SIN-1 and SNAP are strong inhibitors of hypoxia-induced EPO production in HepG2 cells. In contrast, 8-bromo-cGMP does not influence EPO formation, indicating that this inhibition is not the consequence of NO-mediated activation of guanylyl cyclase. Considering the fact that pyrrogaolol and H_2O_2 show similar suppression of EPO synthesis, we suggest that formation of reactive oxygen species associated with NO release are responsible for the reduced EPO release from HepG2 cells.

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DIFFERENCES IN THE KINETICS OF INFLAMMATORY AND RESIDENT MACROPHAGES (M ϕ); A POTENTIAL CONTROL POINT IN THE RESOLUTION OF INFLAMMATION.

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OBJECTIVES: To define the relative rates of emigration of resident and inflammatory M ϕ during the resolution of inflammation.

DESIGN: Placebo controlled semi-allogeneic cell transfer study.

SUBJECTS: 19 CB-1 mice, 10 resident, 7 inflamed and 2 controls injected with PBS and no cells.

METHODS: The peritoneal cavities of normal CB-1 mice [Resident], expressing H-2^{d/k} were injected with 2.5 $\times 10^6$ red fluorescent labelled M ϕ , from normal C3H donor mice expressing H-2^k only. These were sacrificed 7 days later, their peritoneal cavities lavaged and the cells washed. In a parallel study CB-1 mice with a resolving peritonitis [Inflamed] which had received 10 $\times 10^6$ red fluorescent labelled M ϕ from similarly inflamed C3H donor mice were also sacrificed after 7 days. Lavaged cells from both experiments were counted, morphology and viability's determined and cells analysed for expression of H-2 antigen and red fluorescence. Samples of peritoneum and draining lymph nodes were obtained to determine degree of adherence of M ϕ and M ϕ emigration respectively. Using dual colour immunofluorescence donor, recipient and donor M ϕ which had been phagocytosed could be distinguished.

RESULTS AND STATISTICAL ANALYSIS: Results suggested Resident M ϕ persisted in the peritoneal cavity much longer than inflammatory M ϕ . 43.8 $\pm$ 7.9% (Mean $\pm$ SE) of the Resident donor M ϕ transferred were recovered from the peritoneal cavities of Resident mice after 7 days. This was significantly greater than the 6.3% $\pm$ 2.1% recovery of Inflamed donor M ϕ from Inflamed mice ($p < 0.05$ Wilcoxon signed rank non-parametric statistics). Dual colour immunofluorescence showed no phagocytosis of the Resident M ϕ whilst all remaining inflammatory M ϕ had been phagocytosed. There was little evidence that widespread M ϕ adherence to peritoneum accounted for this differential recovery but there was a marked difference in the emigration of fluorescent labelled donor M ϕ to the draining lymph nodes, with all the nodes from Inflamed mice containing labelled cells whilst only 3 of 10 Resident did.

CONCLUSIONS: Inflammatory M ϕ rapidly emigrate from the inflamed site and the kinetics of this emigration are very different from those of Resident M ϕ . The mechanism for this increased emigration rate remains unexplained.

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INHIBITION OF L-ARGININE TRANSPORT WITH L-LYSINE REDUCES NITRIC OXIDE PRODUCTION AND EXERTS BENEFICIAL EFFECTS IN RODENT ENDOTOXEMIA

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An enhanced production of nitric oxide (NO) plays a major role in the pathophysiology of septic shock, and inhibition of NO synthesis could be useful in this setting. Since NO is synthesized from L-arginine, decreasing L-arginine availability might be an interesting way to reduce NO production. **Aim of study:** To assess the effects of L-lysine (LYS), a cationic amino-acid able to inhibit cellular L-arginine transport, in an animal model of septic shock. **Method:** 29 anesthetized rats, instrumented for the measurement of mean blood pressure (MBP) and cardiac index (CI), were challenged with an iv bolus of 10 mg/kg lipopolysaccharide (LPS), given over 15 minutes. Immediately after, rats were randomly assigned to receive an infusion of either LYS (500 μ mol/kg/h; LYS group, n=12) or an equivalent volume of isotonic saline (2 ml/kg/h; SAL group, n=17) given over the next 5 h. NO production was assessed by the measurement of plasma nitrate (NO₃). Arterial blood lactate was measured as an index of global tissue oxygenation. The table gives the results obtained at the end of the 5h observation period.

	MBP (mm Hg)	CI (ml·min ⁻¹ ·kg ⁻¹)	NO ₃ (μ mol/l)	Lactate (mmol/l)
SAL	56 $\pm$ 6	161 $\pm$ 19	271 $\pm$ 18	5.8 $\pm$ 0.5
LYS	88 $\pm$ 3 *	222 $\pm$ 14 *	224 $\pm$ 13 *	3.7 $\pm$ 0.3 *

mean $\pm$ SEM. * $p < 0.05$ vs SAL group (ANOVA).

LYS reduced the fall in MBP and CI induced by LPS, while attenuating the rise in arterial lactate. These effects were associated with lower plasma NO₃ level, indicating an inhibition of the L-arginine:NO pathway. **Conclusion:** In endotoxemic rats, inhibition of cellular L-arginine transport with L-lysine reduces NO production and exerts beneficial hemodynamic and metabolic effects. Such a way to alter NO production might be of great therapeutic value in septic shock.

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SELECTIVE AMELIORATION OF MONOCYTE FUNCTION FROM SEPTIC SHOCK PATIENTS BY GM-CSF IN VITRO
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OBJECTIVES: Some aspects of monocyte function may be impaired following trauma predisposing the patient to infection. We hypothesised that GM-CSF may modulate the function of monocytes from septic shock patients.

DESIGN: Informed consent and Ethics Committee approval was sought. Whole blood and enriched monocytes were stimulated with GM-CSF or control diluent in vitro.

SUBJECTS: Monocyte activation from septic shock patients (n=12) compared with a panel of normal controls (n=64) was studied with/without GM-CSF stimulation in vitro.

METHODS: Blood monocytes or enriched monocytes were stimulated with GM-CSF, LPS (endotoxin), or GM-CSF plus LPS. Membrane-expressed cytokines (TNF α , IL-1 α) cytokine receptors (TNF type I and Type II receptors, IL-1 Type II receptor) and function-associated antigens were enumerated by flow cytometry and enriched monocyte-mediated secretion of immunoreactive cytokines were quantitated by ELISA.

RESULTS AND STATISTICAL ANALYSES. Monocyte expression of CD14, HLA-DR, CD11c and the type II IL-1 receptor from septic patients were lower than normal subjects ($P < 0.01$, Mann-Whitney U-Test) and the expression of CD11a, CD11b, LAM-1, membrane-associated TNF α and IL-1 α were greater ($P < 0.01$). Expression of CD16, CDw32 and CD64 were also greater on monocytes from septic patients compared with normals ($P < 0.001$). Stimulation of monocytes from normal subjects and septic patients with GM-CSF up-regulated the expression of membrane bound cytokines, cytokine receptors and adhesion molecules. The expression of LAM-1 and Fc receptors were down-regulated following GM-CSF stimulation, indicative of normal activation. The secretion of IL-6 by monocytes from septic patients was elevated in response to LPS in vitro compared with normals ($P < 0.01$), however, the secretion of IL-1 β , IL-1 receptor antagonist, TNF α and IL-10 were markedly attenuated ($P < 0.001$). The secretion of TNF receptors from normal and patient monocytes were similar.

CONCLUSION: Monocyte function from septic patients is compromised. While GM-CSF may restore certain parameters of normal monocyte function, GM-CSF may not restore other functions such as the secretion of cytokines.

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GM-CSF RESTORES MONOCYTE MICROBICIDAL FUNCTION FROM SEPTIC PATIENTS AND AUGMENTS TISSUE FACTOR ACTIVITY IN VITRO

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OBJECTIVES: Monocyte function may be impaired following trauma predisposing the patient to infection. Cytokines such as GM-CSF activate monocyte function *in vivo* but may contribute to coagulation disorders mediated by tissue factor (TF) expression. This hypothesis was tested by stimulating monocytes with GM-CSF *in vitro*.
DESIGN: Blood monocytes and enriched monocyte cultures from septic patients and normal controls were stimulated with GM-CSF and/or agonists of monocyte activation.
SUBJECTS: Septic shock patients (n=12) and normal healthy control subjects (n=64) were recruited for the study.
METHODS: Blood monocytes and monocyte cultures were primed with GM-CSF (5ng/ml, 2h, 37°C). Monocyte microbicidal activity was quantitated in a whole blood assay of respiratory burst activity by flow cytometry. Time-dependent monocyte surface TF expression and total cellular activity was quantitated before and after GM-CSF stimulation in the presence or absence of LPS by flow cytometry and a chromogenic substrate assay respectively.
RESULTS AND STATISTICAL ANALYSES: Monocytes from patients with sepsis exhibited a greater respiratory burst than normals in the absence of stimulation ($P<0.001$, Mann-Whitney U Test). The response to secondary stimulation such as LPS or opsonised *Staphylococcus aureus* was attenuated compared with normals ($P<0.001$) but a normal respiratory burst was restored following GM-CSF stimulation. GM-CSF also primed monocytes from these patients to respond to secondary stimulation. The enhanced microbicidal function of monocytes from septic patients was complicated by an increase in TF expression and an accelerated TF activity following LPS stimulation compared with normal controls. TF expression and activity on septic patient monocytes was greater than normals ($P<0.005$) and was augmented by GM-CSF, both before and after exposure to LPS. GM-CSF also accelerated peak TF activity in response to LPS from 6h to 2h in both normal and septic subjects.
CONCLUSION: GM-CSF restored the microbicidal function of monocytes from septic patients which was complicated by an accelerated and elevated procoagulant activity.

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VENTILATOR-ASSOCIATED PNEUMONIA (VAP) IS NOT ASSOCIATED WITH INCREASED CIRCULATING LEVELS OF INTERLEUKIN-6 (IL-6) AND MORTALITY: A CASE-CONTROL STUDY

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OBJECTIVES: VAP is the most frequently observed infection among mechanically ventilated (MV) patients (pts). It is unclear to what extent VAP is associated with inflammatory response and mortality.
DESIGN: We studied associations between VAP, mortality and inflammatory response, assessed by plasma IL-6 levels, in a case-control design.
SUBJECTS: MV pts developing VAP were matched to MV pts not-developing VAP, diagnosed with protected specimen brush or bronchoalveolar lavage. Matching was based on days of MV, diagnosis on admission, renal function, hepatic function, surgical procedures, preceding infections, immunosuppressive therapy and leucocyte count.
METHODS: Prospectively collected plasma samples were analyzed for IL-6 on the day of diagnosis of VAP (D0), and 2 and 4 days prior to D0 (D-2, D-4). Mortality was analyzed on day 28.
RESULTS: In all, 42 cases were matched to 72 controls (30 cases to 2 controls, 12 cases to 1 control). Success of matching was achieved in 486 of 504 (96.4%) variables used. Comparable IL-6 levels were detected in cases and controls on all days studied. However, cases with sepsis (n=10) had higher IL-6 peak levels than cases without sepsis (median 10.89 vs 0.53 ng/mL, $p=.002$). Mortality rates between cases and controls were comparable (33 vs 35%), but non-survivors had higher IL-6 levels than survivors ($p<.05$). In addition, pts developing sepsis had a higher mortality than other pts (8/14 vs 28/100, $p=.03$).
CONCLUSION: The correlations between sepsis, high levels of IL-6 and increased mortality were confirmed. But, VAP, diagnosed with bronchoscopic techniques, was not associated with increased levels of IL-6 or increased mortality.

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sPLA2 HYDROLYSES MICROVESICLES SHED FROM ACTIVATED CELLS IN SEPTIC SHOCK, AND GENERATES LIPID MEDIATORS

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OBJECTIVE: Non pancreatic secretory phospholipase A2 (sPLA2) is a proinflammatory enzyme (J. Lipid Mediators 1993; 8: 1-30) which hydrolyses the sn-2 position of glycerophospholipids. Increased sPLA2 in the plasma of patients suffering from septic shock, is induced by inflammatory cytokines mainly interleukine-1 (IL1) and tumor necrosis factor (TNF α), and is of high diagnostic as well as pronostic value. The precise role of sPLA2 still remains obscure, even if this enzyme probably act on cell membrane to produce inflammatory lipid mediators. However sPLA2 is anable to act on intact cells. Our study (Cell 1995; 80: 919-927) suggest that sPLA2 migh preferentially act on membranes upon loss of phospholipids asymetric. We purpose that microvesicles (MV) shed from activated cells in response to inflammatory cytokines, *Staphylococcus aureus* α -toxin or complement membrane attack complex, provide a preferential substrate for the enzyme, and generates active lipid mediators.
DESIGN: Experimental, *in vitro* studie.

MATERIALS: sPLA2 is a recombinant human enzyme prepared in our laboratory. Preparation of MV: erythrocytes from fresh blood were obtained from normal volonter donors, 32 P labelled, and incubated at 37° C for 45 min with CaCl2 (1mM), ionophore A23187 (2microM). After elimination of intact cells by centrifugation (1500 x g), microvesicle pellet was obtained by centrifugation of the supernatant at 13000 x g for 20 min.

METHODS: Suspension of microvesicles was incubated at 37°C for 60 min in the presence of recombinant sPLA2 (2microg/ml), with CaCl2 (1mM) and sphingomylinase (*Staphylococcus aureus* β -toxin 0.1 U/ml). Lipids were extracted according to Bligh and Dyer. After separation by thin-layer chromatography, the various phospholipids were detected upon iodine exposure vapor and scrapped, or by autoradiography. Phospholipids and lysophospholipids were determined for phosphorus content or for 32 P radioactivity by scintillation counting.

RESULTS AND STATISTICAL ANALYSES: sPLA2 hydrolyses phospholipids from microvesicles, and generates lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA). Activity of LPA generated is confirmed by induction of an extensive aggregation of human platelets. Microvesicles and sPLA2 presence is confirmed in rheumatoid arthritis exsudates and in septic shock plasmas. On the contrary, sPLA2 is not able to hydrolyse phospholipids from intact erythrocytes.

CONCLUSION: Our study allowed us to suggest that sPLA2 preferentially act on membrane upon loss of phospholipid asymetry, like microvesicles shed from activated cells in response to inflammatory cytokines. Hydrolysis generates active lipid mediators like LPA able to induce platelet aggregation.

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INTEREST OF INTERLEUKIN 1 DETERMINATION IN BRONCHOALVEOLAR LAVAGE AFTER INHALATION INJURY: PRELIMINARY RESULTS

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A. VANDERKELEN

Inhalation injury is a major complication of burn injury responsible for severe pulmonary infections and acute respiratory failure in several patients (pts).

Inhalation injury can be assessed by spirometric and radioisotopic tests which are difficult to perform in unstable ICU pts; environmental (open or close space fire), clinical (facial burns, carbonaceous expectorations ...) and fiberoptic criteria are actually used in this population with a poor prognostic value.

Interleukin 1 (IL1) is suspected to be an important mediator in acute respiratory failure pathophysiology.

9 pts requiring artificial ventilation after severe burn injury (mean age 35 $\pm$ 12 - Burn Surface area 46 $\pm$ 22%) were prospectively studied for IL1 level in bronchoalveolar lavage performed at admission time (3 $\pm$ 1.8 hours after injury) in the right basal bronchi. IL1 level were determined by radioimmunoassay (reference <100 pg/ml). These pts were conventionally ventilated (Dräger Evita) and classically reanimated.

In 2 pts, IL1 levels were low (<100 pg/ml), no complication ocured during ventilation period.

In 4 pts, IL1 levels were moderately elevated (112 to 216 pg/ml); all these pts developed a pulmonary infection during artificial ventilation.

In 3 pts, IL1 levels were elevated (187 to 401 pg/ml); all these pts developed an acute respiratory failure (PaO2/FiO2 105 $\pm$ 49, 72 hours after admission) and 1 pt died (MOF on day 3 - IL1 level on admission 289 pg/ml).

This observation suggests that IL1 could be incriminated in the development of respiratory complications following inhalation injury and that IL1 detection in BAL on admission after burn injury could represent an interesting parameter for the diagnostic of inhalation injury.

Further studies are necessary to confirm these results and evaluate the possible relation between IL1 levels in BAL, the severity of inhalation injury and the prognostic value of these determinations.

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