321. Barrier functions in sepsis and acute lung injury

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TNF-α induced septic shock is attenuated in acid sphingomyelinase-deficient mice

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TNF-α plays a major role as mediator of acute inflammation and apoptosis. Surprisingly, little is known about the effects of high plasma levels of TNF-α on the lung. Previous studies revealed that TNF-α causes lethal depression of systemic circulation including hypothermia. The aim of this study was to investigate the pulmonary effects of TNF-α in mechanically ventilated mice. Further, the role of caspases and acid sphingomyelinase (ASMase) was examined.

C57BL/6 wild type and ASMase$^{-/-}$ mice received TNF-α intravenously; in addition, half of the animals were treated with the caspase inhibitor zVAD-fmk. All mice were ventilated for 6h at V_T=8mL/kg and f=180min^{-1} with FiO_2=0.3 and PEEP=2cmH_2O while lung functions were followed by the forced oscillation technique. In order to reduce mortality due to septic shock, saline was given via an arterial catheter and body temperature was stabilized at 37°C. Blood gases, lung histopathology, pro-inflammatory mediators and microvascular permeability were examined.

Fluid support and stabilization of body temperature were sufficient to avoid lethal septic shock. Sepsis was indicated by high serum levels of pro-inflammatory mediators and metabolic acidosis. TNF-α decreased blood pressure and increased heart rate in wild type mice. ASMase$^{-/-}$ mice were protected from the cardiovascular effects, but caspase inhibition had no influence. Although high levels of TNF-α were detected in the lung, no severe pulmonary inflammation or alteration of lung functions was found.

We conclude that septic shock caused by high circulating levels of TNF-α is partly mediated by ASMase. However, TNF-α alone is not sufficient to cause acute lung injury in ventilated mice.

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WITHDRAWN

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Lipopolysaccharide attenuates endothelial barrier function through a pp60^{Src} mediated inhibition of dimethylaminohydrolase (DDAH)

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Acute lung injury is a severe hypoxemic respiratory insufficiency associated with alterations in lung structure and function. Previously, we found that decreases in the activity of DDAH and increased in asymmetric dimethylarginine (ADMA) contributes to the development of ALI in mice exposed to lipopolysaccharide (LPS). In this study, we elucidated the mechanisms involved in the attenuation of DDAH2 by LPS. Utilizing an electric cell impedance sensing apparatus, we found that overexpression of DDAH2 in human lung microvascular endothelial cells (HLMVEC) prevents the LPS (1 endotoxin unit/ml) induced decrease in transendothelial resistance. Further, we found that overexpression of a dominant negative mutant of pp60^{src} attenuated the LPS mediated decrease in DDAH activity and increase in ADMA levels. While, overexpression of a constitutively active mutant decreased DDAH activity and increased ADMA levels without altering DDAH2 protein levels. Further, LPS increased the interaction between DDAH2 and pp60^{src} and also increased its tyrosine phosphorylation. In the LPS treated mouse lung, we found that the decrease in DDAH activity correlated with an increase in pp60^{src} interaction and tyrosine phosphorylation of DDAH2. Finally, the overexpression of DDAH2 in murine lung endothelial cells, using a polyethyleneimine (PEI) derivative transfection reagent, led to an increase in DDAH activity, a decrease in ADMA levels, and the attenuation of the LPS mediated increase in the lung leak as measured by extravasation of Evans blue dye. The prevention of the pp60^{src} mediated decrease in DDAH activity may have clinical utility in the prevention of LPS induced ALI.

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Role of ADAM17 in endotoxin-induced pulmonary inflammation

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Acute lung inflammation is associated with enhanced vascular permeability and leukocyte recruitment. Several proinflammatory, soluble and surface-expressed mediators, including TNFα, TNFR1/2, amphiregulin, IL-6R, IL-1R, L-selectin, CX3CL1, and JAMs may become released by the activity of the metalloproteinases ADAM10 and ADAM17. We examined the role of these proteases in vascular permeability and leukocyte transmigration in vitro by pharmacological inhibition and lentiviral-mediated siRNA knockdown of ADAM10 and ADAM17. The in vivo role of these proteases was studied in a murine model of LPS-induced lung injury by pharmacological inhibition. The relevance of ADAM17 was further analyzed by knockout of ADAM17 in endothelial or smooth muscle cells. The BAL protein levels and the wet/dry-ratio served as markers of vascular permeability and edema formation. Cell recruitment to the alveolar space and lung tissue was analyzed by flow cytometry; cytokines were determined by ELISA. In vitro, transmigration of neutrophils to IL-8 through pulmonary endothelial cells was reduced by pharmacological inhibition as well as knockdown of ADAM10 or ADAM17. LPS-mediated induction of permeability was reduced by pharmacological inhibition, but not by ADAM10 knockdown, indicating a predominant role of ADAM17 in the regulation of endothelial permeability. In vivo, LPS challenge increased the wet/dry-ratio as well as the BAL levels of protein, TNFα, IL-6 and leukocytes. All these effects were largely prevented by inhibitor application or by knockout of ADAM17 in smooth muscle or endothelial cells. These results indicate that local ADAM17 is involved in the onset of inflammation and tissue injury during endotoxin-induced lung inflammation.

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NADPH oxidase isoform 1 is expressed in lung tissue of ARDS patients and decreases hyperoxia-induced ROS production and cell death in pulmonary type II epithelial cells

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We have previously reported that NADPH oxidase 1 (NOX1) deficiency prevented hyperoxia-induced acute lung injury in mice and played an essential role in cell death of mouse alveolar epithelial cells (Carnesecchi, S. *et al.,* AJCCM, 2009; 180: 972-981). In order to determine the mechanisms by which NOX1 induces epithelial cell death during hyperoxia, we specifically knock-down NOX1 in a pulmonary epithelial cell line (MLE-12) using a lentiviral vector strategy. Our results show that NOX1 mRNA was reduced by 35% after hyperoxia compared to scramble siRNA (control cells). Hyperoxia-induced ROS production was inhibited by 36% in transfected MLE-12 compared to control cells. In addition, we demonstrated that NOX1 deletion leads to less hyperoxia-induced cell death analyzed by lactate deshydrogenase release, TUNEL staining and decreased cleaved caspase 3. Hyperoxia-induced ERK phosphorylation, a MAPK involved in cell death signaling was inhibited in NOX1-transfected cells. These data show that NOX1 inhibition decreases hyperoxia-induced ROS production and cell death in an epithelial cell line through ERK signaling pathways.
Furthermore, to determine whether NOX1 is also involved in human, we studied NOX1 expression in lungs of ARDS patients by using immunostaining. We found that NOX1 was highly expressed in alveolar type II cells of patients suffering from ARDS in particularly in the exudative and organizing stages of the disease.
This study is the first direct demonstration that NOX1 is of crucial importance in ARDS and might be responsible for the damage occurring in epithelial type II cells.

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Conditioned medium from human mesenchymal stem cells restores both amiloride sensitive sodium transport and epithelial permeability to protein across alveolar epithelial cell monolayers in an in vitro model of alveolar injury

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Patients with acute lung injury (ALI) have a decreased capacity to reabsorb alveolar edema. Alveolar fluid clearance results from the electro-osmotic gradient created by active sodium (Na) transport across alveolar epithelium. In various models of ALI, MSCs reduce pulmonary edema and increase survival in mice, but in some studies, MSC conditioned medium (MSC-CM) was as effective as MSCs themselves. However, the mechanisms of MSC-CM beneficial effects remain unclear. Thus, in this study our objective was to test the effects of human MSC-CM on vectorial ion transport and epithelial permeability in injured alveolar epithelial cells (AEC). After 18 h of exposure to both hypoxia (3% O2) and 25 ng/ml cytomix (IL-1β, TNFα and INFγ) (CYT-HYX), there was an increase in epithelial permeability measured by radio-labeled albumin over 12 h (1.6±0.40 vs 4.7±2.3%, $p<0.05$) and a marked decrease in transepithelial Na transport (4.2±0.3 vs 1.7±0.1 $\mu A.cm^2$), ($p<0.05$). After permeabilization of the basolateral or apical membrane, we showed no change in Na,K-ATPase activity. We then tested the effect of MSC-CM and found that AEC that were exposed to CYT-HX in the presence of MSC-CM (i) completely prevented CYT-HX-induced increase in protein permeability; (ii) restored the amiloride sensitive-Isc, and iii) increased ENaC activity in the apical membrane. Taken together, these findings indicate that MSC-CM exerts beneficial effects on the injured alveolar epithelium through the preservation of epithelial barrier function and by restoring amiloride-sensitive sodium transport.

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Epigenetic regulation of alveolar ion transport

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The Na,K-ATPase regulates alveolar ion transport and fluid balance in the lung, generating the driving force for alveolar fluid clearance (AFC). Transforming growth factor (TGF)-β is a mediator of acute lung injury (ALI), and impacts AFC. It was hypothesised that TGF-β influences Na,K-ATPase function by controlling subunit expression and stoichiometry. The ATP1B1 subunit of the Na,K-ATPase regulates cell-surface stability of the heteromeric Na,K-ATPase complex. Quantitative RT-PCR analysis revealed downregulation of the *ATP1B1* gene both in the lungs from ALI patients (4-fold; p=0.03) and lungs from bleomycin-treated mice which developed ALI (2.1-fold; p=0.002); as well as in TGF-β-treated A549 cells (2-fold; p=0.02). Moreover, biotinylation and $^{86}Rb^+$ uptake assays revealed that TGF-β depleted Na,K-ATPase cell-surface abundance by 66%, and reduced Na,K-ATPase activity by 85%, respectively, in primary mouse alveolar type II cells (as well as in A549 cells; not shown). *In sillico* and dual-luciferase reporter analyses of the *ATP1B1* promoter identified several putative TGF-β-responsive elements. Gene ablation experiments confirmed that the downregulation of *ATP1B1* expression does not rely on conventional TGF-β transcriptional regulation. Rather, an epigenetic mechanism based on class I histone deacetylase (HDAC) action is involved in the regulation of *ATP1B1* expression by TGF-β. This was clarified using a class I HDAC inhibitor, MGCD0103. Preliminary siRNA knockdown experiments suggest that HDAC1 and HDAC11 are the leading candidates implicated in this process. Together, these data suggest that TGF-β impacts Na,K-ATPase function by epigenetic regulation of subunit stoichiometry in the alveolar epithelium.