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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 VT=8mL/kg and f=180min⁻¹ with FiO₂=0.3. Besides, TNF-α-induced septic shock is attenuated in acid sphingomyelinase-deficient mice. However, TNF-α alone is not sufficient to cause acute lung injury in ventilated mice.

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Lipopolysaccharide attenuates endothelial barrier function through a pp60src-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 DDAH activity and ADMA levels without altering DDAH protein levels. Further, LPS increased the interaction between DDAH and protein levels. While, overexpression of a constitutively active mutant decreased DDAH activity and increased ADMA levels without altering DDAH protein levels. Further, LPS increased the interaction between DDAH and protein levels. While, overexpression of a constitutively active mutant decreased DDAH activity and increased ADMA levels without altering DDAH protein levels. Finally, the overexpression of DDAH2 in murine lung microvascular endothelial cells (HLMVEC) prevented the LPS (1 endotoxin unit/ml) induced decrease in transendothelial resistance. Further, we found that overexpression of a dominant negative mutant of pp60src 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 DDAH protein levels. 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 LPS mediated decrease in DDAH activity may have clinical utility in the prevention of LPS induced ALI.
2942 NADPH oxidase isoenzyme 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; 185: 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 dehydrogenase 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.

2943 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 NaCl+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 MSC-CM (i) completely prevented CYT-HX-induced increase in protein permeability; (ii) restored the amiloride sensitive-Isc, and (iii) increased amiloride sensitive sodium transport and epithelial permeability to protein across alveolar epithelial cell monolayers in an in vitro model of alveolar injury.