association with increased PMN sequestration, P110γ-/- mice also strate greater increase in lung microvascular permeability and edema ion in the initial period after *E. coli* challenge as compared to . These results suggest an important role of PMN PI3Kγ in the e regulation of CD47 expression (a counter-ligand for β1 integrins), reby in modulating PMN adhesion to endothelial cells and PMN ation. NIH grants HL27016, HL46350 and HL45638.

OLVEMENT OF MAPK IN HYPEROXIA-INDUCED MONARY LIPO-TO-MYOFIBROBLAST NSDIFFERENTIATION

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roxia is implicated in the pathogenesis of BPD, however, the lying molecular mechanisms remain unclear. Lipofibroblasts (LFs) an important role in the injury-repair mechanisms in the lung. We ined the effects of hyperoxic exposure on the transdifferentiation of ury LFs to myofibroblasts (MFs), and explored the underlying ular mechanisms. D18 and d21 fetal rat lung fibroblasts were exposed $\%O_2$ or hyperoxia ($95\%O_2$ for 24h) from passage (P)1 to P5, and then zed for lipogenic and myogenic markers expression, using RT-PCR GC/MS. The markers included, adipose differentiation related protein, isome proliferator-activated receptor, α-smooth muscle actin (SMA), vo palmitate synthesis and ^{13}C enrichment of acetyl-CoA. Using ific inhibitors of p38, ERK-1, and JNK, involvement of MAPKs in the differentiation process was assessed. Maintaining cells in $21\%O_2$ ted in modest decreases in various lipogenic markers from P1 to P5, in d18 and d21 cells (d18>d21). In contrast, α-SMA increased on ure to hyperoxia, in both P1 and P5 cells. Our initial results suggest involvement of JNK MAPK in this transdifferentiation process. We lude that hyperoxia accelerates the transdifferentiation of pulmonary to MFs, and it is a critical molecular event underlying the pathogenesis BPD. This transdifferentiation process is likely mediated through JNK way.

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ralty in signaling pathways by human lung fibroblasts following ation by thrombin.

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ivated lung fibroblasts, or myofibroblasts, are metabolically distinctive blasts that express smooth muscle-alpha actin and are associated with us fibrosing diseases including scleroderma. In human lung fibroblasts bin activates PAR-1 and induces a myofibroblast phenotype that nbles scleroderma lung myofibroblasts. PAR-1 can couple both ssis toxin-sensitive and -insensitive heterotrimeric G-proteins, Gi and We have shown that thrombin induces smooth muscle-alpha actin ression and rapid collagen gel contraction through PKC-epsilon and pendent of Gi. In contrast, thrombin-induced DNA synthesis is PKC-ion independent, but Gi dependent. Pertussis toxin completely ishes thrombin-induced DNA synthesis in normal lung fibroblasts, but about 50% in scleroderma. MEK1/2 inhibitor U0121 significantly ces DNA synthesis induced by thrombin in both cell lines. Thrombin ces MAPK phosphorylation and expression of cell cycle-regulatory in cyclin D1; both are completely inhibited by U0121, but not affected pertussis toxin. PI3K inhibitor LY-294002 markedly decreases bin-induced DNA synthesis. This suggests that activation of PI3K is red for the mitogenic effect of thrombin in human lung fibroblasts. er investigations of the role of Gi and PI3K in thrombin-induced lung blast activation may offer new insights in to the pathogenesis of the titial lung fibrosis associated with scleroderma.

PK and MEK inhibition contribute to cellular reoxygenation ry

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epithelial cells produce increased reactive oxygen species (ROS) after ia exposure, and they are more prone to injury by agents that generate oxide in the cytosol (DMNQ) or mitochondria (antimycin A and P). Cellular GSH and MnSOD both decrease in hypoxic lung epithelial cells, altering the redox state. Since ROS may participate in signaling pathways involved in cell death or survival, we tested the hypothesis that MAP kinases were involved in cellular injury during reoxygenation. Human lung epithelial cells (A549 cells) were incubated in hypoxia (<1% O_2 for 24 h) and then reoxygenated by return to air. During reoxygenation, cells were incubated with DMNQ (0-50 μM), a redox cycling quinone that produces superoxide. LDH release was assayed as a marker of cellular injury. Hypoxia preexposure increased epithelial cell lysis by DMNQ. Addition of the p38 MAP kinase inhibitors SB202190 and SB203580 markedly increased cytotoxicity, as did the MAP kinase kinase (MEK) inhibitor PD98059. We also investigated effects of hypoxia preexposure on gene expression using arrays of stress related genes. JNK1, JNK2, p21-activated kinase gamma and MAPK6 all decreased at least two-fold in hypoxia, while no stress related pathway genes appeared to increase. These data suggest that stress related signaling pathways in epithelial cells are modulated by hypoxia, and that inhibition of MAPK and MEK increase cytolysis due to excess superoxide.

LUNG SURFACTANT (865.1-865.8)

865.1

Role of Lung Surfactant in Phagocytic Clearance of Apoptotic Cells by Macrophages During the Development of Pulmonary Inflammation and Fibrosis

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Two of the common features of inflammatory lung diseases are the increased production of lung surfactant and induction cell apoptosis in the lungs. However, the relationship between these two events has not been addressed. To investigate the role of surfactants in pulmonary inflammation and apoptosis, we instilled Survanta (1.5-12 mg) into the lungs of rats and determined the levels of alveolar macrophages (AMs) and apoptotic lung cells by TUNEL assay. Our results show that high-dose treatments of Survanta (> 6 mg) caused an increase in macrophage influx and apoptotic cell number 4 weeks after the treatment. In vitro studies using lavaged AMs showed that Survanta did not enhance apoptosis induced by DMSO over control level. We then examined the ability of AMs to clear apoptotic cells with or without Survanta. AMs were able to clear apoptotic cells more efficiently in the absence of surfactant than in the presence of surfactant at 6 h (75% vs 38%). These results suggest that excessive accumulation of lung surfactants can impair or overwhelm the phagocytic function of AMs and that this impairment may lead to an uncontrolled increase in apoptotic cells.

865.2

Influence of Lung Surfactant on Eustachain Tube Mechanics

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The development of Otitis media with effusion (OME) has been related to abnormal Eustachian tube (ET) mechanics. The ET is a collapsible tube that must be periodically opened to regulate middle ear (ME) pressure and clear ME fluid into the nasopharynx. The ability to perform these functions depends on several ET mechanical properties including the opening pressure, Po, compliance, C, and viscoelasticity, μ. A forced-response protocol was used to determine these mechanical properties in 6 cynomologus monkeys by correlating pressure-flow measurements with a mathematical model of flow in a collapsible tube. These mechanical properties were measured under baseline conditions, after "washing out" the normal mucosal layer, and after instillation of a lung surfactant, Infasurf. Removal of the normal mucosa did not significantly alter Po, but did result in a decrease in C and μ (ANOVA, $p<0.05$). Treatment of the mucosa with Infasurf was effective in reducing Po and increasing C and μ to baseline values ($p<0.05$). Knowledge of how lung surfactant alters ET mechanics may lead to a better understanding of how surfactant therapy could be used to treat OME. Supported by NIH P01 DC1260.

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ABSTRACTS

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