Increasing evidence suggests a role for apoptosis in the maintenance of the alveolar epithelium under normal and pathological conditions. However, the signaling pathways modulating alveolar type II (AT II) cell apoptosis remain poorly defined. Here we investigated the role of lysosomes as modulators of oxidant-mediated AT II cell apoptosis using an *in vitro* model of H$_2$O$_2$ stress led to time-dependent increases in intracellular oxidants, mitochondrial membrane polarization, cytochrome c release, lysosomal rupture and AT II cells apoptosis. Increased apoptosis was prevented by specific inhibition of the caspase 3 inhibitor, or by using the broad-spectrum caspase inhibitor z-VAD-fmk or a caspase 3 inhibitor, or by using functional inhibitors for cathepsin D (pepstatin A) or cathepsin B. Inhibition of cathepsin D also prevented mitochondrial permeabilization and cytochrome c release suggesting that lysosomal rupture precedes and is necessary for the activation of the mitochondrial pathway of cell death.
TGF-Beta Protects Alveolar Epithelial Type 2 Cells (AEC2) from Hyperoxia-Induced DNA Damage

S. Buckley, L. Barsky, K. Weinberg, D. Warburton, Los Angeles, CA

We have previously shown that in vivo inosine, administered during hyperoxic exposure, protects AEC2 from DNA damage and subsequent apoptosis. Inosine, administered in vivo or in vitro, decreases the number of AEC2 in S and G2M phases of the cell cycle, suggesting a G1 cell cycle block. Analysis of BAL from hyperoxic animals + inosine in vivo shows that inosine treatment results in a threefold excess of active TGF-β above already elevated hyperoxic levels. To determine whether TGF-β per se was protective, cultures of AEC2 from normoxic and hyperoxic animals were incubated for 24h with TGF-β, at a concentration equivalent to that seen in the BAL, then DNA damage was assessed by FACS analysis of TUNEL labeling. TGF-β significantly reduced hyperoxia-induced DNA damage, and was associated with Smad2 phosphorylation. The extent of protection was inversely proportional to the initial DNA damage. TGF-β treatment resulted in increased IκB-α phosphorylation, suggesting NFκB activation. Expression of the DNA repair enzyme OGG, which is increased by hyperoxia, was further increased by TGF-β treatment. Taken together, our data suggest that TGF-β can protect AEC2 from hyperoxic DNA damage by slowing the cell cycle for DNA repair. Since inosine treatment of hyperoxic rats also results in increased Smad2 activation and OGG expression in AEC2, coincident with induction of TGF-β into the alveolar milieu, the protective effect of inosine may be mediated through TGF-β. The alveolar macrophage is the source of BAL-derived active TGF-β in this hyperoxic model, and we speculate that tightly regulated secretion of TGF-β by the alveolar macrophage may play a critical role in maintaining integrity of the alveolar epithelium after hyperoxic injury.

Date: Monday, May 23, 2005
Session Info: [**] Poster Discussion Session (Abstract Page: A327) Session: 8:15 am-11:00 am, TO LIVE OR DIE: APOPTOSIS AND CELL SURVIVAL
Presentation Time: 08:15 AM
Room: Room 24 A/B/C (Upper Level), San Diego Convention Center

Close Window
Surfactant Protein A (SP-A) Promotes Alveolar Type II Cell Survival Induced by Keratinocyte Growth Factor (KGF)

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Apoptosis of the alveolar epithelium is a major feature of idiopathic pneumonia syndrome (IPS), a commonly fatal immune-mediated non-infectious pulmonary complication after allogeneic bone marrow transplantation (BMT). In a murine IPS model, we reported that recombinant human (rHu)KGF suppresses lung inflammation and enhances survival of SP-A-sufficient (SP-A+/+) but not SP-A-deficient (SP-A-/-) mice (AJP Lung 285:L602, 2003). To begin to understand mechanisms of KGF and SP-A, rHuKGF (5 mg/kg) or PBS were administered subcutaneously for three consecutive days prior to total body irradiation to SP-A+/+ and SP-A-/- B6 mice given inflammation inducing allogeneic donor spleen T cells at time of BMT. ATII cells were isolated on day 7 after BMT, during time of peak donor T cell-dependent injury. Cells were identified by ATII-specific pro-SP-C staining. ATII cell apoptosis was analyzed by annexin-V and propidium iodide (PI) staining on a FACScalibur. Allogeneic BMT markedly increased ATII cell apoptosis and necrosis. KGF treatment suppressed apoptosis of ATII from SP-A+/+ (unstained cells 17% in PBS-treated vs 33% in KGF-treated BMS SP-A+/+). In contrast, ATII cells isolated from KGF- and PBS-injected SP-A-/- mice exhibited similar levels of apoptosis and necrosis (84% in PBS-treated vs 87% in KGF-treated SP-A-/- mice). In addition, KGF induced phospho-Akt assessed on Western blotting of extracted ATII cellular proteins from SP-A+/+ mice to a greater extent than SP-A-/- mice (n=2, p<0.05). We concluded that one mechanism of KGF protection after BMT is suppression of ATII cell apoptosis and enhanced survival signaling, and that SP-A facilitates this process during donor T cell-dependent inflammation.

Date: Wednesday, May 25, 2005
Session Info: [**] Mini-Symposium (Abstract Page: A897) Session: 1:30 pm-4:15 pm, NOVEL ROLES OF SURFACTANT IN LUNG INJURY AND REPAIR
Presentation Time: 02:35 PM
Room: Marriott Hall 3 (Marriott Pavilion), San Diego Marriott Hotel and Marina
Mechanical Ventilation of Newborn Mice: Effects on Lung Development Genes

R.D. Bland, MD, B.E. Jacobson, BS, E.S. Shinwell, MD, R. Ertsey, MS, Stanford, CA

Rationale: Failed formation of alveoli and lung capillaries is a key histological feature of neonatal chronic lung disease (CLD). CLD typically afflicts premature lungs exposed to lengthy mechanical ventilation (MV) with O2-enriched gas.

Objective: As lung septation and angiogenesis occur mainly after term birth in mice, we did studies to see if MV with 40% O2 would alter lung expression of genes that regulate alveolar and lung vascular development in newborn mice. Methods: We studied 3 groups of 8 pups that were 2-4d old and weighed 2-4g. Group 1 had a tracheotomy followed by MV with 40% O2 for 8h at 60 breaths/min (bpm), peak inflation pressure (PIP) 17±1, mean airway pressure (MAP) 4±1 cmH2O. Group 2 had a tracheotomy followed by MV with 40% O2 for 8h at 180 bpm, PIP 11±2, MAP 4±1 cmH2O. Group 3 (Control-no MV) had sham surgery, then breathed 40% O2 for 8h. Lungs were frozen for later microarray analysis and quantitative RT-PCR to measure mRNA for genes considered important in lung development (Vascular Endothelial Growth Factor A, VEGF-A, and its receptor, VEGF-R2; Tenascin-C, TN-C; and Lysyl Oxidase, LO). Some lungs were processed for histology, which showed no evidence of injury in the 3 groups. Results: Microarrays and qRT-PCR showed 30-35% less (p<.05) lung expression of VEGF and VEGF-R2 and >2-fold more LO in Groups 1 and 2 than in Group 3 (control). TN-C was >50% less (p<.05) in Group 2 than 3, but not significantly different in Groups 1 and 3. Conclusion: A prolonged period of cyclic lung inflation with O2-enriched gas at a critical stage of development, even without apparent lung injury, may impact lung expression of genes that regulate alveolar and lung vascular formation.

Date: Sunday, May 22, 2005
Session Info: [*] Poster Discussion Session (Abstract Page: A276) Session: 1:30 pm-4:15 pm, PATHOGENESIS OF BRONCHOPULMONARY DYSPLASIA: ANIMAL AND HUMAN STUDIES
Presentation Time: 01:30 PM
Room: Room 11 A/B (Upper Level), San Diego Convention Center
Surfactant Protein B (SP-B) is important for the normal functioning of lung surfactant. Early theoretical SP-B models predicted that the disulfide cross-linked, N- and C-terminal domains fold as charged amphipathic helices, and suggested that these adjacent helices may participate in critical surfactant activities. Here, this hypothesis is tested using a disulfide-linked construct (Mini-B) based on the primary sequences of the N- and C-terminal domains. Consistent with theoretical predictions of the full-length protein, both isotope-enhanced Fourier transform infrared (FTIR) spectroscopy and molecular modeling indicated charged amphipathic alpha-helices for the N- and C-terminal domains in the mini-B peptide. Mini-B exhibits in vitro surface activity and produces alterations in the molecular topography of surfactant-lipid dispersions, similar to those achieved by native SP-B. Moreover, in vivo studies of Mini-B show oxygenation and dynamic compliance in ventilated surfactant-deficient rats that compare favorably with that achieved by full-length SP-B. Either Mini-B variants (i.e., reduced disulfides or cationic residues replaced by uncharged residues) or Mini-B fragments (i.e., unlinked N- and C-terminal domains) produced greatly attenuated in vivo and in vitro surfactant properties. Hence, these findings indicate that the unique charge distribution in amphipathic helical N- and C-terminal domains is involved in the function of native SP-B in surfactant lipids.
Lung surfactant is a mixture of lipids and proteins that is indispensable for normal breathing through its ability to reduce surface tension in alveoli. Surfactant protein B (SP-B) is an essential component of lung surfactant, presumably due to its ability to facilitate large-scale rearrangement of lipid material. The structural basis for this ability is not understood. SP-B is an exceptionally hydrophobic protein, making it difficult to address with structural studies. We have characterized partially active sub-fragments of SP-B in order to define the minimal structural features necessary for function, as well as to develop constructs more amenable to NMR studies. A 34 amino acid construct consisting of the N-terminal and C-terminal helices of SP-B linked by a loop, as well as two native disulfide bonds, retains near full activity. NMR has been used to probe the structure of this fragment in detergent and lipid micelles, as well as organic solvent.
GST Gene Polymorphisms and Fruit Intake Modify Lung Function Decline: A Population-Based Study

M. Imboden, PhD, S.H. Downs, PhD, O. Senn, MD, C. Schindler, PhD, U. Ackermann-Liebrich, Prof. MD, G. Maytas, PhD, W. Berger, Prof. Ph, N.M. Probst-Hensch, PhD, SAPALDIA team, Zurich, Basel, Switzerland

RATIONALE
Glutathione-S-transferase (GST) polymorphisms modify lung function decline in smokers. We investigated whether genetic variation of GSTM1, GSTT1, GSTP1 and fruit intake as antioxidative markers modify lung function also in healthy adult never smokers.

METHODS
Participants are derived from the SAPALDIA cohort (18-60 years), which is representative of the Swiss general population. All subjects were genotyped for deletions of GSTM1 and GSTT1 genes and GSTP1 I105V polymorphism by a 5' nuclease TaqMan assay. The impact of polymorphisms and fruit intake on annual decline in forced expiratory volume in one second (FEV1 [ml/yr]) over the 11 year follow-up was assessed using multiple linear regression.

RESULTS
We observed a GSTM1/GSTT1 as well as fruit intake effect on lung function decline (ml/yr) in men (n=1962) but not in women (n=2186). In male never smokers (n=802), the additional decline in lung function for one and two gene deletions was -2.9 (95% CI: -7.0,1.2; p=0.16) and –9.8 (-17.7, -2.0; p=0.01), respectively. In male persistent smokers (n=466) the equivalent changes were –3.2 (-9.0, 2.7; p=0.29) and –9.6 (-18.5, -0.6; p=0.04), respectively. The protective fruit effect on change in FEV1 (6.0 (0.4, 11.6; p=0.04)) was restricted to male persistent smokers.

CONCLUSIONS
The impact of GSTM1 and GSTT1 gene deletions on lung function decline can be expanded to healthy male never smokers. The restriction of the genotype effect to men and the fruit effect to male persistent smokers points to complex interplay of various factors (genes, nutrients, toxicants, and potentially hormones) in respiratory health.

Date: Wednesday, May 25, 2005
Session Info: [**] Poster Discussion Session (Abstract Page: A919) Session: 1:30 pm-4:15 pm, GENE-ENVIRONMENT INTERACTIONS
Presentation Time: 01:30 PM
Room: San Diego Ballroom A (North Tower, Lobby Level), San Diego Marriott Hotel and Marina
Mechanical Stress Upregulates Xanthine Oxidoreductase through a p38 MAP Kinase-Dependent Pathway

R.E.E. Abdulnour, MD, X. Peng, MD, PhD, E.J. Han, E.J. Hasan, U.S. Kayyali, PhD, J.G.N. Garcia, MD, P.M. Hassoun, MD, Baltimore, MD

Rationale: Xanthine oxidoreductase (XOR) plays a prominent role in the pathogenesis of acute lung injury. We have previously shown post-translational modification of XOR by hypoxia involving phosphorylation by p38 MAP kinase (Kayyali, J Biol Chem, 2001). We investigated changes in XOR function in response to mechanical stress and its role in lung permeability related to ventilator-induced lung injury (VILI). Methods: Male C57BL/6J mice were randomly assigned to spontaneous ventilation (controls) or mechanical ventilation (MV) with low (LVT, 7ml/kg) and high tidal volumes (20ml/kg, HVT20, or 35ml/kg, HVT35) for 2 h after which lung XOR function and MAPK activation were assessed. The effect of XOR inhibition by allopurinol (50mg/kg by gavage 16 h prior to MV) on pulmonary leakage (assessed by BAL and the Evans Blue Dye technique, EBD) was also examined. Results: Lung XOR activity correlated with the level of tidal volume MV at 1h (2.11 fold (HVT35) and 1.87 fold (HVT20), P < 0.05) despite no change in XOR protein. Phosphorylation of p38 was evident at 1h MV, with some ERK 1/2 phosphorylation, but no change in JNK. There was a significant increase in both EBD extravasation (1.55 and 1.89 fold increase; P < 0.05) and BAL protein concentration (1.61 and 1.72 fold increase; P < 0.05) in HVT20 and HVT35 at 2 h, which was prevented by allopurinol. Cyclic stretch (18% elongation, 20cycles/min for 1 h) of cultured pulmonary microvascular endothelial cells resulted in p38 phosphorylation and a significant increase in XOR activity which was completely abrogated by pretreatment with the p38 MAPK inhibitor SB203580 (1µM). Conclusion: XOR is significantly upregulated by mechanical stress via activation of p38 MAPK, and plays a critical role in the pathogenesis of pulmonary edema associated with VILI.

Date: Tuesday, May 24, 2005
Session Info: [**] Thematic Poster Session (Abstract Page: A717) Session: 8:15 am-4:15 pm, PULMONARY CIRCULATION
Presentation Time: 08:15 AM
Room: Area G (Hall C, Ground Level), San Diego Convention Center

Close Window
Gene therapy has great potential for the treatment and prevention of lung disease. Although there have been several reports of effective gene delivery and expression using adenovirus vectors, the lung contains several barriers against this vector type, and high levels of inflammation are consistently observed. Additionally, the ability to restrict gene expression to specific cell types remains elusive. In contrast, non-viral DNA vectors represent an effective alternative to viruses. Using DNA nuclear import sequences, we have shown that we can use a selective nuclear import strategy to restrict gene expression to specific cell types. In the present experiments, we have identified a DNA sequence that mediates alveolar type II epithelial cell-specific nuclear import. To assay for nuclear import sequences, the promoters of several alveolar epithelial-specific genes were cloned into a plasmid and microinjected into the cytoplasm of A549, MLE-12, and primary rat ATII cells. DNA localization was detected 8 hours post-injection by in situ hybridization. Plasmids containing a sequence of DNA located within the SP-C promoter were transported into the nuclei of alveolar epithelial cells but not into the nuclei of non-alveolar epithelial cell types. These data suggest that we can restrict gene transfer and expression to alveolar epithelial cells by cell-selective nuclear import. This finding represents a novel strategy in lung gene therapy for restriction of gene expression to alveolar epithelial cells.

Date: Tuesday, May 24, 2005
Session Info: [**] Thematic Poster Session (Abstract Page: A739) Session: 8:15 am-4:15 pm, GENE REGULATION
Presentation Time: 08:15 AM
Room: Area K (Hall C, Ground Level), San Diego Convention Center

Close Window
Rationale: Surfactant protein D (SP-D) is not only important for innate immunity, but also for maintenance of surfactant phospholipid homeostasis. SP-D-/- mice exhibit a progressive alveolar lipoproteinosis with reported decreased surfactant phospholipid catabolism. However, mechanisms of lung synthesis and acyl remodeling of phospholipid molecular species have not been studied in the SP-D-/- mouse and may contribute to the abnormal phenotype.

Methods: SP-D-/- and wild type (C57BL6) mice were injected with [methyl-D9]choline chloride (1 mg i.p.) and killed at 1.5, 3, 6 and 24h (n=5/group). Phospholipids were extracted from bronchoalveolar lavage fluid (BALF) and post-lavage lung tissue. The molecular specificities of endogenous and newly-synthesized phosphatidylcholine (PtdCho), together with the rates of PtdCho synthesis and secretion were determined by electrospray ionization mass spectrometry (ESI-MS).

Results: Total BALF PtdCho was greater in SP-D-/- compared to control mice (163 vs 82 nmoles, p<0.001). By contrast, PtdCho synthesis was consistently lower (48.2±9.3%, p<0.01) in SP-D-/- mice, a difference reflected in the appearance of newly synthesized PtdCho in BALF. SP-D lung tissue was enriched in PC16:0/16:0 (p<0.01) and depleted in arachidonyl-PtdCho species (p<0.001) compared with control mice.

Conclusion: Lower rate of PtdCho synthesis and accumulation of BALF PtdCho confirms previous reports of decreased phospholipid catabolism in SP-D-/- mice. The altered lung PtdCho composition is consistent with increased intracellular accumulation of surfactant lipid in SP-D-/- mice, possibly exerting feedback inhibition on PtdCho synthesis.
Pseudomonas Invasion of Type I Pneumocytes Is Dependent on the Expression and Phosphorylation of Caveolin-2

D.W. Zaas, MD, M.J. Duncan, BS, G. Li, PhD, J.R. Wright, PhD, S.N. Abraham, PhD, Durham, NC

Rationale: Pseudomonas aeruginosa (Pa) is a major cause of pneumonia in immunocompromised and hospitalized patients. The morbidity and mortality of Pa respiratory infections usually results from dissemination of Pa from the upper airways to the alveolar space. We hypothesized that Pa co-opts lipid raft mediated endocytosis to invade the alveolar epithelium.

Methods: Pa invasion was studied in vivo using a rat model of Pa pneumonia as well as in vitro by using primary rat type I-like cells and a murine lung epithelial cell line (MLE-12).

Results: Confocal microscopy of rat lung frozen sections showed co-localization of Pa with type I pneumocytes in vivo. Transmission electron microscopy of type I-like cells in vitro identified intracellular Pa within vacuolar membranes. Pa invasion of type I-like cells and MLE-12 cells was inhibited by removal of membrane cholesterol with methyl-β-cyclodextrin, nystatin, and filipin. Confocal microscopy demonstrated co-localization of intracellular Pa with lipid raft components including caveolin-1 and 2. Using RNA interference to knockdown caveolin-1 and 2, we found that caveolin-2 is required for the optimal invasion of Pa. In addition, Pa invasion was dependent on tyrosine phosphorylation. The number of intracellular Pa was increased by treatment with okadaic acid and decreased by treatment with genistein. Immunoprecipitation experiments revealed that the changes in the tyrosine phosphorylation of caveolin-2 correlated with Pa invasion.

Conclusion: Pa invades type I pneumocytes via a lipid raft dependent mechanism. In addition, Pa invasion is dependent on the expression and tyrosine phosphorylation of caveolin-2.

Date: Tuesday, May 24, 2005
Session Info: [*] Thematic Poster Session (Abstract Page: A683) Session: 8:15 am-4:15 pm, PATHOGEN HOST CELL INTERACTIONS
Presentation Time: 08:15 AM
Room: Area D (Hall C, Ground Level), San Diego Convention Center
Although alveolar surfactant secretion by alveolar type 2 cells occurs by exocytosis of lamellar bodies (LBs), factors that regulate the exocytosis remain inadequately understood. To determine mechanisms, we quantified type 2 cell secretion by real-time alveolar imaging (Ashino, 2000). We held the isolated blood-perfused rat lung at baseline pulmonary artery, left atrial, and airway (Palv) pressures of 10, 5 and 5 cmH2O, respectively. By alveolar microinfusion, we loaded epithelial cells in the alveolar wall with the LB-localizing fluorescent dye, LysoTracker Green (LTG). Then, we tracked cell fluorescence in 10-min periods. Type 2 cells were identified by their bright LTG fluorescence that remained steady at baseline, decreasing less than 10%. However, an increase of Palv to 15 cmH2O for 5 s followed by return to baseline, induced a fluorescence decrease by 92±2% of baseline (mean±SE, P<0.05). Hence, lung inflation induced LB exocytosis.

By contrast, in alveoli which were treated with the mitochondrial inhibitor, carbonyl cyanide p-(trifluoro methoxy) phenylhydrazone (FCCP, 1 μM, 15 min), the exocytosis was inhibited, since a similar inflation decreased LTG fluorescence by 52±4% of baseline (P<0.05). Our findings are the first indication that mitochondria play a role in the regulation of type 2 cell secretion. Under pathological conditions, mitochondrial dysfunction may abrogate surfactant secretion and thereby, promote alveolar collapse.

(Support: HL64895).

Date: Monday, May 23, 2005
Session Info: [**] Thematic Poster Session (Abstract Page: A484) Session: 8:15 am-4:15 pm, SURFACTANT FUNCTION AND REGULATION
Presentation Time: 08:15 AM
Room: Area E (Hall C, Ground Level), San Diego Convention Center