Factor Inhibition Alters Stretch-Induced Gene Transfer and

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...lial cells are continuously subjected to the mechanical forces of cyclic ...ntilation. The intracellular trafficking and expression of exogenous ... by cyclic stretch, as previously shown. Plasmids containing the ... increase gene transfer possibly by increasing nuclear localization of ...ugh binding by a number of transcription factors to the enhancer. ...ow that cyclic stretch in alveolar epithelial cells not only upregulates ...ption factor genes and increases transcription of these transcription ... alters subcellular localization and activation of these transcription ...udy we investigated the effects of inhibiting activation of several tran-..., including NF?B and AP-1, on cyclic stretch-enhanced gene transfer ...helial cells. A549 cells grown on pronectin-coated 6-well Bioflex ...bated with several transcription factor activation inhibitors, including ..., and PDTC. Subsequently, these cells were transfected with pCMV- ... plasmids using electroporation at 160V with 10ms pulse and then ... equibiaxial cyclic stretch at thirty cycles per minute for twenty-four ...tant gene expression was quantified by luciferase assay. After twenty- ...clic stretch, the inhibition of transcription factor activation signifi-...erase expression in stretched alveolar epithelial cells. Thus not only ... factors altered by cyclic stretch, but they, in turn, modulate the effects ... on intracellular DNA trafficking. These results suggest a significant ...tion factors in stretch-enhanced gene transfer.

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...ry Delivery of IgG Via Rat Lungs In Vivo

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...ly reported that IgG is transcytosed across primary rat alveolar epithelial ...s via an FcRn-mediated process. In order to explore transpulmonary IgG ...vivo, male (~400g) Sprague-Dawley rats (Charles River, Wilmington, MA) ...tized by intraperitoneal injection with ketamine and xylazine and intratra-...istered 0.02 mg of biotinylated rat IgG (biot-rIgG, Jackson Immunore-... West Grove, PA) dissolved in 0.2 mL of phosphate-buffered saline (PBS, ...e presence and absence of 0.22 mg of unlabelled rat Fc (Jackson Immu-...abs), using an intratracheal microspray device (Model IA-1B, Penn-...adelphia, PA). Blood samples were subsequently collected at regular inter-... tail vein or at 18 hours by heart puncture and analyzed by enzyme-linked ...t assay using streptavidin-coated microplates (Roche Diagnostics, Indi-...) with peroxidase-conjugated donkey anti-rat $F(ab')_2$ to detect biot-rIgG. ...ed that [biot-rIgG] in the circulation peaked at 18 hours. Co-administration ...abelled rat Fc significantly inhibited biot-rIgG absorption, with ~33 ng of ...L serum observed at 18 hours for rats receiving biot-rIgG alone and ~13 ng ...L serum observed at 18 hours for rats receiving excess unlabelled Fc. Phar-...c analysis using samples taken at 0.5, 1, 2, 4, 8, 18, and 24 hours post-admin-... yielded an absolute bioavailability (compared to intravenous bolus dose) of ...lly administered biot-rIgG of ~5%, which decreased to ~2% in the presence ...d excess rat Fc. These data suggest that exogenously administered IgG is ...d from airspaces to blood in rat lungs in vivo, and that IgG absorption is ... on Fc-mediated processes (most likely FcRn-mediated transcytosis).

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...lucan Stimulates A549 Cells To Release Neutrophil and Monocyte ...c Activity

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...glucan is derived from fungal wall, and its molecular structure is very ...that of lipopolysaccharide(LPS). LPS is a very potent stimulator of many ...ing airway epithelial cells. Because inflammatory cell infiltration is ...th the fungus-induced lung inflammation such as invasive asperugillosis, ...airway epithelial cells participate in the orchestration of acute and chronic ..., we determined whether beta-D glucan might stimulate airway epithe-...(A549 cells) to release neutrophil (NCA) and monocyte chemotactic activity ... cells released NCA and MCA in a dose- and time- dependent manner in ...beta-D glucan. The release of chemotactic activity was inhibited by lipox-...bitors and cycloheximide, and also by a38 MAP kinase inhibitor, ... N-acetyl cystein. NCA was inhibited by leukotriene (LT) B4 receptor ...d interleukin (IL)-8 antibodies. MCA was attenuated by LTB4 receptor ...d anti-monocyte chemoattractant protein (MCP)-1 antibodies. The ...tion and mRNA expression of IL-8 and MCP-1 in A549 cells signifi-...ed in response to beta-D glucan. These data suggest that airway epithe-... release NCA and MCA in response to beta-D glucan and contribute to ...duced lung inflammatory changes.

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Hemoglobin Expression in Alveolar Type II Cells

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Hemoglobin gene expression has been previously reported in activated macrophages from adult mice, and recent studies indicate that alveolar epithelial cells can be derived from hematopoietic stem cells. Our laboratory has now produced strong preliminary evidence that hemoglobin is expressed by alveolar type II (ATII) cells, important stem cells of the pulmonary epithelium and producers of surfactant. RT-PCR was used to measure the expression of nearly all possible transcripts from both the alpha globin (HBA) and beta globin (HBB) gene clusters in several human and rodent ATII cells. Surprisingly, PCR products of the predicted sizes were obtained in human A549 cells (HBA and HBB, the two major globins expressed by adult erythroid cells), mouse MLE-15 cells (HBA ortholog), and primary ATII cells isolated from normal rat and mouse lungs (HBA and HBB orthologs). As controls, these cells also demonstrated expression of genes characteristic of pulmonary epithelial cells (e.g., surfactant proteins), but not other erythroid-specific genes like the erythrocyte anion-exchanger (band 3 protein). PCR products were subjected to DNA sequencing to verify that they were indeed the result of specific amplification of globin gene cDNAs. These cells also expressed hemoglobin protein subunits as determined by probing Western blots with three different anti-hemoglobin antibodies. In addition, tandem mass spectrometry sequencing was used to verify the expression of both alpha and beta globin polypeptides in rat primary ATII cells. The function(s) of hemoglobin expression by cells of the pulmonary epithelium will be determined by future studies, but this novel finding could potentially have enormous implications on the physiology and pathology of the lung.

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Effect of IL-1β, TNFα and Lipopolysaccharide on Elafin and Secretory Leukoprotease Inhibitor (SLPI) Synthesis and mRNA Expression by Primary Human Alveolar Epithelial Type II (ATII) Cells In Vitro

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Pulmonary epithelial cells synthesise and release low molecular weight inhibitors of serine proteinases, Elafin and SLPI. As these inhibitors also exhibit properties of defensins they are likely to be important during infection and inflammation of the respiratory tract. Little is known about the synthesis and secretion of Elafin and SLPI by human ATII cells. We treated confluent primary human ATII cells (n=4 subject samples) with 20ng/ml TNFα, 20 ng/ml IL-1β and 100ng/ml LPS. A parallel group were also treated with 10^{-6} M dexamethasone. SLPI mRNA was constitutively expressed and high levels of SLPI were constitutively released (25,000 to 200,000 pg/ml/24h); in contrast, Elafin mRNA was not constitutively expressed and Elafin was released at very low levels (<50 pg/ml). IL-1β caused a significant ($p<0.05$), 1.25-1.5-fold increase in SLPI release, although this could not be detected by analysis of mRNA expression. However, IL-1β caused a marked significant increase in Elafin, ranging from 15,000 to 50,000 pg/ml/24h ($p<0.01$). This was matched by an increase in mRNA expression, which was previously undetectable in unstimulated cells. TNFα, LPS and dexamethasone showed no consistent effects. These results suggest that constitutive release of SLPI by ATII is an important source of antiproteases in the respiratory unit. Furthermore, increased IL-1β stimulates ATII cell release of low molecular weight protease inhibitors, particularly Elafin, suggesting an important role for low molecular weight protease inhibitors in the peripheral lung during the inflammatory response

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Mechanical Distention of Type II Cells Grown on EHS Matrix Modulates Pulmonary Alveolar Epithelial Phenotypic Expression

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Introduction: We have previously reported that mechanical distention of type II cells in culture favored expression of the type I cell phenotype and inhibited expression of the type II cell phenotype and that mechanical contraction had the opposite effect. In these previous studies, we evaluated primary cultures of type II cells that were cultured on a substratum that promoted attachment, spreading and de-differentiation of type II cells. The objective of the present study was to investigate the effects of mechanical forces on the expression of markers for the type I and type II cell phenotypes in primary cultures of rat type II cells cultured on a substratum that promotes the maintenance of the type II cell phenotype. **Methods:** Adult rat type II pneumocytes were cultured on Engelbreth-Holm-Swarm (EHS) basement membrane matrix for 7 days prior to being subjected to mechanical distention. The content the markers of the type II cell phenotype, (SP-A, SP-B and SP-C) and type II cell phenotype (RTI 40, Aquaporin-5) were assessed by RNAse protection assay or real-time PCR. **Results:** Cyclic distention at an amplitude of 15% at a frequency of one cycle per second for 18 hours resulted in a decrease in mRNA content for SP-A and SP-B of 20% and SP-C of 40% relative to controls. There was an increase in RTI40 of 350% and aquaporin 5 of 200% in cells subjected to distention in comparison to control cells. **Conclusions:** These data demonstrate that in type II cells cultured on a substratum that promotes maintenance of the type II cell phenotype, mechanical distention favors expression of the type I cell phenotype and inhibits expression of the type II cell phenotype.

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