The alveolar epithelium is subjected to considerable stretch during mechanical ventilation of patients with acute respiratory failure. The balance between tensional and tethering forces modulating the alveolar barrier integrity could be modified by changes in cell viscoelasticity during stretch. AIM: To study the viscoelasticity of human alveolar epithelial cells in response to stretching. METHODS: Cells from a human alveolar epithelial cell line (A549) were cultured on collagen-coated flexible substrates. Cell substrate was uniformly and equibiaxially stretched with a vacuum-driven system mounted on an inverted optical microscope. An optical magnetic twisting cytometer was coupled to the stretching device to measure the cell complex elastic modulus ($G = G' + jG''$) during substrate deformation. This technique consists in sinusoidally twisting ferrimagnetic beads specifically bound to cell surface and cytoskeleton. The storage ($G'$) and loss ($G''$) moduli were measured from 0.1 Hz to 100 Hz at baseline and after the application of a single held stretch (30% area increase). RESULTS: Baseline values were $G' = 5390$ Pa and $G'' = 1782$ Pa (0.1 Hz). $G'$ and $G''$ increased with frequency following a power law with a weak exponent ($\alpha = 0.195$) according to the structural damping law. Cell stretching induced statistically significant increases of 50% and 37% in $G'$ and $G''$ respectively (0.1 Hz) and a decrease in the power law exponent ($\alpha = 0.173$). Disruption of the cytoskeleton with latrunculin-A (1 M, 30 min) inhibited the increase in $G'$ and $G''$ with stretch. CONCLUSION: The observed stretch-induced stiffening and structural damping support the hypothesis that cells are stress supported structures and soft glassy materials. The increase in cell stiffness with stretch could play a role in epithelial barrier disruption in ventilator induced lung injury.
Effect of Human Monocyte/Macrophage Serine Esterase-1 (HMSE) on Surfactant Subtype Conversion

C. Ruppert, P. Markart, S. Haendel, W. Seeger, A. Guenther Dept of Internal Medicine, Justus-Liebig-University, Giessen, Germany

Rationale: Surfactant subtype conversion from surface-active large surfactant aggregates (LA) to inactive small surfactant aggregates occurs in dependency of cyclic surface area changes and a carboxylesterase activity, which is thought to be released with surfactant by type II cells. In view of the dramatic reduction in LA in ARDS patients we now investigated the effect of HMSE, an esterase sharing many similarities with mouse lung surfactant convertase (MLSC), on surfactant subtype conversion in vitro.

Methods: A rabbit BALF Bligh&Dyer extract containing PLs, SP-B and P-C was supplemented with SP-A (B&D-SPA) and cycled in the presence of A) a cell extract or B) supernatant of LPS-stimulated U937 cells expressing HMSE, C) a cell extract or D) supernatant of freshly isolated mouse type-2 cells expressing MLSC, E) human BALF from ARDS patients, F) human BALF from healthy controls. In vitro surface area changes (9-fold, 32x/min, 37 C) were performed for 240 min. LA and SA were separated by high speed centrifugation (48,000 x g, 1h) and the relative LA content in the resulting pellet was determined by PL quantification.

Results: Incubation of U937 cells with LPS resulted in a 40fold increase in HMSE expression. In vitro cycling of B&D-SPA resulted in a neglectable LA-to-SA conversion in absence of any enzymatic activity, but pronounced LA-to-SA conversion was induced by LPS-stimulated U937 (HMSE in cell extract or supernatant) or freshly isolated type II cells (MLSC, cell extract). Addition of BALF from ARDS patients resulted in an increased LA-to-SA conversion whereas addition of BALF from healthy controls displayed a lower conversion rate.

Conclusions: Coincubation of B&D-SPA with HMSE resulted in LA-to-SA conversion comparable to that of MLSC suggesting the involvement of this enzyme in surfactant subtype conversion in man. Future studies will have to assess the nature of convertase activity in human BALF.

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[**] Thematic Poster Session (Abstract Page: A681) Session: 8:15 am-4:15 pm, SURFACTANT FUNCTIONS
Cultured Alveolar Type I Cells Display Phenotypic Plasticity

R.F. Gonzalez, J.A. Gutierrez, L.G. Dobbs Cardiovascular Research Institute; Departments of Medicine and Pediatrics, University of California, San Francisco

Although the ability of type II (TII) cells to transdifferentiate into type I (TI) cells is essential for alveolar homeostasis and repair after injury, little is known about what factors regulate alveolar cell phenotype and the extent to which transdifferentiation occurs between both cell types. To study this process in vitro, freshly isolated TI and TII cells were cultured under conditions favoring either the TI or TII cell phenotypes. The differentiated state of each cell type was assessed by measurement of phenotype-specific marker gene mRNAs. Freshly isolated TII cells cultured on EHS matrix in media containing KGF, 8-Br-cAMP and rat serum recovered from enzymatic isolation in a time-dependent manner and expressed 80% of surfactant protein A, B and C mRNA content found in freshly isolated TII cells. As previously observed, TII cells cultured on FN-coated tissue culture plastic (FN-TCP) under conditions favoring TI cell phenotype cease to express surfactant protein genes and start to express RTI-40 and aquaporin5 mRNAs, suggesting inverse co-regulation of TI and TII marker genes. TII cells cultured on FN-TCP expressed caveolin and aquaporin5 mRNAs to a much lesser extent (20% for aquaporin5 and 3% for caveolin expression) than freshly isolated TI cells demonstrating significant differences between freshly isolated TI cells and cultured TII cells. TI cells cultured on EHS expressed surfactant protein mRNAs, demonstrating that differentiated TI cells have a certain degree of phenotypic plasticity.

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[**] Thematic Poster Session (Abstract Page: A680) Session: 8:15 am-4:15 pm, ALVEOLAR EPITHELIAL CELLS