studies were focused on the function of the 5-HT4 receptor in the CNS, none of the studies showed its expression and function in the endothelial cells. In the present study, we provide evidence for the first time that 5-HT4 receptor is expressed in the human umbilical vein endothelial cells (HUVECs). We demonstrate the transcription of 5-HT4 mRNA in the HUVECs using reverse transcription polimerase chain reaction. Additionally, we show 5-HT4 receptor expression in HUVECs by immunoblotting and immunofluorescent analysis with 5-HT4 specific antibody. Importantly, we determine that overexpression of 5-HT4 receptor leads to a pronounced cell rounding and intercellular gap formation in HUVECs. We are currently investigating the mechanism underlying 5-HT4 receptor-induced actin cytoskeleton changes in the endothelial cells. These data suggest that by activating 5-HT4 receptor, serotonin could be involved in regulation of actin cytoskeleton dynamics in the endothelial cells that may affect the endothelial barrier integrity.

## 40

**OVEREXPRESSION OF ANTIOXIDANT ENZYMES IN LUNG EPITHELIAL CELLS PREVENTS DAMAGE FROM AIRBORNE PARTICULATE MATTER–INDUCED OXIDANT INJURY.** S. Soberanes,[1] V. Panduri,[1] H. Wang,[1] G. Mutlu,[1] G.R. Budinger,[1] D.W. Kamp,[1,2]
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Elevated levels of air pollution particles are associated with increased morbidity and mortality from acute and chronic cardiopulmonary injury. One mechanism underlying these effects involves oxidative damage to lung epithelial cells. We previously showed that Düsseldorf particulate matter (DPM) causes alveolar epithelial cell DNA damage and apoptosis by a mitochondria-regulated death pathway. In this work, we used several different types of well-characterized particulates to determine whether mitochondria-derived reactive oxygen species (ROS) are the primary cause of apoptosis. Washington particulate matter (WPM), residual oil fly ash (ROFA), and DPM each increased ROS production (ASSAY HERE) and apoptosis (DNA fragmentation) as compared to inert particulates such as desert dust (DD) and Mount St Helen volcanic dust (MSH) (Table 1). Notably, WPM, ROFA, and DPM did not induce ROS production or apoptosis in ;gr0-A549 cells, which are incapable of mitochondrial ROS production. We also found that overexpression of MnSOD or CuZnSOD using adenoviral expression vectors blocks DPM-induced A549 cell ROS production and apoptosis as compared to null/sham adenoviral controls. We conclude that a diverse group of toxic airborne particulates, unlike inert particulates, induce mitochondria-derived ROS production and lung epithelial cell apoptosis. We propose that strategies aimed at reducing mitochondrial-derived ROS levels will protect the lung epithelium exposed to airborne particulate matter.

**TABLE 1 Elevated Levels of ROS in Airborne Particulate Matter Increases A549 Cell Apoptosis**

| | *DPM* | *WPM* | *ROFA* | *DD* | *MSH* |
|---|---|---|---|---|---|
| ROS (% over control) | 301.3 ± 20.0* | 319.3 ± 8.0* | 146.1 ± 15* | 8.38 ± 2.1 | 9.77 ± 1.7 |
| DNA frag. (% over control) | 70.4 ± 5.9* | 37.4 ± 5.3* | 58.8 ± 9.1* | 0.98 ± 1.1 | 0.98 ± 0.25 |

Mean ± SEM ($n = 3$). *$p < .05$ vs untreated cells.

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## 41

**EXPANSION OF SUBPOPULATIONS OF *BACTEROIDES* THAT HYPERPRODUCE BETA-LACTAMASE PREVENTS CEFTRIAXONE-INDUCED DISRUPTION OF THE HUMAN INTESTINAL MICROFLORA IN A CONTINUOUS-FLOW CULTURE MODEL.** U. Stiefel,[1,2] N.J. Pultz,[2] C.J. Donskey,[1,2] [1]Case Western Reserve University, [2]VA Medical Center, Cleveland, OH.
**Background:** The indigenous intestinal microflora may generate beta-lactamase activity in response to beta-lactam antibiotic selective pressure. We hypothesized that expansion of subpopulations of *Bacteroides fragilis* group organisms that hyperproduce cephalosporinases could prevent disruption of the indigenous microflora in the face of ceftriaxone selective pressure. **Methods:** In vitro mixing studies were performed to determine the ability of *Bacteroides fragilis* TAL3636 (BF3636), a hyperproducer of a broad-spectrum beta-lactamase, to raise the minimum bactericidal concentration (MBC) of susceptible organisms to ceftriaxone. Continuous-flow (CF) cultures of human colonic microflora were established with or without the addition of BF3636 and increasing concentrations of ceftriaxone were added to the inflowing media. Beta-lactamase activity, ceftriaxone concentrations, and densities of bacterial populations were monitored. A mouse model was also utilized to evaluate the ability of BF 3636 to preserve colonization resistance in vivo. Concomitant with injections of ceftriaxone, mice received orogastric inoculation of CF culture containing BF3636, BF3636 culture alone, or normal saline (control group). Mice subsequently received a culture of vancomycin-resistant enterococcus (VRE) by orogastric gavage. Stool samples were collected on succeeding days and plated for the presence of VRE and resistant *Bacteroides* species. **Results:** In mixing studies, BF3636 raised the MBC of a ceftriaxone-susceptible *Escherichia coli* strain from < 0.25 to 64, whereas organisms that did not produce beta-lactamase did not ($p < .001$). The CF culture containing intestinal microflora was not affected when 8 µg/mL of ceftriaxone was added to the inflowing media, but 100 µg/mL resulted in disruption of the microflora and detection of ceftriaxone in the culture. In the CF culture containing BF3636, the population of *Bacteroides* species with high-level resistance to cephalosporins expanded over time and beta-lactamase activity increased; infusion of 100 µg/mL of ceftriaxone did not disrupt the indigenous microflora and ceftriaxone levels remained undetectable. In vivo, ceftriaxone-treated mice that had been orally inoculated with CF culture containing BF3636 did not develop intestinal overgrowth with VRE despite exposure; this was in contrast to ceftriaxone-treated mice that had received an oral inoculation with saline alone. **Conclusion:** Expansion of subpopulations of *Bacteroides* species that hyperproduce cephalosporinases can inactivate high concentrations of ceftriaxone and preserve the indigenous intestinal microflora. CF culture containing beta-lactamase-producing *Bacteroides* species was able to preserve colonization resistance of ceftriaxone-treated mice against VRE.

## 42

**THE N-TERMINUS OF FOG-2 INTERACTS WITH METASTASIS-ASSOCIATED PROTEINS 1 AND 2 TO MEDIATE TRANSCRIPTIONAL REPRESSION DURING CARDIAC DEVELOPMENT.** S.A. Samant, A.E. Roche, E.C. Svensson, Department of Medicine, The University of Chicago, Chicago, IL.
Mutations in several transcription factors that regulate cardiac development have recently been described to cause congenital heart disease in humans. A greater understanding of the molecular mechanisms that regulate heart formation may help identify other genes that when mutated will result in human congenital heart disease. FOG-2 is one such gene that encodes a transcriptional corepressor expressed in the developing heart. It is critical for proper cardiac morphogenesis as mice deficient in this factor die during midgestation of cardiac malformations. We have previously shown that FOG-2 physically interacts with GATA4 and attenuates GATA4's ability to activate cardiac specific gene expression. This repression is mediated by a domain of FOG-2 localized to its N-terminus, termed the FOG repression motif. To gain further insights into the molecular mechanism responsible for this repression, we took a biochemical approach to identify factors that interact with FOG repression motif. Using MALDI-TOF mass spectrometry, we identified seven proteins from rat neonatal cardiac nuclear extracts that copurified with a FOG-2-GST fusion protein. All of these proteins have been previously described to be subunits of a nucleosome-remodeling complex called the NuRD complex. To determine which of the NuRD subunits directly interact with the FOG repression motif, we used a series of in vitro binding assays. We found that MTA-1 and MTA-2 specifically bound to the N-terminus of FOG-2 but not to a mutant form of the N-terminus that is unable to mediate repression. In situ hybridization revealed that both MTA-1 and MTA-2 are expressed in the developing heart during mouse embryogenesis. Taken together, these results suggest that FOG-2 mediates transcriptional repression during cardiac development by the recruitment of the NuRD complex to GATA-dependent promoters leading to the remodeling of the local chromatin structure and the attenuation of gene expression.
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**THE ROLE OF PROTEIN PHOSPHATASE 2A IN THE REGULATION OF ENDOTHELIAL CELL CYTOSKELETON STRUCTURE.** K. Tar,[1,2,#] C. Csortos,[1,2,#] I. Czikora,[2] G. Olah,[1] S-F. Ma,[1] R. Wadgaonkar,[1] P. Gergely,[2] J.G.N. Garcia,[1] A.D. Verin,[1*] [1]Department of Medicine, Division of the Biological Sciences, The University of Chicago, Chicago, IL; [2]Department of Medical Chemistry, Research Center for Molecular Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary.
Our recently published data suggested the involvement of protein phosphatase 2A (PP2A) in endothelial cell (EC) barrier regulation (Tar et al, 2004). In order to further elucidate the role of PP2A in the regulation of EC cytoskeleton and permeability, PP2A catalytic (PP2Ac) and A regulatory (PP2Aa) subunits were cloned and human pulmonary arterial EC (HPAEC) were transfected with PP2A mammalian expression constructs or infected with PP2A recombinant adenoviruses. Immunostaining of PP2Ac or of PP2Aa+c overexpressing HPAEC indicated actin cytoskeleton rearrangement. PP2A overexpression hindered or at least dramatically reduced thrombin- or nocodazole-induced F-actin stress fiber formation and microtubule (MT) dissolution. Accordingly, it also attenuated thrombin- or nocodazole-induced decrease in transendothelial electrical resistance indicative of barrier protection. Inhibition of PP2A by okadaic acid abolished its effect on agonist-induced changes in EC cytoskeleton; this indicates a critical role of PP2A activity in EC cytoskeletal maintenance. The overexpression of PP2A significantly attenuated thrombin- or nocodazole-induced phosphorylation of HSP27 and tau, two cytoskeletal proteins, which potentially could be involved in agonist-induced cytoskeletal rearrangement and in the increase of permeability. PP2A-mediated dephosphorylation of HSP27 and tau correlated with PP2A-induced preservation of EC cytoskeleton and barrier maintenance. Collectively, our observations clearly demonstrate the crucial role of PP2A in EC barrier protection.
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## 44

**AMBIENT PARTICULATE MATTER INDUCES MYOFIBROBLAST DIFFERENTIATION VIA MACROPHAGE-DEPENDENT TRANSFORMING GROWTH FACTOR β TYPE I (ALK5) SIGNALING.** K. Thavarajah, R. Kalhan, A. Nair, M.C. Nlend, N. Wang, P.H.S. Sporn, Northwestern University, Chicago, IL.
**Rationale:** Increased levels of ambient particulate matter have been associated with increased pulmonary morbidity and mortality. To investigate if particulate matter induces airway remodeling, we studied the effects of particulate matter (< 10 µm in diameter) collected from Dusseldorf, Germany (DPM), on fibroblast to myofibroblast differentiation. **Methods:** Human fetal lung fibroblasts (IMR-90) were grown to subconfluence, serum-starved for 48 hours, and exposed to either TGF-β1 (2 ng/mL), DPM, or conditioned medium from human monocytic (THP-1) cells exposed to DPM. The role of TGF-β1 signaling was assessed by the addition of SB431542 (10 µM), a TGF-β type I (ALK5) receptor inhibitor. After 48 hours, cells were lysed and analyzed by immunoblot for α-smooth muscle actin (αSMA), a marker of myofibroblast differentiation. **Results:** Direct exposure of IMR-90 cells to DPM did not increase αSMA expression. However, conditioned medium of THP-1 cells exposed to DPM induced increased αSMA expression in fibroblasts. This increase was blocked by the ALK5 inhibitor SB431542. **Conclusions:** Ambient particulate matter triggers macrophage-dependent induction of myofibroblast differentiation via ALK5 receptor signaling. We speculate that particulate matter induces airway remodeling.

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**REGULATION OF 4-HYDROXY-2-NONENAL–MEDIATED ENDOTHELIAL BARRIER FUNCTION BY FOCAL ADHESION AND ADHERENS JUNCTION PROTEINS.** P.V. Usatyuk, V. Natarajan, School of Medicine, The University of Chicago, Chicago, IL.
4-Hydroxy-2-nonenal (4-HNE), a highly reactive aldehyde generated by peroxidation of membrane lipids, altered endothelial cell (EC) barrier function, resulting in vascular leakiness. Here we report that 4-HNE mediates EC barrier dysfunction by modulating focal adhesion and adherens junction proteins that affect cell-cell and cell-matrix interactions via integrins. Treatment of bovine lung microvascular endothelial cells (BLMVECs) with 4-HNE, in a dose-dependent manner, modulated cell-cell adhesion contacts, enhanced intracellular ROS formation, and increased permeability. Interestingly, 4-HNE did not alter

cell-matrix interactions as determined by transendothelial electrical resistance measurement. Therefore, we hypothesized that 4-HNE-induced permeability changes involved modulation of focal adhesion and adherens junction proteins. Treatment of BLMVECs with 4-HNE resulted in the redistribution of FAK, β-catenin, paxillin, VE-cadherin, and ZO-1 and caused intercellular gap formation. Western blot analyses confirmed that 4-HNE formed Michael adducts with the focal adhesion and adherens junction proteins. Furthermore, 4-HNE decreased tyrosine phosphorylation of FAK without affecting total cellular FAK contents. Flow cytometry and fluorescent microscopy analyses revealed a time-dependent reduction in the surface integrins after 4-HNE treatment. **Conclusion:** These results indicate that 4-HNE affects EC permeability by modulating cell-cell adhesion involving focal adhesion, adherens and tight junction proteins, as well as integrins signal transduction.

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**HYPERCAPNIA INDUCES MUCIN GENE EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS.** N. Wang, M.C. Nlend, A. Nair, J.I. Sznajder, P.H.S. Sporn, Pulmonary and Critical Care Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL.

**Objective:** Mucus hypersecretion is a prominent feature of chronic obstructive pulmonary disease, asthma, and cystic fibrosis. These diseases may be associated with development of hypercapnia, acutely during exacerbations and/or chronically in their advanced stages. In this study, we tested the hypothesis that hypercapnia might contribute to mucus overproduction by increasing expression of genes for mucins produced by airway epithelial cells. **Methods:** The human mucoepidermoid carcinoma cell line (NCI-H292) was used in this study. Cells were cultured in the presence of 5% $CO_2$ (control; equivalent to $PCO_2$ ≈40 mm Hg) or 20% $CO_2$ (hypercapnia; equivalent to $PCO_2$ ≈160 mm Hg), in the absence or presence of phorbol 12-myristate 13-acetate (PMA; 10 nM), an inflammatory agent known to induce mucin gene expression, for 6 to 96 hours. mRNA for the mucin genes *MUC2*, *MUC5AC*, *MUC5B*, and *MUC19* were quantitated by real-time PCR and normalized to expression of 18S ribosomal RNA. **Results:** In the absence of PMA, culture of NCI-H292 cells in 20% $CO_2$, as compared to 5% $CO_2$, increased expression of *MUC5AC* by ≈3-fold. In comparison, PMA (in 5% $CO_2$) increased *MUC5AC* mRNA expression ≈70-fold. Culture of NCI-H292 cells in 20% $CO_2$ augmented the PMA-induced increase in *MUC5AC* mRNA levels by ≈3-fold as compared to stimulation with PMA in 5% $CO_2$. The increases in *MUC5AC* mRNA in response to 20% $CO_2$ peaked at 24 hours. *MUC19* mRNA expression was increased in the presence of 20% $CO_2$ in the absence and presence of PMA to a degree similar to that for *MUC5AC*. Neither 20% $CO_2$ nor PMA increased expression of mRNA for *MUC2* or *MUC5B*. **Conclusion:** Hypercapnia stimulates mucin gene expression in vitro and may be important in driving gene expression and synthesis of mucins in vivo. These results reveal a previously unrecognized stimulus of mucin gene expression. Furthermore, they suggest that correcting hypercapnia may be an important strategy for reducing mucus hypersecretion in patients with acute and chronic lung diseases.

## 47

**INCREASED NOX1 IN NEOINTIMAL SMOOTH MUSCLE CELLS IS ASSOCIATED WITH REDOX-SENSITIVE SIGNALING AND ACTIVATION OF MATRIX METALLOPROTEINASE 9.** S. Xu,[1] D. Jagadeesha,[2] A. Chamseddine,[1] R. Bhalla,[2] F. Miller Jr,[1] [1]Internal Medicine, [2]Anatomy and Cell Biology, University of Iowa, Iowa City, IA.

The vascular response to injury involves redox-dependent activation of smooth muscle cells (SMC) and development of a neointima. Activation of matrix metalloproteinase 9 (MMP-9) appears to be critical in the development of arterial lesions. We hypothesized that increased NADPH oxidase–derived superoxide ($O_2^{\bullet-}$) by neointimal SMC contributes to activation of MMP-9. Two weeks after balloon injury of rat aorta, neointimal SMC showed higher $O2^{\bullet-}$ levels compared with adjacent medial SMC. A role for NADPH oxidase was suggested by the observations that neointimal SMC $O2^{\bullet-}$ levels were reduced by the flavoenzyme inhibitor DPI, and immunostaining for $p47^{phox}$ and $p22^{phox}$ were increased in the neointimal, as compared with medial, SMC. Neointimal and medial SMC were isolated and grown in culture. Consistent with the findings in situ, $O2^{\bullet-}$ levels and NADPH oxidase activity were significantly greater in neointimal SMC. Nox1 mRNA levels were more than 2-fold increased in neointimal SMC, whereas Nox4 expression was similar to that of medial SMC. ERK1/2 kinase activation was greater in neointimal vs medial SMC and was inhibited by tiron and DPI. MMP-9 activity, as measured by gelatin zymography, was higher in neointimal SMC compared to medial SMC in basal conditions and 48 hours after IL-1β stimulation and was reduced by pretreatment with the ERK inhibitor PD98059 or DPI. We conclude that NADPH oxidase-derived $O2^{\bullet-}$ signals activation of MMP-9 following vascular injury. Moreover, Nox1-dependent cell signaling in neointimal SMC may play an important role in the pathogenesis of vascular disease.

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**THE SILENCING MEDIATOR OF RETINOID AND THYROID HORMONE RECEPTORS REPRESSES ADIPOGENESIS VIA ITS DISTAL INTERACTING DOMAIN.** C. Yu, M.M. Sutanto, R.N. Cohen, Department of Medicine, The University of Chicago, Chicago, IL.

Obesity is known to be a risk factor for the development of type 2 diabetes mellitus and the metabolic syndrome. Thus, it is important to understand the factors that modulate adipogenesis. Adipogenesis requires the nuclear hormone receptor (NHR) peroxisome proliferator–activated receptor gamma (PPARgamma). NHRs recruit coactivators (CoAs) in the presence of agonists to increase gene transcription; in addition, some NHRs also recruit nuclear receptor corepressors (CoRs) under certain circumstances. CoRs, such as the nuclear corepressor protein (NCoR) and the silencing mediator of retinoid and thyroid hormone receptors (SMRT), recruit a histone deacetylase complex to repress gene transcription. Using a RNA interference system to decrease SMRT and NCoR expression in 3T3-L1 cells, we have previously shown that CoRs repress adipogenesis and decrease the expression of adipocyte-specific proteins by modulating PPARgamma transcriptional activity. We have additionally shown that the intracellular balance of CoRs and CoAs dictates the ability of thiazolidinediones (TZDs), which are PPARgamma ligands used clinically in the treatment of diabetes mellitus, to stimulate adipocyte differentiation. To define the mechanisms of CoR recruitment by PPARgamma in 3T3-L1 cells, we have now focused on the corepressor SMRT. SMRT contains two interacting domains (IDs) that are required for interactions with NHRs. Interestingly, and contrary to other NHRs, PPARgamma interacts with SMRT specifically via the SMRT C-terminal ID (SMRT ID1). To define the amino acids important in specifying the ability of ID1 to function, residues within this domain were mutated to the corresponding residues in the proximal SMRT ID (ID2). Interestingly, a single amino acid within ID1, which is not conserved in ID2, appears to be vital in the recruitment of SMRT by PPARgamma. In contrast, this specific sequence is not required for SMRT interactions with the retinoic acid receptor (RAR), another NHR that interacts strongly with SMRT. Thus, the ability of PPARgamma to recruit the corepressor SMRT is dependent on specific residues within the SMRT C-terminal ID that are distinct from amino acids important for interactions with other NHRs. We have shown that the ability of TZDs to up-regulate PPARgamma–mediated processes is dependent on the balance of CoRs and CoAs. These data also suggest that it might be possible to specifically alter PPARgamma-SMRT interactions to modify TZD action on adipogenesis.

## 49

**NOVEL ROLE OF PRE-B-CELL COLONY-ENHANCING FACTOR IN PULMONARY ENDOTHELIAL BARRIER REGULATION.** L.Q. Zhang, J. Cepeda, J.G.N. Garcia, S.Q. Ye, Section of Pulmonary/Critical Care, Department of Medicine, The University of Chicago, Chicago, IL.

**Rationale:** Our prior genomic and genetic studies identified pre-B-cell colony-enhancing factor (PBEF) as a potential novel biomarker in acute lung injury (ALI) (Ye et al, 2005). To elucidate the molecular mechanism underlying PBEF in the pathogenesis of ALI, we assessed the role of PBEF in in vitro vascular barrier regulation using confluent human pulmonary artery endothelial cell (HPAEC) or microvascular cells (HMVEC-L) monolayers since increased vascular permeability is a cardinal feature of ALI. **Methods:** Inhibition of PBEF expression was achieved by the PBEF siRNAs. Overexpression of the PBEF gene was accomplished by adeno-PBEF. Endothelial cells (EC) were transfected with PBEF siRNA or infected with adeno-PBEF for 48 hours before treated with thrombin (0.1 U/mL) or S1P (1 mm) for various time. Parameters to evaluate endothelial cell barrier functions include transendothelial electric resistance (TER), actin staining, myosin light chain phosphorylation, calcium influx. **Results:** Reductions in PBEF protein expression (> 70%) by siRNA significantly attenuated EC barrier dysfunction induced by the potent edemagenic agent, thrombin reflected by reductions in TER (60 to 70% reduction). Furthermore, PBEF siRNA blunted thrombin-mediated increases in increases in $Ca^{2+}$ entry, polymerized actin formation, and myosin light chain phosphorylation, events critical to the thrombin-mediated permeability response. Overexpression of PBEF by adeno-PBEF vector significantly increases EC permeability reflected by decreased TER in HMVEC-L by ≈30%, indicating that overexpression of PBEF results in dysfunction. **Conclusions:** These in vitro observations strongly support that PBEF is critically involved in the endothelial cell barrier regulation. It encourages us to further explore the mechanistic insight into PBEF in the murine model of ALI.

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**SPHINGOSINE 1-PHOSPHATE DRAMATICALLY ALTERS THE HUMAN PULMONARY ARTERY ENDOTHELIAL CELLS' LIPID RAFT PROTEOME.** J. Zhao, P. Singleton, S. Dudek, J.G.N. Garcia, Department of Medicine, The University of Chicago, Chicago, IL.

**Rationale:** Lipid rafts are plasma membrane microdomains involved in dynamic membrane signaling and trafficking. Our previous studies (Singleton et al, FASEB J, 2005) have demonstrated that the platelet-derived lipid sphingosine 1-phosphate (S1P) plays a critical regulatory role in maintenance and enhancement of pulmonary vascular barrier function. S1P ligates the S1P receptor 1, which we have previously localized to lipid rafts of human pulmonary artery endothelial cells (HPAECs). Here we use 2-D electrophoresis analysis to characterize changes in tyrosine phosphoprotein content in lipid rafts of HPAECs challenged with S1P. **Methods and Results**: HPAECs were challenged with 1 μM of S1P for 5 minutes, washed with cold PBS twice, and solublized with 1% Triton X-100 at 4°C. The Triton X-100 insoluble material was subjected to discontinuous Optiprep™ gradient centrigugation and the light density (lipid raft) fractions were collected. Isolated lipid rafts were resolubilized in 50 μL of 7 M urea/2 M thiourea and 50 mM DTT. Isoelectric focusing was carried out on a nonlinear electrophoresis gel strip (pH3–10, 7 cm) with the second dimension carried out on 4–20% SDS-PAGE gel. Analytical gels were stained using Imperial blue (Pierce) and Western blotting was performed using antiphosphotyrosine antibody. Protein levels were not significantly altered in lipid rafts isolated from control vs S1P-treated cells (1 μM, 5 minutes). The 2-D gel images revealed over 30 proteins recruited to lipid rafts after S1P treatment (5 minutes) with antiphosphotyrosine blots showing S1P to increase tyrosine phosphorylation of over 20 proteins, including the src kinase family and cortactin. **Conclusions:** These results suggest that tyrosine kinases participate in S1P-induced signaling in lipid rafts and that 2-D proteomic analysis is a powerful tool for studying regulation of pulmonary endothelial cells barrier function.

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**HIGH-MOLECULAR-WEIGHT POLYETHYLENE GLYCOL PROTECTS THROMBIN-INDUCED ENDOTHELIAL BARRIER DYSFUNCTION BY INDUCING ACTIN CYTOSKELETON REARRANGEMENT, WHICH RESULTS IN ROBUST ENHANCEMENT OF ENDOTHELIAL CELL BARRIER INTEGRITY.** E.T. Chiang, S.M. Camp, S.M. Dudek, O. Zaborina, J.C. Alverdy, J.G. Garcia, The University of Chicago, Chicago, IL.

ARDS is characterized by sudden, life-threatening lung failure with diffuse alveolar infiltrate, reduced arterial oxygenation, and pulmonary edema. Acute lung injury (ALI) is the end result of common pathways initiated by a variety of local or systemic insults. Polyethylene glycol (PEG) compounds are inert and nontoxic polymers that act as a surrogate mucin lining providing protection against bacterial infections on intestinal epithelial cells. We hypothesized that PEG may provide similar protective effects on lung endothelium by attenuating endothelial cell (EC) activation that results in barrier dysfunction. This study examines the effects of a high-molecular-weight PEG (PEG15–20) on cultured human pulmonary microvessel EC from barrier disruptive agents. EC were cultured on microelectrodes and changes in transendothelial electrical resistances (TER) were measured to assess alterations in paracellular permeability. PEG induced a rapid, dose-dependent increase in TER similar to barrier-enhancing lipid such as sphingosine 1-phosphate (S1P). Optimal concentration of 7.5–9% PEG induced a robust increase in TER from 2,500 to 5,000 ohm, which was sustained for 40 hours. In comparison to S1P, which induced a 40% increase in resistance in 10–15 minutes, the maximum barrier enhancing effect of PEG was achieved