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**AN ELECTROPHORETIC STUDY ON THE EFFECT OF HEPARIN AND PROTAMINE SULFATE ON THE INTERACTION BETWEEN FACTOR XIa AND ANTITHROMBIN III.**  
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It has been known that factor XIa (XIa) can react covalently with antithrombin III (ATIII) to form two complexes with ratios of 2ATIII:1XIa and 1ATIII:1XIa. In the hands of these investigators, the M.W.s, as measured by SDS-PAGE, were 265 kDa and 225 kDa, respectively. In this investigation it has been observed that the addition of 1 µg or 5 µg heparin (H) to 3.1 × 10<sup>-5</sup> (mol ATIII for 5 minutes prior to the addition of 1.125 µ 10<sup>-5</sup> µmol XIa for a 30-minute incubation led to an increase in the 265 kDa band of 113% and 223%, respectively. These results were statistically significant ( $p < .01$ ). However, when H was premixed with XIa first, before the addition of AT III, statistical increases in the 265 kDa band were also seen (267% and 183%, respectively;  $p < .0005$ ,  $n = 6$ ). Protamine sulfate (PS) statistically significantly ( $p < .05$ ) inhibited the formation of the 265 and 225 kDa XIa-ATIII complexes at the 5 µg PS level when premixed with ATIII or XIa, respectively, the decreases in the 265 kDa band being 39.1 and 34.4%, respectively for [(ATIII/PS) + XIa] and [(XIa/PS) + ATIII] mixtures, and 23.1 and 23.8% for the 225 kDa band with [(ATIII/PS) + XIa] and [(XIa/PS) + ATIII] mixtures. These results with PS indicate that PS inhibits complex formation between XIa and ATIII at the 2ATIII:1 XIa and 1ATIII:1 XIa levels, in contrast with its reported stimulation of complex formation between thrombin and ATIII.

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**PROTECTIVE EFFECTS OF OXIDIZED PHOSPHOLIPIDS ON VENTILATOR-INDUCED LUNG INJURY.** D.O. Burdette,<sup>1</sup> S.A. Nonas,<sup>2</sup> I. Miller,<sup>3</sup> A.A. Birukova,<sup>4</sup> S. Chatchavalanich,<sup>1</sup> J.G.N. Garcia,<sup>1</sup> K.G. Birukov,<sup>1</sup> <sup>1</sup>Department of Medicine, The University of Chicago, Chicago, IL; <sup>2</sup>Department of Medicine, Johns Hopkins University, Baltimore, MD.

**Purpose of Study:** Acute lung injury (ALI) is a devastating syndrome characterized by pulmonary inflammation and vascular barrier dysfunction with protein-rich edema. Mechanical ventilation at high tidal volumes can worsen existing ALI and even cause ventilator-induced lung injury (VILI) de novo. Previous studies have demonstrated that oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (OxPAPC) enhances basal endothelial cell (EC) barrier properties and prevents acute lung inflammation and EC dysfunction in response to bacterial lipopolysaccharide (LPS) via direct Rac-Cdc42-mediated effects on EC cytoskeleton and via competitive inhibition of LPS binding to toll-like receptor 4 (TLR4). **Methods:** Adult male Brown Norway rats (250–350 g) were ventilated at low tidal volume (LTV, 7 mL/kg) or high tidal volume (HTV, 20 mL/kg) for 2 hours, 85 breaths/min. A subset of animals received intravenous OxPAPC (1.5 mg/kg) at the start of mechanical ventilation. Bronchoalveolar lavage (BAL) cell count and protein concentration were measured as markers of inflammation and vascular permeability. IL-6, IL-1β, and hyaluronan levels in BAL were quantitatively assessed by enzyme linked immunosorbance assays (ELISA). **Results:** HTV caused a 70% increase in BAL total cell count ( $p < .05$ ) and a 169% increase in BAL protein ( $p < .01$ ) compared with controls. OxPAPC reduced HTV-induced elevations in total cell count (41% decrease versus HTV alone) and protein (42% decrease versus HTV alone). HTV also caused an increase in the hyaluronan levels found in BAL samples, which was completely abrogated by intravenous injection of OxPAPC. HTV also caused a 223% increase in the IL-6 levels in BAL samples as compared to controls, which was attenuated by OxPAPC treatment (130% versus control,  $p < .05$ ). HTV had only a modest effect on IL-1β up-regulation (45% increase versus control), which still was attenuated by OxPAPC treatment (27% increase versus control). **Conclusion:** These studies demonstrate for the first time the protective effect of membrane-derived oxidized phospholipids on ventilator-induced lung inflammation and barrier dysfunction.

HL75349, HL76259, HL58064.

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**TUMOR NECROSIS FACTOR α EXPRESSION IS REGULATED BY DIFFERENTIAL MKP-1 OXIDATION IN BLOOD MONOCYTES COMPARED TO ALVEOLAR MACROPHAGES.** L.A. Tepfly, A.B. Carter, University of Iowa Carver College of Medicine and Iowa City Veterans Administration Medical Center, Iowa City, IA.

Monocytic cells are involved in the pathogenesis of immune and inflammatory disorders of the lung. A characteristic feature of alveolar macrophages obtained from asbestosis patients is the release of proinflammatory cytokines. We have shown previously that asbestos-induced p38 MAP kinase activity and TNF-α gene expression are mediated by H<sub>2</sub>O<sub>2</sub>. Since kinase activity is tightly regulated by phosphatases, we ask if MKP-1, which is a dual-specificity phosphatase that can be inactivated by H<sub>2</sub>O<sub>2</sub>-induced oxidation, played a role in regulating p38 activity in alveolar macrophages compared to blood monocytes. We hypothesized that MKP-1, in part, regulates p38 activity in alveolar macrophages due to inadequate H<sub>2</sub>O<sub>2</sub> generation in response to asbestos. We found that MKP-1 was constitutively expressed in alveolar macrophages, and its level increased with asbestos stimulation. In contrast, blood monocytes had minimal MKP-1 protein expression. When protein translation was inhibited with cycloheximide or MKP-1 was inhibited with triptolide or NaVO<sub>3</sub>, p38 activity was recovered in alveolar macrophages. In addition, when MKP-1 was immunoprecipitated from whole-cell lysates, an activated p38α remained phosphorylated when exposed to alveolar macrophage lysates, unlike when MKP-1 was present. To determine if MKP-1 oxidation was different in these cells, lysates from blood monocytes and alveolar macrophages were subjected to immunoprecipitation for MKP-1, which was then labeled with the thiol reactive fluorescent dye F5M. We found that MKP-1 in blood monocytes was oxidized at a significantly higher level than in alveolar macrophages. Blood monocytes stimulated with asbestos released TNF-α, while alveolar macrophages did not. These data suggest that MKP-1, through increased expression and lack of oxidation, regulates p38 MAP kinase activity and TNF-α gene expression in alveolar macrophages stimulated with crocidolite asbestos.

VA MERIT and ALA Career Investigator Award.

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**SIGNAL TRANSDUCTION AND GENE EXPRESSION IN MAGNITUDE-DEPENDENT MECHANOCHEMICAL REGULATION OF LUNG ENDOTHELIAL BARRIER.**

S. Chatchavalanich,<sup>1</sup> A.A. Birukova,<sup>1</sup> J.G.N. Garcia,<sup>1</sup> K.G. Birukov,<sup>1</sup> <sup>1</sup>The University of Chicago, Chicago, IL. Ventilator-induced lung injury is associated with activation of inflammatory cytokines and excessive lung distention, which directly affects blood-gas barrier and lung vascular per-

meability. Small GTPases Rho and Rac play an essential role in agonist-mediated endothelial cell cytoskeletal remodeling and permeability, but their role in lung endothelial cell barrier regulation under mechanical stress is less understood. We studied magnitude-dependent effects of cyclic stretch on lung endothelial cell barrier restoration after thrombin challenge and linked these effects with differential activation of Rho and Rac. Consistent with differential effects of 5% cyclic stretch and 18% cyclic stretch on thrombin-induced endothelial cell monolayer disruption, a peak of thrombin-induced Rho activation at 5 minutes was enhanced by endothelial cell preconditioning at 18% cyclic stretch and was attenuated by 5% cyclic stretch. Endothelial cell preconditioning at physiological cyclic stretch (5%) induced more pronounced activation of Rac at 30 minutes compared to endothelial cell exposed to 18% cyclic stretch and caused nearly complete endothelial cell monolayer recovery observed after 50 minutes of thrombin stimulation, which was associated with peripheral accumulation of Rac effector cortactin. Promotion of endothelial cell barrier restoration by physiological cyclic stretch as well as cortactin peripheral accumulation was abolished by siRNA-based depletion of Rac expression or by Rac pharmacological inhibitor NSC-23766. Lung endothelial cell subjected to long-term cyclic stretch (18% elongation, 24 hours) increased expression of procontractile cellular proteins Rho, myosin light chain kinase, ZIP-kinase, and released extracellular bioactive molecules with barrier-disruptive properties. These results show involvement of Rac-mediated mechanisms in the barrier-promoting effects of physiologic cyclic stretch preconditioning and suggest direct link between amplitude-dependent activation of Rho- and Rac-mediated pathways and dynamic regulation of lung endothelial barrier by mechanochemical stimuli.

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**OXIDIZED PHOSPHOLIPIDS REDUCE VASCULAR LEAK AND INFLAMMATION IN A RAT MODEL OF ACUTE LUNG INJURY.** S. Chatchavalanich,<sup>1</sup> S. Nonas,<sup>2</sup> I. Miller,<sup>2</sup> K. Kawkitinarong,<sup>2</sup> I. Gorshkova,<sup>1</sup> V.N. Bochkov,<sup>3</sup> N. Leitinger,<sup>4</sup> V. Natarajan,<sup>1</sup> J.G.N. Garcia,<sup>1</sup> K.G. Birukov,<sup>1</sup> <sup>1</sup>The University of Chicago, Chicago, IL; <sup>2</sup>Johns Hopkins University School of Medicine, Baltimore, MD; <sup>3</sup>University of Vienna, Vienna, Austria; <sup>4</sup>University of Virginia, Charlottesville, VA.

Acute inflammation and vascular leak are cardinal features of acute lung injury and the acute respiratory distress syndrome. Nonspecific tissue inflammation and injury in response to infectious and noninfectious insults lead to oxidative stress and the generation of lipid oxidation products, which, in turn, may inhibit the acute inflammatory response to bacterial components. In this study, we used animal and endothelial cell culture models of lipopolysaccharide-induced lung injury to test the hypothesis that oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine may attenuate the acute lung inflammatory response to bacterial wall lipopolysaccharide (LPS) and enhance lung vascular barrier recovery. Rats received aerosolized LPS (5 mg/kg) or sterile water with concurrent intravenous oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (0.5–6 mg/kg) or saline. At 18 hours, bronchoalveolar lavage was performed and the lungs were removed for histological analysis. Measurements of transendothelial electrical resistance and histochemical analysis of endothelial monolayer disruption were used as an *in vitro* model of LPS-induced lung barrier dysfunction. Intratracheal instillation of LPS induced lung injury with profound increases in bronchoalveolar lavage and tissue neutrophils, protein content, and production of inflammatory cytokines IL-6 and IL-1β. Intravenous injection of oxidized phospholipids markedly attenuated LPS-induced tissue inflammation, barrier disruption, and IL-6 and IL-1β production over a range of doses. *In vitro*, oxidized phospholipids attenuated LPS-induced endothelial permeability and reversed LPS-induced cytoskeletal remodeling and disruption of monolayer integrity. These studies demonstrate *in vivo* and *in vitro* protective effects of oxidized phospholipids on LPS-induced lung dysfunction.

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**EVIDENCE OF OXIDATIVE STRESS IN A RAT MODEL OF PREECLAMPSIA.**

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We have developed a rat model of preeclampsia related to the administration to pregnant animals of desoxycorticosterone acetate (DOCA) and the replacement of their drinking water with 0.9% saline. At 16–19 days of gestation, the rats demonstrate established hypertension, proteinuria, and intrauterine growth restriction (IUGR). Oxidative stress has been reported in various hypertensive models and is considered to play an important role in the pathogenesis of some of the clinical features of preeclampsia. Accordingly, we have studied the urinary excretion of 8-isoprostane (8-IP), an indicator of the oxidative process. Pregnant Sprague-Dawley rats (250–300 g) were rendered "preeclamptic" (PDS,  $n = 7$ ). Determinations were performed prior to pregnancy and at 16–19 days of gestation. Their blood pressures (BPs), 24-hour protein, and 8-IP excretion in urine and pup numbers for PDS rats were compared to those of normal pregnant animals (NP,  $n = 6$ ). BP, obtained with a tail cuff method, increased in the PDS rats from a baseline level of  $114 \pm 3$  to  $136 \pm 2$  mm Hg ( $p < .001$ ). BP in the NP rats fell from a mean baseline value of  $117 \pm 3$  to  $108 \pm 5$  mm Hg ( $p < .05$ ), nearing the end of gestation, much as is the case in the course of human pregnancy. Urinary protein excretion was higher in PDS than in NP animals ( $18.6 \pm 3.6$  and  $4.0 \pm 0.3$  mg protein/24 h, respectively,  $p < .001$  at the end of gestation. Pup number was  $8 \pm 1$  in the PDS rats, which differed significantly ( $p < .005$ ) from the corresponding value for the NP animals ( $15 \pm 1$ ). In addition, malformed pups were common in the PDS rats but were not seen in the NP animals. Urinary 8-IP excretion increased from  $10.0 \pm 1.6$  to  $16.5 \pm 2.3$  pg/min ( $p < .05$ ) in the PDS rats. The mean excretion rates in the NP animals did not change significantly ( $p > .05$ ) from pre-pregnancy to late gestation periods ( $6.6 \pm 0.6$  and  $9.3 \pm 1.3$  pg/min, respectively). Based upon these data, we conclude that oxidative stress is a feature of a rat model of preeclampsia as it is in the human condition.

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**KILLING OF NOSOCOMIAL PATHOGENS BY ACID AND ACIDIFIED NITRITE: IMPLICATIONS FOR INFECTION CONTROL.** A. Rao,<sup>1</sup> M.J. Pultz,<sup>2</sup> R. Jump,<sup>2</sup> C.J. Donskey,<sup>2</sup>

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**Background:** Proton pump inhibitors are considered safe medications, but their use has been associated with colonization or infection with nosocomial pathogens. We used a mouse model to test the hypothesis that inhibition of gastric acid production by proton pump inhibitor treatment may facilitate establishment of intestinal colonization by nosocomial pathogens such as vancomycin-resistant enterococci (VRE) and *Klebsiella pneumoniae*. **Methods:** *In vitro* studies were performed to examine killing of VRE and *K. pneu-*