Total and pneumococcal-specific IgA levels did not change after initiation of HAART. No change was detected in the levels of pneumococcal-specific IgA when expressed as a percentage of total IgA. No significant correlation was found between BAL IL-6 and IgA concentration or BAL percentage lymphocytes and IgA concentrations. **Conclusion:** HAART is not associated with significant changes in total or pneumococcal-specific IgA concentrations in the distal respiratory tract. We speculate that this reflects the more dominant role of IgG in protection of the alveolar space against bacterial pathogens.

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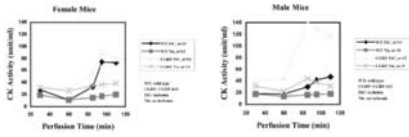
BIOINFORMATIC CLASS DISCOVERY OF POTENTIAL BIOMARKERS IN VENTILATOR-ASSOCIATED LUNG INJURY. S.-F. Ma,[1] D.N. Grigoryev,[2] R.B. Easley,[3] S.Q. Ye,[1] B.A. Simon,[3] J.G N. Garcia,[1] [1]Critical Care, The University of Chicago, Chicago, IL; [2]Clinical Immunology and [3]Anesthesiology and Critical Care, Johns Hopkins University, Baltimore, MD.
Rationale: The unilateral (one lung injured and the contralateral lung intact) model of ventilator-associated lung injury (VALI) allows us to utilize uninjured lung as the systemic VALI effects indicator. We speculated that regulation of genes in uninjured lung will be attributed to blood or lymph carrying effectors produced by an injured lung and the analysis of upstream bioprocesses governed by affected genes will lead to the effectors' discovery. **Methods:** Dogs (n = 4) were intubated, left lung lavaged, and then both lungs were either independently ventilated (total Vt = 15 mL/kg) for 6 hours (injured and uninjured) or immediately harvested (control). Lung mRNA (n = 16 for all three groups) was hybridized to HG_U133A and analyzed by SAM using interspecies probe adjustment. Genes with the lowest false discovery rate (q = 0.124%) that imposed fold change (FC) range from −3.52 to −1.26 and 1.22 to 6.96 with corresponding FC averages −1.59 and 2.01 were considered affected by VALI. Gene ontology filtering for receptor activity term was conducted by MAPPFinder. **Results:** Our analyses revealed 22 receptor-related genes, of which 7 were growth factor receptors including EGFR (FC = −1.54), FGFR1 (FC = −1.59), FGFR2 (FC = −1.56), and PDGFR (FC = −1.30). The overall down-regulation of these receptors was concordant with decreased expression of their corresponding ligands in injured lung including EGF (FC = −1.39), FGF2 (FC = −1.39), PDGFA (FC = −1.41), and PDGFB (FC = −2.00). Fibroblast (Z = 6.17) and epidermal (Z = 4.49) growth factor receptor activity were the first and the third significantly (Z > 1.96) affected pathways. **Conclusions:** Our approaches effectively identify a class of potential biomarkers in VALI. Further investigation of this study may elucidate systemic VALI effects and facilitate the development of new diagnostic tools.



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MYOCARDIAL ISCHEMIA CAUSES HIGHER CREATINE KINASE RELEASE IN CALCITONIN GENE–RELATED PEPTIDE KNOCKOUT MALE MOUSE HEARTS. H. Ma, R Huang, A. Carve, I. Shah, S.C. Supowit,* D.J. DiPette,* G.S. Abela, Department of Medicine, Michigan State University, East Lansing, MI; *Texas A & M University, Scott and White Hospital, Temple, TX.
Background: Calcitonin gene–related peptide (CGRP) influences vasoregulatory activities. We determined the gender-specific effects of CGRP knockout (KO) on creatine kinase (CK) activity following ischemia. **Methods:** Ninety-six mice were studied in a Langendorff preparation using 50 mm Hg perfusion pressure. Myocardial ischemia was induced by stopping flow to the coronary arteries for 20 minutes. The perfusion buffer was collected from a small chamber that housed the heart. Perfusion buffer was collected over 2 hours and CK activity was measured. **Results:** CK activity was significantly greater in CGRP-KO mouse hearts compared to wild-type (see graphs). Male CGRP-KO hearts released 1.5 times more CK than female CGRP-KO hearts 15 minutes after ischemia (130.4 vs 90.2 unit/mL, $p < .003$). **Conclusions:** CGRP contributes significantly to CK release during ischemia. This effect is more prominent in the male compared to the female mouse heart.

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OXIDIZED PHOSPHOLIPIDS MEDIATE INTERACTION BETWEEN ADHERENCE JUNCTION AND FOCAL ADHESION PROTEINS. I. Malyukova,[1] A. A. Birukova,[1] A. Rios,[2] K.G. Birukov,[1] [1] Department of Medicine, The University of Chicago, Chicago, IL; [2] Department of Medicine, Johns Hopkins University, Baltimore, MD.
Introduction: Oxidized phospholipids appear in the pulmonary circulation as a result of acute lung injury or inflammation. We have previously shown that oxidized phospholipids exhibit barrier-protective effects on pulmonary endothelial cell (EC) monolayers. However, effects of oxidized phospholipids on EC focal adhesion (FA) and adherence junction (AJ) remodeling have not been yet fully explored. **Goal:** To study molecular mechanisms of adherens junction and focal adhesion remodeling mediated by oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OxPAPC). **Methods:** All experiments were performed on human pulmonary artery endothelial cells (HPAEC). Intracellular protein localization was analyzed by immunocytochemistry. Subcellular localization of the proteins of interest was determined using subcellular proteome extraction kit. Protein phosphorylation profile was assessed by Western blot analysis. Protein-protein interactions were analyzed by coimmunoprecipitation assays. **Results:** Enhancement of EC barrier in response to OxPAPC was accompanied by dynamic remodeling of focal adhesions and adherence junctions. Immunofluorescent analysis of OxPAPC-stimulated EC revealed dramatic translocation and peripheral and enhanced peripheral staining for AJ proteins beta-catenin and VE-cadherin. In addition, OxPAPC treatment increased tyrosine phosphorylation of FA proteins FAK at Tyr-576 and paxillin at Tyr-118, which was associated with peripheral redistribution of FA and AJ complexes. Furthermore, subcellular fractionation analysis showed increase of VE-cadherin, beta-catenin, and GIT2 in membrane fraction after OxPAPC challenge. Remarkably, coimmunoprecipitation studies indicated increased interaction of paxillin with FA components FAK, vinculin and GIT2, and AJ protein beta-catenin upon HPAEC stimulation with OxPAPC. Complementary experiments with immunoprecipitation beta-catenin followed by probing for paxillin confirmed these results. **Conclusions:** The results of these studies characterize OxPAPC-induced focal adhesion remodeling and determine for the first time the specific interactions between focal adhesion and adherens junction protein complexes in endothelial barrier-protective responses to OxPAPC

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***BACILLUS ANTHRACIS* SPORES STIMULATE CYTOKINE AND CHEMOKINE INNATE IMMUNE RESPONSES IN HUMAN ALVEOLAR MACROPHAGES THROUGH MULTIPLE MAPK PATHWAYS.** K. Chakrabarty, W. Wu, J.L. Booth, E.S. Duggan, K.M. Coggeshall, J.P Metcalf, Pulmonary and Critical Care Division, Department of Medicine, University of Oklahoma Health Sciences Center, and the Program in Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK.
Contact with the human alveolar macrophage plays a key role in the innate immune response to *Bacillus anthracis* spores. Because there is a significant delay between the initial contact of the spore with the host and clinical evidence of disease, there appears to be temporary containment of the pathogen by the innate immune system. Therefore, the early macrophage response to anthrax exposure is important in understanding the pathogenesis of this disease. We examined the initial events after exposure of human alveolar macrophages obtained by bronchoscopy to *Bacillus anthracis (Sterne)* spores. Spores were rapidly internalized as determined by confocal microscopy. Spore exposure also rapidly activated the MAPK signaling pathways ERK, JNK, and P38. This was followed by transcriptional activation of cytokine and primarily monocyte chemokine genes as determined by RNase protection assays. Transcriptional induction was reflected at the translational level as IL-1α and β, IL-6, and TNF-α cytokine protein levels were markedly elevated as determined by ELISA. Induction of IL-6 and TNF-α, and to a lesser extent IL-1α and -β, was partially inhibited by blockade of individual mitogen-activated protein kinases, while complete inhibition of cytokine induction was achieved when multiple signaling pathway inhibitors were used. Taken together, these data clearly show activation of the innate immune system in human alveolar macrophages by *Bacillus anthracis* spores. The data also show that multiple signaling pathways are involved in this cytokine response.

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A REEVALUATION OF EVANS BLUE DYE AS A MARKER OF ALBUMIN CLEARANCE IN MOUSE MODELS OF ACUTE LUNG INJURY. S. Sammani, J. Moitra, J.G.N. Garcia, Department of Medicine, The University of Chicago, Chicago, IL.
Background: Quantifying the amount of albumin conjugated to Evans Blue dye (Alb-EB) fluxing across organ barriers is a popular technique to measure intactness of the physical barrier in rodent models of a variety of diseases. We have reevaluated this technique in terms of the correction factors required in a spectrophotometric assay. **Methods and Results:** Eight- to 10-week-old C57BL6/J mice received either pH-neutral water (controls) or LPS (treatment) by intratracheal instillation, and acute lung injury was allowed to develop for 24 hours. Both control and treatment mice were further injected with Alb-EB via the jugular vein, at doses of either 20 or 30 mg/kg body weight, at 30, 60, 120, or 180 minutes before termination of the LPS treatment (24 hours total). At the end of exposure, formamide extracts of lungs were prepared, and the centrifuged supernatants were measured at 620 and 740 nm in a spectrophotometer. Lungs from control mice that were not injected with Alb-EB were similarly extracted and measured. The linear regression equation between absorbances at 740 nm (X) and 620 nm (Y) in control lung extracts of animals that did not get Alb-EB was considered to be the tissue-specific correction factor. The observed absorbances of the control and treatment samples at 620 and 740 nm were then normalized using this factor. This tissue-specific correction resulted in control samples read as positive integers, as opposed to negative integers when using a serum correction factor commonly used in the literature. We also determined that adjusting the duration of the conjugated dye in circulation is critical for maximizing the signal-to-noise ratio. **Conclusion:** The Evans Blue dye extravasation method to quantify barrier dysfunction can be improved in terms of repeatability and sensitivity by using tissue-specific correction factors and maximized signal-to-noise ratios, respectively.
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NOX4, A HOMOLOGUE OF NOX2, REGULATES HYPEROXIA-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION AND ANGIOGENESIS IN HUMAN LUNG ENDOTHELIAL CELLS. S. Pendyala, I. Gorshkova, B. Gorshko, H. Donghong, R.K. Stern, P. Usatyuk, V. Natarajan, Department of Medicine, The University of Chicago, Chicago, IL.
Rationale: Nox4, a homologue of Nox2 ($gp91^{phox}$), is involved in ROS production and signal transduction in vascular cells. In human pulmonary artery endothelial cells (HPAECs), mRNA expression of Nox4 is several folds higher compared to Nox2 and exposure of cells to hyperoxia (95% O_2, 24 hours) resulted in up-regulation of Nox4 and $p22^{phox}$ but not Nox1 or Nox3. Nox4 siRNA partially reduced ROS formation and blocked cell motility and capillary tube formation in cells exposed to either normoxia or hyperoxia, suggesting a role for Nox4 in angiogenesis. **Methods/Results:** In HPAECs and human lung microvascular ECs, expression of Nox4 was several folds higher, as shown by real-time PCR, and exposure to hyperoxia (24 hours) up-regulated Nox4 mRNA as well as protein expression. The localization of Nox4 in HPAECs, as determined by immunofluorescence microscopy with Nox4 antibody, revealed that a majority of the native Nox4 protein was localized near the perinuclear region that stained positive for Golgi marker and a small fraction extended throughout the cytoplasm in internal membrane and vesicular structures. Exposure of cells to hyperoxia (3 hours) caused the Golgi to assume a rounded appearance from a saucer-shaped structure where in majority of Nox4 was colocalized. As hyperoxia-mediated cell motility was attenuated by Nox4 siRNA and was dependent on ROS production, we studied the role of Nox4 in capillary tube formation using matrigel assay. Exposure of HPAECs grown on matrigel to hyperoxia (24 hours) increased the number of capillary tubes compared to normoxia and Nox4 siRNA attenuated the capillary tube formation. **Conclusion:** Nox4 participates in ROS production and acts as a signaling protein that plays a pivotal role in regulating key EC functions such as migration and capillary tube formation.
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5-HYDROXYTRYPTAMINE 4 RECEPTOR IN THE ENDOTHELIAL CELLS. I. Profirovic, I. Vardya, T. Voyno-Yasenetskaya, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL.
Serotonin (5-hydroxytryptamine [5-HT]) is an important neurotransmitter that regulates multiple events in the central nervous system (CNS). We have recently demonstrated that 5-HT4 receptor couples to G13 protein to induce RhoA-dependent gene transcription, neurite retraction, and neuronal cell rounding (Ponimaskin et al, 2002). Although multiple