American Thoracic Society

EDUCATION

2017 MTDS AWARD WINNING ABSTRACTS

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Program Overview

The American Thoracic Society (ATS) would like to congratulate the 40 Minority Trainee Development Scholarship (MTDS) awardees who are being recognized at the 2017 Diversity forum for their excellent abstracts!!

The MTDS program provides an opportunity for individuals of minority status in science and medicine to travel to the International Conference yearly. A unique aspect of the MTDS is that a trainee at any level (high school and beyond) who submits an abstract is eligible to be considered for this award. On average each year, the ATS receives 60+ applications and a sub-committee chaired by Dr. Yolanda Mageto, ranks applications to select the top applicants. From 2002 to 2013 this program was supported by Merck; however, in the past few years that ATS has funded this program because the Society values advancing the careers of early career professionals.

History and Results:

The MTDS program aims to address the lack of underrepresented minorities in medicine, especially respiratory medicine. The program was created in 2002 under the leadership of Membership and Training Committees spearheaded by Dr. Estelle Gauda in an effort to recruit the best and brightest underrepresented minorities to the field of pulmonary, critical care and sleep medicine. Each year the MTDS recipients are honored at the Diversity Forum and presented with a $1000 scholarship and a certificate of achievement.

- Over the past 17 years, the ATS have given a total of 367 MTDS scholarships to attend the ATS International Conference.
- Out of those 17 years, ATS has received educational support from Merck for 12 consecutive years to provide scholarships for the MTDS.
- This is the third consecutive year that the ATS funds the MTDS scholarships.
- Of the 327 past awardees 155 participants are pursuing a career in Pulmonary, Critical Care and /or Sleep Medicine and 152 participants have remained members of the ATS.
- MTDS Awardees have stated in surveys done from 2011 – 2017 that they would not have attended the ATS International Conference if it were not for the MTDS scholarship.

Criteria:

Each MTDS recipient is an author of an abstract accepted for presentation at the ATS 2017 International Conference. The awards are based on the quality of science, the contribution of the trainee to the project and the potential impact of the award on the trainee's career development. Additional award criteria includes:

- Must be a member of an underrepresented minority group as defined by the NIH (African American, Hispanic, Native American Alaskan Native or Pacific Islander)
- Must not be a recipient of another abstract award to the 2017 ATS International Conference
- Must be a trainee (high school trough post-doctoral fellow) at a US Institution.
- Must be an author (preferably first author) of an abstract accepted for presentation at the ATS IC.

The Minority Trainee Development Scholarship (MTDS) would like to thank the American Thoracic Society for their generosity in support this program!

Single Cell RNA-sequencing reveals distinct effects of inhibition of FENDRR, a long non-coding RNA implicated in fibroblast to myofibroblast differentiation

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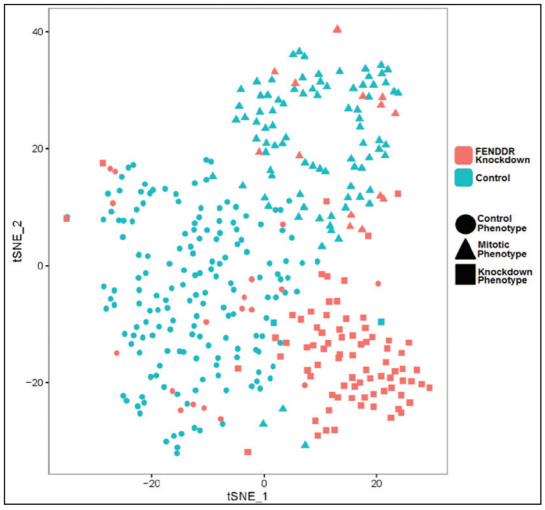
Rationale: FOXF1 adjacent non-coding developmental regulator RNA (FENDRR) is a long non-coding RNA found expressed highly expressed in the lung and relatively limited to mesenchymal cells. We have shown that FENDRR is decreased in idiopathic pulmonary fibrosis (IPF) lungs and that it is involved in regulating fibroblast to myofibroblast transition and senescence in lung fibroblasts through epigenetic effects on chromatin remodeling. Single cell RNA-seq is an increasingly powerful tool for providing insight into gene expression and cell identity in computationally powerful ways. Here we utilize single cell RNA-seq to determine the detailed effects of inhibiting FENDRR at the single cell level.

Methods: Primary human lung fibroblasts were transfected with siRNA targeting FENDRR; fibroblasts transfected with a scrambled sequence were used as a control. We established the Drop-seq protocol [1] in our lab and utilized it to generate single cell barcoded cDNA libraries from each treatment. Both libraries were sequenced (Illumina) at a combined depth of ~300 million reads. Data analysis was conducted in R, the package Seurat was used for cluster analysis and the identification of differentially expressed genes.

Results: 250 control cells were compared with 112 knockdown cells. The average expression of FENDRR in control cells was 7.2 transcripts per 100,00 transcripts, but 21.2% of control cells lacked any expression of FENDRR. In cells transfected with siRNA, 25.9% had FENDRR levels similar to controls and 83 cells had an average decline in expression of 85.97%.

Functional annotation (DAVID) of the differentially expressed genes characterizing the knockdown cells revealed enrichment in smooth muscle contraction (p-value: 2.86E-6) as well as the p53 signal pathway (p-value: 7.7E-6) and cell cycle arrest (p-value: 2.3E-4) confirming our previous results. Additionally, endoplasmic reticulum translocation (p-value: 4.3E-19) was one of the highest enriched biological functions observed, suggesting a new set of targets for FENDRR previously unrecognized.

Cluster analysis revealed 3 distinct groups of cells. A normal fibroblast phenotype (16.1% of the knockdown treated cell population; 64.8% of the control population), a cluster characterized by mitotic cell cycle markers (13.4% knockdown; 34.4% control), and a knockdown phenotype (70.5% knockdown; 0.8% control).



Conclusion: Our data provides a single cell view of the role of FENDRR as an epigenetic regulator of gene expression networks regulating myofibroblast differentiation and senescence. Follow up studies include detailed phenotypic assessment of gain and loss of function and generation of a detailed map of chromatin changes that determine the effects of FENDRR.

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The Use of Branched-DNA In Situ Hybridization to Diagnose Mycobacterial Infection in Formalin Fixed-Paraffin Embedded Tissues

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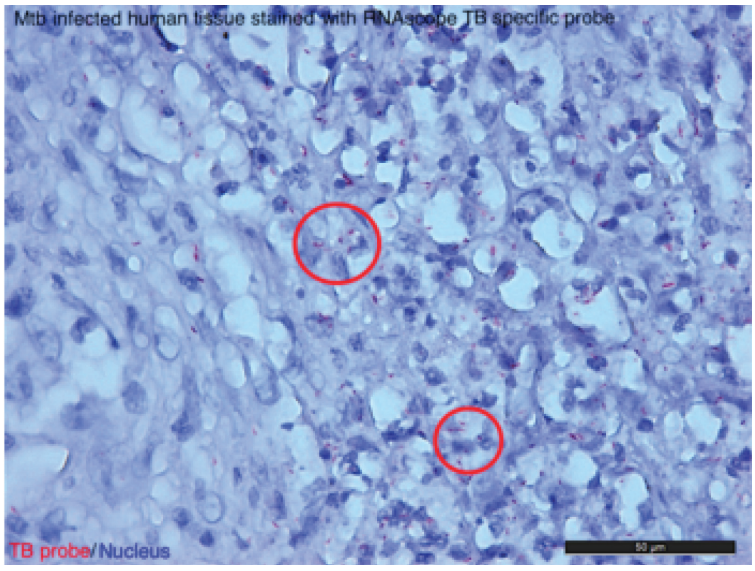
San Francisco, CA/US; Mycobacterium Tuberculosis on Adults

Introduction: *Mycobacterium tuberculosis* (Mtb) the bacteria responsible for Tuberculosis (TB) affects 1 in 3 people worldwide. The most widely used assays for diagnosing infection with Mtb include Ziehl-Neelsen staining, polymerase-chain reaction, and bacterial culture. Moreover, the diagnosis of TB is even more difficult in the setting of coinfection with HIV. *In situ* hybridization (ISH) offers the ability to visually assess biological samples for the presence of mycobacterial nucleic acids. Older generations of ISH are long, technically challenging assays with low signal-to-noise ratios and thus not appropriate as a clinical diagnostic for TB. Branched-DNA (bDNA) based ISH assays have demonstrated improved sensitivity and specificity compared to older ISH technologies. Here we describe the use of RNAscope, bDNAISH technology by Advanced cell Diagnostics (ACD) for the sensitive and specific diagnosis of Mtb in formalin-fixed paraffin-embedded (FFPE tissues).

Methods: Deidentified and banked FFPE human tissues that were known to be infected with Mtb or MycobacteriumAvium Complex (MAC) were donated by the clinical pathology lab at the San Francisco General Hospital and assessed for the presence of mycobacterial nucleic acids using RNAscope. RNAscope was used according to the manufacturers recommendations with the exception of the pretreatment steps. Ribosomal RNA sequences were targeted because their abundance and conservation among species. We used a twostep approach whereby tissues were first assessed for Mtb and nonTuberculous mycobacterium (NTM) using probes targeting 16S rRNA sequences (screening probe), followed by assessment with probes specifically targeting 23S rRNA Mtb sequences (TB specific probe). In this way samples that are 16S+/23Sare NTM, and 16S+/23S+ samples thought to be infected with Mtb. The thick mycobacterial cell wall has posed a significant barrier to ISH based TB diagnostics. Therefore, we tested a panel of pretreatment conditions described in the literature or provided by ACD to find conditions that allow the sensitive and specific detection of mycobacterial nucleic acids. These reagents were varied by concentration, time, and temperature.

Results: RNAscopeISH targeting mycobacterial ribosomal RNA was able to sensitively detect mycobacteria in FFPE tissues and specifically distinguish MAC from Mtb. Tissues infected with MAC compared to those with Mtb required a more aggressive pre-treatment strategy for sensitive detection. The pre-treatmentused for MAC infected samples was also useable for Mtb infected samples.



Conclusion: Branched DNAISH technology offers an alternative approach for the diagnosis of mycobacteria in FFPE tissues. Moreover, this technique is easily scalable, rapid, and transferable to cell suspensions and other sample types.

Optimization of a Protocol for Quantification of the Innate Immune Response in Human Whole Blood

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Introduction: The innate immune response is the first line of defense against infection, and is thought to play a pathophysiologic role in virtually every category of human disease. Toll-like receptors (TLRs) detect microbial molecules and trigger rapid induction of cytokines, which, in turn, promote host defense and inflammation. The magnitude of the innate immune response triggered by specific TLRs varies across individuals, potentially determining risk for disease, but the genetic and environmental determinants of human TLR responses remain poorly understood. In order to better define the inter-individual genetic and environmental determinants of innate immunity, it is important to establish robust, reproducible, and high-throughput methods for quantifying TLR pathway output that are applicable to accessible primary human cells/tissues.

Objective: To optimize a protocol for quantifying the human whole blood ex vivo inflammatory response to microbial molecule stimulation..

Methods: Whole blood was collected from 10 volunteers (3 female, 7 male) for same-day microbial molecule stimulation. Peripheral blood monocytes were quantified using a clinical laboratory to allow for cytokine normalization to cell number. Study exclusion criteria included use of anti-inflammatory or immunosuppressive agents, confirmed/suspected immunodeficiency, autoimmune disease, and recent gastrointestinal illness. Blood was anticoagulated using sodium citrate (0.1mol/L, pH 7.2), diluted 1:1 with RPMI 1640 medium, and added (240ul) under sterile conditions to each well of a 96-well plate pre-spiked with 10ul of selected pathogen-associated molecular patterns. Ligand concentrations tested were 1ng/mL LPS (TLR4), 1-10ug/mL Pam3CSK4 (TLR2), 1-10ug/mL L18-muramyl dipeptide (NOD2), 25-100ug/mL imiquimod (TLR7), 1-10ug/mL poly(I:C)(TLR3), and 1-10ug/mL CL075 (TLR7/8). Serum was harvested after 3h and 6h incubation (37oC, 5% CO2) for measurement of TNF-α by ELISA.

Results: Robust and reproducible TNF-α induction was seen in response to LPS (359.9-1516.0 pg/mL), Pam3CSK4 (9.8-235.2 pg/mL), and CL075 (22.0–1604.4 pg/mL) at all tested concentrations at 3h. TNF-α induction was seen with 100ug imiquimod (0-62.2 pg/mL) at 3h. TNF-α concentrations were higher at 6h than at 3h. Poly(I:C) and L-18 muramyl dipeptide did not demonstrate robust or reproducible cytokine induction at the concentrations tested.

Conclusions: Concentrations were established for LPS, Pam3CSK4, CL075, and imiquimod that elicit robust and reproducible cytokine induction following ex-vivo stimulation of whole blood from healthy subjects. In studies currently underway, we are using these conditions to compare the innate immune response of blood donors with defined single nucleotide polymorphisms in the gene immunity-related GTPase M (IRGM) that have been reported to associate with increased risk for Crohn's Disease and Tuberculosis.

Greater Odds for Cardiovascular Diseases in Uranium Miners than Non-uranium Miners in New Mexico

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Introduction: It is well documented that radiation exposure is associated with increased risk for cardiovascular disease in Japanese atomic bomb survivors. Radiation-related pulmonary fibrosis is described in uranium miners, but current literature is inconclusive about an association between uranium mining and cardiovascular disease presumably mediated by radiation exposure. We hypothesized that uranium miners in New Mexico have a greater prevalence of cardiovascular disease than non-uranium miners.

Methods: Using cross-sectional standardized questionnaire data from the Mining Dust in the United States (MiDUS) cohort that included miners participating in community-based mobile screening clinics in New Mexico between 1998 and 2008, we compared uranium miners to non-uranium miners who worked with coal, metal, nonmetals, and other materials. The multivariable analysis was adjusted for age, sex, race/ethnicity, diabetes mellitus, BMI, cigarette smoking, and snuff exposure. Data were analyzed using SAS software with p-values <0.05 considered significant.

Results: Of the 1,954 miners screened, mining exposure history was known in 1,814 eligible subjects. Most miners were men (95.5%) and of middle-age (53.8 ± 14.9 years). Hispanics, non-Hispanic whites, and Americans Indians constituted 49.4%, 30.9%, and 17.5% of the study population, respectively. 43.8% mined uranium. Most miners worked in surface mining operations (49.2%). As compared to non-uranium miners, uranium miners were older and more likely to be American Indians and diabetic but less likely to currently smoke or use snuff (≤0.009 for all analyses). Uranium miners were more likely to report any cardiovascular disease, including angina, myocardial infarction, and hypertension, than non-uranium miners, even after adjustment for covariates (Table 1). Uranium miners were also more likely to report cerebrovascular accident, but this association was not statistically significant after adjustment for covariates (p=0.053).

Conclusions: New Mexican uranium miners are more likely to report a history of cardiovascular disease than non-uranium miners. The strengths of the study include its large sample size, high proportion of minority subjects, and use of a control population that was occupationally exposed to similar agents except radiation. The weaknesses include absence of occupational radiation exposure measurements and possible environmental radiation exposure from uranium tailings in and near homes. Our data suggest that in addition to screening for pulmonary diseases, uranium industry workers should be screened for cardiovascular diseases.

	Bivariate analysis	Multivariable analysis
Outcome	O.R. (95% C.I.)	O.R. (95% C.I.)
Any cardiovascular disease	2.60 (2.13, 3.18)	1.95 (1.53, 2.49)
Angina	3.16 (2.26, 4.43)	2.27 (1.57, 3.28)
Myocardial infarction	2.80 (1.96, 4.00)	2.02 (1.35, 3.03)
Hypertension	2.22 (1.81, 2.71)	1.66 (1.30, 2.12)
Cerebrovascular accident	2.91 (1.69, 5.01)	1.89 (0.99, 3.60)

Surfactant Protein D deficiency increases survival after CLP by regulating the gut microbiome

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Rationale: Sepsis is a devastating condition that affects nearly 750,000 Americans annually and is associated with substantial morbidity and mortality. Surfactant protein D (SPD) is a member of the collectins family of proteins that plays a key role in innate immunity and pathogen clearance and is expressed at mucosal surfaces of the lung and other organs. Paradoxically, we found that SPD null mice have decreased mortality following cecal ligation and puncture (CLP). We set out to examine inflammatory cell responses and gut microbiome characterization of WT and SPD KO mice.

Methods: SPD null (KO) and wild type (WT) mice were subjected to CLP (23g, 1 hole, 100% ligation). Blood and peritoneal lavage fluid were obtained for bacterial counts post CLP. The microbiome of WT and SPD KO mice was analyzed using standard microbiologic techniques and bacterial 16S rRNA sequencing. In vivo phagocytosis assays were performed by injecting mice with GFP labeled bacteria prior to harvesting peritoneal cells for FACS analysis. Microbiome colonization experiments were performed using oral gavage with GFP labeled bacteria followed by quantitative analysis of gut segments for labeled bacteria.

Methods: Primary human lung fibroblasts were transfected with siRNA targeting FENDRR; fibroblasts transfected with a scrambled sequence were used as a control. We established the Drop-seq protocol [1] in our lab and utilized it to generate single cell barcoded cDNA libraries from each treatment. Both libraries were sequenced (Illumina) at a combined depth of ~300 million reads. Data analysis was conducted in R, the package Seurat was used for cluster analysis and the identification of differentially expressed genes.

Results: Most WT mice had E. coli bacteremia 24 hours after CLP. In contrast, none of the SPD null mice had E. coli bacteremia. FACS assays showed no difference in the ability of SPD WT and KO neutrophils to phagocytose GFP labeled E. coli ex vivo, while in vivo phagocytosis assays revealed a mild increase in neutrophil phagocytosis in SPD KO mice that did not explain the observed findings. Microbiologic analysis of cecal contents revealed substantial differences in the gut microbiome of WT and SPD KO mice including the absence of enteric Gram negative rods in SPD null mice. Administration of exogenous E. coli by peritoneal fibrin clot produced similar mortality in WT and SPD KO mice. Finally, oral gavage with exogenous GFP-labeled E. coli revealed that SPD KO mice had cleared bacteria from all gut segments by 48h, while WT animals had retention of labeled E. coli in gut segments at 48h

Methods: SPD KO mice have decreased mortality following CLP that appears largely due to differences in gut microbiota. Our studies to date support a role for SPD in mediating E. coli colonization of the gut, possibly driving differences in response to CLP. Ongoing studies are characterizing whether reconstitution of SPD KO mice with recombinant SPD protein can modulate the gut microbiome and response to sepsis.

The Circulating Bacterial Microbiome in Pulmonary Sarcoidosis Relates to Racial Disparities

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Rationale: The immunopathogenesis of sarcoidosis is not well defined; however, it is known that phenotypic manifestations and prognosis of sarcoidosis vary by age, gender, and race. Symptoms, pulmonary involvement, fibrosis, and advanced disease are more likely in African Americans (AA) compared to Caucasians (C); all likely contributing factors to higher ageadjusted mortality rates. We hypothesize that a predisposed host harbors an antigenic agent that promotes the dysregulation of inflammatory pathways observed in sarcoidosis. Furthermore, we aim to define distinct microbial signatures that may underlie racial dissimilarities and foster immune disparities in sarcoidosis.

Methods: Serum samples were obtained from AA and C subjects that were age, gender, and clinically matched for pulmonary involvement. DNA was extracted and multiplexed amplicon libraries were prepared using the NEXTflex™ 16S V4 Amplicon-Seq Library Prep Kit 2.0. Metagenomic data was examined utilizing culture and cloning free methods with paired-end sequencing with the Illumina MiSeq® system and aligned against the Greengenes Database. The R statistical environment (*Phyloseq* and *DESeq2* packages) was used for metagenomic data analysis and associations with radiographic findings were examined via Chi-square test for independence.

Results: We identified 108 distinct bacterial and archeal families, as well as mitochondrial rRNA from the serum of all AA females (n=4) and C females (n=4) with pulmonary sarcoidosis. No differences in α-diversity indices were observed among groups. Seven of the 108 families were significantly overexpressed and seven were significantly underexpressed in AA compared to C (Benjamini-Hochberg p-adj<0.1) (Table 1.). Caucasians demonstrated a higher level of Verrucomicrobiaceae, Alcanivoracaceae, and Dietziacea (a bacterial family belonging to the Actinomycetales order which includes bacteria that have been associated with sarcoidosis previously e.g. mycobacteria and propionibacteria). AA exhibited much higher levels of Erythrobacteraceae, Clostridiaceae, and mitochondrial rRNA than C. When considering findings on chest computed tomography, increased mitochondrial rRNA was associated with the presence of pulmonary fibrosis (Chi-square test of independence, p-value = 0.03).

Taxonomic Group (Family)	Benjamini-Hochberg Adjusted p-value	Log2-Fold Change
Verrucomicrobiaceae	1.12E-08	10.90976
Erythrobacteraceae	1.82E-05	-10.0824
mitochondria	7.53E-05	-9.51756
Clostridiaceae	0.000454	-8.83751
Alcanivoracaceae	0.000811	8.165376
Dietziaceae	0.002933	7.782669
Leptotrichiaceae	0.004253	-7.62799
Tissierellaceae	0.006412	7.108513
Beijerinckiaceae	0.006412	7.242472
Saprospiraceae	0.010576	-7.05103
Bacteroidaceae	0.012735	-6.96331
Bacteriovoracaceae	0.013037	6.893225
Promicromonosporaceae	0.017735	6.641658
EB1017	0.018036	-6.55215

Conclusions: Comparison of the circulating metagenome of AA and C females with pulmonary sarcoidosis demonstrates the presence of a core microbiome with notable and significant taxonomic differences. It is possible that these unique taxa contribute to phenotypic variability observed in sarcoidosis among different ethnicities. Exploration of immune pathways activated by significantly higher levels of mitochondrial rRNA is warranted as this could represent a damage associated molecular pattern involved in development of pulmonary fibrosis and thus worse clinical outcomes in African Americans.

Disruption of lung microbiota precedes peak lung injury in a mouse model of acute respiratory distress syndrome

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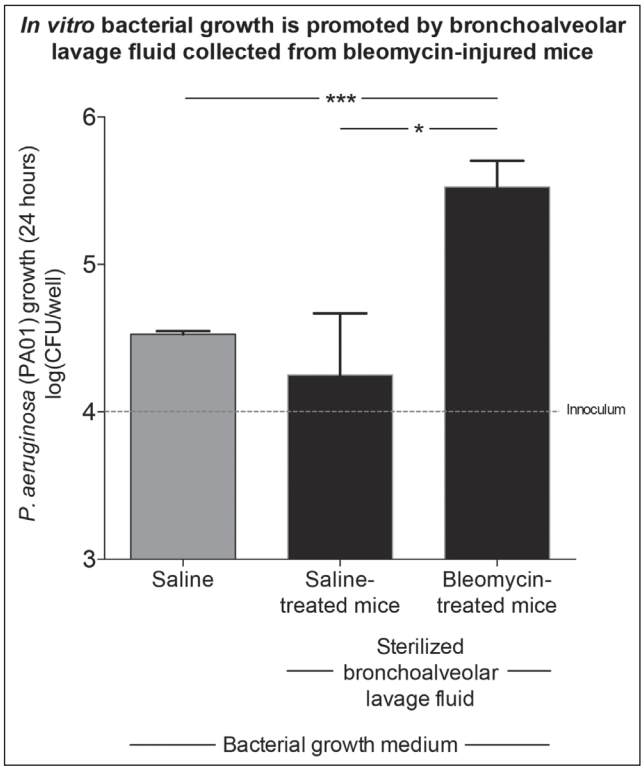
Rationale: The acute respiratory distress syndrome (ARDS) is a life-threatening disease characterized by alveolar inflammation and edema leading to severe hypoxemia. Recent studies have shown that the lung microbiome is altered in humans and animals with ARDS. The temporal relationship between altered lung microbiota and the development of lung injury is unknown.

Methods: We used an established murine model of ARDS, intratracheal instillation of bleomycin in C57BL/6 mice. We sampled microbiota from lungs, tongues, and the lower gastrointestinal tract using a culture-independent approach (community sequencing of the 16S rRNA gene, Illumina MiSeq). We characterized inflammation in bronchoalveolar lavage (BAL) fluid by measuring inflammatory cytokines at various timepoints. We characterized lung injury in BAL fluid using a colorimetric protein assay. We assessed the growth-promotion or growth-inhibition of the BAL fluid by inoculating sterilized BAL fluid with *Pseudomonas aeruginosa* and measuring growth at 24 hours.

Results: Lungs of bleomycin-treated mice had markedly altered microbiota ($P \leq 0.01$, PERMANOVA), with transiently increased diversity ($P \leq 0.01$) and significant enrichment with the Firmicutes ($P \leq 0.01$) and Bacteroidetes ($P < 0.05$) phyla. Changes in lung microbiota were correlated with increased alveolar concentrations of TNF-alpha, IL-17 and KC. Importantly, these alterations in lung microbiota temporally preceded the onset of peak lung injury, which was seen at day 7-post bleomycin treatment. In an *ex vivo* validation assay, BAL fluid from bleomycin-injured lungs enhanced the growth of *P. aeruginosa* compared to sterile saline ($P \leq 0.001$) and BAL fluid from uninjured mice ($P \leq 0.05$) (Figure).



***In vitro* bacterial growth is promoted by bronchoalveolar lavage fluid collected from bleomycin-injured mice**

Conclusions: In a murine model of ARDS, alterations in lung microbiota precede alveolar injury and are concurrent with alveolar inflammation. Our results provide in vitro evidence that the injured lung environment selectively promotes bacterial growth, and in vivo plausibility to the hypothesis that altered lung microbiota contribute to the alveolar inflammation and injury of ARDS.

Transgenic Overexpression of microRNA-25 Regulates Cytokine and Chemokine Production in a Mouse Model of Allergic Inflammation

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Rationale: Asthma is a chronic inflammatory disease of the airways characterized by hyper-responsiveness, airway remodeling and eosinophilic inflammation. Secretion of various cytokines, chemokines and growth factors accompany the inflammatory process mediated by allergic responses in asthma. Prior work in airway smooth muscle (ASM) cells exposed to a proinflammatory stimulus identified miR-25 as a mediator of ASM remodeling and demonstrated that inhibition of miR-25 expression mediated secretion of RANTES, eotaxin and TNFα. Additional studies from our laboratory have shown that smooth muscle-targeted overexpression of miR-25 ($Tg^{SM\text{-}miR25}$) in an ovalbumin (OVA) model of allergic inflammation alleviates asthmatic features. However, the role of miR-25 on cytokines expression remains poorly understood in vivo. The hypothesis tested in these experiments is that miR-25 expression restores cytokine and chemokine expression profiles in OVA-treated mice to alleviate allergic inflammation and thereby contribute to improved lung function seen in mice overexpressing miR-25

Methods: To investigate the role of miR-25 on cytokines and chemokines production *in vivo*, 8-10 week old female or 6-8 week old male mice were treated with OVA using an acute sensitization and challenge protocol. Bronchoalveolar lavage (BAL) fluid was obtained 48 hours after the last OVA-challenge by washing airways eight times with 500 µL of 0.9% sterile saline solution containing 2.6mM EDTA. Luminex multiplex assays were performed by Eve technologies (Calgary, CANADA) using 75 µl of BAL fluid supernatants per duplicate sample

Results: Wild-type (WT) mice treated with OVA displayed higher levels of various cytokines including interleukin-4 (IL-4) and interleukin-5 (IL-5), chemokines including eotaxin (CCL11), monokine induced by gamma interferon (MIG/CXCL9) and macrophage inflammatory protein (MIP-2), as well as growth factors such as transforming growth factor beta-1 (TGF-β1) compared with the WT-Alum control, in contrast, there were lower levels of IL-1α, IL-12-p40 and IL-12-p70 proinflammatory cytokines. miR-25 expression decreased the secretion of eotaxin (by~50%), MIG (by~50%) and MIP-2 (by~20%) chemokines, and restored IL-1α, IL-12-p40 and IL-12-p70 cytokines production to levels seen in control animals in response to OVA treatment. Surprisingly, miR-25 did not influence the levels of key cytokines and growth factors such as IL-4, IL-5 and TGF-β1, respectively.

Conclusions: These results indicate that miR-25 regulates the production of several cytokines and chemokines involved in allergic inflammation. This is the first study that addresses the role of miR-25 on cytokines and chemokines secretion *in vivo*, and provides data crucial to the characterization of miR-25 as potential novel therapeutic target in asthma.

Funding sources: This study is supported by R01 HL127192-01A1 from NIH/NHLBI to CAS

Lung Cancer Screening with Low Dose CT: Two Year Experience at Providence Veteran Affairs Medical Center

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Rationale: Veterans are at higher risk for lung cancer due to greater prevalence of cigarette smoking in this population. The National Lung cancer Screening Trial (NLST) demonstrated reduced mortality among high risk patients with the use of low dose computed tomography scan (LDCT) of the chest. We recently published data from the first year of LDCT screening in our veteran population and showed an increased number and proportion of early stage lung cancers detected. We now analyze the performance of LDCT screening over a two-year period and compare these findings to an historical cohort of veterans with incidental lung nodules before the implementation of LDCT screening (pre-screening group).

Methods: Between December 2013 and December 2015, 3,880 veterans (55-74 years; smoking, or quit within 15 years; and with >/=30 pack-years) were invited to undergo LDCT screening. Nodules > 4mm went on to further evaluation. True positives received a histologic diagnosis of lung cancer. False negatives were defined as having nodules diagnosed after screening stage>1. False positives were either benign nodules resected surgically or diagnosed by other invasive means, or nodules, which were stable or resolved on follow-up. Demographics, smoking history, comorbidities, cancer stage, and invasive diagnostic procedures in positive screens were recorded retrospectively. In our pre-screening group, 538 veterans with nodules larger than 4mm and suspicious for malignancy were entered into our previous surveillance program between December 2012 and December 2013.

Results: Eighty-four lung cancers were detected during the two-year LDCT screening period. Mean age of veterans was 65±5 years. Of these cancers, 60.7% were N0M0 and 14.3% were NxM1. False negatives were 2.38%. Sensitivity and specificity of LDCT screening was 97.6% and 90.8%, respectively. Positive predictive value was 19.5% and negative predictive value was 99.9%. Of the 347 benign nodules detected during screening, 9 were diagnosed surgically and 3 with bronchoscopy. In the pre-screening group, 62 lung cancers were detected (mean age 70±8). Compared to the LDCT screening group, 19.3% were N0M0 and 29% were NxM1; of the 464 benign nodules detected, 2 were diagnosed surgically and 1 with bronchoscopy.

Conclusions: The performance of LDCT screening in veterans was satisfactory, with an acceptable sensitivity, specificity, and number of diagnosed benign lesions. Veterans with positive screens were younger and had an increased proportion of early pathologic stage malignancies compared to veterans with incidental lung nodules in the pre-screening period. Screening resulted in a low false positive rate (0.092).

Characteristics of Interstitial Lung Disease in the Mid-Atlantic Veterans Affairs Regional Network

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Rationale: Interstitial Lung Diseases (ILD) are associated with substantial morbidity, mortality, and healthcare utilization. Despite the clear impact of ILD on human health and the ongoing aggressive efforts to define and treat ILDs, there remain considerable deficits in our understanding of ILD epidemiology. One of the central issues is the need to clearly define ILD characteristics in large patient cohorts using well defined criteria. By using the clinical information from Veterans Administration Medical Center, one of the largest healthcare systems in the US, we can define a large patient cohort of ILD. This can provide valuable insights into the characteristics and outcomes of ILD.

Methods: Using the Corporate Data Warehouse (CDW) for the Mid-Atlantic Veteran Affairs Medical Region (includes North Carolina, Virginia and a portion of West Virginia), we identified Veteran Health Administration (VA) patients who received inpatient or outpatient care for ILD (as defined by ICD 9 code 515: Post-inflammatory pulmonary fibrosis or 516: Other alveolar and parietoalveolar pneumonopathy) from January 2008-2013. We obtained data from the CDW, and performed chart reviews, to define characteristics of this population. Data was collected into a database (Microsoft Access) and summarized using descriptive statistics.

Results: During the pre-specified 5-year period, we identified a total of 2642 unique patients with an ILD diagnostic code. We present here data from the first 371 patients. Of these patients, 15% patients had an ILD diagnostic code but no evidence during the chart review of a specific ILD. 31.8% were coded with a 515 code and 48.8% with a 516 code. Using the information obtained from these subjects, we identified the following aggregate characteristics (Table 1). The ILD cohort is predominantly elderly, white, and male. Our results revealed 16% of patients underwent bronchoscopy, 15.1% of the subjects had evidence of a connective tissue disease related ILD and 24% of the subjects received a form of immunosuppression. Forced vital capacity and carbon monoxide diffusion capacity percent predicted values were comparable to other large ILD cohorts.

TABLE 1. COHORT CHARACTERISTICS

	Total (N= 371)
Age [Median (Range)]	71 (43.0-97.0)
Gender (n,%)	
Male	352 (95%)
Female	19 (5%)
Race (n,%)	
Black	125 (34%)
White	239 (64%)
Hispanic	5 (1%)
Other	2 (1%)
BMI [Median (Range)]	27.5 (17.2-53.0)
Percentage with a Bronchoscopy (n,%)	59 (16%)
Prevalence of Comorbidities (n,%)	
COPD	122 (33%)
Asthma	21 (6%)
Lung cancer	40 (11%)
Gastroesophageal Reflux Disease	107 (29%)
Coronary Artery Disease	111 (30%)
Congestive Heart Failure	63 (17%)
Stroke	43 (12%)
Diabetes Mellitus	138 (37%)
Obesity	64 (17%)
Rheumatologic Disease (n,%)	
Rheumatoid Arthritis	28 (8%)
Polymyositis	1 (0%)
Scleroderma	3 (1%)
Systemic Lupus Erythematosus	3 (1%)
Dermatomyositis	3 (1%)
Mixed Connective Tissue Disease	3 (1%)
Percentage on Immunosuppression (n,%)	86 (24%)
Percentage on Oxygen Therapy (n,%)	165 (44%)
Pulmonary Function at Time of Diagnosis [% Predicted, (Range)]	
Spirometry (n=146)	
FVC	73.4 (30.4-130)
FEV1	82 (30.9-136.3)
FEV1/FVC	78 (46.0-100.0)
Lung Volumes (n=70)	
TLC	73.7 (38.6-108.3)
Diffusion Capacity (n=126)	
DLCO	49 (14.4-97.0)

Conclusions: We present an initial data analysis of the characteristics of ILD in a well-characterized VA patient cohort. Identifying patients with diagnostic codes followed by a detailed chart review and database query offers the opportunity to define the types and characteristics of ILD in a VA population and will enhance our understanding of the epidemiology of these diseases.

Anti-RBP Antibody Positivity in Patients with Idiopathic Interstitial Pneumonitis

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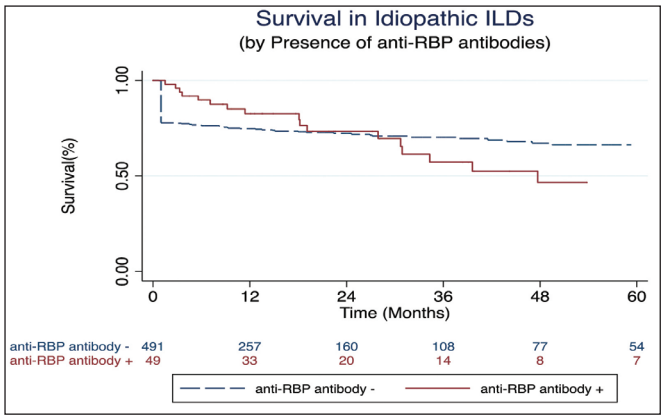
Introduction: Circulating anti-RNA binding protein (anti-RBP) antibodies play a role in the clinical diagnosis and stratification of disease severity in some connective tissue diseases (CTD). These antibodies include anti-SSA, anti-SSB, anti-Smith, and anti-RNP autoantibodies. In systemic lupus erythematosus, anti-RBP antibodies are associated with increased expression of interferon-alpha associated genes. Additionally, anti-SSA is associated with pulmonary fibrosis in the idiopathic inflammatory myopathies. We hypothesized that the presence of positive anti-RBP antibodies in patients with idiopathic interstitial pneumonia (IIP) would be associated with decreased lung function and worsened transplant-free survival.

Methods: The University of Chicago ILD Registry was screened to identify patients with IIP and available serologies. The IIP cohort was then stratified based on anti-RBP antibody seropositivity. Clinical, serologic and pulmonary function data were obtained via chart review of the electronic medical record. Demographic features, disease comorbidities, pulmonary function test results, radiographic fibrosis, antinuclear antibody (ANA) seropositivity and transplant-free survival were compared between the anti-RBP positive and the anti- RBP negative subgroups. Survival analysis was performed using Cox regression.

Results: Five hundred forty patients with IIP were identified. Of these, nine percent (n=49) were anti-RBP positive. Both subgroups were similar with regard to age, gender, body mass index, smoking status, disease comorbidities, and ANA seropositivity. When compared to anti-RBP negative subjects, those who were anti-RBP positive were more likely to be African-American or Asian (n=9, 18.4% vs n=47, 9.6%; and n=5, 10.2% vs n=16, 3.3% respectively; p=0.016), require oxygen therapy (n=29, 59.0% vs n=266, 26.1%; p<0.001) and have radiographic honeycombing on high-resolution computer tomography imaging (n=24, 49.0% vs n=173, 35.2%, p<0.001). Anti-RBP positive patients also demonstrated a trend towards lower percent predicted forced vital capacity (60.9% vs 64.5%, p=0.184), and lower percent predicted forced expiratory volume in 1 second (74.1% vs 78.4%, p=0.167). There were no differences in transplant-free survival between subgroups (log rank p =0.559).



Conclusions: In our IIP cohort, anti-RBP positive subjects were more likely to require oxygen therapy, have radiographic honeycombing, and have reduced pulmonary function than anti-RBP negative subjects. Further studies are needed to determine the clinical impact of RBP autoantibodies in patients with IIP.

Peripheral Blood Monocyte Surface Markers are Associated with Pulmonary Dysfunction In HIV Infection.

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Rationale: HIV-infected persons in the current era of highly active anti-retroviral therapy (HAART) have increased prevalence and early onset of chronic obstructive pulmonary disease (COPD). Both HIV and COPD are independently characterized by chronic inflammation and immune activation, and monocytes are key innate immune cells implicated in both diseases. Given that circulating monocytes replenish alveolar macrophages, whose number and function are altered during inflammatory lung diseases, the immune phenotype of monocyte subsets in the course of HIV-associated lung dysfunction warrants further study. Differential distribution of circulating monocyte subsets and surface marker expression may predict lung dysfunction in HIV-infected persons.

Methods: HIV-infected and uninfected persons with viably cryopreserved peripheral blood mononuclear cells (PBMCs), as well as clinical and pulmonary function test (PFT) data were included in the study. Monocyte subsets: classical (CD14++CD16-), intermediate (CD14++CD16+) and non-classical (CD14+CD16++) were quantified and phenotyped by flow cytometry for expression levels of monocyte activation (CX3CR1, CD11b, HLA-DR) markers. Relationships between the frequencies of monocyte subsets, their respective phenotypes and PFTs were tested by Spearman correlation or Ranksum. Multivariable models were used to adjust for clinically relevant variables.

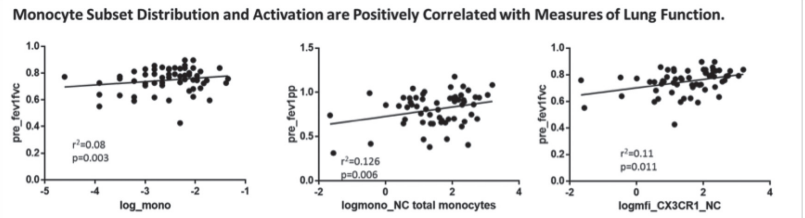
Results: 79 participants were enrolled of whom; 61(77%) were HIV infected; mean age of 52 years and 28% were female. HIV infected persons had more tobacco exposure than HIV-uninfected (median pack-years 16.87 vs. 0.15 p=0.009). 95% of HIV-infected persons used HAART and 74% were virologically-suppressed. PFTs were normal on average, and there were no differences in PFTs by HIV status in this cohort.

The intermediate (IM) monocyte subset, and certain inflammatory phenotypes (CD11b+ IM and CD11b+ CX3CR1+ NC) were more frequent in HIV-infected persons. In the HIV-infected group only, positive correlations were found between the number of circulation monocytes and FEV1 percent-predicted [β =1.34 p =0.003]. The frequency of NC-monocytes was correlated with pre-bronchodilator FEV1/FVC [β =3.95 p =0.006]. Correlations were also found between CX3CR1 expression on NC-monocytes and FEV1/FVC: pre-bronchodilator FEV1/FVC [preβ =3.94 p =0.006], post-bronchodilator FEV1/FVC [β =3.87 p =0.011] (Figure 1).

Methods: Monocyte subset distribution and activation vary with certain measures of lung function in HIV-infected individuals. The positive associations suggest an adaptive expansion of monocyte subsets that may initially compensate for lung dysfunction in HIV-infected individuals. Functional studies are required to correlate monocyte surface marker expression with disease phenotype in HIV-associated lung diseases.

SUPPORT: NIH K24 HL123342, R01 HL120398 (AM)

Figure 1:



The Role of Mycobacteria in the Pathogenesis of Sarcoidosis.

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Sarcoidosis is an inflammatory disease characterized by the presence of noncaseating granulomata in the affected organs, particularly the lungs and lymph nodes. Currently, the etiology of sarcoidosis is unknown. While there is compelling evidence that infectious agents particularly mycobacteria are present in sarcoid granulomas, the inability to detect the bacteria by culture and staining techniques presents a problem. Previous observations from our laboratory have identified the presence of mycobacterial DNA in the lymph node and lung biopsies of patients with sarcoidosis, compared with no detection in healthy controls. However, there is little knowledge about the role of mycobacteria in the pathogenesis of sarcoidosis.

We hypothesize that mycobacteria have virulence attributes that play a role in the pathogenesis of sarcoidosis.

To test this hypothesis, we conducted molecular analysis on 10 sarcoidosis lung biopsies for the presence of mycobacterial genes. Our studies identify the presence of several mycobacterial virulence genes in lung biopsies of sarcoidosis patients, which may play a role in the survival and persistence of mycobacteria in sarcoid granulomas.

We detected 16srRNA (80%), and other mycobacterial virulence genes such as Superoxide dismutase SodA (10%), ESX-1 secretion-associated protein A, (30%), and ESX-1 secretion-associated protein EspC (10%), Rv 1077C (50%) as well as Mce-family protein; Mce4D (70%), which is thought to be involved in host cell invasion. These genes were not detected in the control lung tissue. We did not detect the presence of other virulence genes such as Mce-family protein; Mce4A, Heat shock protein HspX, or the conserved hypothetical integral membrane protein YrbE3A.

Taken together, our findings on the virulence factors in mycobacteria expressed in sarcoidosis tissues will contribute to our understanding of the role of mycobacteria in the pathogenesis of sarcoidosis.

A Comparison of the Transcriptional and Translational Inflammatory Profiles of Primary Airway Epithelial Cells from CF and Non-CF Donors

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Introduction: The mutation of CFTR in the CF genotype contributes to several changes in the airways, including reduced mucus clearance, a heightened chance of infection, and inflammation. However, it is disputed whether inflammation is a severe response to a compromised lung microenvironment, or if the mutation in CFTR itself is sufficient to elicit this response. To further understand the direct role of CFTR in inflammation, we sought to examine basal differences in the inflammatory and anti-inflammatory pathways directly related to the CF genotype.

Hypothesis:: The profile of the common pathways in human primary airway epithelial cells (AEC) from CF patients in comparison to "normal" (non-CF) patients reveals distinct transcriptional and translational differences in inflammatory gene expression outside lung microenvironment.e.

Methods: The transcriptional and translational profiles were assessed between two groups of primary cultured cells: (1) airway epithelial cells with homozygous F508 deletions (CF-AEC; n=3) and (2) Normal airway epithelial cells (AEC; n=3). Both types of cells were obtained from a commercial source and grown under sterile conditions. Activity of 96 transcriptional factors (TFs) was assessed with a TF Plate Array based on quantification of luminescence. Inflammatory gene profile was done using a qPCR 96 Gene Array. Additionally, western blots were performed to analyze protein expression of common inflammatory pathways, such as NF- κB and GR.

Results: Analysis of inflammatory pathways by both arrays revealed basal differences between CF-AEC and Normal-AEC. There was a statistically significant upregulation in CF-AEC in receptors (IFNγR1, TLR3), cytokines (IL-6, IL-23, TNF), co-stimulatory molecules (complement component 3, ICAM-1, CASP1), and a trend of upregulation for TFs (Stat1, NF-κB). There was also an increased presence of cell differentiation and stem cell markers in CF-AEC (Nkx3-2, SATB1, SF-1). Conversely, there was significant downregulation in CF-AEC of receptors (RORC, TLRs 1, 2, 4, 9), cytokines (IFNα1, IFNγ, MHC-1E, ILs-1, 2, 5), co-stimulatory molecules (CD14, TRAF6, JAK2, Tyk2, AP3), and TFs (Stat3, IRF3, IRF7, MyD88, CREB). Additionally, western blot analysis revealed a significant downregulation of glucocorticoid receptor (GR) in CF-AEC; however no difference was detected in the downstream signaling such as NF-κB, MAPK, or MyD88 expression.

Conclusions: Analysis of inflammatory pathways reveals a significant difference in CF-AEC when compared to Normal-AEC growing under uniform and sterile conditions, particularly the increased expression of key receptors (TRLs) that transmit inflammatory cascades. In addition, anti-inflammatory GR was significantly downregulated in CF-ACE. This study suggests that CFTR mutation directly influences the impaired inflammatory signaling in the absence of infection. Our data also indicates that the basal profile differences between CF and non-CF are sufficient to contribute to lung disease. Repairing the inflammatory cascade could be a promising target for CF therapy.

Lung function in patients with chronic allograft dysfunction (CLAD) following lung transplantation: Patterns of decline.

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Background: The clinical course of chronic lung allograft dysfunction (CLAD) following lung transplantation is heterogeneous. Forced vital capacity (FVC) loss has been shown to be the most important determinant after CLAD onset, independent of other clinical factors.

Objectives: Objectives of this pilot study were to examine patterns of decline in FVC and Forced expiratory volume in the first second (FEV1) in a retrospectively identified cohort of single, double, and lobar transplant (SLTx, DLTx, and lobar Tx, respectively) recipients.

Methods: Clinical and physiologic data of patients aged 18-70 years receiving SLTx, DLTx, and lobar Tx from 2000 through 2016 were obtained. CLAD was defined as a sustained > 20% decline in FEV1 compared with the average of the two best post-transplant FEV1 measured ≥3 weeks (absent clinical confounders). FVC loss was defined as, if at CLAD onset (in the restrictive form of CLAD) was defined as, if at CLAD onset, FVC/FVCbest was <0.8. The FVCbest was defined as the average of two post-transplant FVC1 measurements matched to the two best post-transplant FEV1 measured used in the CLAD calculation. Spirometry was recorded at 6-month intervals until most recent measurement available or death. Patients with malignancy, uncontrolled infection, pneumonectomy, recurrent sarcoidosis and pleural disease were excluded.

Results: Data for 349 patients (129 SLTx, 190 DLTx, 30 lobar Tx) were available. Of these, 120 (34%) exhibited spirometric criteria for CLAD. An additional 25 patients were excluded based on criteria listed above. Ninety one (76%) patients with spirometric decline expired during the study period. The rate of FEV1 decline in SLTx, DLTx, and lobar Tx were were 30, 62, and 60 mL/mo, respectively; FVC decline was 33, 57, 62 mL/mo respectively (declines in FEV1 and FVC significantly different between SLTx and DLTx, and between DLTx and lobar Tx, both P<0.01, ANOVA, Tukey test). Mortality rates during the study period for DLtx, SLTx and lobar Txs were 38/57 (67%), 22/29 (76%) and 5/8 (63%), respectively.

Conclusions: In our experience, CLAD occurs in one-third of patients undergoing lung transplantation, of which those who underwent double lung and lobar transplant exhibited the fastest decline in lung function. These findings may be explained by the diagnoses for which the patient's were transplanted. With the addition of histopathologic and imaging data, we will be able to classify the type of CLAD for double, single, and lobar transplant, namely restrictive versus obstructive, or mixed, and correlate findings with associated histopathologic and imaging findings.

Thioredoxin Reductase Inhibitors Induces Pulmonary Heme Oxygenase-1 By Nrf2-independent Mechanisms

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Rationale: The thioredoxin reductase-1 (TrxR1) inhibitor aurothioglucose (ATG) attenuates hyperoxic lung injury in neonatal and adult murine models. TrxR1 inhibition activates nuclear E2-related factor (Nrf2), the master regulator of antioxidant responses. We have consistently observed a disproportionate induction of heme oxygenase-1 (HO-1) following TrxR1 inhibition. The contribution of Nrf2 toward increased HO-1 expression by TrxR1 inhibitors is unclear. The present studies tested the hypothesis that TrxR1 inhibition induces HO-1 via Nrf2-dependent mechanisms. Methods: Nrf2-/- murine club cells (mtCC) were generated using CRISPR/Cas9 gene editing techniques and Nrf2 deletion was confirmed using qRT-PCR and western blot. Nrf2-/- or wild-type (WT) mtCCs were treated with 5μM ATG or vehicle control for 4h or 24h. Four day-old Nrf-/- or WT C57Bl/6 mice were treated with 25mg/kg ATG or equal volume saline and lungs were harvested 24h later. Cell lysates and lung homogenates were collected and TrxR1 activity and HO-1 protein expression determined by enzymatic assay and western blot. Results: There was no detectable Nrf2 mRNA or protein in gene-edited mtCCs confirming successful generation of Nrf2-/- cells. In vitro, TrxR1 activity in Nrf2-/- cells was 15% less than in WT mtCC. ATG treatment decreased TrxR1 activity by 50% in WT and Nrf2-/- cells compared to vehicle-treated controls. Baseline HO-1 protein expression was significantly lower in Nrf2-/- mtCCs when compared to WT cells at 4h and 24h. ATG increased HO-1 expression in WT at 24 but not 4 h; however ATG did not alter HO-1 expression in Nrf2-/- mtCCs at either time point. In vivo, TrxR1 activity was 40% lower in Nrf2-/- lungs than in WT mice. ATG treatment decreased TrxR1 activity by ~80% in WT and Nrf-/- lungs when compared to saline-treated controls. Baseline HO-1 expression was greater in Nrf2-/- than in WT lungs. ATG treatment increased lung HO-1 protein levels in both WT and Nrf2-/- mice when compared to saline-treated controls. Conclusion: Our in vitro data suggest that Nrf2 is required for HO-1 induction by ATG. In contrast, our in vivo data using whole lung homogenates reveal Nrf2-independent induction of HO-1 as evidenced by ATG-mediated increases in HO-1 expression in Nrf2-/- lungs. These discrepancies highlight the complexities of working in cultured cell systems and whole animals but nonetheless reveal the presence of Nrf2-independent mechanisms of HO-1 induction by TrxR1 inhibitors. Future studies will focus on identifying the pathway(s) by which HO-1 is induced in the absence of Nrf2.

Aerosolized TLR Agonists Suppress Acute Sendai Virus Burden and Chronic Asthma-Like Lung Disease in Mice

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Rationale: Persistent activation of immune pathways in mice can transform an acute respiratory viral infection into chronic lung disease that resembles asthma. The Toll-like receptor (TLR) agonists oligonucleotide M362 and lipopeptide Pam2CSK4 (O/P), given in combination by aerosol, have been shown to attenuate respiratory viral infections in mice, rats and guinea pigs. The purpose of this study was to determine whether treatment with O/P at the time of Sendai parainfluenza virus (SeV) challenge can protect mice from developing chronic lung disease.

Methods: Adult C57BL6 mice were treated with aerosolized O/P (1 μM and 4 μM in 8 ml water) around the time of oropharyngeal challenge with 2.4x106 PFU of SeV. On day 5 after infection, SeV burden was measured by qPCR of lung homogenates. On day 49 after infection, inflammation was measured by enumeration of leukocytes in bronchoalveolar lavage fluid, mucous metaplasia was measured by quantitative image analysis of airway epithelial cells stained with PAFS, and airway hyperresponsiveness to methacholine was measured by forced oscillometry.

Results: Pretreatment with O/P 24 hours before infection resulted 5 days later in 72% reduction in lung SeV levels, and 49 days later in 88% reduction in eosinophil numbers, 52% reduction in airway mucin content, and 53% reduction in total respiratory resistance in response to 30 mg/ml methacholine. In dose-response studies, there were parallel reductions in SeV levels at 5 days and in eosinophil numbers and airway mucin content at 49 days. In time-course studies, there similarly were parallel reductions in SeV levels, eosinophil numbers, and airway mucin content, with maximal effects of O/P observed within 3 days of treatment before or after SeV challenge. No tachyphylaxis to O/P occurred with 5 treatments every 3 days. The effects of O/P on SeV levels, eosinophil numbers, and airway mucin content were comparable to treatment with 400,000 units of aerosolized IFN-β, though there was no increase in IFN-β in response to O/P

Conclusions: Suppression of acute SeV burden by O/P in a mouse model results in attenuation of chronic asthma-like lung disease. The benefits of O/P are similar to those of IFN-β, which has been shown to reduce lung virus burden in preclinical models and human subjects. Since respiratory viral infections are a major cause of asthma exacerbations and disease progression, preemptive treatment with O/P might reduce the severity of exacerbations and disease progression.

Effects of refractory hypoxemia protocol in timing of proning position in patients with acute respiratory distress syndrome

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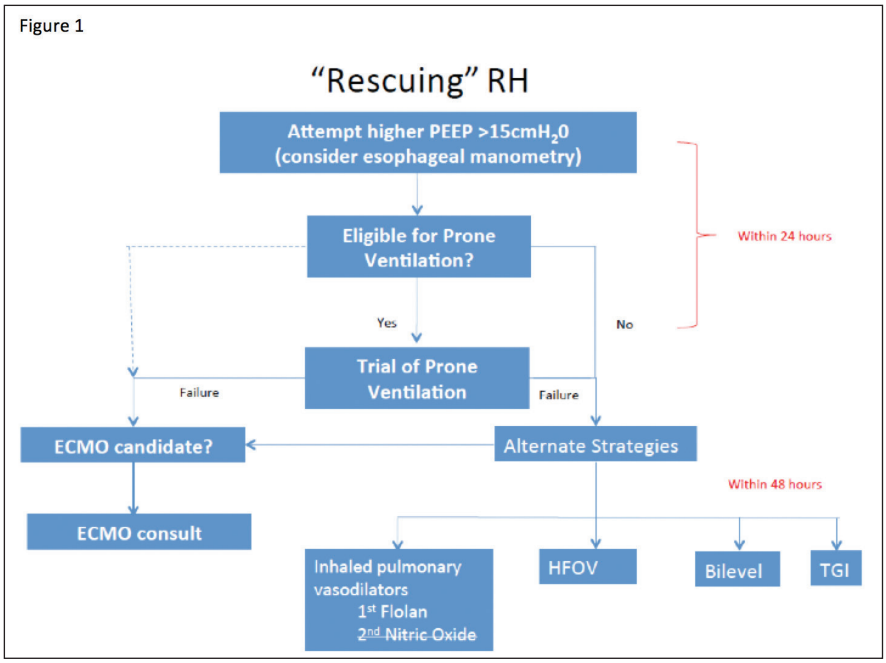
Rationale: Acute respiratory distress syndrome (ARDS) has a high mortality, ranging from 27-45%, depending on its severity. Prone positioning for ARDS has been demonstrated to improve oxygenation and lung recruitment in ARDS. Commonly, its use varies by provider experience, type, and location of intensive care unit (ICU). In an attempt to standardize the care for patients with ARDS at our institution, a refractory hypoxemia (RH) protocol was developed and adopted in April of 2015 (Figure 1). We aim to compare time to prone before and after the introduction of the protocol.

Methods: 23 patients who had an order for a Rotoprone® bed recorded in their medical records from April of 2014 to April of 2016 were included in the retrospective chart review. Of those, 18 were really proned and represent our cohort.

Results: At time of proning median age was 64 years (IQR 46-70), median PaO2/Fio2 ratio was 77 (IQR 25-75= 53-97) and median time from diagnosis of hypoxia to prone was 19.9 hours (IQR 15.7-45.4). Median ICU length of stay (LOS) was 7.5 days (IQR 4.75-14.25) and duration of proning was 25 hours (IQR 10-5-46.1). When comparing patients proned before (7 patients, 38%) and after (11 patients, 62%) the initiation of the RH protocol, time to prone was significantly shorter in the RH protocol group, 17.4 hours versus 42.1 hours ($\chi 2$ 6.6, p=0.009). There was no difference between ICU LOS, duration of proning, P/F ratio or age at proning between groups. Fifteen patients died during the ICU stay.

Conclusions: The implementation of a RH protocol significantly decreased the time from hypoxia to prone at our institution. Mortality, ICU LOS and time in the prone position were not affected by the RH protocol.

Figure 1



National Rates of 30-day Hospital Readmissions for Adults with Asthma Exacerbation

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Introduction: Approximately one-in-twelve adults in the U.S. have asthma and it is extremely important to recognize the populations at greater risk for hospitalizations to improve overall health care and reduce the economic strain on the U.S. Our primary objective was to compare the 30-day rates of readmissions for asthma exacerbations in adults by demographics and comorbidities in 2013.

Methods: We determined the 30-day readmission rates in the 2013 by using the U.S. Agency for Healthcare Research and Quality's National Inpatient Sample – National Readmission Database (NIS – NRD). The NRD contains discharge data from all-payer hospital inpatient stays in 21 geographically dispersed states. We initially extracted all admissions for asthma in adults 18 or above and then determined which patients were readmitted within thirty days. The rates and risk of readmission were then calculated and analyzed based on specific demographics and comorbidities.

Results: In 2013 there were 29,242,439 adult admissions in the NIS-NRD database, which included 527,592 (1.8%) for asthma. Of the admissions with asthma 70,186 (13.3%) were readmitted within 30-days for asthma. Among those readmitted for asthma, we noted a higher rate of readmission for males verse females (14.9% and 12.6%, respectively). Specifically, the readmission rates for asthma in patients with comorbidities such as drug abuse (19.40%), obesity (14.49%), psychiatric disorders (19.09%), anxiety (15.89%), and GERD (14.77%) was increased compared to non-asthma readmissions in the general population. Patients with Medicaid had a higher readmission rate for asthma compared to non-asthma readmissions in the general population (16.28% and 13.86%, respectively). Multivariate logistic regression showed increased risk of readmissions in patients with drug abuse, CHF, DM, and renal failure (OR: 1.63, 1.96, 1.76, 1.64, 1.835, respectively ($p<0.05$)).

Conclusions: Asthma is a serious health and economic problem in the United States. A patient admitted for asthma has an overall 30-day readmission rate for asthma of 13.3% and specific subsets of patients in our data increase this rate. To conclude, our data represents the first time a nationwide analysis of 30-day asthma readmissions has been compiled and the specific populations that are effected and contributing to our economic misfortunes have been identified. By promoting proper education and care we can help reduce the number of hospitalizations and improve the livelihood of our patients.

Control of Mast Cell Regulated Exocytosis by Munc18 proteins

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Rationale: Upon stimuli, mast cells (MCs) release inflammatory mediators stored in secretory granules (degranulation) which contribute to the pathophysiology of anaphylaxis and asthma. This process involves granule to plasma membrane fusion (regulated exocytosis) and granule to granule fusion (compound exocytosis). The SM (Sec/Munc18) protein family includes essential regulators of vesicular trafficking and three isoforms of Munc18 (-1, -2, and -3) are involved in exocytosis in many mammalian cells. Since MCs express all three isoforms, we attempted to identify which functions were unique, redundant and complementary to each Munc18 and how they participate in regulated and compound exocytosis using mature MCs and in vivo models.

Methods: The global deletions (-/-) of Munc18-1, -2 and -3 are lethal, therefore we studied heterozygote (+/-), conditional KO (F/F), and exclusive MC-deletant (Δ/Δ) mice. Expression in peritoneal MCs was assessed by qPCR and immunoblot. Degranulation was measured as an increase of membrane capacitance in patch-clamped MCs stimulated with a pipette solution containing GTPγS and calcium. With electron microscopy (EM), we studied ultrastructural changes of stimulated peritoneal MCs. We evaluated regulated, constitutive and non-exocytic effector responses using populations of MCs. We determined the number, distribution, migration and differentiation of MCs. We tested the pathological consequences of our mutants in vivo with a passive systemic anaphylaxis (PSA) model.

Results: Munc18-2 was the main isoform expressed in MCs followed by Munc18-3 and -1. Electrophysiological recordings showed impaired exocytosis only in the MCs from Munc18-2Δ/Δ, and an intermediate defect in the Munc18-2+/- MCs, indicating that Munc18-2 is a limiting factor in this process and ruling out a redundant function of the three proteins in regulated exocytosis. A severe defect in granule to plasma membrane and granule to granule fusion was observed in EM studies of Munc18-2Δ/Δ MCs, meaning that Munc18-2 plays a significant role in compound exocytosis also. The secretion assays showed a selective defect in regulated exocytosis on the Munc18-2Δ/Δ. Although mice lacking Munc18-2 in their MCs showed a normal development and distribution of their MCs, they had a blunted response to the PSA model, corroborating that this MC-exclusive pure functional defect has an important impact on the anaphylactic reaction.)

Conclusions: Although other isoforms are expressed in MCs, Munc18-2 is the only one involved in MC degranulation probably by its interaction with Syntaxin-3. Munc18-2 is critical for regulated exocytosis, including compound exocytosis, but not constitutive exocytosis in MCs. MCs regulated exocytosis is required for a full anaphylactic response.

In Severely Obese COPD Patients, Obesity May Contribute to Exercise Intolerance More Than Lung Function

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Introduction/Rationale: In the US, many patients with COPD are obese. Whether obese severity has an impact on six-minute walk distance, severity of dyspnea and quality of life before these patients start a program of pulmonary rehabilitation remains unclear.

Methods: 111 older men (age=65.5±7.9 yr.) with moderate-to-severe COPD (FEV1= 44.0±14.4% predicted) completed standard pulmonary function testing and a symptom-limited metabolic treadmill test. In addition, all participants filled out dyspnea and quality of life questionnaires (Chronic Respiratory Disease Questionnaire and Rand Short-form 36). Body mass index (BMI) was calculated using a wall-mounted stadiometer and a weight scale (BodPod). A practice six-minute walk was completed during one visit and the six-minute walk used in this analysis was completed at the subsequent visit.

Results: 26 patients were neither overweight nor obese (BMI 18.5 to 24.9), 41were overweight (BMI 25 to 29.9), 22 were obese (BMI 30 to 24.9) and 22 were severely obese (BMI ≥35). No differences were identified on major outcome variables when the non-obese patients were compared with the overweight, obese and severely obese patients combined. In contrast, severely obese patients were less obstructed than the other patients (FEV1 percent predicted 52.8±10.5% vs. 42.0±14.6%, p=0.001) yet perception of dyspnea and quality of life in the severely obese patients were not better than in the other patients. Despite less airway obstruction, severely obese patients tended to have less maximum oxygen uptake (15.1±2.7 ml/kg/min vs. 16.5±3.2 ml/kg/min, p=0.07), and had a shorter six-minute walk distance (391±101 m vs. 454±104 m, p=0.018). As expected, percentage of body fat was higher in the severely obese patients than in the other patients: 37.4±3.9% vs. 28.5±4.7% (p<0.001).

Conclusions: Despite less severe obstruction, severe obesity was associated with worse exercise performance but not worse quality of life and dyspnea than non-severely obese patients with COPD. These findings suggest that in this group of patients, body mass may play a more significant role in functional performance than lung function.

Effects of Air Pollution Particulate Matter on Adaptive Antimycobacterial Immunity

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Introduction: Tuberculosis (TB) and urban air pollution both contribute significantly to the global burden of disease. Epidemiological studies have shown that indoor air pollution increases the risk of new infections with Mycobacterium tuberculosis (M.tb) and development of TB. The mechanisms by which exposure to ambient PM adversely affects M.tb-specific adaptive immunity and T cell functions have not been studied. We explored the effects of "real-world" ambient urban PM on adaptive human antimycobacterial immune mechanisms.

Methods: PBMC were obtained from healthy adult donors in New Jersey that were selected based on IFN-γ release assay (IGRA) results. Ambient PM2.5 and PM10 (aerodynamic diameters <2.5μm and <10μm, respectively) were collected during three weather seasons (cold dry, warm dry, rainy) in 2012 with high-volume samplers (GMW Model 1200, VFC HVPM10, airflow rate 1.13m3/min) at the National Center for Environmental Research and Training (CENICA), Mexico City). PBMC from IGRA-positive (n=5) and IGRA- negative (n=7) donors were exposed to PM2.5 or PM10 at final concentrations of 0, 1, and 5μg/mL and incubated at 370 C for 18 h followed by infection with M.tb (multiplicity of infection of 1). On days 0 (1h), 1, 4, and 7, cells were lysed and serial dilutions of cell lysates plated on M.tb growth media in triplicate and incubated at 370 C for 21 days for colony forming unit (cfu) assays. PM2.5 or PM10 induced changes to cellular viability was assessed by lactate dehydrogenase (LDH) assay.

Results: M.tb growth control assays on days 1, 4 and 7 showed significantly ($p < 0.05$) higher M.tb cfu numbers in PM10-exposed (IGRA-negative) and in PM2.5 and PM10-exposed (IGRA-positive) M.tb-infected cells than in unexposed cells. Effects of PM exposure on M.tb growth control were independent of (1) the seasonal source of PM and (2) PM-induced decreases in cellular viability.

Examination of intracellular IFNγ and TNFα expression in M.tb-infected PBMC (CD3, CD4, CD8) and monocyte-derived macrophages, respectively, by flow cytometry, showed a decrease in M.tb-induced IFNγ and TNFα production upon PM exposures. PM exposures also downregulated the expression of the early T-cell activation markers (CD69 and CD25) in M.tb-infected PBMC.

Conclusions: PM exposure leads to loss of intracellular growth control of M.tb in human immune cells. Increased ambient air pollution exposures from growing vehicular traffic and industries in cities of TB endemic countries may impact on TB control efforts. Funding: NIEHS 5RO1ES020382-05

Undiagnosed sleep disorders in African Americans, The Jackson Heart Study

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Background: Despite the high prevalence of sleep disorders, a large proportion of the affected population remains undiagnosed, particularly African Americans. Undiagnosed sleep disorders can lead to daytime sleepiness which impacts daily functioning and increases risk of cardiovascular disease. Using data from Jackson Heart Study (JHS) Sleep Study, we determined the prevalence of undiagnosed sleep disorders and identified factors related to diagnosis of a sleep disorder in 825 African Americans.

Methods: Between 2012 and 2015, JHS participants underwent an in-home sleep apnea study with measurement of nasal pressure, abdominal and thoracic inductance plethysmography, oximetry, position, ECG and completed standardized measurements and questionnaires. Sleep apnea was defined as an apnea-hypopnea index (AHI) >5 (mild), >15 (moderate), and >30 (severe). The Women's Health Initiative (WHI) Insomnia Rating scale >10 was used to assess insomnia. Physician-diagnosis of sleep disorders were self-reported. Probabilities of physician-diagnosed sleep apnea and insomnia conditioned on AHI defined sleep apnea and WHI derived insomnia were calculated. Logistic regression models were fit to determine the associations of age, sex, body mass index (BMI), daytime sleepiness (Epworth Sleepiness Scale > 10), self-reported history of hypertension and diabetes with undiagnosed and diagnosed sleep disorders.

Results: The sample was mostly female (66.6%) with a mean age of 63.0 (standard deviation=10.7) years and a mean BMI of 32.1 (7.0) kg/m2. The median AHI was 10.7 events/hr and the median WHI insomnia score was 5.0. Approximately 75% of the sample had AHI>5 (38.4%(mild), 21.3%(moderate), 15.8%(severe)); whereas 2.1% reported a physician-diagnosis of sleep apnea. Approximately 22.2% had a WHI>10; whereas 2.4% reported a physician-diagnosis of insomnia. Among those with AHI>5, 2.4% had a physician-diagnosis of sleep apnea. Only 6.7% of participants with WHI>10 had a physician-diagnosis of insomnia. Male sex, older age, higher BMI, hypertension, and diabetes were independently associated with undiagnosed moderate sleep apnea, $P<0.05$. Similar associations were observed for mild and severe sleep apnea. A higher AHI and BMI were associated with a physician-diagnosis of sleep apnea, $P<0.05$. Higher reported daytime sleepiness was associated with undiagnosed insomnia, while younger age was associated with a physician-diagnosis of insomnia, $P<0.05$.

Conclusions: The prevalence of undiagnosed sleep disorders among individuals with clinically significant levels of insomnia and sleep apnea were 93.3% and 97.6%, respectively. These results suggest that there is a significant burden of undiagnosed sleep disorders in African Americans, and emphasize a need to improve recognition and treatment across population groups.

Plasma sRAGE is a Genetically Regulated Risk Factor for Sepsis-Associated ARDS

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Rationale: Plasma concentrations of the soluble receptor for advanced glycation end products (sRAGE) strongly associate with Acute Respiratory Distress Syndrome (ARDS) risk and impaired alveolar epithelial fluid clearance. However, whether plasma sRAGE contributes causally to ARDS is unknown. We used a genetic instrumental variant analysis technique, Mendelian Randomization (MR), to determine if plasma sRAGE concentration in sepsis is genetically regulated and whether genetically predicted plasma sRAGE is associated with sepsis-related ARDS.

Methods: We enrolled 326 critically ill patients with sepsis and measured plasma sRAGE concentration collected at ICU admission. Patients were genotyped using an Affymetrix Tx v1 array and were followed for 6 days for ARDS, which was phenotyped by Berlin criteria. We used logistic regression to test the association between risk factors and ARDS. For MR, plasma sRAGE levels were regressed on genome-wide genotypes in genetic ancestry-specific populations. We selected 2-5 single nucleotide polymorphisms (SNPs) strongly associated with sRAGE ($p<10\text{-}5$) that jointly explained at least 10% of sRAGE variance as the genetic instrument for MR. A 4th SNP, rs2070600 in the gene encoding RAGE (AGER), was included in the genetic instrument based on prior reports. Post-estimation analysis was used to segment the variance in sRAGE into genetic and residual components. We then determined the association of the genetic component of measured plasma sRAGE with ARDS adjusting for potential confounders. Plasma sRAGE was inferred to have a true causal effect on ARDS risk if the association between genetically predicted sRAGE and ARDS risk remained significant.

Results: Plasma sRAGE was strongly associated with ARDS, with an overall adjusted odds ratio of 2.05 (95% CI [1.53, 2.76]; $p=1.02x10\text{-}6$). By principle components analysis, there were 170 European and 156 African ancestry patients. For each population, we selected the 3 SNPs most strongly associated with sRAGE at $p<10\text{-}5$ and jointly explaining 28% of the variance in plasma sRAGE. In both populations, genetically predicted sRAGE remained strongly associated with ARDS risk after accounting for the non-genetic residual as well as potential confounders (Table). Rs2070600, an exonic variant in AGER previously associated with sRAGE, replicated its association in European ancestry subjects ($p=0.0190$).

Cluster analysis revealed 3 distinct groups of cells. A normal fibroblast phenotype (16.1% of the knockdown treated cell population; 64.8% of the control population), a cluster characterized by mitotic cell cycle markers (13.4% knockdown; 34.4% control), and a knockdown phenotype (70.5% knockdown; 0.8% control).

Conclusions: Our findings demonstrate that early sepsis-evoked plasma sRAGE is genetically regulated and genetically predicted sRAGE is associated with ARDS risk. These results indicate that plasma sRAGE is a causal intermediate in sepsis-related ARDS and suggest that plasma sRAGE should be investigated as a novel target for preventative or therapeutic intervention in ARDS.

Table: Odds ratios for the association between plasma sRAGE and ARDS risk

Logistic Model by Ancestry*	OR (95% CI) for ARDS	
	Measured sRAGE	Genetically predicted sRAGE
European Ancestry	1.84 (1.26, 2.71) p=0.002	2.58 (1.28, 5.20) p=0.001
African Ancestry	2.10 (1.38, 3.21) p=2.3x10-4	3.30 (1.58, 6.92) p=0.002

*Each logistic regression model was adjusted for age, gender, APACHE-III score, and pulmonary source of sepsis

Predictors of clinical outcomes in chronic critical illness

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Brown University

Rationale: Up to 10% of patients surviving critical illness subsequently require long-term care at a long-term acute care hospital (LATC) and are termed "chronically critically ill" (CCI). CCI patients are a highly vulnerable population, with approximately 50% 1-year mortality. We recently developed a new care model for CCI patients that uses a multidisciplinary team to improve continuity of care between the acute hospital (AH) and LTAC. We examined our patient cohort to determine risk factors for discharge to home and death. We hypothesized that mechanical ventilation (MV) and/or renal replacement therapy (RRT) at the time of transfer to LTAC would be risk factors for death in CCI patients.

Methods: We analyzed 241 consecutive patients transferred between August, 2013 and May, 2016 from BWH to LTAC. We collected information regarding demographics, length of stay (LOS), clinical outcomes, and disposition after LTAC. Age and pre-transfer albumin were analyzed in a subgroup of 75 patients. Analysis for categorical variables used Chi-squared and Fisher's exact tests and logistic regression was used for continuous variables, with $P < 0.05$ accepted for statistical significance. Multivariate logistic regression analysis (MLRA) was used to develop model to predict death and discharge to home.

Results: Patient characteristics are provided in Table 1. Home discharge was significantly associated with AH LOS ($P < 0.02$) and absence of early readmission (<30 days) from LTAC to AH ($P < 0.01$). Only absence of early readmission remained significant in MLRA. Death was associated ($P < 0.05$) with AH LOS, >1 transfer from AH to LTAC, MV, and early readmission from LTAC to AH. MLRA model to predict death included MV, early readmission from LTAC to AH, and >1 transfer from AH to LTAC. There were no associations identified between age, pre-transfer albumin, or RRT and home discharge or death.

Conclusions: MV and early readmission from LTAC are significant predictors of clinical outcome in CCI. The association between absence of early readmission and home discharge suggests that uninterrupted rehabilitation is important to recovery in CCI. The lack of association between age or albumin and death or discharge to home outcomes supports the distinction between patients with chronic and acute critical illness. Our findings can enhance patient and family counseling, and identifies areas for quality improvement efforts in CCI.

Na,K-ATPase inhibition inhibits influenza A viral replication

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Rationale: Influenza is a highly contagious disease with significant morbidity and mortality. Currently, there are limited therapeutic strategies to reduce the risk of severe illness and death. The development of new drugs is a necessity, preferably targeted to modulate the host response which should avoid the development of virus resistance. Na,K-ATPase inhibitors, such as cardiac glycosides, have been shown to inhibit viral replication. Here we set out to explore mechanisms by which the inhibition of Na,K-ATPase prevents influenza A virus replication.

Methods: Cell culture experiments: A549 cells were infected for 1 h with influenza A virus (IAV: A/WSN/33, H1N1) at 1 MOI and incubated with ouabain at different time-points. Western blots were used to assess host and viral protein expression, and plaque assays were performed to evaluate viral titers. Real time PCR was used to determine viral mRNA in infected cells. Inductively coupled plasma/mass spectrometry was used to measure intracellular sodium and potassium. *Ex vivo experiments:* Lung slices from transgenic mice expressing an ouabain-sensitive Na,K-ATPase were infected ex vivo and treated with ouabain and images were taken using a confocal microscopy. *In vivo experiments*: Transgenic mice mentioned above were infected with IAV by intra-tracheal instillation and treated with ouabain or saline solution by intraperitoneal injection and bronchoalveolar lavage (BAL) was collected and lung homogenates were used to measure viral titer.

Results: In vitro experiments revealed that influenza viral protein expression was blunted when Na,K-ATPase was inhibited. The effect of Na,K-ATPase inhibition on influenza replication was determined to be 4 to 6 h post infection and independently of mRNA transcription, increased protein degradation, the Src signaling pathway and intracellular calcium changes. We found that Na,K-ATPase inhibition led to decrease in the intracellular potassium affecting the cell translational machinery and consequently viral protein synthesis. Mice infected with IAV and treated with ouabain for 2 days post infection had decreased inflammation markers in BAL and lower viral titer in lung tissue compared to saline injected mice.

Conclusion: Na,K-ATPase inhibition by decreasing intracellular potassium concentrations inhibits influenza viral protein synthesis post-transcriptionally. Accordingly, Na,K-ATPase inhibition impairs influenza virus replication and appears to decrease e lung inflammation and viral titers of influenza infected mice.

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A Novel PatientCentered Metric of U.S. Lung Transplant Center Performance

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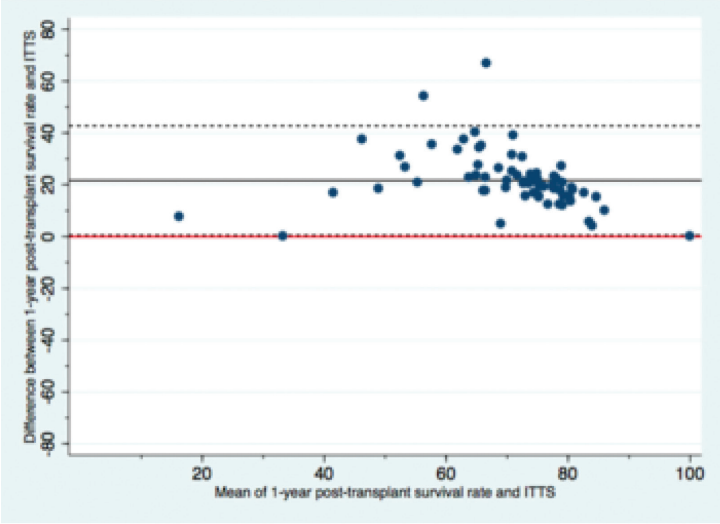
Rationale: Publically available center outcome data influence lung transplant center selection by patients and physicians, and policymakers use 1year posttransplant survival rates in accreditation decisions. While important, this metric ignores the outcomes of those listed but never transplanted. Therefore, we developed a novel, patientcentered, "intentiontotreat" center performance metric that encompasses both the likelihood of transplantation and the likelihood of survival oneyear after transplantation. We hypothesized that this metric, termed "intentiontotreat 1year survival" (ITTS), would exhibit poor agreement with 1year posttransplant survival rates within and across U.S. centers.

Methods: We performed an ecologic study of 68 U.S. lung transplant centers between 2006 and 2012 using Organ Procurement and Transplantation Network data. During the study period, 13,401 candidates were placed on the active waiting list and were followed through March 2015. ITTS was calculated as the percentage of candidates who were both transplanted and survived to 1year after transplantation. ITTS failures were those who were removed from the list before transplantation, those who died before transplantation, and those who died less than one year after transplantation. We excluded one center that listed only one candidate during the study period. We calculated centerspecific rates of 1year posttransplant survival, waiting list mortality, transplantation, and ITTS. We examined ITTS agreement with 1year posttransplant survival by calculating the mean difference and 95% limits of agreement, and we examined ITTS correlation with traditional metrics by measuring Pearson correlation coefficients.

Results: The median centerspecific ITTS was 63.2% (range 12.5% to 100%). The median centerspecific 1year posttransplant survival was 84.2% (range 20.0% to 100%). The correlation coefficient between ITTS and 1year posttransplant survival was 0.70 ($p<0.001$), but there was substantial disagreement between ITTS and 1year posttransplant survival within centers (Figure): on average, the oneyear posttransplant survival rate overestimated ITTS by 22% (mean absolute difference 21.6%, 95% limits of agreement 0.5%42.7%). Centers with >90% 1year posttransplant survival rates had ITTS rates ranging from 33.3% to 100%; centers with 8090% 1year posttransplant survival had ITTS rates of 29.4% to 82.0%, and centers with <80% 1year posttransplant survival had ITTS rates of 12.5% to 66.7%. Centerspecific ITTS was weakly associated with a higher transplant rate (r=0.26, p=0.03) but was not associated with waiting list mortality (r=0.01, p=0.92).



Conclusions: Centerspecific 1year posttransplant survival rates vastly overestimate the experience of lung transplant candidates after listing. We propose a novel center performance metric that may be of greater value to patients and policymakers.

In utero second-hand smoke exposure aggravates elastase-induced emphysema in mice: pivotal roles of Mmp12 and IL-17a.

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Rationale: Despite *in utero* second-hand smoke (SHS) exposure being a risk factor for several pulmonary diseases, including asthma, the association between in utero SHS and development or exacerbation of emphysema remains unclear. Emphysema is a progressive and irreversible obstructive lung disease characterized by airspace enlargement. We previously showed that lung functions were significantly decreased and pulmonary alveoli significantly enlarged in 15-week old mice exposed solely to in utero SHS. Additionally, Serpina1a, the mouse ortholog of the human gene alpha-1 antitrypsin (α1AT) a known genetic risk factor for emphysema, was significantly down-regulated in SHS exposed mice. These results strongly suggested that in utero SHS exposure alone can affect emphysema development in adulthood. Here, we hypothesized that in utero SHS exposures will predispose adult mice to the development of elastase-induced emphysema.

Methods: Pregnant Balb/c mice inhaled filtered air or 10 mg/m3 of SHS from gestation days 6-19. At 17 weeks of age, male offspring were instilled intratracheally with 0.035 U/g of elastase or saline. At 20 weeks, we assessed emphysema phenotypic endpoints, including lung function, morphometric analysis, and gene expression.

Results: Mice exposed *in utero* to SHS and treated as adults with elastase showed up-regulation of the Mmp12 gene (3.2-fold), who's protein product is involved in tissue destruction; and had significantly increased mean linear intercept values (98.9 μm vs 80.9 μm) for the lung parenchyma, indicating that overexpression of Mmp12 plays a key role in in utero SHS-aggravated airspace enlargement. Consistent with these findings, *in utero* SHS + elastase caused significant increases in lung compliance, as well as decreased elastance and minute volume compared to controls, suggesting that *in utero* SHS-altered lung structure precedes and contributes to decline lung function. Furthermore, the expression of IL-17a, a cytokine produced by activated T cells and associated with cigarette smoke-related emphysema in humans, was up-regulated 9.6-fold in in utero SHS + elastase mice, suggesting that *in utero* SHS exposure primes the lungs for increased adaptive immune responses later in life..

Conclusions: These experimental findings demonstrate that *in utero* SHS increases the lungs' susceptibility to develop emphysema-related responses as adults and suggest that there is a pivotal imprint of in utero SHS exposure on the lungs.

Innate host factors present in serum of healthy volunteers control growth of carbapenemase-producing *Klebsiella pneumoniae*.

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Rationale: Klebsiella pneumoniae (KP) remains an important cause of nosocomial multidrug-resistant gram-negative bacterial lung infections but also community-acquired invasive disease worldwide. A primary mechanism by which KP evades host immunity is through the expression of a polysaccharide capsule that enables escape from serum killing and phagocytosis. Less clear are host factors that dictate KP pathogenicity. KP virulence is associated with hypermucoviscous strains expressing capsular serotypes K1 and K2 that cause invasive syndromes in Asia. On the other hand, nosocomial carbapenemase-producing KP (KPC) isolates in the U.S. generally lack K1, K2 capsular serotypes, and do not induce lethality in mouse septicemia models. We hypothesized that soluble host factors present in serum fail to control hypermucoviscous KP but are sufficient to control KPC growth..

Methods: KP strain 396 (K1 serotype, hypermucoviscous clinical isolate), KP strain 43816 (K2 serotype, American Type Culture Collection research strain), and carbapenemase-producing KP ST258 clinical isolates (non K1/K2 serotypes) were utilized. Serum from healthy volunteers (n=7) was obtained from peripheral whole blood following venipuncture of 20 mL blood. All subjects underwent venipuncture after informed written consent and ethnicities and gender were identified by self-reporting. C4, C3/C4, Properdin, and Factor B-depleted sera were utilized in select experiments. KP strains in early logarithmic phase were incubated at 37oC in 5% sterile TSB, and 85% human serum. Bacterial growth in human serum was determined by optical density at 600 nm and, in select experiments, confirmed by CFU bacterial serial plating.

Results: We evaluated host serum killing of KP hypermucoviscous K1and K2 isolates compared to three multidrug-resistant KPC isolates. Serum from all healthy volunteers failed to kill hypermucoviscous K1 and K2 isolates. In contrast, KPC isolates were easily killed by serum from all healthy volunteers tested. Serum killing of KPC was abrogated by heat inactivation or serum depletion of C3/C4 complement proteins. Specifically, depletion of Properdin or Factor B alone enabled KPC growth in serum, indicating an alternative complement pathway mediated process.

Conclusions: While hypermucoviscous KP strains escape serum killing, soluble factors found in serum of healthy subjects are sufficient to control KPC growth. Moreover, depletion of components of alternative complement pathway attenuated serum killing of KPC. These findings suggest the possibility that individuals with susceptibility to multidrug-resistant KPC infection may harbor acquired defects in alternative complement pathway.

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Impaired Lymphatic Flow Leads to Increased Pulmonary Inflammation in Mice

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The pulmonary lymphatics play a major role in lung fluid homeostasis in both physiologic and pathologic conditions. In addition, the pulmonary lymphatics are likely to play an active role in regulating the inflammatory milieu of the lungs due to their role in the trafficking of antigens and antigen presenting cells. Here, we investigated the functional role of flow in pulmonary lymphatic vessels using mice deficient for the platelet-specific receptor C-type lectin–like receptor 2 (CLEC2). We have previously shown that blood backfills the lymphatic network and impairs lymph flow in these mice. The effect of this impaired flow on the pulmonary lymphatics was unknown. We found that CLEC2-deficient animals exhibited abnormal, dilated, and blood-filled pulmonary lymphatics. In addition, the pulmonary lymphatics in Clec2-deficient animals had abnormal and excessive smooth muscle cell coverage. Furthermore, Clec2-deficient mice had increased inflammatory cell infiltrate within the lung parenchyma, which was associated with abnormal collagen deposition. These studies indicate that impaired lymphatic flow leads to increased pulmonary inflammation, and implicates the lymphatic vasculature in regulating the inflammatory response in the mouse lung.

Anatomic Gradients, Cell Specificity and Smoking Modulation of Human Airway Epithelium Expression of ADH7, the Rate Limiting Enzyme for Generation of Retinoic Acid

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Rationale: During embryonic development of the lung, retinoic acid (RA) acts as a morphogen with regionspecific retinoid signaling in the induction and growth of primary lung buds, making anatomic distribution of retinoid levels essential for proper development and maintenance of the lung. Little is known, however, regarding the control of RA metabolism in the adult human airway epithelium, a cell population under constant stress from inhaled contaminants. Based on the knowledge that alcohol dehydrogenases (ADH) are the ratelimiting enzymes in the conversion of retinol to retinoic acid, and that alcohol dehydrogenase 7 (ADH7) is the most highly expressed efficient ADH for retinol oxidation in the human airway epithelium, we explored the expression pattern of ADH7 in nonsmokers and smokers from proximal to distal airway epithelium.

Methods: Paired trachea, large airway (3rd–4th), and small airway (10th–12th) epithelium was recovered by fiberoptic bronchoscopy and brushing of healthy nonsmokers (n=12) and healthy smokers (n=15). Immunostaining for ADH7 was performed on human airway epithelium of the trachea, large airways, and small airways of nonsmokers. Microarray (Affymetrix U133 plus 2.0) with RNAseq validation was used to quantify ADH7 expression along the human airway epithelium. Basal cells purified from trachea epithelial brushings were seeded on airliquid interface (ALI) culture to determine ADH7 expression during basal cell differentiation over 28 days.

Results: ADH7 was expressed in basal cells and upregulated during airway basal cell differentiation on ALI culture. In vivo, ADH7 was expressed in basal and ciliated cells with colocalization of ADH7 in KRT5+ and betatubulin IV+ staining respectively, with no expression in CC10+ and MUC5AC+ secretory cells. There was a linear decrease ($p<104$) in ADH7 mRNA expression along the airway epithelium (tracheal > large airways > small airways) in healthy nonsmokers. ADH7 expression in healthy smokers significantly increased throughout the epithelium [trachea, ($p<2x104$); large airways ($p<2x104$); and small airways ($p<3x105$)]. The smoking modulation of ADH7 expression gradient along the airways resulted in a uniform upregulated expression of ADH7 with an average foldchange of 3.5 ($p<105$), a finding supported by RNAseq.

Conclusions: The control of ADH7 expression in the human airway epithelium is complex, with differences in cell specificity, proximal to distal anatomic regions and responses to the stress of smoking. In the context that retinoids play a central role in lung growth and differentiation, these observations have implications for adult lung regeneration.

Streptococcus Pneumoniae Induce Necroptosis in the Myocardium of NonHuman Primates with Severe Pneumonia

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Background: *Streptococcus pneumoniae* is the most common isolated bacterial pathogen in patients with communityacquired pneumonia (CAP) worldwide. It is well established that adverse cardiovascular events (ACE) occur during and after CAP with important impact on mortality. Recently, our research group has described in mice that S. *pneumoniae* is capable of invading the myocardium and disrupting cardiac function during invasive pneumococcal disease. Yet, the underling mechanism of CAPinduced ACE is still unclear; therefore, we hypothesize that S. *pneumoniae* is capable of inducing necroptosis, a highly proinflammatory cell death pathway in cardiomyocytes. To test this hypothesis, we assessed the development of necroptosis in the heart of nonhuman primates with severe pneumococcal pneumonia.

Methods: We evaluated the heart of six baboons intratracheally challenged with S. *pneumoniae* TIGR4 and two uninfected controls. Animals with severe pneumococcal pneumonia were stratified in two groups: natural disease cohort (n=3) and antibiotic treated cohort (n=3); to determine the effect of antibiotic treatment in the development of necroptosis in cardiomyocytes. Translocation of the pneumococcus into the myocardium was determined by immunofluorescence staining and transmission electron microscopy (TEM). Cardiomyocyte death was determined by Hematoxylin & Eosin (H&E) and CardioTAC staining. To determine the activation of necroptosis, we used western blots and immunocytochemistry for receptorinteracting protein 3 (RIP3), mixed lineage kinase domainlike protein (MLKL) and phosphorylated MLKL (pMLKL). Cytokines and chemokines were measured in homogenized heart tissue using a multiplex assay.

Results: Cytoplasmic changes of contraction bands, focal vacuolation, myocytolysis, karyolyis, edematous interstitium containing increased small mononuclear cells and neutrophilic invasion were observed in the heart of infected animals using TEM and H&E. S. *pneumoniae* was isolated in cluster conformations in all the hearts of baboons with severe pneumonia but not in the controls. Increased levels of RIP3 and pMLKL (necroptosis) were detected in all the hearts of baboons with severe pneumonia versus controls ($p=<0.05$). Higher levels of interleukins (IL) 6, 8, 1Ra, 1β, macrophage inflammatory protein (MIP) 1a and tumor necrosis factor (TNF) a were identified in the heart of all infected animals compared to controls, independent of the antibiotic treatment ($p=<0.05$).

Conclusions: S. pneumoniae is capable of reaching the heart during severe pneumonia and induce necroptosis independently of the antibiotic treatment. This is of great importance as necroptosis inhibition might be potential therapeutic target during CAP.

Rhinovirus induces dysmaturity and unjamming of normal and asthmatic human bronchial epithelial layers

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Rationale: Human Rhinovirus (RV) is implicated in asthma pathogenesis and is a known asthma trigger[1],[2], but mechanism remains unclear.[3] The maturing layer of primary human bronchial epithelial cells (HBECs) derived from healthy or asthmatic donors undergoes a transition from a migratory, fluid-like, unjammed phase to non-migratory, solid-like jammed phase. [4] But in the mature jammed HBEC layer, mechanical compression as occurs during bronchospasm provokes dysmature layer unjamming [4] and airway wall remodeling (ref). Using the mature jammed HBEC layer, here we test the hypothesis that RV infection, too, can provoke dysmature layer unjamming.

Methods: HBECs from asthmatic and normal donors were grown in air-liquid-interface (ALI) culture for 14 days. Well-differentiated HBECs were infected on ALI days 7 and 14 with RV-A16 (MOI from 0.01 to 10). Time-lapse images were taken for 24 hrs. starting at 2 hrs. after RV infection on ALI day 14, and Particle Image Velocimetry (PIV) was used to measure cell motility. Protein lysates were collected for western blot (WB) analysis to determine vimentin and E-cadherin levels.

Results: In normal donors, HBECs infected with RV-A16 at MOI 0.1-10 underwent a transition from a solid-like jammed state toward a fluid-like unjammed state. With infection, average cellular speed typically increased to roughly 3-5 μm/hr, but velocities slowed by 24 hours post-infection. In asthmatic donors, likewise, infected HBECs underwent a transition to the unjammed state. With infection, average cellular speed typically increased to roughly 10-15 μm/hr., however, velocities did not slow by 24 hours post-infection. In addition, infection with RVA16 at a submaximal dose (MOI 0.01) induced unjamming in the asthmatic cells but not in normal cells. In all cases, RV-A16 infection caused no induction in vimentin and no reductions in E-cadherin or other epithelial cell marker proteins.

Methods: In the mature jammed HBEC layer derived from either normal and asthmatic donors, RV infection caused unjamming that approximated a dysmature layer phenotype. At any level of MOI, the unjamming response of cells from asthmatic donors was far greater than those from non-asthmatic donors, suggesting that asthmatic cells are more susceptible to RV-mediated layer unjamming. Our data reveal an unanticipated effect of RV infection upon the migratory dynamics within the HBEC layer and suggest, further, a convective pathway for virus spreading within the layer. Taken together, these observations suggest a new physical picture of RV infection and its role in asthma exacerbations and pathogenesis

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Associations between *in utero* ambient fine particulate matter exposure and childhood wheeze in Mexican children: Effect modification by prenatal psychosocial stress

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Rationale: Studies report synergistic effects between stress and ambient fine particulate matter (PM2.5) on incidence and exacerbation of asthma and decrements in lung function in older children, however this interaction has not been examined in pregnancy. We examined associations between in utero exposure to PM2.5 and wheeze in preschool-aged children and whether associations were modified by increased exposure to prenatal stress.

Methods: Analyses included 546 mothers and children enrolled in the longitudinal Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) birth cohort study in Mexico City. Daily prenatal PM2.5 exposure was estimated based on residence during pregnancy using a validated satellite-based spatio-temporally resolved prediction model and then averaged over trimesters. Mothers reported negative life events (NLEs) occurring in the past 6 months in pregnancy and a summed NLE score was derived. NLE scores were dichotomized at the median as low (NLEs<3) and high (NLEs>3) stress.

Caregiver-report of ever wheeze and wheeze in the past 12 months for children were obtained using the International Study of Asthma and Allergies in Childhood (ISAAC) survey administered at 48 months. The association between PM2.5 exposure averaged over trimesters and wheeze outcomes was analyzed using a modified Poisson regression and stratified by low/high stress. Models were adjusted for child's sex, exposure to a smoker in the home during the prenatal period and at 48 months, maternal age at delivery, maternal asthma, birth weight Fenton-Z-score and co-adjusted for other trimester exposures.

Results: Among children, half were male; a minority were exposed to a smoker in the home pre-(36%) and postnatally (14%); 25% had ever wheezed and 12% had wheezed in the past 12 months. Just over a third of mothers (37%) reported high stress during pregnancy. While no significant associations were seen for ever wheeze, higher PM2.5 exposure during the first trimester was significantly associated with increased risk of wheeze in the past 12 months among children born to mothers reporting high prenatal stress (RR: 1.08, 95%CI [1.00, 1.17]) but not among those born to women reporting low stress (RR: 0.96, 95% CI [0.89, 1.04]) (pinteraction=0.03).

Conclusions: Increased prenatal stress enhanced the association between PM2.5 exposure in early pregnancy and child wheeze at age 48 months. These data add to a growing literature underscoring the importance of considering chemical and non-chemical stressors together in order to more comprehensively characterize children's environmental risk.

Enhanced Endothelium-Dependent Pulmonary Vasodilation and eNOS Expression Limit Increased Vasoconstrictor Sensitivity in Neonatal Chronic Hypoxia

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Endothelial dysfunction may contribute to chronic hypoxia (CH)-induced pulmonary hypertension in neonates. However, whether basal and stimulated nitric oxide (NO)-dependent pulmonary vasodilation are similarly impaired in neonatal CH is unclear. We hypothesized that neonatal CH enhances basal tone and pulmonary vasoconstrictor sensitivity by limiting NO-dependent pulmonary vasodilation. To test this hypothesis, we assessed effects of the NO synthase (NOS) inhibitor N⍵-nitro-L-arginine (L-NNA) on baseline pulmonary vascular resistance (PVR) and vasoconstrictor sensitivity to the thromboxane mimetic U-46619 in saline-perfused lungs (in situ) from 2-wk-old control and CH (2 wk at 0.5 atm) rats. Basal tone was defined as that reversed by exogenous NO (spermine NONOate). Lungs from CH rats showed greater baseline PVR, basal tone and U-46619-mediated vasoconstriction compared to controls in the absence of L-NNA. L-NNA increased baseline PVR, basal tone, and reactivity to U-46619 in lungs from CH neonates, further augmenting vasoconstrictor sensitivity compared to control lungs. Exposure to CH additionally augmented NO-dependent vasodilatory responses to arginine vasopressin (AVP), pulmonary expression of NOS III (eNOS), and eNOS phosphorylation at activation residue serine-1177. We conclude that, in contrast to our hypothesis, enhanced basal tone and agonist-induced vasoconstriction following CH is limited by increased NO-dependent pulmonary vasodilation resulting from greater eNOS expression and phosphorylation at activation residue serine-1177.

FBXO17 Regulates Glycogen Synthase Kinase-3b (GSK3b) Polyubiquitination andProteasomal Degradation in Lung Epithelial Cells

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Rationale: Glycogen synthase kinase-3β (GSK3β) is a highly conserved serine-threonine kinase that is a critical regulator of cell differentiation, metabolism, development, and inflammation. GSK3β-mediated phosphorylation is a key step in targeting substrates of Skp1/Cul1/F-box protein (SCF) E3 ubiquitin ligases to the proteasome for degradation. Recent data suggest that GSK3β has critical roles in propagating inflammation in murine models of acute lung injury. The goal of our study was to understand how GSK3β protein stability is regulated and how these mechanisms may influence inflammation in murine lung epithelial cells..

Methods: Site-directed mutagenesis was used to generate lysine to arginine point mutations in GSK3β. Plasmids expressing hemagglutinin (HA)-tagged wild-type, K183R, and K205R mutant GSK3β were transfected into murine lung epithelial cells (MLE-12). After cells were cultured for 48 h, a cycloheximide chase assay (40 μg/mL) was performed to evaluate the half-life of GSK3β. Lysates were collected at 0, 2, 4, and 8 h and immunoblotted for HA-tagged GSK3β. Plasmids were also transfected into MLE-12 cells and after 48 h of culture, cells were treated with proteasome inhibitor MG132 (20 μM) to allow accumulation of polyubiquitinated proteins. Lysates were immunoprecipitated for HA-GSK3β and immunoblotted with antibody for K48-linked ubiquitin. Lysates from a HEK293 expression library of over 30 F-box proteins were immunoblotted for endogenous GSK3β. Plasmid expressing histidine (V5)-tagged FBXO17 was also transfected into MLE-12 cells. Lysates were prepared after 48 h and immunoblotted for GSK3β.

Results: We identified lysine 183 as the primary acceptor site for K48-linked ubiquitin chains in GSK3β. K183R mutant GSK3β protein had a longer half-life and significant reduction in polyubiquitination than wild-type or K205R-GSK3β. Finally, we identified FBXO17 as a subunit of an SCF E3 ubiquitin ligase complex that targets GSK3β for polyubiquitination and proteasomal degradation in lung epithelial cells.

Conclusions: Our study characterizes a previously unknown mechanism for GSK3β degradation by the proteasome. We have identified FBXO17 as a subunit of an SCF E3 ligase complex that targets GSK3β. Future studies will focus on characterizing how FBXO17 modulates inflammation in lung epithelial cells. Our data suggest a critical role for the ubiquitin-proteasome pathway in regulating active and inactive pools of GSK3β which may influence the severity of lung inflammation.

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Incidence of Iatrogenic Complications Following Thoracentesis in an Academic Medical Center

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Rationale: Thoracentesis is a crucial procedure in the evaluation of pleural effusions with over 170,000 procedures performed annually by pulmonologists, interventional radiologists and internists.

The incidence of iatrogenic complications is variable and dependent on such factors as operator experience, patient characteristics and the use of ultrasound guidance.

We present findings of a quinquennial review of over 1400 adult patients who had ultrasound guided thoracentesis at a large tertiary medical center, reporting the incidence of iatrogenic pneumothorax and hemothorax.

Methods: Patients with pleural effusions who underwent thoracentesis between October 2010 to September 2015 were identified by querying billing records for international classification of diseases code 34.91 (Thoracentesis). Iatrogenic complications were identified using ICD codes 512.1 (iatrogenic pneumothorax) and 511.89 (hemothorax as a complication of a procedure). Procedures were stratified by provider (pulmonary/critical care versus non-pulmonologists) using national provider identification codes (NPI). A comprehensive chart review was performed including a review of chest x-ray images before and after the procedure to ascertain the veracity of the coding. Hemothorax was defined by a hematocrit ratio >0.5 and/or chest tube insertion. This study was reviewed and approved by the University of Massachusetts Medical School IRB (IRB # H00010460).

Results: Of 1494 patients who underwent the procedure, the mean age was 66 years (SD±17), 55 % were male, and 75 % were Caucasian. 27 patients (1.8 %) had a radiographically confirmed iatrogenic pneumothorax. Out of these, 17 patients required chest tube insertion due to size and/or clinical symptoms resulting in an incidence rate of 1.1 %.

There was a non-significant difference in pneumothorax incidence for pulmonary fellows as compared to other providers- mostly comprised of interventional radiology physicians (1.4 % compared to 0.83 %, p=0.35). In the last decade the reported iatrogenic pneumothorax rate is estimated at 0.16 % to 6 %.

Only 6 patients had a hemothorax, resulting in an estimated incidence rate of 0.4 %, which compares favorably to the incidence rates reported in the literature (0.2 % - 1 %).

Complication	Pulmonary/Critical Care physicians n=799	Other Provider (n=695)	p-value
Iatrogenic pneumothorax	11 (1.38 %)	6 (0.86 %)	0.35
Hemothorax	4 (0.5 %)	2 (0.29 %)	0.52

Conclusion: Thoracentesis is a safe procedure with an iatrogenic pneumothorax and hemothorax complication rate that is low and did not differ significantly among pulmonary critical care fellows compared to other physicians. We attribute these findings largely to the routine use of ultrasound guidance as a standard of care.

TUMORIGENIC CELLS OF LYMPHANGIOLEIOMYOMATOSIS ARE OF A NEURAL CREST ORIGIN AND SUSCEPTIBLE TO TYROSINE KINASE INHIBITORS

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Lymphangioleiomyomatosis (LAM) is a rare neoplasm characterized by the proliferation of atypical melanocytic and smooth muscle-like cells (LAM cells) causing cystic destruction in the lungs, and the development of renal angiomyolipomas (AMLs) and lymphangioleiomyomas in the axial lymphatics. LAM occurs mostly in women in a sporadic manner, or as part of the Tuberous Sclerosis Complex (TSC) disorder caused by mutations in the TSC1/2 genes, which activates the mTOR signaling complex. Inhibition of the mTOR pathway temporarily stymies tumorigenesis, however, given disease recurrence upon discontinuation of therapy, there remains no sustainable cure for LAM. Importantly, the source of LAM cells is also not known. Given the multi-systemic manifestation of the disease using pluripotent lineage cells, we tested the hypothesis that LAM and AML cells are mesenchymal and derive from the neural crest. Using immunohistochemistry and semi-quantitative cell imaging techniques, we defined the expression of early stage pre-lamination and migratory neural crest markers activating protein 2 (AP2α) and β-1,3-glucoronyltransferase 1 (CD57), and melanoma-initiator nerve growth factor receptor (NGFR), annotating the origin of AML cells to a cranial neural crest lineage. We also identified mesenchymal PDGFRβ, melanocytic HMB45 and endothelial CD146 marker expressions on both LAM and AML cells and demonstrated pluripotency by successfully stimulating the differentiation of AML cells into smooth muscle cells, melanocytes, adipocytes and lymphatic endothelial cells. We also resolved an alternative therapeutic strategy for LAM by successfully attenuating AML proliferation ($p<0.05^*$) and diminishing tumor cell viability ($p<0.005^*$) using a combination of sirolimus (0.05μM) and co-adjuvant tyrosine kinase inhibitors (Nilotinib – (1-5μM); Imatinib (20μM)) that target the PDGF pathway. In delineating the ontogenetic basis of LAM, we propose LAM and AML cells as embryonic migrants of the neural crest, domiciled in various organs and quiescent in activity until environmental triggers stimulate tumorigenesis. An ongoing clinical trial at our center based on these findings is testing the therapeutic efficacy of these agents on the disease.

Follistatin-Like Protein 1 Conditional KO Mice Develop Spontaneous Lung Emphysema

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Background: Follistatin-Like protein 1 (fstl-1) is crucial for lung organogenesis. Germline fstl-1 knockout mice show malformed proximal airways, abnormal saccular maturation, defective production of surfactant protein C, and die shortly after birth. However, the postnatal role of fstl-1 in the lungs is unknown. This secreted protein interacts with TGF-beta, increasing its activity. TGF-beta signaling is known to suppress matrix metalloproteinase 9 (MMP9) gene expression, decreasing extracellular matrix degradation. We hypothesized that mice lacking fstl-1 in postnatal life will have parenchymal abnormalities due to increased expression of MMP9.

Methods: We used a conditional murine KO for fstl-1 using a global tamoxifen inducible UBC-Cre/lox recombinase. We treated 8-week-old mice with intraperitoneal injections of tamoxifen and euthanized at several time-points up to 24 weeks post-treatment. We assessed pulmonary function by pressure-volume loop curves and force oscillation techniques, collected bronchoalveolar lavage fluid (BALF), and harvested lung tissue to perform HE staining, qPCR and western blot for MMP9.

Results: We found that mice lacking fstl-1 for 24 weeks compared to age-matched tamoxifen-treated controls demonstrated: i) lung parenchymal abnormalities characterized by increased airspaces in HE stained lung sections; ii) decreased lung elasticity, increased total lung capacity, and decreased airway resistance by pulmonary function tests; iii) decreased number of cells per mL with similar cell differentials in the BALF analysis; iv) increased protein abundance and gene expression of MMP9 by Western blot and qPCR analysis.

Conclusion: These results suggest that loss of fstl-1 for 24 weeks during postnatal life induces spontaneous changes compatible with lung emphysema in mice. These findings might be explained by increased MMP9 and not by an increased number of inflammatory cells in the airways. Future research will be required to elucidate the role of fstl-1 in pulmonary emphysema.

The Median is Not the Message in the Recovery for Pneumonia

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Rationale: Pneumonia hospitalizations are common. However, little is known about variation in patterns of recovery among patients who are discharged alive from pneumonia hospitalizations. We sought to characterize the variation in patterns of recovery in the year after a pneumonia hospitalization among patients in three different health systems.

Methods: This is an observational study of 3 cohorts of patients hospitalized with pneumonia: (1) Health and Retirement Study (HRS) participants enrolled in Fee-for-service Medicare (1998-2009), (2) Veterans Administration (VA) Healthcare system (2013-2014), and (3) Kaiser Permanente of Northern California (2010-2014). We identified pneumonia hospitalizations using International Classification of Diseases, 9th Edition, clinical modification (ICD-9-CM) coding. We then determined the median experience after pneumonia by cohort, and measured the proportion of patients who had this median experience. To begin to understand potential contributors to the different patterns of recovery, a multinomial logistic regression was performed with the patients being grouped into those who did better and those who did worse as compared to the median experience.

Results: We identified 2731, 23536, and 39147 pneumonia hospitalizations in HRS, VA, and KPNC, respectively, of whom 88.1%, 92.8%, and 89.7% survived to hospital discharge. The median patient survived to 1 year and was re-hospitalized once (VA and KPNC) or twice (HRS). Just 9%, 14.1%, and 9.1% of patients experienced the median outcome in HRS, VA, and KPNC, respectively. Of the patients who survived the hospitalization, 33.3% (HRS), 30.2% (VA), and 26.8% (KPNC) died during the subsequent year—an outcome worse than the median. Of those who survived, 29.8 % (HRS), 35.9% (VA) and 46.1% (KPNC) were never re-hospitalized—an outcome better than the median. 11.9 % (HRS), 11.9% (VA), and 11.7% (KPNC) had greater than 3 hospitalizations. Age, race, gender, comorbidity, ICU use, and hospital length stay collectively explained very little of the variation in the subsequent recovery pattern..

Conclusions: The median experience after pneumonia hospitalization hides wide variability in individual experiences. In 3 separate cohorts of pneumonia hospitalizations, we found that 1/3 live to 1 year and are not re-hospitalized, 1/3 die, and 1/3 experience varying degrees of recurrent hospitalizations. Older age, increasing co-morbidity, and length of stay are associated with a poor recovery, but provide little predictive power. There may be important opportunities to better classify these heterogeneous recovery pathways, to guide both prognostication and interventions.

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