13th Annual Lung Day 2022

POSTER SESSIONS AND ABSTRACTS
Poster Session 1
Rooms 1 - 3
11:45 a.m. - 12:45 p.m.
Abstracts Presentations 1 - 16

Poster Session 2
Rooms 1 - 2
12:45 p.m. - 1:45 p.m.
Abstracts Presentations 17 - 33

Hybrid Poster Session
Room 3 and Zoom
1:00 p.m. - 1:40 p.m.
Abstracts Presentations 34 - 38
## POSTER SESSION 1  
(11:45 a.m. - 12:45 p.m.)

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## HYBRID POSTER SESSION  
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13TH ANNUAL LUNG DAY

**Poster Session 1**

Rooms 1 - 3

11:45 a.m. - 12:45 p.m.

Abstracts Presentations 1 - 16

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(11:45 a.m. - 12:45 p.m.)
Abstract # 1 page 1

A CASE OF DISSEMINATED TB WITH GENITOURINARY INVOLVEMENT

Katyayini Aribindi¹, Namita Sood¹

¹University of California – Davis

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Introduction: A 23-year-old woman presented to the emergency department with shortness of breath and twenty-five-pound weight loss over four months. She emigrated to the United States from Nepal a year prior to her admission to the hospital. She had a chronic worsening cough that she attributed to allergies, and amenorrhea for the last four months, with normal prior menstruation. She denied any exposure to tuberculosis, or sick contacts, or sexually transmitted illnesses.

Results: On Examination: Chest x-ray showed diffuse nodular opacities. Computed tomography of the chest showed innumerable upper lobe predominant pulmonary nodules, including some cavitating lesions with mediastinal and hilar lymphadenopathy. Computed tomography of the abdomen and pelvis showed a fluid filled uterine wall with diffuse necrotic abdominopelvic lymphadenopathy (Figure 1 A-D). Transabdominal pelvic ultrasonography showed a fluid filled uterus with a 6 cm x 2.4 cm x 3.8 cm echogenic mass within the endometrium. Differential diagnosis included malignancy, infection, and granulomatous disease such as sarcoidosis.

Sputum stain returned positive for acid-fast bacilli and Mycobacterium tuberculosis PCR. Sputum and urine cultures grew Mycobacterium tuberculosis. She was initiated on rifampin, isoniazid, pyrazinamide, and ethambutol with sputum and AFB stain negativity after two weeks.

Discussion: Mycobacteria tuberculosis (TB) remains a major cause of morbidity and mortality. Pulmonary tuberculosis is the most common manifestation, however genitourinary TB accounts for 20% of extrapulmonary TB, and can affect 2 to 20% of individuals following pulmonary tuberculosis. It affects men more frequently with involvement of the kidneys or epididymis following hematogenous spread from the lungs. TB affecting the endometrial cavity is extremely rare but has been associated with 0.2 to 21% of infertility, with higher incidences among women without access to reconstructive surgery. While primary genitourinary TB is possible after sexual intercourse with an individual with TB of the penis or epididymis, hematogenous spread from the lungs or kidneys is more common. We suspect this was the case in our patient. Genitourinary tuberculosis can present as pelvic or abdominal pain or mass, and menstrual disorders. or with infertility. The diagnosis should be suspected in patients with the clinical manifestations and epidemiology and is made via endometrial or fallopian tube biopsy stain and culture. Treatment may also include surgical reconstruction in cases of infertility. Conclusion: Genitourinary tuberculosis is a rare manifestation of tuberculosis and should be considered in patients from an endemic area with abdominal or pelvic masses, menstrual disorders, especially with signs of disseminated disease.

See next page for Figure 1
A CASE OF DISSEMINATED TB WITH GENITOURINARY INVOLVEMENT

Katyayini Aribindi¹, Namita Sood¹

Figure 1: A) CXR with diffuse bilateral nodules. B) CT chest demonstrating multiple nodules with some cavitary lesions. C) Fluid filled uterus with pelvic mass on CT abdomen and pelvis. D) Endometrial mass visualized on pelvic ultrasound.
A CASE OF COVID-19 RELATED DEMYELINATING POLYNEUROPATHY

Jacob Blount, MD,1 and Florence Chau-Etchepare, MD1

1University of California Davis, Pulmonary Critical Care and Sleep Medicine

Introduction: We present a unique case of COVID-19 related auto-immune demyelinating polyneuropathy.

Case presentation: A healthy 30 year old male was diagnosed with COVID-19 in an outside hospital emergency department after developing typical symptoms of fevers, chills and malaise. Four days later he returned to the same ED with urinary retention. His initial COVID symptoms had improved. A Foley catheter was placed and he was discharged to follow-up with Urology. Two days later, he presented to our ED with progressive lower extremity weakness, upper extremity numbness, and hypercarbic respiratory failure requiring emergent intubation. On exam, he had upper and lower extremity bilateral paralysis as well as diaphragmatic paralysis with an initial negative inspiratory force of -2.7cm H2O. LP was performed revealing cell count with 54 WBC, 16 RBCs with a differential of 70% neutrophils, 22% lymphocytes, 7% macrophages, glucose 84, protein 99. An MRI of his brain and spinal cord revealed abnormal enhancement with a demyelinating process from the cervical cord tapering to the T2 level. Serum and CSF studies ultimately revealed a myelin oligodendrocyte glycoprotein (MOG) targeted antibody. He was diagnosed with acute disseminated encephalomyelitis. He received 7 days of high dose methylprednisolone and underwent plasmapheresis. He underwent percutaneous tracheostomy placement and his strength and mobility was starting to recover at the time he left the ICU for long term ventilator weaning and rehabilitation.

Discussion: This demonstrates several unique aspects in regards to the presentation, incidence and clinical trajectory. First he had findings consistent with an immune directed demyelinating disease leading to an ascending paralysis with an initial symptom of urinary retention prior to development of any peripheral sensory deficits or weakness. This is atypical for an ascending demyelinating condition. Second, he had antibody confirmed disease with a MOG antibody which in the limited number of case reports of COVID-19 associated encephalomyelitis accounts for less than 10% of cases. Finally, our patient survived to hospital discharge after treatment with high dose steroids followed by plasmapheresis. Additionally, he was showing signs of improvement in his strength and respiratory mechanics at time of ICU discharge and ultimately weaned from the ventilator and was on trach mist at the time of hospital discharge. The long term for steroid sparing therapy was initiation of rituximab as an outpatient. Most patients reported in the literature demonstrate limited improvement or have died on short-term follow-up.
VALIDATION OF CLINICAL CRITERIA FOR PROGRESSIVE FIBROSING INTERSTITIAL LUNG DISEASE

Janelle Vu Pugashetti¹, Ayodeji O. Adegunsoye², Zhe Wu³, Cathryn T. Lee², Anand Srikrisnan⁴, Sahand Ghodrati¹, Christine Kim Garcia⁵, Felix Chua³, Chad Newton⁴, Phil L. Molyneaux³, Justin M. Oldham¹

¹Pulmonary, Critical Care and Sleep Medicine, University of California at Davis, Sacramento, CA, United States, ²Section of Pulmonary and Critical Care Medicine, University of Chicago, Chicago, IL, United States, ³Imperial College London, London, United Kingdom, ⁴UT Southwestern Medical Center, Dallas, TX, United States, ⁵Medicine, Columbia University, New York, NY, United States.

Introduction: Criteria for progressive fibrosing interstitial lung disease (PF-ILD) or progressive pulmonary fibrosis (PPF) have been proposed, but their prognostic value beyond categorical decline in forced vital capacity (FVC) remains unclear. To determine natural history for PF-ILD criteria in the absence of ≥10% FVC decline among patients with non-idiopathic pulmonary fibrosis (IPF) ILD.

Methods: A retrospective, multi-center cohort analysis was performed. Patients diagnosed with fibrotic connective tissue disease associated ILD, chronic hypersensitivity pneumonitis and non-IPF idiopathic interstitial pneumonia from three US centers and one UK center comprised test and validation cohorts, respectively. Cox regression was used to assess transplant-free survival for ≥10% categorical decline in FVC and twelve additional proposed and potential PF-ILD criteria satisfied in the absence of ≥10% FVC decline.

Main Results: One thousand three hundred and forty-one patients met inclusion criteria. The proportion of patients satisfying each PF-ILD criterion was variable, with substantially smaller proportions satisfied in the absence of ≥10% relative FVC decline. Categorical declines in FVC of ≥10% (relative and absolute) and six additional criteria were associated with differential survival in test and validation cohorts.

Conclusions: FVC decline of ≥10% and several criteria satisfied in the absence of 10% FVC decline identify non-IPF ILD patients at increased risk of death or lung transplant. It should be noted that only a subset of criteria predict progression, and our results highlight several areas that deserve consideration before broadly implementing the proposed PPF criteria.
A NOVEL, HAND-HELD, EXHALED BREATH CONDENSATE SAMPLER FOR THE CLINICAL RESEARCH MARKET; APPLICATIONS FOR ASTHMA, PULMONARY INJURY, AND INFLAMMATION

Alexander J. Schmidt1, Borras Eva1, Thomas H. Turpen2, Nicholas J. Kenyon3,4,5, Cristina E. Davis1,*

1Department of Mechanical and Aerospace Engineering, University of California Davis, One Shields Avenue, Davis, CA 95616, USA; 2SensIT Ventures, Inc. 720 Olive Dr, STE B, Davis, CA 95616, USA; 3Department of Internal Medicine, 4150 V Street, Suite 3400, University of California, Davis, Sacramento, CA 95817, USA; 4Center for Comparative Respiratory Biology and Medicine, University of California, Davis, CA 95616, USA; 5VA Northern California Health Care System, 10535 Hospital Way, Mather, CA 95655, USA.

*cedavis@ucdavis.edu

Exhaled breath is one of the most non-invasive human effluents that can be captured and analyzed, and yet it has minimal presence as a common clinical endpoint measurement in any branch of modern medicine. There has been a persistent technology gap for both breath sampling and breath analysis, despite the many vulnerable patient populations and age groups that could be aided by its use. Exhaled gasses include the major respiratory gasses from the alveolar interface with the blood and hundreds of small volatile organic compounds (VOCs). Additionally, exhaled breath condensate (EBC) includes respiratory aerosols, extracellular vesicles, viral particles, and non-volatile compounds that originate from the liquid lining deep inside the lungs. Our group and others have shown EBC contains thousands of human metabolites and has tremendous diagnostic and therapy-monitoring potential. A portable breath sampler could also monitor drug regimen therapy compliance, which would substantially aid clinical trials where participants sometimes fail to appropriately take their treatments. This can contribute to an increase in statistical “noise” and overall cost and duration of a clinical trial. We have selected asthma for further demonstrations of clinical research utility because of the broad need to better understand the plurality of phenotypes of individual patients arising from the complex interplay of environmental triggers, genotypes, epigenetic/somatic expression, and pharmaceutical response. This project will further the commercialization of a palm sized hand-held EBC sampler that can be used by patients in their own homes to sample their breath for analysis.

Our technology rests on a patented micro-condenser microfabricated “chip” (μCON) that efficiently samples the breath. The device has a disposable mouthpiece and can be cleaned and reused by the patient. Upon exhaling into the device for a short period of time, we obtain enough EBC for mass spectrometry analysis for clinically relevant information. We have previously demonstrated detection of over ~3,000+ untargeted metabolites, 30 inflammatory biomarkers as well as drugs and drug metabolites in EBC. Our first objective for this proposed work is to conduct proof-of-concept EBC testing to detect compliance in taking intervention drugs for asthma. We will select drugs categorized by different uses and mechanisms of action (e.g., β2-Agonist, inhaled corticosteroid, anticholinergic/antimuscarinic therapy, antileukotriene therapy). Healthy human EBC spiked with drug standards will be tested for method development and to demonstrate detectability in a native background. Secondly, we aim to improve the durability and extended use of our breath sampler by exploring alternative manufacturing methods. For this, we will develop innovative design strategies to consider machine choices to scale up for rapid manufacturing, interchangeable parts for a streamlined manufacturing pipeline, and innovations for device assembly and pre-commercial quality assurance / quality control (QA/QC) testing.
Abstract # 5

TLR3 IS REQUIRED FOR FULL STRENGTH EXTRAFOILLCULAR B CELL RESPONSES IN EARLY INFLUENZA A INFECTION

Emma Keller¹, Jonathan Lam¹, Nicole Baumgarth¹

¹University of California, Davis

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Introduction: In response to respiratory tract infections, such as Influenza A virus (IAV) and SARS-COV2, virus-specific antibodies are produced in two distinct B cell responses: early extrafollicular (EF) plasmablast (PB) responses, and later-forming germinal center (GC) responses. While the GC response is often considered superior for immune protection, the EF response is the only humoral response rapid enough to control viral dissemination in acute IAV infections. Furthermore, EF serum antibodies are sufficient to protect naïve mice from lethal IAV infection. New strains of respiratory viruses like IAV and SARS-COV2 can quickly impact societal health, however the EF response critical to controlling viral dissemination is poorly understood.

Methods/Results: Our lab previously established that the EF response takes place in the mediastinal lymph node (MedLN) of mice as early as day 4 post-IAV infection, peaking at day 7, and subsiding by day 14. EF B cells are characterized as CD19low B220low and CD24hi. We recently showed that toll-like receptor (TLR) adaptor proteins, MyD88 and TRIF, are required to mount an adequate EF response, as mice lacking both adaptors have significantly decreased EF responses to IAV. Most TLRs using the adaptor MyD88, while TLR3 and TLR4 utilize TRIF, TLR3 exclusively so. We have previously observed that TLR4 is not required for EF responses to influenza and that B cells are unresponsive to TLR3 agonist, poly I:C, raising the question of what role, if any, TLR3 plays in EF response regulation. Infecting mice double-deficient in MyD88 and TLR3 (DKO) with IAV, we found reduced EF responses compared to wildtype mice. The DKO mice had significantly reduced populations of EF B cells, as assessed by flow cytometry, as well as trends towards decreased populations of EF-derived plasma blasts and IAV-specific plasma blasts and plasma cells, and cells expressing the transcriptional master regulator of plasma cell differentiation, IRF4. In contrast, the pre-GC compartment appeared intact, showing that signaling through TLR3 supports generation of EF responses.

Conclusion: TLR3 signaling via TRIF is required for a full-strength EF response during IAV infection. The role of TLR3 is specific to the EF response, as the pre-GC response in MyD88 TLR3 DKO mice is not significantly altered. Future directions are to study whether TLR3 supports EF response development via B cell intrinsic or extrinsic signaling.
Abstract # 6

EFFECTS OF LOW LEVEL HYDROGEN SULFIDE EXPOSURE ON THE PATHOGENICITY OF INFLUENZA A VIRUS IN A SWINE MODEL

Cristina M. Santana2, Phillip Gauger2, Amber Vegter2, Drew Magstadt2, Dong-Suk Kim1, Wilson K Rumbeiha1

1Molecular Biosciences, University of California, Davis, CA; 2Dept. of Veterinary Diagnostic & Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA
dskkim@ucdavis.edu, wkrumbeiha@ucdavis.edu

Introduction: Hydrogen sulfide (H2S) is a toxic gas that affects the respiratory, cardiovascular and central nervous systems. In intensive swine confinement operations, H2S is a hazard for both humans and swine. It has been shown that H2S is an upper and lower respiratory tract irritant. Influenza A virus (IAV) is a zoonotic disease of public health significance. However, the effects of repeated low level exposures of H2S on the pathogenicity of IAV or toxicity of H2S have not been investigated. We hypothesized that repeated exposure to low concentrations of H2S increases the pathogenicity of IAV.

Methods/Results: Thirty pigs were exposed daily to H2S ranging from 0 to 50 ppm for 6 hours daily for 12 days. Five controls were exposed to breathing air (BA). Pigs were exposed to H2S for 7 days before challenge with approximately 3x10^5 TCID50/ml H3N2 IAV (C) or placebo (NC) on day 0. Pigs were weighed upon arrival, and on days 0 and 5 post-inoculation (dpi). Body temperature and clinical observations were collected daily including coughing, respiratory distress, lethargy and eye irritation. All pigs were euthanized after exposure on dpi 5. The lungs were removed, weighed, and scored for percent lesion severity. Sections of lungs were collected for histopathology and biochemical endpoints. 50 ppm H2S reduced growth rate compared to other groups. Group3 pigs experienced the most significantly elevated body temperature. Pigs exposed to both H2S and influenza exhibited significantly more severe clinical signs compared to inoculated groups without H2S exposure. Grossly, pigs in group6 had the greatest percentage of IAV-induced pneumonia and microscopic lung lesions and severity consisting of necrotizing bronchiolitis and interstitial pneumonia. The IAV titers in the nasal swabs and lungs were elevated in H2S/C compared to BA/C, indicating that H2S exposure increased nasal shedding of IAV. Cytokines were lowest in group6 suggesting a suppression of these while group2 had the highest concentration followed by group4.

Conclusions: Pigs inoculated with IAV and exposed to H2S exhibited more severe clinical signs and body temperature. 50 ppm H2S decreased growth rate of pigs. H2S exposure changed the viral shedding pattern suggesting a broader window of infectivity in H2S + IAV groups. Pigs exposed to H2S and IAV manifested more severe lung pathology than IAV alone group. H2S + IAV suppressed pro-inflammatory cytokines. Overall, these results suggest that H2S exposure worsened IAV infection in swine.
Abstract # 7

SCREENING MYCOBACTERIUM TUBERCULOSIS MUTANTS IN VIVO TO IDENTIFY PHYSIOLOGICALLY RELEVANT PROTEIN-PROTEIN INTERACTIONS

Abigail E. Ray¹,², Bennett H. Penn¹,²

¹Microbiology Graduate Group, University of California, Davis; ²Department of Internal Medicine, University of California, Davis

Introduction: Mycobacterium tuberculosis (Mtbo) is the causative agent of tuberculosis (TB) and the leading cause of death by an infectious disease worldwide. Infection begins when the aerosolized bacilli are phagocytosed by the resident alveolar macrophages. Unlike other microbes, Mtbo can survive and proliferate in this harsh environment. Mtbo permeabilizes its phagosome and introduces bacterial effector proteins and nucleic acids into the host cell that prevent fusion of the phagosome to the lysosome and alter multiple other macrophage responses. However, how these secreted bacterial effectors actually disrupt host immunity remains poorly understood. The central hypothesis of this project posits that Mtbo secretes protein virulence factors into the macrophage that interact with host proteins to disrupt immunity.

Methods/Results: To identify functionally relevant interactions, we used a genetic approach to disrupt components of the interactome. We began by evaluating whether loss of any of the individual secreted proteins resulted in a loss of virulence. For each mutant, we created a pair of isogenic strains from a single bacterial mutant expressing either a wild-type copy of the disrupted gene under the control of its endogenous promoter on a single-copy, integrated plasmid ('complemented' strain) or an empty control plasmid ('mutant' strain), and inserted a unique DNA sequence tag. We then performed an in vivo competition assay, in which mice were inoculated with a pool of complement and knockout strains for four effectors. During infection, spleen and/or lung tissue was harvested to recover bacteria, and plated in serial dilution to a) enumerate bacteria through CFUs, and b) prepare genomic DNA used to amplify the barcodes and quantify the proportion of strains by qPCR.

To date, we have screened through half of the interactome and found one hit, LpqN and its host factor, CBL, (Penn, et al 2018). The factors subsequently screened have not had a phenotype. For the effectors not yet screened, the mutant strains need to be generated. Thus, the focus has been on optimizing the technically challenging Mycobacterial genetic editing options. After testing several approaches, we have successfully generated mutants in M. smegmatis, M. marinum, and M. tuberculosis. This will advance the screening of the remaining effectors.

Conclusions: We have established an efficient means of screening mutants in vivo and have screened half of the bacterial proteins from the interactome. We have now optimized the most efficient method of generating Mtbo mutants which will expedite the screening of the remaining interactome.
EFFECT OF NEONATAL ANTIBIOTIC TREATMENT ON THE RESPIRATORY MICROBIOME: ALTERED DEVELOPMENT AND SEXUAL DIMORPHISM

Noah A. Siegel1,2, Taylor Westmont1,2, Matt Ralston1, Alexa Rindy2, Hitesh Deshmukh2, Lisa A. Miller1,2

1California National Primate Research Center, Davis, 2University of California, Davis, 3Cincinnati Children’s Hospital and Medical Center, Cincinnati

Abstract

Introduction: Early life administration of antibiotics can result in dysbiosis of the gut microbiome, but it is unknown whether the respiratory tract microbiome is also affected by treatment. We hypothesized that a single course of antibiotic treatment in neonates can persistently alter the microbiota of the upper respiratory tract. To test this hypothesis, we used the rhesus macaque monkey as a pediatric animal model to progressively measure development of the nasopharyngeal microbiome during the first six months of life.

Methods/Results: Monkeys were co-housed with their dams indoors and continuously breastfed until weaning. Infant monkeys received a daily antibiotic cocktail consisting of ampicillin, gentamicin, and vancomycin (targeting both gram-positive and negative bacteria) for 7 days during the first week of life (n=10; males=5, females=5); control animals received saline (n=8; males=3, females=5). Microbial DNA was extracted from nasopharyngeal swabs collected at birth and monthly. 16S rRNA sequencing was conducted on V3-V4 amplicons. Reads were analyzed using Qiime2 to assess for longitudinal effects and differences in microbiome diversity. The infant rhesus monkey nasopharyngeal microbiome consisted primarily of the phyla Actinobacteriota, Firmicutes, Proteobacteria, and Fusobacteriota. Overall, Actinobacteriota was the most abundant phyla in the nasopharyngeal microbiome during infancy. The relative abundance of taxa in the nasopharyngeal microbiome fluctuated throughout the first 6 months of life for all animals; a stable phenotype was not reached for the study duration. Following antibiotic treatment, animals had a significant increase in Fusobacteriota abundance in the nasopharyngeal microbiome relative to controls. The nasopharyngeal microbiome of antibiotic-treated male animals contained a significant reduction in Firmicutes at six months of age, while age-matched females showed no difference for this taxa. Alpha diversity metrics (Shannon and Pielou’s evenness) were also decreased in antibiotic-treated monkeys at six months of age relative to controls. Linear discriminant analysis Effect Size (LEfSe) demonstrated the phylum Firmicutes (LDA=4.83, p<0.01) and class Clostridia (LDA=4.76, p<0.01) were responsible for a significant proportion of nasopharyngeal microbiome differences observed in antibiotic-treated and control animals.

Conclusions: To the best of our knowledge, this study is the first to progressively characterize the development of the nasopharyngeal microbiome during the first six months of life in a primate species. We observed shifts in Firmicutes abundance, with sex-dependent differences detected in antibiotic-treated and control infant monkeys. Despite the lack of stable phenotype, our findings suggest early life antibiotic treatment can influence the development of the upper respiratory tract microbiome in a sexually dimorphic manner.
THE KEY ROLE OF LIPOGENESIS IN HYPER-PROLIFERATION AND SURVIVAL OF HUMAN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS IN PULMONARY HYPERTENSION

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Abstract

Rationale: Pulmonary arterial vascular smooth muscle cells (PAVSMC) proliferation is a key pathological component of pulmonary arterial hypertension (PAH). Lipogenesis is linked with proliferative diseases, including cancer, but its role in PAVSMC proliferation in PAH is understudied and underlying mechanisms remain to be elucidated.

Methods and results: Immunoblot and cell growth analyses demonstrated that key fatty acids synthesis enzymes ATP-citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) were significantly upregulated in early-passage human PAH PAVSMC compared to controls, which was associated with increased unstimulated cell growth. Using immunocytochemical analysis with fluorescent probe BODIPY, cell growth (cell counts) and proliferation (Ki67) assays, we found that accumulation of intracellular lipids and increased proliferation of PAH PAVSMC were preserved in lipid-free conditions but suppressed by non-metabolizable analog of glucose 2-Deoxy-D-glucose and partially restored by addition of pyruvate. In agreement with published data, key glycolytic enzymes phosphofructokinase and hexokinase II were upregulated in human PAH PAVSMC compared to controls. Importantly, 5-tetradecyloxy-2-furoic acid, an allosteric ACC inhibitor, significantly decreased proliferation and induced apoptosis in PAH PAVSMC, demonstrating that lipogenesis is required for PAH PAVSMC hyper-proliferation and survival. Akt was upregulated in human PAH PAVSMC, and its inhibition with Akt inhibitor VIII suppressed phosphorylation of ACLY and ribosomal protein S6, increased inhibitory phosphorylation of ACC, reduced proliferation and promoted apoptosis in PAH PAVSMC, suggesting that Akt supports increased cell proliferation and survival in the PAH via promoting lipogenesis. Next, we tested whether NAD+-dependent deacetylase SIRT7 and c-Jun N-terminal kinase (JNK), known upstream regulators of Akt in other cell types, modulate Akt and lipogenesis in PAH PAVSMC. We found that SIRT7 was overexpressed in PAH PAVSMC compared to controls, and its shRNA-mediated depletion reduced Ser473, but not Thr450 Akt phosphorylation, while pharmacological inhibition of JNK significantly reduced both Ser473 and Thr450 Akt phosphorylation in PAH PAVSMC. shRNA SIRT7 and JNK inhibitor bentamapimod downregulated lipogenic enzymes ACLY and ACC in human PAH PAVSMC.

Conclusion: Human PAH PAVSMC have up-regulated lipogenesis supported in Akt- and glycolysis-dependent manner to sustain increased cell growth. Inhibition of Akt-lipogenesis axis reduces proliferation and induces apoptosis of human PAH PAVSMC. In aggregate, our data provide a link between glycolysis, lipogenesis and proliferation of human PAH PAVSMC and call for further studies to determine potential attractiveness of SIRT7/JNK-Akt-lipogenesis axis as a target pathway for therapeutic intervention.
PITAVASTATIN POTENTIATES β2-AGONIST-INDUCED BRONCHODILATION
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INTRODUCTION: We previously established that the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) inhibitor, pitavastatin, inhibits contractile force generation in cultured human airway smooth muscle (ASM) cells. Here, we hypothesize that this pitavastatin effect is (1) sufficient to inhibit bronchoconstriction in mouse and human precision-cut lung slices (PCLS), and (2) potentiates the ASM relaxing and bronchodilatory effects of β2-agonists.

METHODS/RESULTS: Methods: (1) Mouse (C57BL/6J, 8-10 weeks old) and human (IIAM) lungs were sliced, cultured, and imaged using established methods. Airways were pre-constricted with 0.2 μM methacholine (MCh) (in mouse lung slices) or 10 μM histamine (in human lung slices) for 20 min and post-treated with pitavastatin (3, 10, 25, 50 μM) alone or together with the β2-agonist formoterol (10 nM). (2) Primary human ASM cells were examined for contractile force changes using Traction Force Microscopy (TFM). Force values after pitavastatin and/or the β2-agonist isoproterenol were normalized to histamine (@30 min) and represented as a “%”. (3) Primary human ASM cells were examined for potential pitavastatin-induced cytotoxicity using ATPLiteTM (PerkinElmer). Results: Pitavastatin (3, 10, 25, 50 μM, for 20 min) acutely i.e. within 5-10 min and dose-dependently dilates MCh-constricted mouse airways by ~35.6% (p<0.05), with the greatest effect observed at the highest statin dose. This acute bronchodilatory pitavastatin effect (50 μM, 5 min; p<0.0001) on MCh pre-constricted mouse airways is independent of the mevalonate (MA) pathway, i.e. independent of HMGCR inhibition. Pitavastatin (10 μM, 35 min) also enhances formoterol-induced bronchodilation (p<0.0001) in pre-constricted mouse airways (p<0.05). Similar findings were observed using human tissues where pitavastatin (3 μM, 4.5 hrs) dilates histamine-constricted human airways by 2-fold (p<0.05) and further enhances the bronchodilator effects of formoterol (p<0.05). In human ASM cells, pitavastatin treatment (10 and 50 μM, 5 min) acutely relaxes histamine pre-constricted ASM by 25% at 5 min and by 50% at 10 min (p<0.05), without any evidence of cytotoxicity at the higher dose. Pitavastatin (10 and 50 μM, 30 min) treatment of histamine pre-constricted human ASM cells also markedly enhances both the magnitude and duration of β2-agonist-induced ASM relaxation (p<0.05).

CONCLUSIONS: Pitavastatin acutely, non-toxically, and dose-dependently reverses agonist-induced bronchoconstriction. This pitavastatin–induced acute bronchodilatory response occurring over minutes was unaffected by MA co-treatment, indicating a MA-independent mechanism. Pitavastatin enhances the magnitude and duration of β2-agonist-induced ASM relaxation. These data support the development of inhaled statins alone or in combination with existing inhaled β2-agonist therapy for the treatment of asthma.
Abstract # 11

BPIFB1 REQUIREMENT FOR ENDOTOXIN RESPONSIVENESS IN PRIMATE AIRWAY EPITHELium

Christopher M. Royer1, Lisa A. Miller1, 2

1California National Primate Research Center, 2UC Davis School of Veterinary Medicine

Introduction: Airway epithelia secrete numerous proteins into the airway protecting the lung from the environment. The most abundant constitutively expressed proteins are bactericidal/permeability-increasing fold-containing (BPIF) proteins, BPIFA1 and BPIFB1. BPIF proteins have antimicrobial and anti-inflammatory activities, as well as surfactant activity and maintain airway surface liquid layer hydration. Macaques recapitulate the anatomy and diversity of epithelia in human airways and were used to examine the role of BPIF proteins in airway mucosal protection.

Methods/Results: Macaque primary airway epithelia were cultured at air-liquid interface. The apical surface was washed at different time points prior to exposure to LPS (at 1 or 10 mcg/ml from P. aeruginosa), giving variable accumulation of secreted factors interacting with LPS. This approach was repeated following CRISPR-based knockout BPIFB1. Apical washes were assessed by western blot specific for BPIFA1 and BPIFB1. Cells were harvested 6hrs post-exposure and assessed by qPCR for BPIFA1 and BPIFB1 and for inflammatory response by interleukin (IL) 6 and IL8 production. BPIFB1 functions were assessed by LPS capture assay for BPIFB1 from the apical washings and assessment of deficient cells for changes in transepithelial resistance and airway surface liquid volume by light refraction microscopy.

Macaque airway epithelia only produced measurable BPIFB1, which was the focus of these experiments. IL6 was suppressed by 7 days of secreted factor accumulation, relative to 1 day, following 10mcg/ml LPS exposure. IL8 was suppressed with 4 and 7 days of accumulation at both 1 and 10mcg/ml of LPS exposure. BPIFB1-deficient cultures displayed reduced IL6 and IL8 with 10mcg/ml LPS exposure but only IL8 was significantly reduced with 1mcg/ml LPS exposure after 1 day of secreted factor accumulation. BPIFB1 from normal apical washings directly bound LPS. BPIFB1-deficient cultures did not differ from controls in transepithelial resistance or airway surface liquid volumes.

Conclusions: Airway mucosal epithelial secreted factors were anti-inflammatory with respect to LPS exposure. BPIFB1 makes up a major proportion of the secreted factors from the airway mucosa and displayed LPS binding activity. However, BPIFB1-deficient cells displayed decreased inflammation upon LPS exposure. The deficient cells did not show differences in transepithelial resistance or airway surface liquid volume. Our data suggest that while BPIFB1 may serve as a binding reservoir for LPS it is necessary to elicit the inflammatory response to LPS. Experiments are underway to determine if BPIFB1 facilitates extracellular recognition of LPS or if it directly modulates intracellular inflammatory cascades and expression of proteins, such as mucins.
VPS34 ACTIVATION PROMOTES PROLIFERATION AND SURVIVAL OF SMOOTH MUSCLE CELLS IN PULMONARY ARTERIAL HYPERTENSION

Yuanjun Shen1*, Dmitry Goncharov1, Lifeng Jiang1, Derek Lin1, Theodore Avolio2, Evelyn Okorie2, Ana L Mora3, Aisha Saiyed1

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*Corresponding Author

Introduction: Pulmonary arterial hypertension (PAH) is a progressive and deadly disease with no cure. PAH manifests by small PA remodeling, leading to increased PA pressure, elevated right ventricular (RV) afterload, heart failure and death. Pulmonary vascular remodeling is characterized by cancer-like hyper-proliferation and increased survival of resident PA vascular cells, the mechanisms of which are not entirely understood. Class III phosphatidylinositol 3-kinase (PI3K) vacuolar protein sorting 34 (Vps34), a member of PI3K/Akt/mTOR network, promotes cell hyper-proliferation in cancer. The status and mechanisms of regulation and function of Vps34 in PA vascular cells in PAH remain to be elucidated.

Methods/Results: Immunohistochemical analysis showed that inhibitory phosphorylation of Vps34 at Ser164 (P-Ser164-Vps34) was significantly decreased in smooth muscle alpha-actin (SMA)-positive areas of small remodeled PAs from subjects with PAH (OD: 0.622±0.026, n=3) compared with PAs from non-diseased donors (OD: 1.307±0.207, n=3; p<0.05 vs. PAH). Immunoblot analysis of early-passage human PA smooth muscle cells (PAVSMC) from small (<1.5 mm outer diameter) PAs demonstrated that P-Ser164-Vps34 is significantly decreased in human PAH PAVSMC compared to controls (1.000±0.331 vs. 0.198±0.039, control vs. PAH, n=5 subjects/group, p<0.05), suggesting that Vps34 is activated in PAVSMC from human PAH lungs. Similar to human PAH, we detected a significant decrease of P-Ser164-Vps34 in SMA-positive area of small remodeled PAs from mice and rats with SU5416/hypoxia-induced PH, which was associated with increased PA medial thickness, RV pressure and RV hypertrophy, suggesting that similar mechanisms with human PAH are shared. Treatments with soluble pro-PH factors did not reduce Vps34 phosphorylation in non-diseased PAVSMC, indicating other mechanisms may be involved. Treatment of Akt Inhibitor VIII and knockdown of Akt by specific siRNA transfection in human PAH PAVSMC significantly increased P-Ser164-Vps34, showing that Vps34 activation in PAH PAVSMC is Akt dependent. Further, Vps34 activation in human PAH PAVSMC was associated with deficiency of TSC2, over-accumulation of Vps15, and increased growth and proliferation. Pharmacological inhibition of Vps34 in PAH PAVSMC by selective inhibitors SAR405 and VPS34-IN1 significantly decreased proliferation (Ki67) and induced apoptosis (TUNEL). Additionally, two-week treatment of SAR405 (10mg/kg/day, 5 days/week) significantly attenuated SU5416/hypoxia-induced pulmonary vascular remodeling in male mice. The above data suggest a therapeutic potential of Vps34 inhibition to reduce PAVSMC hyper-proliferation in PAH.

Conclusions: Vps34 is activated and supports proliferation and apoptosis resistance of PAVSMC from PAH lungs. Further studies are needed to evaluate benefits of Vps34 inhibition as a potentially attractive anti-remodeling therapeutic option in PAH.

Funded by AHA Postdoctoral Fellowship #826806.

See next page for Figure 1
VPS34 ACTIVATION PROMOTES PROLIFERATION AND SURVIVAL OF SMOOTH MUSCLE CELLS IN PULMONARY ARTERIAL HYPERTENSION

Yuanjun Shen¹*, Dmitry Goncharov¹, Lifeng Jiang¹, Derek Lin¹, Theodore Avolio², Evelyn Okorie², Ana L Mora³, Aisha Saiyed¹
Abstract # 13 page 1

HUMAN AIRWAY EPITHELIAL CELL TEMPORAL KINASE SIGNALING DYNAMICS: CONNECTING CELLULAR METABOLISM, INFLAMMATION, AND KINASE BIOLOGY

Nicholaus DeCuzzi1,2, Devan Murphy2, Abhineet Ram2, Justa Ferguson2, Daniel Oberbauer2, Kenneth Chmiel1, Michael Pargett2, Amir A. Zeki1, and John Albeck2

1University of California, Davis School of Medicine; UC Davis Lung Center; Department of Internal Medicine; Division of Pulmonary, Critical Care, and Sleep Medicine; University of California, Davis, CA; 2Department of Molecular and Cellular Biology, University of California, Davis

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Introduction: Extracellular signal-regulated kinase (ERK) and AMP-activated protein kinase (AMPK) are essential kinases regulating airway epithelial cell growth, proliferation, and metabolism; however, these kinases are dysregulated in airway inflammation. Signal Transducer and Activator of Transcription 3 (STAT3) is an important downstream transcription factor triggered by select pro-inflammatory cytokines that involve kinase signaling. However, the link between airway epithelial cells’ individual and temporally dynamic signaling profiles, and their ultimate physiological effects has not been well-described. We hypothesize that cell microenvironment metabolic and inflammatory stimuli produce unique spatially localized single-cell signaling responses that impact STAT3 activation in human airway epithelial cells.

Methods/Results: We used fluorescent biosensors and live-cell imaging to track single-cell kinase signaling activity continuously (up to 48 hrs) in our Human Bronchial Epithelial cell lines (HBE1 and 16HBE14) and primary human bronchial epithelial cells (pHBE), both in submerged and Air-Liquid Interface (ALI) culture conditions. Computational image analysis extracts kinase signaling activity profiles in response to growth factors (EGF), inflammatory cytokines relevant to both asthma and COPD (IL6, IL1β, TNFα), and metabolic modulators (Insulin, 2-DG, Oligomycin). After 24 hours of ligand exposure, cells were fixed and stained for nuclear pSTAT3 immunofluorescence to measure active cellular inflammatory response(s). All airway epithelial cells displayed heterogeneous and dynamic single-cell ERK responses to inflammatory ligands, with frequency, duration, and onset of activity dependent on both ligand and concentration (figure 1a). In response to inflammatory cytokines, HBE1 displayed increased frequency of discrete ERK activity pulses and localized pSTAT3 clusters. These spatially localized behaviors of ERK and STAT3 increased with insulin but were attenuated in the presence of metabolic perturbation and AMPK activation (figure 1b&c). In contrast, duration rather than amplitude of ERK activity increased in pHBE and 16HBE14 in submerged culture in response to inflammatory cytokines. In ALI culture, pHBE displayed increased duration of ERK activity, but the initial response was delayed by ~2 hours as compared to submerged cells.

Conclusion: These results support our central hypothesis and highlight a previously underappreciated mechanism by which local cellular microenvironment influences the airway epithelial signaling response to inflammatory cytokines. We propose a model in which local metabolic conditions and paracrine cytokine signaling determine cell-specific ERK and STAT3 activation programs. Identifying these programs with greater spatial and temporal specificity will enhance our ability to manipulate inflammatory and metabolic factors. This will in turn enhance our understanding of chronic airway disease pathogenesis.

See next page for Figure 1
HUMAN AIRWAY EPITHELIAL CELL TEMPORAL KINASE SIGNALING DYNAMICS: CONNECTING CELLULAR METABOLISM, INFLAMMATION, AND KINASE BIOLOGY
Nicholaus DeCuzzi$^{1,2}$, Devan Murphy$^2$, Abhineet Ram$^2$, Justa Ferguson$^2$, Daniel Oberbauer$^2$, Kenneth Chmiel$^1$, Michael Pargett$^2$, Amir A. Zeki$^1$, and John Albeck$^2$
Abstract # 14

CYTOKINE-MEDIATED DYSREGULATION OF SINGLE CELL ERK SIGNALING DYNAMICS AND WOUND RESPONSE IN AIRWAY EPITHELIAL CELLS

Justa Ferguson¹, Nicholaus DeCuzzi¹,², Abhineet Ram¹, Kenneth Chmiel², Michael Pargett¹, Amir A. Zeki², and John Albeck¹

¹Department of Molecular and Cellular Biology, University of California, Davis; ²University of California, Davis School of Medicine; UC Davis Lung Center; Department of Internal Medicine; Division of Pulmonary, Critical Care, and Sleep Medicine; University of California, Davis, CA.

Introduction: Chronic obstructive pulmonary disease (COPD) and Asthma are associated with impaired resolution of damage to the airway epithelium from exposure to air irritants such as smoking and microorganism's proteases. Proper wound healing of the epithelial layer depends on inflammatory and growth signaling ligands surrounding the wound and the coordinated response of the epithelial cells adjacent to wound edge. In other model systems coordinated cell migration into the wound is, in part, regulated by Extracellular signal-regulated kinase (ERK). Notably, ERK activity is dysregulated in airway diseases, and we have established airway epithelial cells display heterogeneous and dynamic ERK signaling activity unique to exposure to inflammatory cytokines frequently elevated in COPD and Asthma. However, the role of ERK signaling dynamics in coordinating the airway epithelial wound healing and its dysregulation by the presence of inflammatory cytokines, has not been established. We hypothesize the presence of inflammatory cytokines limit the coordinated ERK signaling response to wounding and impair airway epithelial wound resolution.

Methods/Results: Using our Human Bronchial Epithelial cell line (HBE1) stably expressing fluorescent biosensors we track live-cell ERK signaling activity with single-cell resolution, continuously, for up to 24hrs. Computational image analysis extracts kinase signaling activity profiles and cell migration into the wound area in the presence of growth factors (EGF), inflammatory cytokines relevant to both asthma and COPD (IL6, IL1β, and TNFα, and/or IFNy), and kinase or receptor inhibitors (gefitinib, tocilizumab, and PD-0325901). Preliminary results demonstrate that, under inflammatory conditions HBE1 cell migration into the wound area was delayed by ~2 hours compared to control conditions (EGF) and had diminished rates of wound closure, averaging 5.6um/hr and 13.9um/hr, respectively for 12 hours following wounding. Pretreatment with gefitinib and tocilizumab (together) or MEK inhibitor (PD) further limit wound closure rate to 4.2um/hr and 2.6um/hr. Additionally, TNFα and IFNy pretreated cells display a decreased (25.4%) single cell ERK response to wounding, but an increased duration of ERK activation of responding cells compared to control.

Conclusion: These preliminary results support our central hypothesis that inflammatory cytokines dysregulate the airway epithelial ERK responses to wounding at the single cell level and ERK’s role in airway epithelial wound resolution, warranting further investigation. Future work includes: 1) identify which subpopulations of cells ERK activity is most dysregulated by cytokines (at wound edge or adjacent). 2) assess how pharmacological agents alter activity profiles to modulate wound healing. This will reveal kinase signaling mechanisms that mediate cell fate relevant to lung health and disease.
A NOVEL FUNCTION OF INOSITOL IN REDUCING PULMONARY FIBROSIS PROGRESSION

Linhui Li¹, Ji-Min Li¹,², David C. Yang¹,², Angela Linderholm¹, Lisa Franzi¹, Ssu-Wei Hsu¹, Ching-Hsien Chen¹,²

¹Division of Pulmonary and Critical Care Medicine and Center for Comparative Respiratory Biology and Medicine, Department of Internal Medicine, University of California Davis, Davis, California, USA, ²Division of Nephrology, Department of Internal Medicine, University of California Davis, Davis, California, USA

Rationale: Idiopathic pulmonary fibrosis (IPF) is a fatal interstitial lung disease with poor prognosis and limited therapeutic options. The invasive phenotype of IPF-derived fibroblasts is well-known to result in the progressive and irreversible lung parenchyma scaring. We recently reported that IPF lung fibroblasts display a dysregulation of ASS1 expression, a major enzyme in arginine biosynthesis, and this deficiency promoted the aggressiveness of lung fibroblasts. Herein, we aimed at developing a therapeutic strategy to eradicate these ASS1-deficient lung fibroblasts for mitigating fibrotic progression.

Methods: Metabolites extracted from various primary lung fibroblasts were subject to untargeted metabolomics analysis and metabolic set enrichment analysis (MSEA) in order to identify the top-ranked metabolic pathways. The antifibrotic activity of inositol was examined on IPF and normal primary lung fibroblasts and confirmed by western blots, invasion, and migration assays. The effect of inositol-containing control diet or inositol-free diet was evaluated in a mouse model of bleomycin-induced lung fibrosis. The fibrotic status of the mouse lungs was demonstrated by collagen content assays and histology analysis.

Results: MSEA data identified that a number of metabolites that were mainly involved in amino acid-metabolic pathways between normal and IPF fibroblast cells. Among these amino acid-metabolic pathways, we noticed inositol phosphate metabolism and phosphatidylinositol signaling system were the top-ranked metabolic pathways. Surprisingly, the inositol phosphate metabolism pathway was noted in ASS1- knockdown (KD) fibroblasts compared to normal lung fibroblasts. Given a critical role of inositol-derived metabolites in cellular signaling events, the effect of inositol on IPF fibroblast cell responses were assessed. We demonstrated that treatment with inositol downregulated the expression level of alpha smooth muscle actin (α-SMA), a myofibroblast marker, and EGFR, AKT and STAT3 phosphorylation, which were associated with cell aggressiveness. Both cell-based assays showed an inhibitory effect of inositol on cell invasiveness and motility in multiple IPF fibroblasts with ASS1 deficiency.

Conclusions: Our findings not only demonstrate a novel function of inositol metabolism in pulmonary fibrosis but also provide a new anti-fibrotic metabolite, suggesting inositol supplement as a promising therapeutic approach for IPF.
Abstract # 16

A SEX-DEPENDENT TRANSCRIPTOME PROFILE IN THE NEONATAL LUNG: POTENTIAL ROLE OF THE METALLOPROTEINASE ADAMDEC1

Taylor Westmont¹, Chris M. Royer¹, Alexa Rindy¹, Noah A. Siegel¹, Hitesh Deshmukh², Lisa A. Miller¹

¹California National Primate Research Center, Davis, CA, United States, ²Cincinnati Children’s Hospital and Medical Center, Cincinnati, OH, United States.

Rationale: Prevalence of respiratory disease during postnatal development is often associated with male infants. Using the rhesus macaque monkey to model the pediatric lung, we previously showed that early life antibiotic treatment resulted in reduced lung function in male but not female infant animals. Based upon our findings, we hypothesize that normal maturation of the infant lung occurs in a sexually dimorphic manner. To test this hypothesis, we characterized the infant monkey lung transcriptome using bulk RNA-seq, comparing male and female tissues obtained from 6-month-old animals.

Methods/Results: Lung samples collected from 6-month-old male (n=3) and female (n=5) infant monkeys were evaluated for differentially expressed genes and protein. The snap frozen right cranial lobe from animals was used to assess the transcriptome via RNA-seq (n=2 per group) and protein expression via Western blot. RNA-seq differential expression analysis was conducted using limma-voom. KEGG pathway and Gene Ontology (GO) analysis were used to examine pathway enrichment. Differential gene expression was confirmed by qRT-PCR of the right cranial lobe as well as microdissected airways (proximal, midlevel, respiratory bronchioles) from the left cranial lobe. Of the 11,732 genes identified in lung samples by RNA-seq, we detected 7 differentially expressed genes, including 5 Y-linked genes (adjusted p<0.05). The metalloproteinase ADAMDEC1 was significantly increased in the male infant lung relative to females, which was confirmed via qRT-PCR. Conversely, female infant lungs showed a significant increase in ADAMDEC1 protein relative to males. Female lung was enriched for GOs related to growth and metabolism, such as cellular response to insulin stimulus (1.84-fold-enrichment (FE)) and Wnt signaling pathway (1.79 FE). KEGG pathway analysis also showed significant enrichment for Ras and VEGF signaling between male and female lungs. Because metalloproteinases are involved in extracellular matrix remodeling, we further investigated ADAMDEC1 expression in an adult rhesus monkey bleomycin model of lung fibrosis. Bleomycin-treated (n=6) animals showed significantly increased lung ADAMDEC1 mRNA and protein expression compared to untreated controls (n=2).

Conclusions: To the best of our knowledge, this is the first report of a sex-dependent transcriptome profile in the developing primate lung. We found increased ADAMDEC1 gene expression in male neonatal airways relative to females. While the function of ADAMDEC1 is unclear, it may be speculated to play a role in extracellular matrix remodeling based upon elevated lung expression in bleomycin-treated animals. Collectively, our results suggest sexually dimorphic lung function may be associated with a differential transcriptome in the male and female infant lung.
# 13TH ANNUAL LUNG DAY

## Poster Session 2

Rooms 1 - 2

12:45 p.m. - 1:45 p.m.

Abstracts Presentations 17 - 33

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Abstract # 17

MACHINE LEARNING AND SIGNAL PROCESSING ASSISTED DIFFERENTIAL MOBILITY SPECTROMETRY (DMS) DATA ANALYSIS FOR CHEMICAL IDENTIFICATION

Pranay Chakraborty1, Maneeshin Y. Rajapakse1,2, Mitchell M. McCartney1,2,3, Nicholas J. Kenyon2,3,4, Cristina E. Davis1,2,3,*

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Introduction: Differential mobility spectrometry (DMS)-based detectors are being widely studied to detect chemical warfare agents, explosives, chemicals, drugs and analyze volatile organic compounds (VOCs). The dispersion plots from DMS devices are complex to effectively analyze through visual inspection. In the current work, we adopted machine learning to differentiate pure chemicals and identify chemicals in a mixture. In particular, we observed the convolutional neural network algorithm exhibits excellent accuracy in differentiating chemicals in their pure forms while also identifying chemicals in a mixture. In addition, we propose and validate the magnitude-squared coherence (msc) between the DMS data of known chemical composition and that of an unknown sample can be sufficient to inspect the chemical composition of the unknown sample. We have shown that the msc-based chemical identification requires the least amount of experimental data as opposed to the machine learning approach.

Methods/ Results: Dispersion plot data of individual chemicals and mixtures were generated from a MicroAnalyzer DMS (Sionex Corp, Bedford, MA) with a 5 mCi, 63Ni ionization source. We introduced the chemical samples with a concentration of 500 ppb into the inlet of the DMS device. Chemical standards were obtained from MilliporeSigma (Missouri, USA). We used ultra-pure nitrogen with ~1 ppm humidity as the carrier gas (200 mL/min) for the device, and the carrier gas temperature was maintained at 80 ℃. We considered the DMS dispersion data of positive polarity; however, the approach of this study is equally valid for negative polarity. For differentiating DMS dispersion plots of chemicals, we implement the convolutional neural network (CNN) within an updated AnalyzeIMS (AIMS) software for differentiating the DMS plots of different chemicals. In the current work, we complete all the data visualization and analyses with this updated version of AIMS that operates in MATLAB r2021a.

Conclusions: In this work, we have shown that the convolutional neural network (CNN) model shows excellent accuracy in differentiating differential mobility spectrometry (DMS) data of pure chemicals. We also observed that the CNN model outperforms other previously reported models while identifying chemicals in binary and ternary mixtures. We updated the custom AnalyzeIMS (“AIMS”) software with a user-friendly implementation of the CNN model. Moreover, we have shown that the calculation of magnitude-squared coherence (msc) of the DMS data is very effective in identifying the chemical identity of a sample. Chemical library building with standard DMS plots and msc analysis can significantly reduce the burden of collecting substantial DMS data to train machine learning models for constituent chemical identification in a sample.
DATA DRIVEN QUALITY ASSURANCE QUALITY CONTROL (QA/QC) MEASURES FOR CHIP-BASED PRECONCENTRATORS SCALED FOR PRODUCTION

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Introduction: Conducting a metabolomic analysis on exhaled breath volatiles has shown a great deal of promise as a technique for point of care human medicine [1]. Prior to chemical analysis, breath preconcentration is a necessary step in improving the metabolomic limits of detection and instrument sensitivity. Furthermore, having an inventory of quantifiably similar devices enables large scale biomarker discovery experiments to be performed as part of disease fingerprinting and ultimately, translated to clinical diagnosis. Here we have developed a high-yield, high-uniformity process for the fabrication of a gas micro preconcentrator chip (Figure 1). We extensively report the statistical distributions of parameters of each process step and their individual contributions to the chip chemical performance testing. A micro preconcentrator chip consists of a sealed sorbent bed cavity, connected microchannels for gas transport, and through glass vias to interface the chip to external equipment. We fabricated a total of 141 chips, with a functional yield rate of 94%.

Methods: Characterization was performed with two different classifications of measurements: (1) process measurements, data collected passively by tooling during the manufacturing process; and (2) product measurements, data collected in between discreet process steps. Laser wet etched channel dimensions are nominally 710 μm wide and 350 μm deep verified with profilometry to have a variance of 717 ± 4.1 μm and 347 ± 6.6 μm, respectively. Next two complimentary wafers have a layer of silicon nitride deposited to assist in a glass-to-glass anodic bonding process. Over the 30-wafer pair batch the total collected charge, a measurement proportional to the bond quality [2] between the two wafers was reported to be 1902.38 ± 58.92 coulombs. Finally, the resistivity of the Tungsten heaters and RTDs, was measured to be 114.8 ± 21.6 ohms and 191.2 ± 35.0 ohms, respectively. After micro preconcentrator chips are produced, they leave the cleanroom facility to be post processed and chemically tested. Post processing consists of packing the chips with a Tenax TA sorbent material and a chemical standardization test. An example spectrum produced by the micro preconcentrator chip and gas chromatography is shown.

Conclusion: Unlike traditional MEMS devices bio and chemical detection MEMS diverge in fabrication processes. In this project we claim to propose a fabrication paradigm to reliably produce such devices. This is demonstrated in the mass production of a gas micro preconcentrator.
DATA DRIVEN QUALITY ASSURANCE QUALITY CONTROL (QA/QC) MEASURES FOR CHIP-BASED PRECONCENTRATORS SCALED FOR PRODUCTION

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REFERENCES:


Figure 1. A realized micro preconcentrator chip showing (1) microchannels (2) sorbent cavity (3) through glass vias (4) heater and RTD traces (5) electrical contact pads (6) QR code identification pad.
A LOW COST, EASY-TO-ASSEMBLE, OPEN-SOURCE MODULAR MOBILE SAMPLER DESIGN FOR THERMAL DESORPTION ANALYSIS OF BREATH AND ENVIRONMENTAL VOCs

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Introduction: For studies of exhaled volatile compounds, one common method for offline chemical analysis is performed using a thermal desorption gas chromatography-mass spectrometry (TD-GC-MS) technique. Study participants exhale directly into bags of inert plastic, such as Tedlar, in volume ranges from 0.5 to 10 l. The breath sample is loaded by vacuum pump onto a sorbent-packed tube, which preconcentrates breath volatiles to increase detection limits. Few commercially available chemical sampling vacuum pumps exist for preconcentration. Many of which are expensive and lack volumetric sampling capabilities. We propose, construct, and qualify a low-cost volumetric sampling pump for conducting exhaled breath volatile analysis. The pump operates in a volumetric sampling mode with tunable flowrate. Additionally, the pump uses a user-friendly interface and robust hardware optimized for a clinical setting.

Methods: The VOC sampler was designed in two separate mechanical parts: a volume and flow sensing computer-controlled base station, and a modular quick changing thermal desorption tube holder. The modularity of the design will allow for future work with different sampling media using the same volume sampling hardware. The base station houses a vacuum pump, flow control valve and electronic microcontroller. A sample media module physically interfaces with the thermal desorption tube to the volume sampling hardware, allowing the user to quickly process samples. To qualify the device the flow rate and volumetric sampling capabilities were benchmarked against a calibrated mass flow control sensor. In addition to single unit calibration, chemical testing with a TO-15 standard VOC mix was performed across four different devices to quantify device-to-device repeatability – a critical feature for wide spread clinical studies. Finally, the sampler was used to extract human breath which was compared to a baseline room air sample.

Conclusion: We found the sampler had high precision and accuracy for flow rate and volumetric sampling. We report at various flow rates, the average flow rate accuracy variance is 1.5% and the precision is 0.12%. For volumetric accuracy at various flow rates, the accuracy was 0.06% and precision 0.05%. For a given VOC from the TO-15 mixtures sampled on multiple devices the mass spectrometry abundance was, on average, 6.5%. This rate is higher than the volumetric accuracy due to the variance induced by the thermal desorption mass spectrometry. In a latent variable analysis of breath data compared to room air we show there is clear separation between the two groups.
Abstract # 20

EFFECTS OF DEVELOPMENTAL 3,3’-DICHLOROBIPHENYL (PCB 11) EXPOSURE ON LUNG MATURATION

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Introduction: 3,3’-Dichlorobiphenyl (PCB 11) is a contemporary industrial byproduct and ubiquitous environmental contaminant to which humans are exposed via inhalation and ingestion. PCB exposure is thought to influence risk of childhood disease. Pre and postnatal chemical exposure is known to alter lung development, and many cell types in the lung mature throughout childhood. Recent studies demonstrate PCB 11 from dietary exposures distributes to the lung, however, it is not known if exposure to PCB 11 during lung maturation alters typical development of the conducting airway epithelium.

Methods/Results: Dams were exposed daily to PCB 11 mixed in peanut butter at 0.1 mg/kg (low dose), 1.0 mg/kg (high dose) or vehicle beginning 14 days prior to mating and through postnatal day (PND) 21. Lungs were collected from offspring at PND 4, 21, and 60. Each litter was a unit of statistical measure. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to analyze either Muc5ac or Muc5b (major surface mucins) expression in whole lung lobes of all ages or in microdissected airway regions at PND 60. Analysis of sex-combined animals using a Kruskall-Wallis test with Dunn’s multiple comparisons showed a significant 2x decrease in whole-lobe Muc5ac expression at PND 21 following a low dose exposure. One-way analysis of variance (ANOVA) of female PND 60 microdissected airways revealed gene expression of Muc5b was significantly 3x higher in the high dose PCB 11 group. Alcian Blue Periodic Acid-Schiff’s stain showed mucin expression increased in large airways of adult mice after gestational and lactational PCB 11 exposure.

Conclusions: Developmental PCB 11 exposure may alter mucin expression in developing mouse lungs. Supported by R01ES014901-09S1 and T32HL007013.
Abstract # 21

DEVELOPMENTAL IMPACT OF PERINATAL EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE ON INFANT Rhesus MONKEY LUNGS

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Introduction: The impact of secondhand smoke exposure contributes to be a global health issue as 41% of children worldwide are exposed to the effects of tobacco smoke. This study was designed to understand the perinatal development effects in maturation and cellular component activity of the lungs for non-human primates. The objective was to investigate site-specific alterations of the airways in morphology, cellular content, and xenobiotic-metabolizing enzymes of the airways, vasculature, and alveoli following direct exposure to ETS.

Methods: Gravid rhesus monkeys and their offspring were exposed to aged and diluted sidestream cigarette smoke as a surrogate for ETS. Exposure began at approximately gestation day 100 and continued through 2.5 months postnatal age.

Results: Nicotine and cotinine levels were measured in the amniotic fluid during gestation and postnatally in the blood. At necropsy, bronchoalveolar lavage was performed. Histologically, airway epithelium was observed and pulmonary cytochrome P450 (CYP) 1A1, 2E1, and glutathione S-transferase (GST) isozymes were measured in site-specific lung subcompartments. Exposure to ETS resulted in eliciting a shift in pulmonary immune cells as well as increased basal cell volume in proximal airways, and increased mucin volume in more distal airways.

Conclusions: Significantly elevated levels of CYP1A1 activity were noted in infants exposed to ETS in the proximal and mid-level airways, respiratory bronchioles, and the lung parenchyma increased above control levels, respectively. Exposure during critical windows of maturation in the neonatal nonhuman primate may compromise lung development and biological response in later life such as increased asthma incidence and susceptibility to infection.
Abstract # 22

DEVELOPMENT OF A NONHUMAN PRIMATE ALVEOLOSPHERE MODEL TO MEASURE E-CIGARETTE TOXICITY IN THE MATURING LUNG

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Introduction: Electronic cigarette (e-cigarette) and vaping product use-associated lung injury (EVALI) is of significant health concern in adolescents as rates of juveniles utilizing e-cigarette/vaping products continue to increase. Determining EVALI pathogenesis has been problematic because thousands of different liquids with varying chemical constituents have been marketed in vaping devices. To our knowledge, a high-throughput method to test the toxicity of e-cigarette liquid constituents on maturing alveolar airway epithelium has not been developed. We hypothesize that nonhuman primate alveolar progenitor cells cultured into organotypic alveolospheres can be effectively used for toxicity testing of e-cigarette liquids and model the detrimental cellular effects of EVALI.

Methods/Results: To test this, we will determine the impact of e-cigarette exposure on toxicity and growth in nonhuman primate (NHP) alveolosphere cultures as a model for the maturing lung epithelium. Bulk lung cells were isolated from NHP lung tissue, and culture conditions directed proliferation toward a distal airway organoid phenotype. Alveolospheres were validated for alveolar type II (AT2) epithelial markers via RT-qPCR and immunofluorescent staining. Cultures will be exposed to e-cigarette liquid, and toxicity will be assessed via cell viability assays, metabolic profile analysis, and gene expression analysis.

Conclusions: RT-qPCR analysis conveyed that NHP alveolospheres expressed high levels of AT2 transcripts while expressing low levels of transcripts for upper airway, basal, and alveolar epithelial type I cells. Immunofluorescent staining showed that alveolospheres express surfactant protein C, uniquely expressed by AT2 cells, and did not express basal cell marker keratin-5 but further validation is required. Alveolosphere cultures are ongoing in preparation for e-cigarette liquid exposures and toxicity studies. Comparative studies using an established human induced pluripotent stem cell model differentiated towards alveolar airway progenitor cells will also be conducted in the future and may serve as a high-throughput model for e-cigarette liquid toxicity screening.
INHALATION OF ZINC OXIDE ENGINEERED NANOMATERIALS LEADS TO NO SIGNIFICANT MICROGLIAL ACTIVATION

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Introduction: Zinc Oxide (ZnO) engineered nanomaterials (ENMs) are widely used in medical and consumer applications, including antibacterial, antifungal, anti-corrosive, and UV filtering agents. Increased use has created concerns for the potential risk of exposure by inhaling these ENMs during synthesis and commercial application. Nanoparticles are capable of nose-to-brain translocation via the olfactory epithelium and nerve fascicles. Therefore, the primary objective of this study was to determine if ZnO ENM translocation to the olfactory bulb (OB) would activate microglia, resident macrophages of the central nervous system, to induce an immune response.

Methods/Results: Male Sprague Dawley rats were randomly assigned to filtered air (control; n=6) or ZnO inhalation (n=20) groups. The rats were exposed to aerosolized 50-nm diameter ZnO for a single 6-hour period, during which aerosolized ENMs were collected for characterization by gravimetric, x-ray fluorescence (XRF), cascade impactor, and transmission electron microscopy (TEM). The rats were necropsied 0-, 1-, 7-, and 21-days following exposure. The nose and cranium were fixed in 4% paraformaldehyde, decalcified, and embedded in paraffin in a sagittal orientation. The paraffin-embedded blocks were then sectioned at 5- and 10-µm thickness for histopathological and immunohistochemical staining, respectively. Hematoxylin and eosin staining was used to assess histopathology. Anti-ionized calcium-binding adapter molecule-1 (anti-Iba-1) immunohistochemical staining was used to identify microglia in the OB.

Conclusion: The mass concentration of the ZnO aerosol was 4.23 +/- 1.27 mg/m³. XRF analyses revealed the Zn concentration in the aerosol was 2.5 +/- 0.2 mg/m³. TEM showed the average aerosolized ZnO ENM size was 118 nm (range 50–340 nm), with clear and varied agglomeration. These findings demonstrated aerosolized ENMs were inhalable, with a high deposition efficiency in the nose and potential for translocation to the brain. However, no statistically significant changes in OB histopathology or microglial activation were observed. These findings suggest a single 6-hour exposure to aerosolized ZnO ENMs does not induce an immunological reaction in the OB by microglial activation.
ACUTE AND DELAYED EFFECTS OF HYDROGEN SULFIDE EXPOSURE ON THE BRAINSTEM AND LUNGS

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Introduction: Hydrogen sulfide (H2S), a systemic toxicant, targets the cardiovascular, respiratory, and central nervous systems. Acute H2S exposure causes acute death by inhibiting the breathing center in the brainstem. We have previously shown that acute H2S exposure causes sequalae in the thalamus but there is a knowledge gap on the long-term consequences of acute H2S exposure in the brainstem and the respiratory tract. We hypothesized that acute H2S exposure injures the brainstem, home to the breathing centers, as well as the lungs with long-term implications to the lung-brain axis.

Methods/Results: Male C57BL/6J mice were exposed to 1000 ppm H2S once for 50 min. Surviving mice were euthanized at 5min, 12h, 24h, 72h, 7d, 14d, 21d, and 28d. Endpoints included plethysmography, neurotransmitters, enzymatic activity, histology, immunohistochemistry, cytokine concentrations, and immunoblotting. Significant reduction in respiratory rate and minute ventilation was observed. In the brainstem H2S increased dopamine and its metabolites, immediately after acute exposure while norepinephrine (NE) was decreased. Glutamate was decreased during and after H2S exposure. Monoamine oxidase A activity was increased. Glutamate concentrations significantly decreased starting at 72h. Neurodegeneration in the medullary reticular formation was observed starting at 72h post-exposure. Pulmonary edema was evident immediately following exposure and normalized at 24h. Surfactant protein D was also significantly increased. Trans-4-hydroxyproline, a collagen biomarker was increased on day 28. Lung histology showed edema, arterial thrombus, and eosinophilic infiltration.

Conclusions: 1000 ppm H2S induced reduction of breathing rate and volume, dysregulation of neurotransmitters in the brainstem. Monoamine oxidase activity was increased during H2S exposure, which might impact the regulation of neurotransmitters. Brainstem neurodegeneration was observed starting at last 72h. Pulmonary edema, vascular thrombosis and inflammation were notable early events while increased collagen deposition biomarker was a delayed event. These results suggest both short- and long-term effects of acute H2S poisoning on brainstem and lungs.
EXPOSURE TO VITAMIN E ACETATE IN C57BL/6 MICE

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Introduction: In 2019, there was a rise in hospitalizations and deaths in what is now classified as E-cigarette associated lung injury (EVALI). Vitamin E acetate (VEA) became a chemical of interest as it was found in the bronchioalveolar fluid of patients and in illicit vaping devices for tetrahydrocannabinol (THC) as a cutting agent. The purpose of this study was to examine if inhalation exposure to VEA for differing lengths of time would induce lung inflammation and/or injury in male and female mice.

Methods/Results: Male and female C57 BL67 mice were exposed to filtered air or VEA (100%) for 3 h/day for 3 or 10 days. Exposure of mice for 3 and 10 days was carried out in four separate experiments (male vs. female) with of total of 48 mice/sex with 12 mice exposed to filtered air and 12 mice exposed to VEA for 3 and 10 days. In each of the 10-day studies, a pulse oximeter (STARR Life Sciences) was used to measure blood oxygen levels, heart and respiratory rate. Necropsy was performed on the last day of the exposure. Bronchoalveolar lavage (BAL) was used to determine total cell number, viability, differentials and lung protein levels. Male mice exposed to VEA for 10 days, showed significant increases in total cell numbers compared to control mice. A significant increase was noted with 10-day VEA exposure in males compared to 3-day VEA male and 10-day VEA female mice. After 10 days of VEA exposure, males had significantly increased macrophage number compared to 3-day VEA males, 10-day VEA females, and respective controls. All treatment groups demonstrated a significant increase in neutrophil count, compared to control. Neutrophils in 3-day VEA males were significantly decreased compared to 3-day VEA females and 10-day VEA males. 10-day VEA males had significantly increased lymphocyte number compared to control. 10-day VEA males and 10-day VEA females had a significant increase in the percentage of non-viable cells compared to control. 3-day VEA males, 10-day VEA males, and 3-day VEA females had a significant increase in BAL protein level compared to respective controls. BAL protein in 3-day and 10-day VEA males was significantly higher compared to female mice at these same time points.

Conclusion: In summary, progressive exposure of VEA resulted in a significant increase in inflammatory and cellular changes compared to control mice with the most significant changes occurring in 10-day VEA male mice.
The Location of Mesoporous Silica Nanoparticles Over Time in Mouse Lungs Following Acute Inhalation

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Introduction: Mesoporous silica nanoparticles (MSNs) are a new and effective method of drug delivery in the body, with the ability to be tailored for continuous or triggered drug release. Past research has shown MSNs to be highly efficient at targeting specific cells in the body, with greater specificity than chemotherapy treatments for cancer. Previous research has also shown that once MSNs are taken up by the body through inhalation, the particles do not leave the lung one (1), seven (7), and twenty-one (21) days after exposure.

Methods/Results: To identify the location of MSNs in the lung once inhaled, and the potential consequences of these MSNs on the body, immunofluorescent staining of the lung epithelium coupled with confocal scanning fluorescence microscopy was done to identify the precise nature of MSN uptake and retention in the lungs over a period of 21 days post-exposure. Researchers at the Center for Health and the Environment at UC Davis exposed mice (n = 53) to MSNs and removed the lungs for histological sampling. These lung samples were embedded and sectioned for staining and subsequent confocal microscopy imaging. To determine the cell type associated with MSNs, staining with antibodies for CDH1 (epithelial cell surface protein), CD11c (macrophage and dendritic cell surface protein), and Siglec F (macrophage-specific surface protein) was performed. No MSNs were found to be present in the epithelial cells of the lung, but instead were found in cells outside of the epithelium and in the airspaces of the lungs.

Conclusions: This suggests MSNs are most likely uptaken by cells of the immune system, macrophages or dendritic cells. Future investigation is needed to confirm this theory.
Abstract # 27 page 1

REDUCED SYMMETRIC DIMETHYLATION STABILIZES VIMENTIN AND PROMOTES METASTASIS IN MTAP-DEFICIENT LUNG CANCER

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Introduction: The aggressive nature and poor prognosis of lung cancer led us to explore the mechanisms driving disease progression. Utilizing our invasive cell-based model, we identified a major purine- and methionine-metabolizing enzyme, methylthioadenosine phosphorylase (MTAP), as a potential metastasis suppressor in lung cancer. Although MTAP deficiency was reported to be associated with poor survival in a broad range of malignancies, the mechanisms underlying tumor progression due to MTAP loss are yet to be elucidated.

Methods/Results: In a screen of 101 primary tumor specimens from lung cancer patients, we show that patients with low MTAP expression displayed worse overall and progression-free survival. Bio-functional assays confirm that MTAP knockout elevated invasion and migration abilities in lung cancer cells compared to control cells, while overexpression of MTAP inhibited both phenotypes. Mechanistically, accumulation of methylthioadenosine substrate in MTAP-deficient cells reduces the level of protein arginine methyltransferase 5 (PRMT5)-mediated symmetric dimethylarginine (sDMA) modification on proteins. We identify vimentin as a dimethyl-protein whose dimethylation levels drop in response to MTAP deficiency. The sDMA modification on vimentin reduces its protein abundance but trivially affects its filamentous structure. In MTAP-deficient cells, lower sDMA modification prevents ubiquitination-mediated vimentin degradation, thereby stabilizing vimentin and contributing to cell invasion. MTAP and PRMT5 negatively correlate with vimentin in lung cancer samples.

Conclusions: Taken together, we present a novel mechanism for lung cancer metastasis involving vimentin post-translational regulation and provide a promising molecular model for developing new anticancer strategies.

See next page for Figure 1
REDUCED SYMMETRIC DIMETHYLATION STABILIZES VIMENTIN AND PROMOTES METASTASIS IN MTAP-DEFICIENT LUNG CANCER

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P73α1, A NOVEL P73 C-TERMINAL ISOFORM, REGULATES TUMOR SUPPRESSION AND INFLAMMATION VIA NOTCH1

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Introduction: Since it was discovered over 40 years ago, p53 has been recognized as a critical tumor suppressor. p53 belongs to the p53 family of proteins, which also includes p63 and p73. Since the discovery of p73, research efforts have been focused on determining whether it has similar functions to that of p53. p73 exists as multiple variants that are known as the N- and C-terminal isoforms. Full-length p73α is the most abundant C-terminal isoform and recent studies have identified a role for it in hippocampal development and multiciliogenesis. However, the role of the C-terminal isoforms in tumor suppression/oncogenesis is largely undiscovered.

Methods/Results: We generated multiple human cancer cell lines and a mouse model wherein p73 exon 12 (E12) was deleted, leading to C-terminal isoform switch from p73α to p73α1. We used the cell lines to determine the biological activity of p73α1 because it is a novel p73 C-terminal isoform. Moreover, we used the mouse model to determine if p73α1 had a physiological function. Here, we found that p73α1 is endogenously expressed in multiple human cancer cell lines. Moreover, isoform switch from p73α to p73α1 inhibits cell growth and migration in cancer cells and promotes cellular senescence in mouse embryonic fibroblasts. Similarly, we found that partial loss of E12 in mice does not promote tumor formation, but rather leads to prolific chronic inflammation. Through RNA-seq analysis, we identified Notch1, a regulator of the inflammatory response, as a direct target of p73α1. Furthermore, we found that Notch1 was critical for p73α1-mediated tumor suppression and inflammation.

Conclusions: Here, we have identified p73α1 as a novel p73 C-terminal isoform that results from exclusion of E12. We found p73α1 to be expressed in multiple cancer cell lines, indicating a potentially important role in the pathology of these malignancies. Moreover, we have shown that p73α1 regulates tumor suppression and the inflammatory response via Notch1. While the function of the p73 C-terminal isoforms remains predominantly unknown, a shift in p73 C-terminal isoform expression is found in cancers that overexpress p73. As such, elucidating the function of these isoforms may uncover targetable pathways for future therapeutic approaches.
Introduction: The advent of immunotherapy with immune checkpoint blockade (ICB) has led to significant advances in lung cancer management; unfortunately, many issues remain to be resolved, especially regarding a low objective response rate. Through integrated large-scale omics data and biomarkers from published immune ICB trials, we identified the PIP2-binding protein, myristoylated alanine-rich C-kinase substrate (MARCKS), and the AXL receptor as top-ranked therapeutic targets in mediating immunotherapy resistance.

Methods/Results: Both immunofluorescence and immunoprecipitation data demonstrated that MARCKS forms a molecular complex with AXL in aggressive lung cancer cells. In a screening of 200 patients using immunohistochemistry staining, we observed a positive correlation between AXL phosphorylation and MARCKS expression in advanced lung cancer. MARCKS knockdown abated the activity of AXL and its downstream pathways including PI3K/AKT and STAT3/Src signaling. Data from our cytokine arrays showed upregulation of anti-tumoral cytokines including GM-CSF and CXCL10 in MARCKS-knockout cells. We further discovered that MARCKS acts in accordance with PD-L1 to modulate T cell activity and killing. Upon co-culture with MARCKS-expressing lung cancer cells, cytotoxic T cells expressed higher PD-1 levels on cell surfaces, whereas T cell killing activity was potentiated in the MARCKS-knockout group. In mouse xenograft models, mice bearing Lewis lung carcinoma tumors displayed increased sensitivity to MPS and anti-PD-1 combined treatment.

Conclusions: Our data suggest an oncogenic role of the AXL-MARCKS axis in modulating the tumor immune microenvironment. Therapeutic targeting of MARCKS may warm up an immunologically “cold” microenvironment, sensitizing tumors to immunotherapy.
VAGAL REGULATION OF LUNG RESIDENT IMMUNE CELL ACTIVATION

Kaitlin Murray1, Michael Cremin1, Sierra Schreiber1, Colin Reardon1

1University of California, Davis

Introduction: The nervous system has the unique ability to regulate immune cell activity in various organs, preventing immunopathology and consequently improving disease outcomes. As such, therapeutics inducing neuronal activation to regulate inflammation are becoming more prevalent, despite poor mechanistic definitions regarding cell types involved and tissue specific effects. Neural stimulators are typically used to target the peripheral nervous system, particularly the vagus nerve, which can reduce immune cell activation. Much of the literature to date has analyzed the utility of vagus nerve stimulators (VNS) across models of septic shock or autoimmunity, but little is known about whether VNS can regulate viral insults. Additionally, the lung is extensively innervated however it is unknown whether VNS can regulate immunity at this mucosal site, as it can in other organs such as the gut. We sought to determine the efficacy of VNS in the context of viral insult to the lung.

Methods/Results: Lung inflammation was induced in C57BL/6 mice by inhalation of the TLR7 agonist resiquimod (R848, 0.25mg/kg). Electrical or sham stimulation of the isolated vagus nerve was performed for 20 minutes (5 V, 5 Hz). One hour post R848 inhalation, tissues and sera were collected for analysis. By qPCR analysis, we determined that right but not left vagus nerve stimulation significantly reduced R848-induced pro-inflammatory cytokine release within the lung. Flow cytometry revealed alveolar and interstitial macrophages, as well as neutrophils, were activated by inhaled R848 and subsequently reduced with VNS but not sham control mice. Bronchoalveolar lavage fluid and serum demonstrated that VNS significantly increased release of the catecholamine epinephrine, but not norepinephrine or dopamine. In line with these findings, removal of the adrenal glands, the primary source of epinephrine, eliminated the anti-inflammatory effects of VNS.

Conclusions: Here, we identify a novel anti-inflammatory circuit elicited by vagus nerve stimulation (VNS) within the lung. VNS induces adrenal gland-derived epinephrine which in turn can reduce alveolar and interstitial macrophage activation and subsequent pro-inflammatory cytokine release in the context of a viral mimetic. These studies ultimately shed light on a mechanism that can be harnessed and targeted either electrically or pharmacologically within the lung to combat viral-induced inflammation and subsequent tissue injury.
Abstract # 31

VARIABILITY OF RESPONSIVENESS TO THE BNT162b2 mRNA COVID-19 VACCINE RECEIVED DURING WILDFIRE SMOKE EXPOSURE IS ASSOCIATED WITH ALTERED NATURAL KILLER (NK) CELL PHENOTYPE

Gursharan Kaur Sanghar¹, Resmi Ravindran², Melissa J. Teuber¹, Pedro A. Hernandez¹, Angela Linderholm¹, Vivian Vo¹, Gabrielle Echt¹, Kaelyn Tuemer-Lee¹, Maya Juarez¹, Timothy E. Albertson¹, Imran Khan², Angela Haczku¹

¹Pulmonary, Critical Care and Sleep Medicine, University of California, Davis, Davis, CA, United States; ²Pathology and Laboratory Medicine, University of California, Davis, Davis, CA, United States.

INTRODUCTION: The individual innate immune determinants of vaccine responsiveness are not well understood. Our previous studies implied the role of natural killer (NK) cells in mediating the effects of environmental exposures on the immune system. Human NK cells are identified by CD56 expression. The CD56bright NK cells are major producers of inflammatory cytokines while the CD56dim cells (a more mature subset in the periphery), are important in effector NK cell function. Here we investigated the phenotypic changes of NK cells in subjects that received the Pfizer vaccine or placebo, during a period of wildfire smoke exposure.

METHODS/ RESULTS: From the Sacramento region we recruited 39 age- and sex-matched healthy subjects (26-82 years of age) who also participated in the Pfizer vaccine trial in August 2020. This was a period of heavy wildfires with high PM2.5 and other pollutants in the air with an AQI ranging between “moderate” and “unhealthy” levels over several weeks. The second visit of these subjects occurred outside of the wildfire season in October-November 2020. Peripheral blood was drawn of the subjects divided into placebo (n=18) and vaccine (n=21) groups. Cells were stained using standard panels and multicolor assessment was performed. FACS data was analyzed by FlowJo®. Serum samples were assessed by Elecsys® and quantitative multiplex testing for SARS-CoV-2, SARS-CoV, MERS, and four common coronaviruses. Wilcoxon matched-pairs signed rank test was used to compare the effects of the vaccine and the wildfire.

The Elecsys® results showed all vaccinated subjects positive for antibodies against the SARS-CoV-2 S-RBD, maintained over one year. Two subjects appeared infected with SARS-CoV-2 as they were also positive for antibodies against the SARS-CoV-2 Nucelocapsid protein although they were asymptomatic and tested negative by PCR test. The subjects were negative for SARS-CoV and MERS. In comparison with placebo, vaccinated subjects had a trend to decreased CD56bright over CD56dim NK cells ratio in the peripheral blood. By wildfire smoke exposure however this trend was significantly reversed across all 39 subjects (p=0.036). In addition, we detected a decrease in the proportion of circulating CD56bright IL-13+ NK cells by wildfire smoke in the placebo injected subject population (p=0.001).

CONCLUSION: During the vaccine response, peripheral blood CD56bright NK cells differentiate into the more mature CD56dim population. Wild-fire smoke inhalation reverses this process. We propose that wildfire smoke inhalation sequesters activated, pro-inflammatory NK cells to the affected tissue compartments and may interfere with vaccine effectiveness.
Abstract # 32

TOBACCO SMOKE ACTIVATED FIBROGENIC MARCKS/AXL COMPLEX PROMOTES MACROPHAGE REPROGRAMMING AND PULMONARY FIBROSIS

David C. Yang1,2, Jun Zhang1,2, Ji-Min Li1,2, Chih-wei Chu1,2, Ssu-Wei Hsu1,2, Ching-Hsien Chen1,2*

1Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of California Davis, Davis, California, USA; 2Division of Nephrology, Department of Internal Medicine, University of California Davis, Davis, California, USA

Introduction: Macrophages and tobacco smoke (TS) exposure have been demonstrated to play significant roles in modulating pulmonary fibrosis (PF). However, the mechanisms of how TS exposure modulates pro-fibrotic macrophage polarization and drives lung fibrosis is unclear.

Methods/Results: In our study, we investigated how TS modulates macrophage polarization and the functional consequences of this polarization. Multicolor flow cytometric data indicated that markers of M2 macrophage polarization were elevated in both human and mouse macrophage cells and tissues upon TS exposure. In addition, multiple primary lung fibroblast cells demonstrated elevated pro-fibrotic markers and aggressive phenotypes upon interacting with TS-exposed macrophage cells in a co-culture system. Elucidation of the signaling pathways activated by TS exposure through a receptor tyrosine kinase array screen revealed AXL receptor as a novel smoke-responsive molecule in macrophage cells. We noted elevated secretion of AXL ligand, Gas6, and AXL activity in TS exposed cells and tissues. Prior work had demonstrated an interaction between MARCKS, a smoke-responsive protein, and AXL in promoting a pro-fibrotic phenotype in lung fibroblasts. Similarly, we observed AXL activity positively correlated with MARCKS phosphorylation in macrophage cells. Pharmacologic and genetic targeting of the MARCKS/AXL signaling complex reduced M2 markers and profibrotic cytokine production in macrophages and reduced fibrotic changes in the co-culture model and in an animal model of smoke-mediated lung fibrosis.

Conclusion: In all, our work suggests that the MARCKS/AXL fibrogenic complex is a potential target in attenuating macrophage activity in TS-mediated fibrosis.
TET1 PROTECTS THE LUNGS FROM DEP-INDUCED INFLAMMATION

Stephanie Henson¹, Tao Zhu¹, Steven Palomares¹, Lucy Cai¹, Sweeney Elston¹, and Hong Ji¹,²

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Introduction: Air pollution is a critical risk factor in the development and exacerbation of lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). Previous studies have shown that exposure to diesel exhaust particles (DEP), a major component in traffic-related air pollution, can promote airway inflammation and mucus secretion, as well as exacerbate lung function impairment and disease severity in both asthma patients and asthmatic animal models. This may be due to the oxidative stress DEP induces. We have previously shown that Tet1 (Tet Methylcytosine Dioxygenase 1) protected against house dust mite (HDM)-induced lung inflammation, possibly through upregulation of detoxication enzymes and downregulation of proinflammatory cytokines (IL33). Therefore, we sought to examine the role of Tet1 in DEP-induced lung inflammation in mice.

Methods/Results: In this study, we used Tet1+/- mice (HET) and their wildtype littermates (Tet1+/-, WT) in a DEP-induced lung inflammation model, in which the mice were intratracheally challenged with saline or DEP (150µg×9 times). Our results demonstrated that Tet1 deficiency led to increased airway hyperresponsiveness (AHR) and increased number of neutrophils in bronchial alveolar lavage fluid (BALF). Consistently, an increase in chemokines associated with neutrophil infiltration, CXCL5 and CXCL15, was observed in the BALF of HET mice compared to WT. The expression levels of Il17a and Il17f in the lungs were also significantly increased, suggesting an increased Th17 response.

Conclusion: Altogether, our findings indicate that Tet1 deficiency significantly enhances DEP-induced lung inflammation in mice, which may be due to the upregulation of a Th17 response. Further studies will be performed to explore the underlying mechanisms involved in the protective role of Tet1 against DEP-induced lung inflammation.
Hybrid Poster Session

Room 3 and Zoom

1:00 p.m. - 1:40 p.m.
Abstracts Presentations 34 - 38

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IMPACT OF COVID-19 ON SEASONALITY OF PEDIATRIC OSA: AN EXPERIENCE FROM A UNIVERSITY-LEVEL SLEEP LAB

Sukhkaran S. Aulakh¹, Jamie L. Funamura M.D., M.P.H.², Roberto N. Solis M.D.², Farrukh R. Virani M.D.², and Kiran Nandalike M.D.³

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Introduction: Building on previous work demonstrating greater severity of obstructive sleep apnea (OSA) in children in winter and spring months due to increased upper respiratory infection (URI) and allergens, along with reports of decreased frequency of URIs with universal infection precautions during the COVID-19 pandemic, this study examines seasonality in pediatric OSA and the effects of the current pandemic at a university-level sleep laboratory.

Methods/Results: In this retrospective chart review we included children under 18 years of age without significant medical co-morbidities, who underwent polysomnography (PSG) at University of California, Davis sleep lab. We compared the variability in apnea hypopnea index (AHI) between the seasons and compared the demographics, PSG parameters, seasonal variation in AHI between the pre-pandemic (December 2017 - March 2020) and pandemic (March 2020 - September 2021) periods. Of the 625 studies, 423 pre-pandemic and 202 pandemic studies, there were no differences in the total number of OSA cases, number of mild to severe OSA cases or obstructive AHI variability between seasons in years before or during the pandemic. Multivariate analysis demonstrated that obesity and age less than 5 years have a significant association with total obstructive AHI over seasonal or pandemic timing.

Conclusions: Seasonal pattern did not exist in our referred population either before or during the pandemic. Delaying surgical intervention for retesting in a favorable season may not be warranted based on our study results. Obese children and children less than 5 years should continue to be referred for PSG on suspicion of OSA.
Abstract # 35

IMPACT OF ERGOTHIONEINE PRE-TREATMENT ON NAPHTHALENE TOXICITY IN THE DEVELOPING AND ADULT MOUSE LUNG

Veneese J Brown,1 Kyle Tran1, Liang Ding,2 Lei Yin,2 Patricia Edwards,1 Xinxin Ding,2 Laura Van Winkle1

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Introduction: With the incidence of large, intense fires increasing in the Western United States, there has been widespread human exposure to wildfire smoke. Naphthalene (NA), a major component of wildfire smoke, has a well characterized pattern of acute toxicity in the epithelium of the mouse lung following conversion to NA oxide. Few compounds have been identified as effective protective agents against environmental pollutants such as NA. Ergothioneine (ET), an antioxidant found in mushrooms, has been hypothesized to be protective against tissue injury. The aim of this study was to determine whether pretreatment with ET can protect against NA-induced acute airway injury in mice of different ages.

Methods: Male and female C57BL/6J juvenile (1 month) and adult (2-3 months) mice were pre-treated with 70 mg/kg of ET, or saline control (SA), by gavage for five consecutive days prior to a single ip dose of 150 mg/kg of NA or corn oil (CO) vehicle. There were 4 treatment groups (SA/CO; ET/CO; SA/NA; ET/NA). At 24 hours post injection, the lungs were analyzed for ET concentration using HPLC-MS/MS. Acute lung toxicity and oxidative stress was assessed using histopathology, a total antioxidant capacity assay, and gene expression of SLC22A4 (ET transporter), Foxj1 (ciliated cell marker) and CCSP (club cell secretory protein).

Conclusion: When comparing the gene expression of male and female juvenile mice in the control group (SA/CO), the males had 3-fold more CCSP expression in the proximal airways than females (p value= 0.0006). The male juvenile CCSP gene expression level was not significantly different than adult males, however the juvenile had a greater expression of CCSP. SLC22A4 was shown to have greater expression in the proximal airway of both the male and female juveniles compared to adults (p value= 0.0594). The total antioxidant capacity in the lungs of juvenile female mice increased when treated with ET, and slightly dropped when treated mice were exposed to NA, compared to untreated. In summary, the results support that there are sex and age-based differences in both Club cell differentiation and CCSP expression as well as NA toxicity and ET effects. In addition, findings support the potential of ET to protect against NA oxidative stress in the lungs of juvenile and adult mice. Support: T32 ES007059, R01 ES020867 and P30 ES023513.
OXIDATIVE STRESS ACTIVATES FREE RADICALS AND GENE EXPRESSION IN PULMONARY SMOOTH MUSCLE AND EPITHELIAL CELLS

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¹University of California at Davis, Davis, CA, USA

Introduction: Environmental exposures such as ozone can lead to damage in pulmonary tissue function through oxidative stress. We previously showed that expression of both pro-inflammatory and antioxidant genes is altered with O3 induced airway hyperreactivity in mice. However, the effects of oxidative stress to the anti-inflammatory effects of glucocorticoids is unclear. Tert-Butyl hydroperoxide (TBHP) mimics the effects of ozone, causing oxidative stress to pulmonary tissues, which is expected to be seen through upregulation of free radicals and gene activation.

Methods/Results: We investigated the effects of oxidative stress on human Airway Smooth Muscle cells (hASM), adenocarcinomic human alveolar basal epithelial cells (A549), and human Bronchial Epithelial cells (hBE1). Cells were placed into serum free media for 10 hours and then treated with 0, 0.05, 0.1, 0.25 mM of TBHP for 2 hours. hASM and A549 cells were additionally treated with 0, 10, or 100 nM of dexamethasone for an additional 12 hours. Reactive oxygen species (ROS) expression was assessed by cellROX assay 0.5, 2, and 3 hours following TBHP exposure. Cell viability and mRNA expression of oxidative stress and eotaxin genes were evaluated.

in vitro TBHP treatment of hASM induced a time and dose dependent increase in ROS. qPCR analysis demonstrated an increase in Sod2 but not Sod1 gene expression with mRNA for the pro-eosinophilic CCL26 gene, also upregulated by 0.25 mM TBHP at a 12 hour time point. Dexamethasone inhibited CCL26 mRNA expression in a dose dependent manner. A549 cells showed an increase in CCL26 mRNA gene expression with the addition of 0.5 mM TBHP at 100 nM dexamethasone. The inhibitory effects of dexamethasone were reversed by TBHP. hBE1 cells demonstrated an increase in mRNA gene expression for Sod2 and Txn1 at 0.1 mM TBHP.

Conclusions: Oxidative stress was induced in hASM cells treated by TBHP, in a dose and time dependent manner. Our data suggest that oxidative stress affects hASM, A549, and hBE1 lung cells by upregulating gene expression levels relevant to airways disease.
DEFINING VOC SIGNATURES OF AIRWAY EPITHELIAL CELLS POST ENVIRONMENTAL EXPOSURE TO IMPROVE RESPIRATORY HEALTH OF EXPOSED VETERANS

Angela M. Linderholm¹, Richart W. Harper¹, Nicholas J. Kenyon¹

¹Division of Pulmonary, Critical Care and Sleep Medicine, University of California, Davis, Sacramento, CA

**Introduction:** We proposed to define volatile organic compound biomarkers produced by the lung upon exposure that would provide an exhaled breath signature for different toxicants. We have developed a reliable method to measure the VOCs emitted from well-differentiated tracheobronchial epithelial cells in vitro. In our experiments, we cultured and differentiated primary epithelial cells from eight different subjects, derived from small airway epithelium and bronchial epithelium. We performed a dose response curve utilizing traffic related air pollutants and particulate matter collected from the Caldecott tunnel obtained in a collaboration with the UC Davis NIEHS-funded Environmental Health Sciences Center to model mixed diesel exhaust exposures experienced by Veterans. These experiments allow understanding of the effects of traffic-related air pollution (TRAP) on veterans. We anticipate that these experiments will be able to model “real-life” sample exposures that veterans experience.

**Methods/Results:** Human bronchial/tracheal airway epithelial (BAE) cells and small airway epithelial (SAE) cells were obtained from Lifeline Cell Technology (Frederick, MD). The BAE and SAE cells were plated on Transwell (Corning Costar, Corning, NY) chambers (12 mm) at 1–2 × 10⁴ cells/cm² coated with 0.05mg/mL type IV collagen (Sigma) in the PneumaCult-Ex medium (Stemcell Technologies). Once BAE and SAE cultures were confluent, they were transferred to ALI culture conditions in their respective media (Stemcell Technologies) for 1 month. The transwells containing confluent cells were placed into glass jars filled with 5mL of the appropriate media and capped with lids that had Twisters magnetized to them. The VOCs were extracted from the Twisters and analyzed using mass spectrometry. We also extracted RNA to look at gene expression and measured inflammatory marker IL6 secreted by the cells using an ELISA. Interestingly, we saw very unique responses. The twisters were grouped per individual subject indicating a unique response to exposures. Our gene expression and ELISA data also indicated trends in responses to particulate matter that were different from each other when SAE was compared to BAE. IL6 from BAE cells increased with particulate dose while in SAE it did not. SOD1 and IL33 expression decreased with increasing particulate dose in BAE while in SAE they did not.

**Conclusions:** We conclude that VOC production in response to particulate matter is unique and possibly related to a phenotype we have yet to uncover. We will further explore this phenomenon in future studies.
CLINICAL CHARACTERISTICS AND POSTOPERATIVE OUTCOMES IN CHILDREN WITH VERY SEVERE OBSTRUCTIVE SLEEP APNEA

Nancy Saied, MD¹, Roberto Solis, MD², Joy Chen, BS², Jamie Funamara, MD, MPH², Cathleen Lammers, MD¹, Kiran Nandalike, MD*³

¹University of California, Davis, Department of Anesthesiology and Pain Medicine, Sacramento, CA; ²University of California, Davis, Department of Otolaryngology, Sacramento, CA; ³University of California, Davis, Division of Pediatric Pulmonology, Sacramento, CA.

Introduction: Obstructive sleep apnea (OSA) affects 2-5% of general pediatric population. Severity of OSA is defined by the apnea hypopnea index (AHI), and AHI of more than 25 events/hour is very severe OSA. Children with very severe OSA are not well studied. In this study we have evaluated the clinical characteristics and polysomnography (PSG) parameters that predict the post operative outcomes in children with very severe OSA.

Methods/Results: A retrospective chart review from a single tertiary care center was performed that identified patients with very severe OSA who underwent adenotonsillectomy (T&A) between January 2016 to September 2021. Demographics, polysomnography studies, and hospitalization records were evaluated. A total of 51 children were studied, 33/51 were male and 20/51 being white. 53% of the children (28/51) were diagnosed with a comorbidity with obesity being the most common comorbidity (25%). 13/51 experienced post operative respiratory events and needed supplemental oxygen (13/51), oxygen and HFNC (4/51), intubation and mechanical ventilation (3/51) and 3/51 children needed multiple interventions. Those requiring postoperative respiratory interventions were younger (4.4± 5.2 vs. 8.2±5.2; p=0.03) with higher pre-operative AHI (73.6± 27.4 vs. 44.5± 24.6; p=0.02) and lower SpO2 nadirs (70.0± 13.0% vs. 83.0± 7.0; p=<0.01) and had lower body metabolic index Z score (-0.51± 2.1 vs. 0.68± 1.5; p<0.03) compared to the children without respiratory events. There were no significant differences noted between both groups in terms of sex, race, and comorbidities. Children under two years of age and children with AHI >50 /hr. were at the highest risk for post-operative complications. 70% of children had moderate to severe residual OSA, younger children had better PSG outcomes compared to the older children and children with co-morbidities.

Conclusions: Our study suggests that younger children with higher AHIs, and lower SpO2 nadirs, and nutritional failure are at higher risk for post operative respiratory related complications. The families and the care providers need to be aware of these risk factors and these children should be monitored in a higher care setting postoperatively. A thorough pre-operative screening for nutritional assessment, co-morbidities such as PHTN, as well as respiratory infections, and addressing them appropriately may mitigate some of the post-operative complications in these children. In our cohort, younger children demonstrated better resolution of OSA post operatively compared to older children and children with co-morbidities, these patients and families need to be counseled about the risks of significant residual OSA prior to surgical intervention.

See next page for Figure 1
CLINICAL CHARACTERISTICS AND POSTOPERATIVE OUTCOMES IN CHILDREN WITH VERY SEVERE OBSTRUCTIVE SLEEP APNEA

Nancy Saied, MD1, Roberto Solis, MD2, Joy Chen, BS2, Jamie Funamara, MD, MPH2, Cathleen Lammers, MD1, Kiran Nandalike, MD*3

Table 2: Univariate Analysis of Baseline Characteristics Among Children with Pre-operative AHI ≥ 25, Stratified by Post-operative Respiratory Events

<table>
<thead>
<tr>
<th>Lack of Post-operative Respiratory Events</th>
<th>Presence of Post-operative Respiratory Events</th>
<th>P Value</th>
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<td></td>
<td>N = 38</td>
<td>N = 13</td>
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Age in years, mean (SD) 8.2 (5.2) 4.4 (5.2) 0.03
Sex, No. (%) Female 13 (34.2) 5 (38.5) 0.78 Male 25 (65.8) 8 (61.5)
Race/Ethnicity, No. (%) Asian 5 (13.2) 2 (15.4) 0.57 Black 7 (18.4) 3 (23.1)
Hispanic 9 (23.7) 1 (7.7) Neurorstriculous disease 0 (0) 0 (0)
White 15 (39.5) 5 (38.5) Pulmonary HTN* 1 (2.6) 1 (7.7) 0.27
Other 2 (5.3) 2 (15.4) Comorbidities, No. (%) Z score weight, mean (SD) 0.68 (1.50) -0.51 (2.07) 0.03

Intervention (%) T&A alone 34 (89.5) 11 (84.6) 0.64 T&A with other 4 (10.5) 2 (15.4)

Opioid administration post-PACU 2 (5.2) 0 (0) 1.00

Preoperative sleep study data, mean (SD)

<table>
<thead>
<tr>
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<th>N = 38</th>
<th>N = 13</th>
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<tr>
<td>Total sleep time, min</td>
<td>352.4 (86.2)</td>
<td>328.2 (124.0)</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>80.9 (14.8)</td>
<td>76.3 (17.3)</td>
</tr>
<tr>
<td>REM duration, min</td>
<td>48.5 (34.8)</td>
<td>49.4 (46.7)</td>
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<tr>
<td>Stage N1 duration, min</td>
<td>29.7 (48.3)</td>
<td>36.7 (50.8)</td>
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<tr>
<td>Stage N2 duration, min</td>
<td>161.2 (68.3)</td>
<td>152.6 (70.2)</td>
</tr>
<tr>
<td>Stage N3 duration, min</td>
<td>113.0 (65.7)</td>
<td>89.5 (58.3)</td>
</tr>
<tr>
<td>Arousal index</td>
<td>22.3 (22.4)</td>
<td>44.2 (33.0)</td>
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<tr>
<td>AHI, events/hr</td>
<td>44.5 (24.6)</td>
<td>73.6 (27.4)</td>
</tr>
<tr>
<td>SpO2 nadir, %</td>
<td>83.0 (7.0)</td>
<td>70.0 (13.0)</td>
</tr>
<tr>
<td>Hypoventilation, No.</td>
<td>16 (47.0)</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td>(%)</td>
<td>1 (2.6)</td>
<td>10 (76.9)</td>
</tr>
<tr>
<td>Length of stay &gt; 1 day, No. (%)</td>
<td>1 (1-1)</td>
<td>2 (2-3)</td>
</tr>
<tr>
<td>Opioid administration post-PACU</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: AHI: apnea-hypopnea index; HTN: hypertension; T&A: tonsillectomy and adenoidectomy; *Supraglottoplasty, inferior turbinate reduction and/or lingual tonsillectomy ; c Chi-squared test; f Fisher exact test ; t t-test ; m Mann-Whitney U test ; *Only 16/51 underwent diagnostic ECHO to r/o PHTN, prior to the procedure and one underwent ECHO post procedure
13th Annual Lung Day

Philip Thai Memorial Award and Research Presentation

Best Clinical Science Abstract

A Shocking Solution to Shock

Jeremy Jung-Soo Kim,

William E. Leon, Amir A. Zeki
A SHOCKING SOLUTION TO SHOCK

Jeremy J. Kim MD1, William E. Leon MD2, Amir A. Zeki MD1

1Division of Pulmonary, Critical Care, and Sleep Medicine, University of California, Davis. UC Davis Lung Center. Sacramento, California, 2Department of Internal Medicine, University of California, Davis. Sacramento, California

Introduction: Anaphylaxis is a routine cause of shock; however, persistent shock due to anaphylactoid reactions is far less common and should be considered in patients with known or suspected systemic mastocytosis presenting in shock.

Methods/Results: A 74-year-old man with recently diagnosed systemic mastocytosis with associated chronic myelomonocytic leukemia presented to the Emergency Department. He had recently initiated treatment for systemic mastocytosis with midostaurin; but subsequently developed nausea, vomiting, abdominal pain, and hematemesis. Vital signs on initial presentation: temperature: 37.0°C, BP: 104/54, HR: 90 bpm, RR: 20, and SpO2 of 95% on high-flow nasal cannula (40L/min, FiO2 100%). Physical exam revealed an ill-appearing elderly man with periorbital ecchymosis, subconjunctival hemorrhage, and acute respiratory distress prompting intubation for hypoxic respiratory failure. The patient developed severe shock and was treated with intravenous crystalloid and colloidal volume resuscitation, broad spectrum antibiotics, epinephrine, vasopressin, norepinephrine, and corticosteroids. On hospital day (HD) 2 he continued to have hypotension (BP 70/49) and developed multiorgan failure despite full supportive measures; however, an intravenous injection of 50 mg diphenhydramine resulted in a substantial and rapid increase in blood pressure, BP increased from 73/49 to 109/65 (20 MAP points) over a few minutes (see Figure). However, this effect was short-lived prompting initiation of a diphenhydramine infusion resulting in blood pressure improvement and stability for 18 hours. The patient then developed acute renal failure necessitating hemodialysis. Despite these supportive measures, the patient suffered persistent and progressive electrolyte derangements, worsening acidemia, and multi-organ failure leading to his death on HD3.

Conclusion: Shock in patients with mastocytosis can result in a mixed pattern due to inappropriate and massive histamine release which can worsen other underlying causes of shock. Diphenhydramine is a first-generation antihistamine which competitively inhibits the histamine-1 receptor (H1). It has a higher binding affinity than other antihistamines but also has a relatively short half-life (2.4 to 4 hours). Therefore, intermittent dosing creates a “seesaw” of therapeutic levels followed by subtherapeutic serum levels. In our case, overwhelming histamine release resulted in worsening shock physiology refractory to multiple vasopressors that was transiently rescued with intravenous push doses of diphenhydramine. We then successfully utilized a protocol for continuous diphenhydramine infusion originally designed for the outpatient management of refractory mast cell activation syndrome. This treatment approach maintained a steady-state concentration of diphenhydramine that significantly improved blood pressure. This approach could serve as a bridge to treat critically ill patients in refractory shock.

See next page for Figure 1
Figure 1: Patient bedside monitor demonstrating a profound change in vitals by arterial wave form monitor after four minutes. Note that the cuff pressure has not been re-cycled yet demonstrating the previous blood pressure. The only intervention between these two time points was the injection of 50mg of diphenhydramine into a peripheral IV.
EARLY-LIFE EXPOSURE TO WILDFIRE SMOKE RESULTS IN DYSREGULATED PULMONARY IMMUNE RESPONSES IN Rhesus Macaques

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Introduction: Wildfires are a public health concern due to the increased frequency of events and generation of particulate matter < 2.5 microns in diameter (PM_{2.5}). The long-term health consequences of wildfire smoke exposure on susceptible human populations are unknown. We have previously reported that rhesus macaque monkeys exposed to ambient wildfire smoke during infancy exhibited decreased lung function and dysregulated innate immune responses in adulthood. The objective of this current study is to investigate whether perturbations of the pulmonary mucosal immune system are detectable in juvenile monkeys following exposure to ambient wildfire smoke during infancy. We hypothesize that early life exposure to wildfire smoke results in dysregulation of pulmonary immunity that persists with maturity.

Method/Results: Lung tissues were collected from two different cohorts of male monkeys that were infants and housed outdoors during the 2018 Camp Fire and 2020 wildfire season in Northern California (6-8 months old, n=4 for 2018 cohort and 3-5 months old, n=3 for 2020 cohort). Lung tissues collected from age-matched male monkeys from Oregon National Primate Research Center served as controls (n=4 for 2018 controls and n=3 for 2020 controls). Gene expression between control and wildfire-exposed animals were assessed by qRT-PCR. Protein expression of proSP-C (immature intracellular version of the SP-C, a protein known to enhance lipid uptake and change surface tension in the lungs) was assessed by western blot and immunofluorescence staining. 2018 Camp Fire resulted in 315% increase in average PM_{2.5} compared to the historical average PM_{2.5} in 2010-2017. The peak of 2020 wildfire season resulted in 358% increase of PM_{2.5} compared to the previous year. A significant decrease in TGF-β mRNA levels was detected in cohorts of wildfire-exposed monkeys compared to the controls. Similarly, IL-10 and IL-1β mRNA levels were downregulated in the wildfire-exposed monkeys compared to the controls. Densitometry analysis of western showed that proSP-C was significantly downregulated at 14 and 16 kDa levels in wildfire-exposed lungs compared to the controls, which correlated with reduced immunofluorescence staining of alveolar type II cells with exposure.

Conclusion: Our results demonstrate that wildfire smoke exposure results in suppression of pulmonary immunity and altered lung development in young rhesus monkeys. Importantly, findings were consistent between two different exposure cohorts of infant rhesus monkeys exposed to ambient smoke from wildfire events. This study suggests wildfire smoke exposure in pediatric populations may impair lung development and immune responses, resulting in long-term deleterious effects on the respiratory system.