Dartmouth CF Retreat Schedule

Thursday, March 12, 2019

Hanover Inn, Dartmouth College Campus

Hanover, NH

8:00-8:55 AM **Registration and Continental Breakfast**

8:55-9:00 AM **Opening Comments: Bruce Stanton**

Session I. Moderator: Jane Hill, Mingming Zhang

9:00-9:20 AM Jane T. Jones, Geisel School of Medicine at Dartmouth. "Impact of Contemporary CFTR modulators on Aspergillosis Disease Progression"

9:20-9:40 AM Giulia Orazi, Geisel School of Medicine at Dartmouth. "*Pseudomonas aeruginosa* alters *Staphylococcus aureus* sensitivity to antiseptics and antibiotics"

9:40-10:00 AM Matthew J. Wargo, Department of Microbiology & Molecular Genetics, Larner College of Medicine, University of Vermont. "*Stenotrophomonas maltophilia* responses to synthetic CF sputum"

10:00-10:20 AM Michael Gebhardt, Boston Children's Hospital, Harvard Medical School, "An Atypical Two-Component System Coordinates Aminoglycoside Resistance in *Pseudomonas Aeruginosa"*

10:20-11:00 AM Coffee Break

11:00-12:00 PM **Keynote**:
"Human Enteroids as a Model to Understand Human Intestinal Physiology, Pathophysiology and Drug Development"

Mark Donowitz, M.D.
LeBoff Professor of Medicine and Physiology
Johns Hopkins University School of Medicine

12:00-1:20 PM **Lunch (provided by the Hanover Inn)**

Session II. Moderators: William Rigby, Lynn Theprungsirikul

1:20-1:40 PM Sara Rolandsson Enes, Larner College of Medicine, University of Vermont
"Bronchoalveolar lavage fluid Gliotoxin induces mesenchymal stromal cell death: a potential explanation to the decreased protective capacity of MSCs in clinical trials"

1:40-2:00 PM Lynn Theprungsirikul, Geisel School of Medicine at Dartmouth, "Breaking of tolerance to BPI in CF patients"

2:00-2:20 PM Emanuela Bruscia, Yale University School of Medicine, "Targeting the HO-1/CO pathway to treat non-resolving airway inflammation and infection in CF"

2:20-2:40 PM Coffee Break

2:40-3:00 PM Katharina Ribbeck, MIT, "Investigating the roles of extracellular polymers in Pseudomonas aeruginosa virulence"

3:00-3:20 PM	Ratnakar Potla, Wyss Institute for Biologically Inspired Engineering at Harvard University, “Cystic fibrosis Airway on a Chip for Modeling Pseudomonas Aeruginosa Infection”
3:20-3:30 PM	**Closing Comments: Bruce Stanton**

Parking: Valet Park at Hanover Inn ($18 for the day). Hanover Town Parking Garage (41 S Main Street).

Impact of Contemporary CFTR modulators on Aspergillosis Disease Progression

Jane T. Jones, Ko-Wei Liu, Joshua J. Obar, and Robert A. Cramer, Geisel School of Medicine at Dartmouth, Hanover, NH

Mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) protein cause Cystic Fibrosis (CF). Recently a small molecule therapeutic, SYMDEKO (VX-770 and VX-661), was developed to restore function of endogenous CFTR. However, it is not known how these molecules impact microbial infections in the dysregulated immune/inflammatory environment observed in CF patients. CF patients commonly test positive for the pathogenic fungus, *Aspergillus fumigatus*, and a subset of CF patients develop Allergic Broncho-pulmonary Aspergillosis (ABPA), a condition in which patients colonized with *A. fumigatus* present with a hyper-inflammatory, Th2-mediated allergic disease. We aimed to determine whether the VX-770/661 combination affects *A. fumigatus*-induced inflammation in the lung and impacts fungal persistence. Combination treatment of VX-770/VX-661 reduced *A. fumigatus*-induced IL-8 production in CF airway epithelial cells and *A. fumigatus* induced airway inflammatory neutrophil numbers in mice. To test these drugs and their impact on *A. fumigatus* mediated disease in the context of CF, we have developed a novel murine model of ABPA that was made possible by the discovery of a clinical *A. fumigatus* strain that persists in the lungs and induces ABPA disease. Future directions include defining the mechanism(s) in which VX-770/VX-661 combination affects *A. fumigatus* induced inflammation in airway epithelial cells and utilizing our mouse model of APBA to determine the effects of VX-770/VX-661 and other contemporary CF therapies on dysregulated immune responses and fungal persistence observed in CF patients. Our results are anticipated to help better inform use of new therapeutic approaches for CF patients fighting persistent lung damaging *A. fumigatus* infections.

***Pseudomonas aeruginosa* alters *Staphylococcus aureus* sensitivity to antiseptics and antibiotics**

Giulia Orazi, Kathryn L. Ruoff, George A. O’Toole, Geisel School of Medicine at Dartmouth, Hanover, NH

The thick mucus in the airways of cystic fibrosis (CF) patients predisposes them to frequent, polymicrobial respiratory infections. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the most common pathogens in the CF lung. Both organisms form biofilms, which are often difficult to eradicate and promote chronic infection. In this study, we have shown that *P. aeruginosa*-derived exoproducts can increase the efficacy of compounds that alone have low bactericidal activity against *S. aureus* biofilms. Exposure to 2-n-heptyl-4-hydroxyquinoline N-oxide (HQNO), a component of the *P. aeruginosa Pseudomonas* quinolone signal (PQS) system, increases *S. aureus* biofilm sensitivity to the antiseptics chloroxylenol and triclosan, and the fluoroquinolone norfloxacin. Treatment of *S. aureus* with chloroxylenol alone did not decrease *S. aureus* biofilm cell viability; however, the combination of chloroxylenol and HQNO led to a 4-log reduction in *S. aureus* biofilm viability compared to exposure to chloroxylenol alone. We found that the electron transport chain (ETC) inhibitor Antimycin A increased *S. aureus* biofilm sensitivity to chloroxylenol. Antimycin A and HQNO both inhibit cytochrome C reductase; therefore, we hypothesize that HQNO-mediated sensitization of

S. aureus to chloroxylenol occurs through the inhibition of this ETC component. Furthermore, we observed that HQNO shifted the lipid profile and fluidity of the *S. aureus* membrane, and that manipulation of these properties impacted antibiotic sensitivity. We propose a model whereby HQNO-mediated ETC inhibition alters *S. aureus* membrane lipid composition, thereby explaining the observed increased sensitivity to antiseptics. Our works shows that polymicrobial interactions can have dramatic impacts on antibiotic efficacy.

Stenotrophomonas maltophilia responses to synthetic CF sputum

Lauren A. Hinkel, Graham G. Willsey, Korin Eckstrom, and Matthew J. Wargo, Department of Microbiology & Molecular Genetics, Larner College of Medicine, University of Vermont

Stenotrophomonas maltophilia is a Gram-negative bacterium that infects people with cystic fibrosis (CF). The highly viscous mucus in the CF lung, expectorated as sputum, serves as the primary nutrient source for microbes colonizing this site and has been shown to induce virulence-associated phenotypes and gene expression in several CF pathogens. We characterized the transcriptional responses of three *S. maltophilia* strains during exposure to synthetic CF sputum media (SCFM2) to gain insight into how this organism interacts with the host in the CF lung. These efforts led to the identification of 881 transcripts differentially expressed by all three strains, many of which reflect the metabolic pathways used by *S. maltophilia* in sputum, as well as altered stress responses. The latter correlated with increased resistance to peroxide exposure after pre-growth in SCFM2 for two of the strains. We also compared the SCFM2 transcriptomes of two *S. maltophilia* CF isolates with the SCFM2 transcriptome of the acute infection strain, *S. maltophilia* K279A, allowing us to identify CF isolate-specific signatures in differential gene expression. Some of the genes induced in SCFM2 are related to biofilm formation and surface motility, including Type 4 pili and predicted Type V secreted adhesins. We identified that two of the latter are important for *S. maltophilia* biofilm formation.

An Atypical Two-Component System Coordinates Aminoglycoside Resistance in _Pseudomonas Aeruginosa_

Michael Gebhardt, Ian Hill, and Simon L. Dove, Division of Infectious Diseases, Boston Children's Hospital, Harvard Medical School

The Gram-negative human pathogen, *Pseudomonas aeruginosa*, survives and replicates under diverse conditions, including the lungs of patients afflicted with cystic fibrosis (CF), and this opportunistic pathogen is a leading cause of morbidity and mortality in CF patients. Given the continued emergence of antibiotic resistance amongst *P. aeruginosa* clinical isolates, a greater understanding of how this pathogen senses and responds to environmental stimuli, including antibiotic exposure, is needed. This study focuses on the characterization of an atypical two-component regulatory system composed of an orphan Response Regulator and a small STAS domain-containing protein. Strikingly, mutant strains lacking either component of this signaling pair demonstrate a dramatic increase (~1,000-fold) in sensitivity to tobramycin, a front-line therapeutic option for CF patients with *P. aeruginosa* infections. Suppressor analyses identified several genetic interactions including MexT, a transcription factor controlling the expression of a multi-drug efflux pump, and multiple components of the Nitrogen Phospho-Transferase System (PTS-Ntr), a phopsho-relay system linked to the regulation of nitrogen metabolism in other bacterial species. Follow-up biochemical experiments uncovered a direct interaction between a novel MexT-regulated peptide and Enzyme I of the PTS-Ntr. Overall, our research has identified a critical signal transduction pathway that may couple aminoglycoside resistance to the metabolic state of the bacterial cell via the widely conserved PTS-Ntr.

Bronchoalveolar lavage fluid Gliotoxin induces mesenchymal stromal cell death: a potential explanation to the decreased protective capacity of MSCs in clinical trials

Sara Rolandsson Enes[1,2]*, Soraia C. Abreu[1,]*, Thomas H. Hampton[3], Robert A Cramer[3], Bruce A. Stanton[3], Matthew Wargo[1], Daniel J Weiss[1]

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Background: Growing evidence demonstrate that human mesenchymal stromal cells (MSCs) tailor their *in vivo* anti-inflammatory response depending on the specific inflammatory environment they encounter. Understanding the anti-inflammatory mechanisms of MSCs in CF is crucial to refine MSC-based cell therapies. The aim of this study was to determine the effects of the CF lung environment on MSC behavior.
Methods: Clinically utilized MSCs were exposed to bronchoalveolar lavage fluids (BALF) obtained from patients with CF or from healthy volunteers. Following exposure, MSCs and conditioned medium were assessed for cytotoxicity, levels of immunomodulatory cytokines, and the presence of apoptotic markers. BALF samples were assessed for pro- and anti-inflammatory cytokines, osmolality, protease activity, gliotoxin concentrations, and double-stranded DNA content.
Results: A sub-set of CF BALF samples induced MSC death within 5 hours of exposure. Utilizing RNAseq, upregulation of *Aspergillus fumigatus (Asp)* associated BCL-2-stimulated apoptosis was observed. Furthermore, Gliotoxin, the major toxin produced by *Asp*, itself induced MSC death. Importantly, gliotoxin-exposed MSCs were shown to have less therapeutic effect compared to untreated MSCs in an endotoxin-induced acute lung injury mice model. Asp- CF BAL and normal volunteer BALF did not either induce MSC death or evidence up-regulation of apoptosis pathways.
Conclusions: MSCs exposed to BALF from *Aspergillus fumigatus* infected CF-patients displayed an increased cellular death, and exposure to the major fungal toxin resulted in a decreased therapeutic capacity of MSCs *in vivo.* These results highlight the need to understand the effect of the *in vivo* inflammatory environment on systemic or locally administered MSCs.

Breaking of Tolerance to BPI in CF Patients

Theprungsirikul, J. [a], Skopelja-Gardner, S. [a], Meagher, R. E. [a], Clancy, J. P. [b], Zemanick, E. [c], Ashare, A. [a,e], Gifford, A. H. [e], Rigby, W. F. C [a, d, e].

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Recurrent airway colonization by *Pseudomonas aeruginosa* afflicts ~80% of cystic fibrosis (CF) patients and is associated with respiratory compromise, morbidity, and mortality in CF. The remarkable predilection of chronic *P. aeruginosa* infection for CF patients is not understood. Our study investigated the relationship between autoimmunity to bactericidal/permeability-increasing protein (BPI) and *P. aeruginosa* infection in CF. We confirmed that a large subpopulation of adult CF patients develops autoantibodies to BPI. Autoantibodies to BPI were detected specifically in the patients who have developed an antibody response to *P. aeruginosa*. We showed that the BPI autoreactivity was more prevalent in adults, was associated with diminished lung function, and was independent of CFTR mutation in CF patients. Interestingly, anti-BPI IgG demonstrated high avidity, suggesting that anti-*P. aeruginosa* and anti-BPI antibodies arise via different immunologic mechanisms. Our

data shows that anti-BPI IgA was present in the BAL samples of CF patients, was strongly correlated with anti-*P. aeruginosa* IgA, and was strongly associated with presence of cleaved BPI. This strong association between presence of cleaved BPI and autoantibodies to BPI IgA suggests that anti-BPI antibodies emerge from immune system exposure to BPI cryptic epitope in the lungs of CF patients with chronic *P. aeruginosa* infection. Understanding the pathogenic role of these autoantibodies is important for treatment and symptoms management for *P. aeruginosa* infection in CF.

Targeting the HO-1/CO pathway to treat non-resolving airway inflammation and infection in CF

Di Pietro C[1], Öz HH[1], Zhang P[1,2], Jubin R[3], Abuchowski A[3], Krause DS[2], Egan ME[1,4], Murray TS[1] and Bruscia EM[1]
Departments of Pediatrics[1], Laboratory Medicine[2], Cellular and Molecular Physiology[4], Yale University School of Medicine, New Haven CT, USA; Prolong Pharmaceuticals, South Plainfield, NJ, USA.[3]

Macrophages (MΦs) mutated at the CFTR contribute to the non-resolving hyper-inflammation and defective host defense in CF lung disease. We have previously demonstrated that the **heme oxygenase-1/carbon monoxide (HO-1/CO)**, a key signaling pathway involved in resolving inflammation, is dysregulated in CF MΦs and murine CF lungs in response to infection. HO-1 is an inducible enzyme abundantly expressed in activated MΦs. This enzyme catalyzes heme groups producing anti-inflammatory and bactericidal mediators, such as carbon monoxide (CO), that help re-establish cellular homeostasis. As such, the HO-1 pathway is an attractive target for disrupting the hyper-inflammatory response in CF. PEGylated carboxyhemoglobin bovine (Sanguinate®, **SG**) is a CO-releasing molecule which has been proved to be safe in clinical setting. **The goal of this study was to evaluate whether SG improves resolution of inflammation while strengthening host defense in CF by acting on HO-1/CO pathway.**
We found that SG is a potent dose-dependent inducer of the HO-1 protein in human and murine CF MΦs, increasing CF HO-1 levels to those found in non-CF cells. SG's mechanism of action for inducing HO-1 expression relies on activation of PI3K/AKT signaling. SG decreases the hyper-inflammatory response in activated CF MΦs, and strengthens the MΦ host defense against PA by improving intracellular and extracellular bacterial killing. We also established that systemic delivery of a single dose of SG in CF mice is sufficient to induce HO-1 expression and improve resolution of the inflammatory response to LPS in lung tissues.
In summary, our results suggest that SG could help decreasing hyper-inflammation, improving host defense, and, ultimately, minimize CF lung disease.

Investigating the roles of extracellular polymers in Pseudomonas aeruginosa virulence

Katharina Ribbeck, MIT

The principle cause of mortality in Cystic Fibrosis patients is persistent lung infection by the bacterium Pseudomonas aeruginosa (P.a.), which forms dense colonies in the dehydrated epithelial mucus. One central goal for the field is to develop effective therapies that prevent infection by this pathogen. A major barrier to this goal, however, is that we do not understand the factors and mechanisms that allow the bacteria to persistently infect epithelial mucus. In particular, two questions are pressing in the field: 1. What is the role of secreted mucins and extracellular DNA, the two major polymers in CF mucus, in infection? 2. What is the role of extracellular polysaccharides expressed abundantly by mucoid clinical isolates in infection? We have begun to tackle these key questions with an interdisciplinary biochemistry-microbiology approach. With a combination of biochemical purification, high-end live microscopy in mucus, structure-function analyses of mucus polymer networks, and the thorough analysis of clinical isolates inside mucus, we have reconstituted simplified and complex mucus gels which allows us to test both host and bacterial factors that permit microbial colonization. Together, our strategy promises to enable identification of the factors important for P.a. infection, and raises hope for new treatment strategies that target both sides of the interaction; including

combination treatments that create lung conditions that limit colony formation and apply antibiotics to kill the cells that remain.

Cystic fibrosis Airway on a Chip for Modeling Pseudomonas Aeruginosa Infection

Ratnakar Potla, Rachelle Prantil-Baun, Donald E. Ingber, Wyss Institute for Biologically Inspired Engineering at Harvard University

Primary human airway on chips (Airway Chips) are small microfluidic devices that recapitulate airway epithelium function in vitro. We are using this Airway Chip to co-culture lung airway epithelial cells from cystic fibrosis patients with primary human lung endothelial cells under physiological vascular perfusion. Culture conditions were optimized and cellular subtypes of primary human cystic fibrosis airway on chip (CF chip) were characterized, as well as airway epithelial functions, such as mucus production and mucociliary clearance. To understand whether CF microenvironment causes a change in P. aeruginosa behavior, we introduced GFP labeled Pseudomonas aeruginosa into normal and CF chips and measured airway function and cytokine responses. Our aim is to study the interplay between CFTR modulation and Pseudomonas aeruginosa infection. These studies will help us identify better biomarkers and novel targets to improve host response and induce tolerance to recurrent infections in CF.
