Journée Recherche Fibrose Kystique 2017
2017 Cystic Fibrosis Research Day

Lundi 3 avril 2017 / Monday April 3rd 2017

George O'Toole
Geisel School of Medicine at Dartmouth, Hanover, USA
To build a biofilm: polymicrobial interactions in CF

Luigi Maiuri
European Institute for Research in Cystic Fibrosis, Milan, Italy
Protein misfolding and protein malfunction: targeting autophagy to circumvent F508del-CFTR defect

Catherine Paradis-Bleau
Université de Montréal, Montréal, Canada
Novel approaches for the development of antibacterial agents against *Pseudomonas aeruginosa*

Emmanuelle Brochiero
Centre de Recherche du CHUM, Montréal, Canada
The importance of counteracting the deleterious impact of infection in CF airways

Research Institute of the McGill University Health Centre
1001 Boulevard Décarie, Block E Atrium & Auditorium (Room ES1.1129)
Montreal, Quebec, H4A 3J1

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We would like to welcome you to the 2017 Cystic Fibrosis Research Day. This event is an exciting opportunity for research groups from across the Montreal area and beyond, to gather, hear and discuss CF research. We look forward to seeing our colleagues from Montreal, Sherbrooke, Quebec city and Ottawa, renewing ties and developing new ones.

This year's event is co-hosted by the Meakins Christie Laboratories at the Research Institute of the MUHC, and the McGill Cystic Fibrosis Translational Research Centre (CFTRc). It will feature an exciting line up of guest speakers from the USA and Europe, and local experts from the Université de Montréal and Centre de Recherche du CHUM, who will share their recent work in CF research. In addition to presentations, we will also hold two panel discussions, one on "New Insights in CF Microbiology" and one on "The Impact of modulating CFTR activity on airway function: from biology to therapy" to foster stimulating and lively discussions with our panel of topic experts. Trainees will present posters and we encourage them to meet the speakers, present their work and obtain expert feedback. We hope that this event will stimulate your research and promote collaboration between laboratories.

We would like to acknowledge Drs. Annick Guyot for her tremendous efforts and Inga Murawski for her assistance in organizing the event, Drs Simon Rousseau and Larry Lands for their help putting together this research day. We also thank the Meakins Christie Laboratories, the McGill Faculty of Medicine and our corporate sponsors Vertex, Fisher Scientific and Traffick Therapeutics for making the event possible.

We wish you an enjoyable and productive day with us,

Dao Nguyen and John Hanrahan,
On behalf of the Meakins Christie Laboratories and CFTRc.

8h00 Inscription & Petit Déjeuner Continental / Continental Breakfast & Registration
Installation des Affiches / Poster Set-up

8h45 Mots d'Ouverture / Opening Words
Dao NGuyen, *RI-MUHC* ; **John Hanrahan**, *McGill CFTRc*

Séance 1 / Session 1:
Sous la Présidence de / Session Chair: Dao NGuyen, *RI-MUHC*

9h00 **George O'Toole**, *Geisel School of Medicine at Dartmouth, Hanover, USA*
To build a biofilm: polymicrobial interactions in CF

10h00 **Catherine Paradis-Bleau**, *Université de Montréal, Montréal, Canada*
Novel approaches for the development of antibacterial agents against Pseudomonas aeruginosa

10h20 Pause Santé avec les Exposants & Session d'Affiches
Health Break with the Exhibitors & Poster Session

Séance 2 / Session 2:
Sous la Présidence de / Session Chair: Larry Lands, *RI-MUHC*

10h40 **Emmanuelle Brochiero**, *Centre de Recherche du CHUM, Montréal, Canada*
The importance of counteracting the deleterious impact of infection in CF airways

11h00 **Luigi Maiuri**, *European Institute for Research in Cystic Fibrosis, Milan, Italy*
Protein misfolding and protein malfunction: targeting autophagy to circumvent F508del-CFTR defect

12h00 Diner avec les Exposants & Session d'Affiches
Lunch with Exhibitors & Poster Session

13h30 Table Ronde I / Thematic Panel Discussion I
New insights on CF microbiology
Dao Nguyen, George O'Toole, Roger Lévesque, Francois Malouin

14h30 Table Ronde II / Thematic Panel Discussion II
Impact of modulating CFTR activity on airway functions: from biology to therapy
Simon Rousseau, Luigi Maiuri, Manon Ruffin

15h30 Remerciements / Closing Words
Simon Rousseau, *RI-MUHC*
Réception & Session d'Affiches / Cocktail & Poster Session

George O'Toole, Ph.D.

Professor
Department of Microbiology and Immunology
Geisel School of Medicine at Dartmouth
Hanover, USA
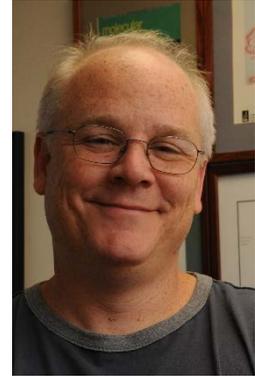
To build a biofilm: polymicrobial interactions in CF

Growing evidence suggests that interspecies interactions can have a profound impact on how microbes persist during human infections by competing for space and nutrients, and/or altering response to antimicrobial agents. My lab has focused on the polymicrobial infections associated with the fatal, inherited disease cystic fibrosis (CF). Progressive decline in pulmonary function, resulting from persistent polymicrobial infections of the airway and the resulting inflammation, is the predominant cause of morbidity and mortality for CF patients. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the most prevalent and problematic pathogens in CF. We and others have identified an association between *P. aeruginosa*-*S. aureus* respiratory coinfection and poor patient outcome, including decreased lung function. Our studies predict these pathogens interact during infection, as pathogens isolated from the same patient coexist more successfully in the laboratory than those strains from individually infected patients. This hypothesis is further supported by *in vivo* data showing *P. aeruginosa* and *S. aureus* can occupy the same airspace during infection. Thus, we seek to understand the mechanisms that these organisms utilize to compete/cooperate during coinfection, and how these interactions drive clinically relevant phenotypes and spatial structuring of these microbial communities. We hope that by acquiring a detailed understanding of interspecies community dynamics, we can identify candidate targets to modulate these interactions and to understand how these interactions alter the efficacy of current and new therapeutics.

📖 I joined the Geisel School of Medicine as an Assistant Professor in 1999 and was promoted to Professor in 2010. I received my Ph.D. from the University of Wisconsin-Madison with Dr. Jorge Escalante-Semerena and performed postdoctoral studies at Harvard Medical School as a Damon-Runyon Fellow with Dr. Roberto Kolter. My awards and honors include the NSF Career Award, Dupont Young Investigator Award and Pew Scholar in the Biomedical Sciences, election as a fellow of AAAS and the American Academy of Microbiology, and serving as an Editor for the Journal of Bacteriology. I was recently named as the co-Director for the Microbial Diversity Course at the Marine Biological Laboratory. I have worked in bacterial systems for ~20 years, including studying pathogens such as *Salmonella, Pseudomonas and Staphylococcus*. My laboratory has made important contributions to our understanding of bacterial biofilm formation by Pseudomonads, and the role of c-di-GMP in this process, and microbial surface sensing. I have also explored the impact of CRISPR/Cas systems on biofilm formation in *P. aeruginosa*, and my lab has also worked extensively in the area of *P. aeruginosa*-host interactions in the context of CF. My lab also studies the microbiota of infants, children and adults with CF to address the impact of the microflora on exacerbation, changes in the microbiota in response to clinical interventions and the association of the microbial communities with health outcomes.

Luigi Maiuri, M.D.

Professor of Pediatrics, Department of Health Sciences, University of Piemonte Orientale, Novara
Scientific Director European Institute for Research in Cystic Fibrosis (IERFC Onlus)
Division of Genetics and Cell Biology
San Raffaele Scientific Institute
Milan, Italy

Protein misfolding and protein malfunction: targeting autophagy to circumvent F508del-CFTR defect

A complex derangement of proteostasis takes place in human bronchial F508del-CFTR homozygous epithelial cell lines as well as in the lungs from F508del-CFTR homozygous (*CftrF508del*) mice. Dysfunctional F508del-CFTR protein induces a complex alteration of the post-translational network with increased generation of reactive oxygen species (ROS) that lead to the activation of transglutaminase 2 (TG2), a versatile multifunctional protein that catalyzes several post-translational modifications of target proteins. TG2 overactivation in the CF epithelial cells leads to functional sequestration of Beclin 1 (BECN1), a protein essential for autophagy, a mechanism required for cell survival, and involved in the pathogenesis of several human diseases. Autophagy is pivotal in promoting cellular clearance of protein aggregates and removal of ROS sources, such as damaged mitochondria and results in the lysosomal degradation of cytoplasmic organelles or cytosolic components after their sequestration in two-membraned vesicles (autophagosomes). Human and mouse CF airways exhibit a defect in autophagy, as indicated by reduced autophagosome formation, and the accumulation of sequestrosome 1 (SQSTM1), a major autophagic substrate also known as p62. Genetic depletion of p62, transgene-enforced BECN1 overexpression, or addition of autophagy-stimulatory proteostasis regulators, such as cystamine (or its reduced form cysteamine), can increase the expression level of F508del-CFTR protein and restore its function at the PM, either in CFTR homozygous bronchial epithelial cells or in primary nasal epithelial cells freshly collected from CF patients bearing F508del-CFTR mutation, as well as in the lungs from *CftrF508del* mice. As a consequence, restoration of autophagy reduces lung inflammation in *CftrF508del* mice. Two phase 2 clinical trials have evaluated the combination of two proteostasis regulators, cysteamine, already approved for patients with cystinosis, and the over-the-counter nutraceutical epigallocatechin gallate (EGCG) for the treatment of patients bearing F508del-CFTR or other non-F508del class II CFTR mutations, either in homozygosis or in composite heterozygosis with a class I CFTR allele. In these phase 2 trials, cysteamine plus EGCG restored autophagy and improved CFTR function while rescuing the expression of mature (band C) CFTR protein from the nasal epithelial cells in vivo. Notably, the functional rescue of F508del-CFTR is coupled to, and correlated with, a decrease of chloride concentrations in sweat, as well as a reduction of the abundance of inflammatory cytokines in the sputum. These findings indicate that such a combination treatment acts "on target". They also indicate that it is feasible to correct the F508del-CFTR defect by manipulating the proteostasis network, thus restoring autophagy, rescuing the mutant CFTR protein and then normalizing its PM stability.

📖 Luigi Maiuri, MD, is Professor of Pediatrics at the University of Piemonte Orientale, Novara and Scientific Director of the European Institute for Research in Cystic Fibrosis (IERFC) at the San Raffaele Scientific Institute in Milan. Prof. Maiuri is a pediatric gastroenterologist and immunologist by training. He was research fellow at the Department of Medical Biochemistry and Genetics, Panum Institute, University of Copenhagen, in 1989-1990. Since 2002 to 2007, he was appointed as Honorary Senior Lecturer at the Institute of Child Health and Great Ormond Street Hospital, University College of London and from 2008 to 2011 as Visiting Professor at the Cancer Sciences Division of the University of Southampton. In 2008 he was appointed as Scientific Director and Research Director of IERFC. From the early stage of his scientific career he focused on the mechanisms of diseases. He was the first to identify the sequential activation of innate and adaptive mucosal immune responses in celiac patients (Lancet, 2003) and first unveiled the role of IL15 in inducing immune/autoimmune disease phenotype in celiac disease (Gastroenterology, 2000). In 2004, he discovered how degenerate self-reactive human T cell receptor causes spontaneous autoimmune disease in mice (Nature Medicine, 2004). During last years, his scientific interest has mainly focussed on cell biology and inflammation and, in particular, on understanding how autophagy can influence human disease, in particular Cystic Fibrosis (CF). He first discovered the role of Tissue Tranglutaminase in CF and that defective autophagy plays a key role in the pathogenic cascade of CF lung disease. Through a translational cascade of pre-clinical models, from cell lines to transgenic CF mice and finally to primary nasal cells directly collected from CF patients he provided the first evidence how manipulating proteostasis can reverse F508delCFTR defect through restoring autophagy (Nature Cell Biology, 2010). His pioneering studies on autophagy are generally considered as a milestone of CF research. While as a clinician he is interested in discovering new therapeutic approaches to control disease evolution, his research is focussed in understanding the cellular autonomous dysfunctions associated with non-functional CFTR (Cell Death and Differ 2013; Nature Medicine 2017). In collaboration with Professor Valeria Raia, University of Naples Federico II, and Professor Guido Kroemer, University Paris Descartes, INSERM, he translated to the clinic the first molecules identified by his translational research. He demonstrated that a combination treatment with two proteostasis regulators is highly effective in correcting the defect of the most common CFTR mutant and ameliorating disease phenotype in CF patients (Autophagy 2014, Cell Death and Differ 2016). In February 2014, the Orphan Drug Designation for the use of cysteamine in CF was granted to Prof. Maiuri's lab by the European Medicines Agency. He is inventor in 3 patents on Celiac Disease and Cystic Fibrosis. He has published more than 90 scientific articles. His impact factor is >750, his h-index is 35. He has given more than 40 invited talks at international conferences. He has supervised many MD students, Post-graduate MD and PhD students. He served as an External Member in Committees for the assignment of the PhD title.

Catherine Paradis-Bleau, Ph.D.

Professeure adjointe
Département de Microbiologie, Infectiologie et Immunologie
Université de Montréal
Montréal, Canada

Novel approaches for the development of antibacterial agents against *Pseudomonas aeruginosa*

The Gram-negative bacterium Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen. While healthy individuals do not generally develop infectionscaused by P. aeruginosa, the genetic, physiological and environmental conditions of cystic fibrosis patients make them vulnerable to the establishment of chronic P. aeruginosa lung infections. These infections are extremely difficult and they remain the principal cause of morbidity and mortality in cystic fibrosis patients. As the envelope of P. aeruginosa cells is both a major factor leading to natural resistance to antibiotics and a validated source of antibacterial targets, we aim to develop new antibacterial agents that directly perturb envelope biology. To do so, we are undertaking two complimentary approaches. We designed an assay to detect physiological defects in the Gram-negative bacterial envelope. Using this assay, we proceeded to high throughput screening of small molecule libraries. We identified 3 compounds of interest that permeabilize the bacterial envelope in a concentration-dependent manner and inhibit the growth of P. aeruginosa cells. We are now characterizing the mode of action of these compounds and are screening a larger library of molecules. We also optimized the technique of phage display to select for peptides that inhibit a group of essential P. aeruginosa envelope synthetic enzymes. These multi-target inhibitors should have a minimized likelihood of target-mediated resistance development in vivo. Overall, our work should lead to the discovery of candidate molecules for the development of new therapies to treat P. aeruginosa infections. These molecules could have an antibacterial potential or affect the integrity of the envelope without killing P. aeruginosa cells. These "permeabilizers" would allow the usage of clinically approved antibiotics that are currently not active against P. aeruginosa because they do not cross its impermeable envelope.

 After a B.Sc. in Biology with a major in Microbiology at the Université de Sherbrooke (1998-2001), Catherine Paradis-Bleau did a M.Sc. and Ph.D. in Microbiology-Immunology at the Université Laval under the supervision of Dr. Roger C. Levesque (2002-2007). She then studied bacterial cell division proteins and cell wall biogenesis enzymes from the Gram-negative opportunistic pathogen Pseudomonas aeruginosa as bacterial targets, and phage lysis proteins as new antibacterial agents. Catherine Paradis-Bleau wan the Canadian graduate student microbiologist of the year award from the Canadian Society of Microbiologists (CSM) for her thesis work. She then moved to Boston for a first postdoctoral training at Harvard Medical School to study the host innate immune response to P. aeruginosa pulmonary infections in the genetic background of cystic fibrosis in the lab of Gerald B. Pier (2007-2008). She joined the lab of Thomas G. Bernhardt at Harvard Medical School for a second postdoctoral training (2008-2012). She then strengthened her training in molecular microbiology and bacterial genetics, made important discoveries in fundamental

bacteriology and wan the Raymond-Blais medal for her accomplishments. Catherine Paradis-Bleau established her scientific niche and expertise in the field of Gram-negative bacterial envelope biology, and opened her lab in the Department of Microbiology, Infectiology and Immunology of the Université de Montréal in 2013 with the aim of understanding the function of the novel envelope factors she had discovered. Her laboratory also works toward the development of antibacterial strategies targeting vulnerabilities in the Gram-negative bacterial envelope. As an independent researcher, Catherine Paradis-Bleau wan the Fisher Scientific Award from the CSM and the Dr. Donald Woods Young Investigator award.

Emmanuelle Brochiero, Ph.D.

Professeure titulaire sous octroi
Département de Médecine,
Université de Montréal
Centre de Recherche du CHUM
Montréal, Canada

The importance of counteracting the deleterious impact of infection in CF airways

The persistent presence of bacterial infections and chronic inflammation in the airways of cystic fibrosis (CF) patients is responsible for a progressive lung damage, which remains the main cause of mortality. Unfortunately, our work unveiled that the capacity of CF airways to repair after injury is less efficient than in healthy subjects, due to both the basic CFTR defect and the presence of infection, especially with *Pseudomonas aeruginosa* (PA). There is now compelling evidence, including from our laboratory, that PA infections also alter the ability of correctors to rescue CFTR. Our main objective is thus to define, and counteract, the factors responsible for the deleterious effect of PA on CFTR rescue and airway repair and to identify the most efficient strategy to promote airway integrity. Using primary airway epithelial cells as well as various laboratory and clinical PA strains and mutants, we investigated the mechanisms and bacterial exoproducts responsible for the observed impairment in CFTR rescue and airway epithelial repair. We found that proteases, under LasR quorum sensing (QS) control, are involved in the deleterious impact of infection. Interestingly, QS inhibitors abrogated its negative effect. Moreover, our data suggest that airway repair and regeneration may be improved by targeting bacterial products and using combinations of CFTR and K^+ channels modulators. Our study gives novel insights in an attempt to improve CFTR rescue and to restore epithelial integrity and functionality, despite the presence of infection, using combined treatments.

📖 Emmanuelle Brochiero is a Professor at the department of Medicine of the University of Montreal and researcher at the Centre de Recherche du CHUM (CRCHUM). Her researches focus on the pathology of lung diseases, especially cystic fibrosis, acute respiratory distress syndrome and primary graft dysfunction after lung transplantation. Dr. Brochiero is supported by research grants from the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC), the Association Vaincre le Mucoviscidose, the Canadian National Transplant Research Program, the Fonds Merck Sharp & Dohme Corp de la Faculté de Médecine de l'Université de Montréal and the Respiratory Health Network of FRQ-S. The main goal of her research program in CF is to identify novel therapeutic strategies to promote airway epithelial integrity and function by targeting CFTR defect and bacterial infection. Based on her expertise, she has been invited to chair the Advisory Committee of Respiratory Heath Network (RHN) and she is a member of the CF Translational Research Center (CFTRc). She is also the Director of the CF Strategic Group of the RHN as well as the director of the Respiratory Cell and Tissue Biobank of CRCHUM.

Notes

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1 - Superoxide dismutases mediate multidrug tolerance in stationary phase *Pseudomonas aeruginosa*
Dorival Martins

2 - Abnormal intragranular mucin packaging in cystic fibrosis airway epithelial cells
Olga Ponomarchuk

3 - Association between glucose intolerance and bacterial colonisation in an adult population with cystic fibrosis
Catherine Lehoux Dubois

4 - Canaux potassiques en tant que cibles thérapeutiques dans la réparation de l'épithélium des voies aériennes en fibrose kystique
Damien Adam

5 - Genetic background or ecological niche - what matters more for adaptation and diversification?
Nicole Filipow

6 - Impact of urban particulate matter on normal and cystic fibrosis primary human bronchial epithelial cells
Victor Dumitru

7 - Interleukin-6 trans-signalling in cystic fibrosis
John Lin

8 - International *Pseudomonas aeruginosa* consortium: the 1000 plus genomes project
Irena Kukavica-Ibrulj

9 - Light Microscopy Resource Platforms: Cystic Fibrosis Translational Research Centre (CFTRc), Advanced BioImaging Facility (ABIF), Cell Analysis and Imaging Network (CIAN)
Erika Wee

10 - Loss of LasB in *Pseudomonas aeruginosa* leads to a hyperinflammatory response in a mouse sub-acute lung infection model
Pierre-André Casgrain

11 - Neutrophil-bacterial interactions and their associations with eradication of *Pseudomonas aeruginosa* in cystic fibrosis
Kelly Kwong

12 - Physical activity patterns and exercise capacity in adults with cystic fibrosis
Daniel Aintabi

13 - Prevalence of glucose abnormalities in Canadian and French cystic fibrosis patients: a population-based comparison
Valérie Boudreau

14 - Primary Airway Cell Biobank (PACB)
Julie Goepp

15 - Relative affinity of tomatidine and analogs for the *Staphylococcus aureus* ATP synthase
Charles Isabelle

16 - The identification of genes in *Pseudomonas aeruginosa* clinical isolates responsible for the induction of a differential inflammatory response
Mellissa Gaudet

17 - Variability in the antagonistic effect of *Pseudomonas aeruginosa* toward *Staphylococcus aureus* from cystic fibrosis lung infections suggests an adaptive evolution of co-isolates
Guillaume Millette

1 - Superoxide dismutases mediate multidrug tolerance in stationary phase *Pseudomonas aeruginosa*

Dorival Martins[1,2], Geoffrey McKay[1], Gowthami Sampathkumar[1], Malika Khakimova[1], Cajetan Neubauer[4], Diane Newman[4], Dao Nguyen[1,2,3]

[1]McGill University Health Centre, [2]Meakins-Christie Laboratories, [3]McGill University, [4]Division of Biology and Biological Engineering, Caltech

Introduction: In adult CF patients, *Pseudomonas aeruginosa* (*Pa*) is the main pathogen in chronic lung infections where it avoids antibiotic eradication due to multidrug tolerance, a phenotype that allows bacteria to survive antibiotic challenge even in absence of genetic resistance. Improving antibiotic therapy requires overcoming *Pa* tolerance, but its mechanisms are poorly understood. Besides their conventional target-specific toxicity, bactericidal antibiotics promote metabolic changes that result in increased respiration and reactive oxygen species levels (ROS), which lead to oxidative damage that contribute to the antibiotic toxicity. We posit that antioxidant defenses that metabolize ROS drive multidrug tolerance, and redox perturbations can modify antibiotic efficacy. Since superoxide is the primary ROS produced during antibiotic challenge, we sought to determine whether manipulations of superoxide dismutases (SODs) activity, the major line of superoxide metabolism in bacteria, alter antibiotic tolerance in *Pa.*

Methods: We compared antibiotic susceptibility of wild-type (WT) *Pa* to a SOD-null mutant (*sodAB*), which was validated to have no measurable SOD activity and ~3-fold higher superoxide levels than WT. To assess multidrug tolerance, the strains were grown to stationary phase where a high proportion of the population becomes tolerant to bactericidal antibiotics, challenged with two different classes of antipseudomonal bactericidal drugs (quinolones and carbapenems) and their survival was determined through plating and colony forming units (CFU) counts after drug exposure. Prior to the antibiotic challenge, the SOD activity of individual samples was also measured to determine its correlation with antibiotic tolerance. To get mechanistic insides on how superoxide metabolism disruption alters antibiotic tolerance, we measured membrane permeability using ethidium bromide uptake and β-lactamase leakage methods. Fatty acid composition of the membranes was assayed by gas chromatography coupled with electron impact mass spectrometry (GC/MS).

Results: Stationary phase cultures of the *sodAB* mutant had 2- to 3-$\log_{10}$ CFU lower survival to quinolone and carbapenem challenge than its isogenic WT, and we found a strong positive correlation between tolerance and SOD activity in individual cultures. This suggests that SOD activity is strong contributor to multidrug tolerance in *Pa*. We observed that the *sodAB* mutant displays a 3-fold increase in ethidium bromide uptake and β-lactamase leakage, suggesting a high overall and outer membrane permeability, respectively. Thus, depletion of SODs may impair drug tolerance via increased drug influx. The increased membrane permeability in *sodAB* was associated with 2-fold lower levels of cyclopropane fatty acids (CFAs), which have been reported to contribute to general stress tolerance of stationary phase cells. Deletion of the gene encoding CFA synthase (*cfa* mutant) resulted in full depletion of CFA levels in stationary-phase, 2-fold increased membrane permeability and 3- to 4-$\log_{10}$ CFU lower tolerance to quinolones and carbapenems, reinforcing that modulation of CFA levels by SODs is a critical determinant of drug uptake and tolerance in stationary phase *Pa.*

Conclusions: SODs play a pivotal role in stationary phase multidrug tolerance. This is partly through keeping outer membrane integrity by modulating CFA levels. Targeting SODs or redox homeostasis may be a useful adjuvant strategy to bypass drug tolerance in *Pa* and improve bacterial eradication in CF patients. We are currently determining whether SOD and CFA synthase inhibitors can synergize with antipseudomonal drugs to overcome *Pa* antibiotic tolerance.

2 - Abnormal intragranular mucin packaging in cystic fibrosis airway epithelial cells

Olga Ponomarchuk[1], Sebastian Raquena[2], Rafal Fudala[2], Ignacy Gryczynski[2], Sergei Dzyuba[3], Zygmunt Gryczynski[4], Emmanuelle Brochiero[1,5], Ryszard Grygorczyk[1,5]

[1]CRCHUM, University of Montreal, [2]University of North Texas Health Science Center, Center for Cancer Research, Institute for Molecular Medicine, [3]Department of Chemistry and Biochemistry, Texas Christian University, [4]Department of Physics and Astronomy, Texas Christian University, Fort Worth, TX, USA, [5]Department of Medicine, Université de Montréal, Montreal, Quebec, Canada

The airway mucus is formed by secretion and hydration of gel-forming polymeric mucin macromolecules (MUC5AC, MUC5B) that are synthetized by secretory epithelial cells. Prior to secretion, exceedingly large mature mucin molecules are stored in a highly-condensed form in intracellular membrane-enclosed secretory granules. Their content is released by exocytosis, followed by rapid (<1-s) and massive (up to 1000-fold) mucin gel swelling/hydration. Swelling process and viscosity of released mucus are critically affected by extracellular airway surface liquid (ASL) pH and Ca^{2+} concentration both of which are modulated by CFTR-dependent HCO_3^- secretion. One of the most prominent clinical manifestations of CF is abnormally viscous and sticky mucus that obstructs lungs and other organs. At the microscopic level, CF mucus abnormalities include preexocytotic granule swelling, slow swelling rate and release of partially decondensated mucus of increased viscosity/diffusivity. While acidic ASL pH may explain some of CF mucus abnormalities, the existence and contribution of defects intrinsic to the stored granules, such as alkaline intragranular pH or altered mucin packaging, remain unclear.

In this study, we used fluorescence life time imaging microscopy (FLIM) and fluorescent BODIPY-based molecular viscometers to investigate intragranular mucin matrix viscosity. Machine learning algorithm was employed to segment images and analyze large number of mucin granules. In mucus producing CF cells (ΔF508 defect), we found a subpopulation of mucin granules with a significantly reduced viscosity of the intragranular matrix when compared to control cells from a healthy subject. Lower viscosity may suggest abnormal packaging/condensation of mucin macromolecules in the CF granules that may impact their release and swelling process. The data suggest that clinically observed abnormalities in CF mucus may be already reflected in the mucin properties stored in the granules prior to their secretion.

3 - Association between glucose intolerance and bacterial colonisation in an adult population with cystic fibrosis

Catherine Lehoux Dubois[1,2], Valérie Boudreau[1,2], François Tremblay[1,3], annick lavoie[1,3], Yves Berthiaume[1,2,3], Adèle Coriati[2]

[1]Université de Montréal, [2]Institut de Recherches Cliniques de Montréal, [3]CHUM

Background and objectives: Cystic fibrosis (CF) is a multisystemic disease that mainly affects the pulmonary and digestive systems. A particular mucus structure accumulates in the lungs and facilitates the bacterial colonisation of the respiratory tracts. In some patients, the bacterial colonisation expands overly and generates an acute deterioration of the usual symptoms of CF, also known as pulmonary exacerbations (PEx), leading to the most frequent cause of morbidity, mortality and respiratory failure. The improvement of life expectancy has led to the emergence of new comorbidities; the most common one is CF-related diabetes (CFRD). When blood glucose is $\geq$ 8 mmol/L, glucose is detected in the airway, creating a favourable environment for bacterial growth in the lungs of CF patients. We investigated the relationship between dysglycemia and lung pathogens in CF.

Methods: This is a cross-sectional observational and prospective analysis of adult CF patients, without known CFRD. All patients (N=260) underwent a 2 h-Oral Glucose Tolerance Test with glucose measurements each 30 min. Pulmonary bacterial colonisation of airway mucus, forced expiratory volume in 1 sec (FEV1), age, gender, nutritional status and the number of PEx requiring intra-venous antibiotics were collected at baseline and three years later for the follow-up. Statistical analysis using the Mann-Whitney U-Test and Chi^2 non-parametric T-Test for normally distributed variables and Independent Sample T Test for normally distributed variables. A linear regression analysis was done to evaluate if OGTT levels changed overtime pre and post colonisation by comparing the slopes of both curves using GraphPad Prism.

Results: *Stenotrophomonas maltophilia* (*S. maltophilia*) was the sole bacteria increased in dysglycemic (AGT: 20.2%, CFRD: 21.6%) patients compared to normotolerants (NGT: 8.7%). *S. maltophilia* positive patients with dysglycemia had more pulmonary exacerbation events compared to NGTs (1.22 vs 0.63, P = 0.003). The interaction between *S. maltophilia* colonisation and glucose tolerance status significantly increases the risk of lower lung function (P=0.003). Its growth was not affected by the evolution of the glucose tolerance after three years follow-up.

Conclusions: Prevalence of *S. maltophilia* was higher in dysglycemic patients, supporting the idea that *S. maltophilia* is a marker of disease severity in CF.

4 - Canaux potassiques en tant que cibles thérapeutiques dans la réparation de l'épithélium des voies aériennes en fibrose kystique

Damien Adam[1,2], Manon Ruffin[1,2], Émilie Maillé[2], Claudia Bilodeau[1,2], Dao Nguyen[3], Simon Rousseau[3], Martin Desrosiers[2], Christelle Coraux[4], Emmanuelle Brochiero[1,2]

[1]Université de Montréal, [2]CRCHUM, [3]McGill University, [4]Inserm UMR-S 903 (France)

Introduction: La défaillance respiratoire liée au dommage et remodelage progressif de l'épithélium des voies aériennes est la principale cause de mortalité chez les patients fibrose kystique (FK). Or, nos précédents travaux ont démontré que l'épithélium FK présentait un retard et un défaut de réparation/régénération suite à ces lésions. Il est donc primordial de développer des stratégies favorisant sa réparation et sa régénération afin de restaurer ses fonctions. De façon intéressante, nous avons précédemment observé que la correction de CFTR et l'activation des canaux potassiques (K^+) permet de stimuler les processus de réparation.

Objectifs: Notre but était d'étudier le rôle des canaux K^+ KvLQT1 et K_{ATP} dans la réparation de l'épithélium des voies aériennes non-FK et FK et d'identifier les composés activateurs de ces canaux, en combinaison ou non avec les modulateurs de CFTR, capables d'améliorer efficacement ces processus dans un contexte exempt ou non d'infection bactérienne par *Pseudomonas aeruginosa*, bactérie prédominante chez les patients FK.

Méthodologie: La vitesse de réparation a été mesurée *in vitro* sur des cultures de cellules épithéliales non-FK et FK non-différenciées (cultures en monocouches) et différenciées pendant 35 jours en interface air-liquide (IAL). Les lésions ont été réalisées sur des cellules soumises pendant 24 heures à des traitements pharmacologiques (inhibiteur des canaux KvLQT1 : clofilium, 5 µM; activateur des canaux KvLQT1 : R-L3, 4 µM et activateur des canaux K_{ATP} : pinacidil, 100 µM) en absence ou en présence des modulateurs de CFTR (correcteur : Vx-809, 5 µM et potentiateur : Vx-770, 1 µM) dans un contexte normal ou infectieux mimé par une exposition à des exoproduits *de Pseudomonas aeruginosa* (PsaDM). L'intégrité de l'épithélium a été évaluée par des mesures de la résistance transépithéliale (RTE) avant lésions et après lésions à différents temps (0h, 24h, 36h, 72h et 1 semaine après lésions). La quantification du nombre des cellules ciliées présentes dans ces épithélia réparés a été effectuée par un immunomarquage de la tubuline-βIV une semaine après les lésions.

Résultats: Nous nous sommes assurés au préalable que les canaux potassiques KvLQT1 et K_{ATP} étaient exprimés dans nos modèles cellulaires de cultures de cellules non-FK et FK indifférenciées ou différenciées en IAL. Nous avons ensuite observé que la modulation des canaux KvLQT1 impactait la vitesse de réparation des lésions en présence ou non d'infection. Dans les cultures FK, la correction et potentialisation du CFTR (avec le Vx-809 et le Vx-770) améliore la réparation, mais cet effet est altéré en présence d'infection. De façon intéressante, l'activation du KvLQT1 seule ou en combinaison avec l'activation des canaux K_{ATP} et/ou la modulation de CFTR permet de stimuler la réparation épithéliale FK malgré la présence d'exoproduits bactériens. Nous avons également pu noter que l'épithélium non-FK et FK réparé retrouvait son intégrité 72h après lésions dans les conditions contrôles et en présence des modulateurs des canaux K^+ et CFTR et ce malgré la présence de l'infection. Enfin, nos résultats préliminaires montrent que l'activation des canaux K^+ et CFTR prévient la diminution du nombre de cellules ciliées observé dans les cultures exposées au *Psa*DM une semaine après lésion.

Conclusions: Nos résultats montrent que la fonction des canaux KvLQT1 et K_{ATP} est cruciale pour la réparation des épithélia des voies aériennes. De façon intéressante, nous montrons pour la première fois que l'activation de ces canaux permet de favoriser la réparation des lésions de l'épithélium non-FK et FK, et ceci malgré la présence d'infection.

5 - Genetic background or ecological niche - what matters more for adaptation and diversification?

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Pseudomonas aeruginosa (PA) is the major contributor to declining lung function and early mortality in people with cystic fibrosis (CF). It is generally believed that individuals are initially infected from environmental reservoirs, as PA genotypes are often unique to their patient. However, sources of these infections, and the generalizability of all PA strains being able to infect have been relatively understudied. Consequentially, prevention measures are difficult to implement, and by 18 years of age, 80% of CF patients harbor chronic strains of PA.

My MSc work has used a selection experiment and phenotypic analyses to identify the underlying drivers of adaptation in PA, and understand the extent that genetic factors and ecological niche of origin contribute to the capability of PA to adapt to a CF lung. Analysis of the results has enabled both a comparison between the evolution of clinical and environmental strains within a synthetic CF lung sputum medium, and the ability to identify a possible phylogenetic signal in determining a strains evolutionary outcome.

The implications of this study could confirm if ancestral genotype or ecological niche confines the evolutionary trajectory of closely related strains to converge on CF pathoadaptive phenotypes, whereby these genotypes, or strains, are resultantly more pathogenic. In contrast, divergent isolates may undergo a different adaptive path, leading to a diversity of less virulent strains. Future studies would then be able to determine the mechanisms that enable some strains to be primed for causing infection over others. Importantly, infection control measures could focus on the prevalence in location, and inevitably prevention, of this subset of strains.

6 – Impact of urban particulate matter on normal and cystic fibrosis primary human bronchial epithelial cells

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This study will improve the health of cystic fibrosis (CF) patients by examining the effects of urban air pollutants on CF bronchial cells. Exposure to particulate matter (PM) from urban air pollution is associated with CF exacerbation. However, the impact of PM on cystic fibrosis transmembrane conductance regulator (CFTR) expression, trafficking and function, along with its mechanisms of action are poorly understood. Increased susceptibility to airborne pollutants is likely to remain a serious problem for CF patients even as novel drugs become available. Therefore, it is essential to understand how air pollution affects CFTR correction and airway epithelial function. For example, elevated reactive oxygen species (ROS) production is a central mechanism of PM cytotoxicity, and the ROS response to PM is 5-fold larger in CF cells compared to non-CF cell lines; however, the mechanisms and functional consequences are not known. The study objectives are to 1) study the effects of air pollution derived PM on well-differentiated human bronchial epithelial (HBE) cells from healthy donors and individuals with CF 2) assess the effects of air pollution on CFTR following correction/potentiation by clinically approved CF drugs 3) determine the mechanisms of pollutant ROS production so that potential therapeutic targets for the treatment of CF can be identified. Ussing chamber experiments show that HBE cells from both healthy and CF donors maintain CFTR function in the presence of PM up to $150\mu g/cm^2$. CFTR function decreases in the presence of PM with the addition of an oxidative stressor. Within air pollution, PM is combined with oxidative gasses. Increasing negative effects in the presence of oxidative stress is relevant for susceptible populations such as those with cystic fibrosis.

7 - Interleukin-6 trans-signalling in cystic fibrosis

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Loss-of-function CFTR mutations increase levels of IL-6, a multifunctional cytokine, which lead to inflammation (Beaudoin et al., 2013; Bérubé, Roussel, Nattagh, & Rousseau, 2010). Previous studies have described IL-6 to have dual roles in inflammation, both as a pro-inflammatory and an anti-inflammatory cytokine. There are two described signalling pathways for IL-6: classic signalling, and trans-signalling. In classic signalling, IL-6 binds to IL-6 receptor alpha (IL-6Rα), a transmembrane receptor that is tissue-specific, and gp130, a ubiquitously-expressed cytokine receptor, activating the JAK-STAT3 pathway. Classic signalling is important for host defense in mice models and ciliated epithelial cell differentiation (Calabrese & Rose-John, 2014; Tadokoro et al., 2014; Zhong, Wen, & Darnell, 1994). On the other hand, in trans-signalling, IL-6 binds to soluble IL-6Rα (sIL-6Rα), which then binds to gp130 and activates the JAK-STAT3 pathway (Tadokoro et al., 2014). This pathway is much stronger and more rapid than classic signalling, and is strongly correlated with inflammation (Calabrese & Rose-John, 2014; Rakemann et al., 1999).

We have shown that IL-6 trans-signaling in bronchial epithelial cells correlates with higher levels of STAT3 phosphorylation and might contribute to upregulation in immune-related genes such as ICAM1 and IRF1 compared to classic signalling.

In CF, IL-6 levels correlate with pulmonary exacerbations (PE), which reduce lung function (Wojewodka et al., 2014). IL-6 trans signalling in PE might contribute to worse prognoses and determining its effects may lead to future treatments that preserve classic signalling while alleviating excessive inflammation caused by trans-signalling.

8 - International *Pseudomonas aeruginosa* consortium: the 1000 plus genomes project

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The International *Pseudomonas aeruginosa* Consortium 1000 plus genome project consists in collecting and sequencing over 1000 genomes using an analysis pipeline for the study of *Pseudomonas* genome evolution, antibiotic resistance and virulence genes. Metadata, including phenotypic as well as genomic data, for isolates of the collection are provided through the International *Pseudomonas* Consortium Database (IPCD), available at https://ipcd.ibis.ulaval.ca/. Currently the collection contains 1588 *P. aeruginosa* isolates, generously provided by an international community of research scientists from 31 institutions, spanning 125 years back to 1880, and covering about 35 countries, on 5 continents. The strain collection was assembled with the aim of representing maximal genomic diversity. To this end, various criteria were taken into consideration, including geographic origin, previous genotyping, phenotype, and *in vivo* behavior. To date almost 1000 genomes were sequenced and analyzed. Results confirm three major groups of *P. aeruginosa* strains and demonstrate the existence of new sub-groups with as yet unmatched resolution. Our approach will allow us to draw potential links between environmental strains and those implicated in human and animal infections, understand how patients become infected and how the infection evolves over time as well as identify novel therapeutic targets and prognostic markers for better evidence-based decisions on patient care.

9 - Light Microscopy Resource Platforms: Cystic Fibrosis Translational Research Centre (CFTRc), Advanced BioImaging Facility (ABIF), Cell Analysis and Imaging Network (CIAN)

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The Light Microscopy Resource Platforms at CFTRc, ABIF, CIAN, have been assisting researchers across Montreal for 10 years running, provide the research community at McGill University and the Montreal scientific community with access to advanced imaging equipment for cellular and tissue imaging.

The facility aims to support researchers on all aspects of their research projects from planning, sample preparation to publication. With over 35 years of microscopy experience combined, the facility offers 20 microscope platforms as: Laser Scanning Confocal, Soining-Disk Confocal , Total Internal Reflection Fluorescence (TIRF) microscopy, LightSheet Z1, Live Cell Imaging, Fluorescence Lifetime imaging (FLIM), Spectral Imaging, Fluorescence Correlation Spectroscopy (FCS), High Content/Throughput automated imaging (HCS/HTS), Super Resolution Microscopy, automated image processing assays (cell cycle, mitotic index, translocation), and advanced image processing and analysis (Imaris, MetaMorph, ImagePro, AutoQuant).

Every training request begins with a one-on-one consultation with one of our microscopy experts. All you have to do is bring your information and an ABIF consultant will advise you on sample preparation, imaging protocols and advanced image processing and analysis. Our in-depth, hands-on training in conjunction with expert advice will accelerate any light microscopy research project. Join us for a consultation about your specific needs and become part of the microscopy community of students, scientists, and staff. To learn about the facility, please visit our poster for more information.

10 - Loss of LasB in *Pseudomonas aeruginosa* leads to a hyperinflammatory response in a mouse sub-acute lung infection model

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Pseudomonas aeruginosa are gram-negative bacteria that frequently infect the lungs of cystic fibrosis (CF) patients. Isolates recovered from chronic infections are often deficient in the production of acute virulence factors such as pyocyanin or type III secretion system. *P. aeruginosa* produces several secreted proteases, which include LasB, LasA and AprA. The loss of protease activity in CF-adapted *P. aeruginosa* isolates has been mainly attributed to loss-of function genetic mutations in the lasR quorum sensing regulator gene, although it may also result from other mutations. LasB has an unusually broad substrate range and has been primarily recognized as a major acute virulence factor capable of causing direct host tissue damage by degrading components of connective tissue matrix or epithelial barrier. Chronic *P. aeruginosa* infections in CF lung disease are characterized by an exuberant neutrophil-dominant inflammation that causes tissue damage and decline in pulmonary function. Since LasB is also capable of degrading different components of the immune system and inflammatory mediators such as immunoglobulins and cytokines, thus dampening immune and inflammatory host responses, the loss of LasB function could paradoxically lead to more exuberant inflammation. In this study, we demonstrate loss of elastase activity in *P.aeruginosa* coming out of CF patient sputum examined the impact of lasB mutants on airway inflammation in vivo using a murine model of chronic airway infection with *P. aeruginosa*.

11 - Neutrophil-bacterial interactions and their associations with eradication of *Pseudomonas aeruginosa* in cystic fibrosis

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Pseudomonas aeruginosa (Pa) is the predominant pathogen causing chronic lung disease in adult patients with cystic fibrosis (CF). Failure to clear Pa by innate host defenses leads to persistent Pa infections in the CF airways, which results in progressive lung tissue damage. However, studies have demonstrated that early antibiotic treatment shortly after new Pa detection has increased the rate of Pa eradication in children. In efforts to prevent or delay the onset of chronic Pa infections in children, recent clinical trials (ELITE and EPIC) have assessed the efficacy of inhaled tobramycin treatment to eradicate early Pa infections in CF children and found that 28% to 42% of patients failed Pa eradication despite bacterial susceptibility to tobramycin. The reason for eradication failure remains unclear but may be associated with specific bacterial phenotypes. Subsequent analysis of bacterial phenotypes of Pa isolates colonizing patients enrolled in these clinical trials showed that Pa eradication failure was significantly associated with loss of motility, wrinkly colony morphology, and irregular colony edges. Since the adequate recruitment and function of neutrophils is a key step for successful Pa eradication, we hypothesized that Pa isolates that are not eradicated at the time of early infection and persist in CF patients elicit dysregulated neutrophils responses, leading to impaired bacterial clearance. In this study, we compared the in vitro neutrophil phagocytosis and intracellular bacterial killing in response to eradicated vs persistent Pa isolates. We observed a higher phagocytic index and more rapid intracellular killing of Pa from the eradicated group compared to those from the persistent group. This suggests that persistent Pa isolates are more likely to be resistant to neutrophil phagocytosis and intracellular killing via a specific mechanism. In addition, we also found that phagocytosis was significantly correlated with bacterial twitching motility. These results highlight the potential role in Pa strain specific host-pathogen interactions that contribute to bacterial eradication in CF patients. Future experiments will be involved with dissecting the mechanism of bacterial persistence in neutrophil and the cause of phagocytic resistance.

12 - Physical activity patterns and exercise capacity in adults with cystic fibrosis

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Introduction: Forced expiratory volume in 1 second (FEV_1) is the main prognostic factor used in the cystic fibrosis (CF) population to assess lung function. Patients with CF are associated with greater airflow limitation in comparison to healthy individuals of similar height and age (FEV_1%predicted). Lung function is a significant predictor of exercise capacity in the CF population. Performing more habitual physical activity can significantly attenuate the rate of decline in FEV_1 in CF patients. Time spent performing moderate-vigorous physical has been shown to have a positive correlation with exercise capacity in CF. While physical activity patterns are well documented for CF children, few studies have assessed these patterns in CF adults. This study aimed to assess physical activity patterns and its impact on exercise performance in CF adults.

Methods: As part of an international prospective study to increase physical activity through motivation using a 6-month training program, patients with CF were recruited from the McGill University Health Centre (MUHC). Inclusion criteria consisted of participants having an FEV_1 $\geq$35%predicted and performing $\leq$4 hours/week of vigorous physical activity. Physical activity was subjectively assessed using The Habitual Activity Estimation Scale (HAES) (time spent "somewhat active" and "very active") and the 7-day Physical Activity Recall (7D-PAR) ("moderate", "hard", "very hard" and "strength" physical activity). Skinfold measurement was used to assess body composition. FEV_1 and forced vital capacity (FVC) were obtained using spirometry. Incremental cycle ergometry following the Godfrey protocol was used to assess peak oxygen capacity (VO_2peak) and maximal workload (Wmax).

Results: Baseline data of 2 female and 4 male CF adults (age= 36.67$\pm$15.36) was collected. Participants had a mean weight of 66.01$\pm$11.92kg, height of 171.2$\pm$10.3cm, and BMI of 22.5$\pm$3.3. Mean fat free mass (FFM) and %body fat were 53.23$\pm$9.92kg and 19.26$\pm$5.18% respectively, all within normal values. Mean FEV_1%predicted was 68.04$\pm$18.8 and FVC%predicted was 91.08$\pm$20.26. A mean of 41.82hrs/week of habitual physical activity was observed using the HAES questionnaire, 0.32hrs/week of which was spent as very active and 41.5hrs/week as somewhat active. A mean of 18.5hrs/week of physical activity was observed using the 7D-PAR questionnaire (15.86hrs "moderate", 1.98hrs "hard", 0.40hrs "very hard" and 0.26hrs "strength"). While different mean values were obtained, a positive correlation was identified between the reported total amount of physical activity using the HAES and 7D-PAR questionnaires (r=0.84, p<0.05). Participants achieved a mean VO_2peak%predicted of 88$\pm$15.7 and Wmax%predicted of 97.5$\pm$28.1. Total time spent performing physical activity using the HAES and 7D-PAR questionnaires both strongly correlated with VO_2peak%predicted (r=0.96 p<0.005, r=0.92 p<0.05). Total time spent performing more than moderate physical activity for the 7D-PAR questionnaire also significantly correlated to VO_2peak%predicted (r=0.95 p<0.005). No significant correlations with VO_2%predicted were seen for FEV_1%predicted, %body fat or FFM.

Conclusion: Using the HAES and 7D-PAR questionnaires, this study provides baseline data on physical activity patterns in CF adults while validating the use of these two questionnaires. The results of this study suggest that increasing physical activity in CF adults may improve their exercise capacity.

13 - Prevalence of glucose abnormalities in Canadian and French cystic fibrosis patients: a population-based comparison

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Aim: We compared the clinical status of two large cystic fibrosis (CF) cohorts (Montreal, Canada and Rhône-Alpes region, France) according to their glucose tolerance classification.

Methods: 267 Canadian and 147 French adult CF patients ($\geq$ 18 years) without known CF-related diabetes (CFRD) were included. We compared clinical data, glucose tolerance status (normo-tolerant [NGT], abnormal glucose tolerance [AGT], and *de novo* CFRD [CFRD]), fasting (G0) and 2-h (G2) glucose values, and glucose area under the curve (AUC) values at study inclusion in both cohorts.

Results: Sex ratio and proportion of F508del homozygous patients were similar (p = 0.6 and p = 0.4). Canadian patients were older (mean age of 26.3 $\pm$ 7.9 vs 24.9 $\pm$ 6.7, p = 0.03), and their age adjusted clinical status was better with higher %FEV1 (72.9 $\pm$ 21.8 vs 62.9 $\pm$ 22.2, p < 0.0001) and higher BMI (21.8 $\pm$ 3.0 vs 20.1 $\pm$ 2.2, p < 0.0001). The prevalence of *de novo CFRD* diagnosis (16.5 vs 10.0%, p = 0.01), as well as G0 and G2 values were higher in the Canadian cohort (respectively 5.5 $\pm$ 0.8 vs 4.8 $\pm$ 0.5mmol/L, p < 0.0001 for G0; 8.0 $\pm$ 3.3 vs 7.1 $\pm$ 2.7mmol/L, p = 0.004 for G2). Across all glucose tolerance groups, Canadian patients displayed higher glucose AUC values (p = 0.001, > 0.001 and 0.008 for NGT, AGT and CFRD groups, respectively).

Conclusion: Despite higher glucose levels and incidence of *de novo* CFRD, Canadian CF patients have a better pulmonary and nutritional status.

14 - Primary Airway Cell Biobank (PACB)

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First of its kind in Canada, PACB is a non-for-profit facility providing researchers worldwide with **standardized, high-quality primary airway cells** from **normal** and a large variety of airway **diseased** donors.

Highly differentiated primary epithelial cells are essential for studies of airway physiology/pathophysiology, cell biology, inflammation, mucosal immunity, and for translational research to develop new therapeutics. While cell lines are not representative of genetic variation and fail to differentiate into the various cell types found *in vivo* such as ciliated and goblet cells. Therefore, primary cells are considered as **"the gold standard"** when testing hypotheses regarding **disease pathogenesis** and preclinical **development of new therapeutics.**

The reduced accessibility to primary human bronchial epithelial cells (**pHBE**) resulting from high cost and low variability discourages investigators, which has a negative impact on the progress of basic research and drug development, while there is a critical need for drugs for CF patients in particular.

The cells distributed by PACB, isolated from a **large variety of donors with different genetic backgrounds**, are essential for improving our understanding of CF and other respiratory diseases and for **translational research** to develop new therapeutic agents.

PACB is committed to **provide top-quality airway epithelial cells to researchers**.

This platform involves the establishment of a **procurement network** throughout Canada, with several physicians and surgical teams, following all ethical requirements and best practices.

15 - Relative affinity of tomatidine and analogs for the *Staphylococcus aureus* ATP synthase

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Staphylococcus aureus (SA) is one of the most prevalent lung pathogen in cystic fibrosis (CF) patients. Respiratory-deficient SA small-colony variants (SCVs) produce large amounts of biofilm and can reside within host cells, two properties associated with persistent infections like seen in CF lungs. Tomatidine (TO), a steroidal alkaloid, is a potent bactericidal agent against SCVs (minimal inhibitory concentration, MIC: 0.03-0.06 μg/mL) although it lacks antibacterial activity against the normal phenotype of SA (MIC >64 μg/mL). Based on mutagenesis studies, the mode of action of TO has been associated with the bacterial ATP synthase (ATPase). However, the interaction between TO and the ATPase remained to be demonstrated. Hence, a structure-activity relationship (SAR) study has been conducted in order to understand the mode of action of TO. Based on the hypothesis that the steroidal backbone of the compound acts as a scaffold to orient functional groups, modifications were made to those groups of TO in order to understand the principal interactions between TO and the bacterial ATPase.

ATP synthesis was assayed using inverted membrane vesicles from a laboratory-derived SCV (Δ*hemB*). Vesicles were energized with NADH and the amount of ATP produced was measured using a luciferin/luciferase system after addition of ADP. TO and analogs as well as known inhibitors of the ATPase were tested and dose-response curves allowed calculation of the inhibitory concentration 50% (IC50) representing the concentration of compound needed to reduce the production of ATP by 50% compared to that measured for untreated vesicles. Finally, to establish a SAR, the MIC of TO derivatives against Δ*hemB* were determined by a microdilution method in 96-well plates and then used in the ATPase assay.

The IC50 of TO for the Δ*hemB* membrane vesicles was 18.0 μg/mL. The IC50 of known ATP synthase poisons were 1.37 and 1.47 μg/mL for DCCD and CCCP, respectively, whereas the IC50 of bedaquiline (a specific inhibitor of the *Mycobacterium* ATPase; MIC against Δ*hemB* >128 μg/mL) was very high (IC50 >1024 μg/mL). TO analogs with low or no antibiotic activity were also tested in the assay. The 3α-hydroxyl stereoisomer of TO (TO is 3β) showed a weaker antibacterial activity against Δ*hemB* (MIC at 4 μg/mL) and its IC50 consequently increased to 87 μg/mL. On the other hand, no MIC (>128 μg/mL) or inhibition on ATP synthesis was observed (IC50 >1028 μg/mL) for a TO analog in which the spiroaminoketal moiety has been opened.

The ATPase assay is discriminatory and can measure the relative activity of inhibitors. Valuable SAR data can be obtained by combining IC50 values to MIC against the TO hypersensitive Δ*hemB* mutant. As such, results suggest that an intact aminoketal moiety and the orientation of functional groups are both important for the interaction of TO against the SA ATP synthase. Extending on the SAR studies initiated here should help designing compounds with both improved ATPase inhibition and antibacterial activities against SA. Development of TO as a drug could help improve the quality of life of CF patients.

16 - The identification of genes in *Pseudomonas aeruginosa* clinical isolates responsible for the induction of a differential inflammatory response

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In Canada 55% of adult patients with Cystic Fibrosis (CF) are infected with *Pseudomonas aeruginosa* [1]. This pathogen is largely responsible for the deterioration of the patient's health and greatly contributes to the morbidity and mortality of CF patients [2]. This is because chronic pulmonary infections with *P.aeruginosa* can elicit significant inflammatory responses that are more detrimental to the patients' lung tissue [3]. Better insight on the host-pathogen interactions in these patients could potentially lead to the control or perhaps the eradication of *P.aeruginosa* in CF patients.

An extensive screen of over 150 clinical isolates of *P. aeruginosa* from different CF patients was initiated. Thirteen isolates from six different CF patients were observed to induce differential inflammatory responses. More specifically, isolates found colonizing the same patient showed differential IL-8 expressions in lung epithelial cells. These isolates also have differential effects in an acute in vivo infection model using *Hydra magnipapillata*. These observations confirm that these bacteria do not exclusively induce differential responses in lung epithelial but also in other organisms.

Phenotypic characterization of the thirteen *P. aeruginosa* clinical isolates was conducted, followed by a comparative single-nucleotide polymorphism (SNP) analysis of each co-isolated strains. This investigation provided 948 discordant genes between the thirteen isolates and 58 of these corresponding genes were found to be common in at least two patients. Of these genes, the following six were retained for a more in depth characterization: fiuA, alkA, aprF, chpA, fusA1 and gyrB. *P. aeruginosa* PA01 transposon mutants for the genes of interest will determine if its disruption could be responsible for the heightened IL-8 induction. The construction of over expression *P. aeruginosa* mutants for the genes of interest will also provide insight as to how gain of function may affect the inflammatory response profile of the pathogen.

The inflammatory responses of these loss and gain of function mutants will allow the identification of regulatory genes that significantly influences the production of virulence factors in *P. aeruginosa.* This brings us one step closer to targeting gene products linked with virulence ultimately leading to improved personalized management and treatment of CF patients who are infected with *P. aeruginosa.*

17 - Variability in the antagonistic effect of *Pseudomonas aeruginosa* toward *Staphylococcus aureus* from cystic fibrosis lung infections suggests an adaptive evolution of co-isolates

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Staphylococcus aureus (SA) and *P. aeruginosa* (PA) are the two most retrieved lung pathogens in cystic fibrosis (CF) patients. Some reports have associated co-infections by both pathogens with a more deleterious outcome in CF patients than that seen in infections by each individual pathogen, with decreased pulmonary functions, more frequent exacerbations and increased mortality. Among factors facilitating PA prevalence, its virulence-modulating quorum-sensing system (QS) allows PA to coordinate the actions of every cells of its population. SA can thrive in CF patients by modulating virulence factors expression, biofilm formation, antibiotics resistance and by adopting the small-colony variant (SCV) phenotype.

SA and PA are often co-isolated. With prototypical strains, PA acts as an antagonist toward SA. One of PA QS molecules, 4-hydroxy-2-heptylquinoline N-oxide (HQNO), either selects the SA SCV phenotype, or acts upon the normal phenotype SA to express SCV-like properties. HQNO-sensitized SA will have a reduced growth, produce more biofilm and less toxins. However, we previously reported that such a SA-PA interaction is not necessarily observed between strains co-isolated from CF patients (Fugère et al, 2014, PlosONE, 9: e86705).

To further assess the specific SA-PA interactions between co-isolates, growth kinetics were followed in co-cultures for five clinical pairs. Some SA-PA pairs behaved as prototypical strains and thus affected SA growth. SA strains for which biofilm production was unaffected by PA did not show a reduced growth in presence of their co-isolated PA. No effect of SA on PA growth was observed. To complement the growth kinetics observed in liquid co-cultures, an agar co-culture model was established. SA strains showing a modest but still reduced viability toward their co-isolated PA revealed a SCV morphotype, while SA strains unaffected by PA kept their normal colony appearance. Finally, using prototypical strains in a murine lung infection model, a significant increase in SA colonization was observed when lungs were co-infected with PA, while PA colonization was slightly reduced but remained significant. Thus, long-term interactions between SA and PA could result in an adaptive evolution for both strains, resulting in a reduced antagonistic effect of PA toward SA and an increased viability of SA.

Notes

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