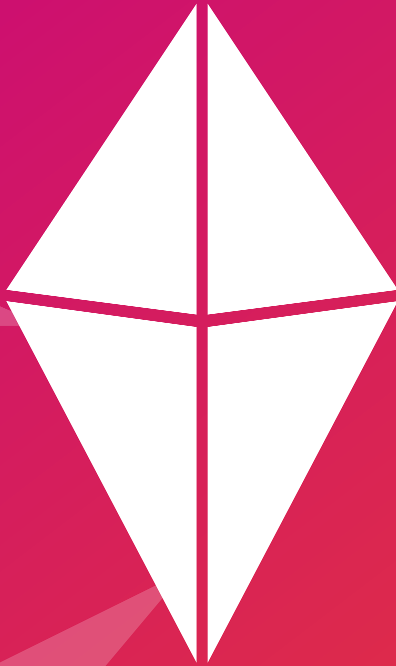

UN SOUFFLE DE COLLABORATION

Symposium québécois des chercheurs et cliniciens FK

Cystic Fibrosis
Fibrose kystique™
Canada

Journée scientifique
Jeudi 10 mai 2018

Cocktail et souper
Jeudi 10 mai 2018 en soirée

Hôtel Le Bonne Entente
3400, chemin Sainte-Foy, Québec (Qc) G1X 1S6

Organisateurs

I would like to extend a warm welcome to the 2018 CFC-CFTRc symposium « An Era of Collaboration».

This symposium will bring us up to date on CF research being carried out here in Quebec and hopefully will foster collaboration between different labs with common interests and complementary expertise. It also provides us with an opportunity to invite a leading researcher from outside Quebec, and this year we are very fortunate to have Dr. Frédéric Becq from Poitiers visit as our plenary speaker. We are looking forward to his talk and also to other exciting presentations throughout the day on diverse topics ranging from infection, inflammation and cell biology to CF related diabetes. There will also be many interesting posters presented and we strongly encourage students and postdocs to discuss their work with other participants including speakers to benefit from their insights and feedback.

This symposium is an annual event for the CF Translational Research centre (CFTRc), a virtual CF centre that was formally established in the fall of 2015. It is based at McGill but includes many other institutions. The main goal of the CFTRc is to accelerate the development of a cure for CF by providing access to state-of-the-art equipment, CF cells and other materials, by promoting collaboration between laboratories, and by organizing symposia, workshops, seminars, and travel awards for trainees to present their work at conferences.

This is my opportunity to acknowledge the tremendous efforts of Drs. Annick Guyot, Larry Lands, Dao Nguyen and Simon Rousseau in putting together this symposium. I also thank Cystic Fibrosis Canada – Division Quebec for their helpful collaboration and our corporate sponsor Vertex, for making the symposium possible.

I wish you all an interesting and productive meeting.

John W. Hanrahan, Ph.D.
Director of CFTRc
Professor of Physiology
McGill University

Schedule / Horaire

8h30	**Inscription, Déjeuner & Installation des Affiches** **Registration, Breakfast & Poster Set-up**
9h15	**Mots d'Ouverture / Opening Words** **Yannick Brouillette** *(Directeur Général Québec - Fibrose Kystique Canada)* **John Hanrahan** *(Director CFTRc, McGill University)*
9h30	**Roger Lévesque,** *IBIS, Université Laval, Québec* ***3D cystic fibrosis lung chip: next generation 3D bioprinting of normal and CF lung tissues***
9h50	**Chris Moraes,** *McGill University, Montréal* ***Tissue-on-chip technologies: microscale engineered models for disease***
10h10	**Dao Nguyen,** *Meakins-Christie Labs, RI-MUHC, Montréal* ***Probing host-pathogen interactions to understand the persistence of Pseudomonas aeruginosa in the CF lung***
10h30	**Pause santé & session d'affiches** **Health break & poster session**
11h00	**Frédéric Becq,** *STIM Laboratory, University of Poitiers, France* ***A better understanding of CFTR is needed for precision medicine***
12h00	**Emmanuelle Brochiero,** *CRCHUM, Université de Montréal, Montréal* ***The complex interplay between bacterial infections, inflammatory response, airway epithelial repair processes and CFTR rescue with Orkambi***
12h20	**Diner & session d'affiches** **Lunch & poster session**
13h20	**François Malouin,** *Université de Sherbrooke, Sherbrooke* ***New antibiotic combination for Staphylococcus aureus, its small-colony variant (SCV), and Pseudomonas aeruginosa in cystic fibrosis***
13h40	**Danuta Radzioch,** *RI-MUHC, Montréal* ***The role of derangements in lipid metabolism in lung inflammation and CFTR dysfunction in cystic fibrosis***
14h00	**André Cantin,** *Université de Sherbrooke, Sherbrooke* ***CFTR, mucins and neutrophils in cystic fibrosis airway defence***

14h20 **Simon Rousseau,** *Meakins-Christie Labs, RI-MUHC, Montréal*
Identification du gène bactérien aprF comme modulateur de la réponse inflammatoire des cellules épithéliales humaines

14h40 **Gergely Lukacs,** *McGill University, Montréal*
Contribution of the Janus-faced chaperones in peripheral protein quality control of CFTR

15h00 **David Thomas,** *McGill University, Montréal*
Can combinations of correctors of F508del-CFTR trafficking provide an effective CF therapy?

15h20 **Pause santé & session d'affiches**
Health break & poster session

15h50 **Larry Lands,** *Meakins-Christie Labs, RI-MUHC, Montréal*
CFTR correction by Lumacaftor-Ivacaftor effects on stimulated interleukin-8 in cystic fibrosis bronchial epithelial cells

16h10 **John Hanrahan,** *McGill University, Montréal*
Precision medicine for cystic fibrosis (CF): Validation by analysis of variance and implications for prospective assays

16h30 **Guillaume Bouvet,** *IRCM, Montréal*
The YKL-40 project: what we learn from since 2014

16h50 **Rémy Rabasa-Lhoret,** *IRCM, Université de Montréal, Montréal*
CFRD: Pathophysiology exploration and current treatment

17h10 **Adèle Coriati,** *St. Michael's Hospital, Toronto*
Clinical impact of hyperglycemia in CF patients

17h30 **Valérie Boudreau,** *IRCM, Université de Montréal, Montréal*
Current and emerging CFRD screening methods

17h50 **Questions CFRD session**

18h10 **Remerciements, Réception & session d'affiches**
Closing Words, Cocktail & poster session

19h00 **Souper / Dinner**

Notes

11h00 - Guest Speaker / Conférencier Invité

A better understanding of CFTR is needed for precision medicine

Frédéric Becq

STIM Laboratory, University of Poitiers, France

After 30 years of intense basic research on the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) ion channel, we are now witnessing the winds of change. First generation precision orphan drugs for Cystic Fibrosis (CF) patients are on the market and several other candidate drugs are progressing through clinical trials and preclinical studies.

New medications such as Kalydeco (class 3 patients), Orkambi and Symdeco (class 2 patients) are paving the way to treatments for CF by targeting the activity and/or the biogenesis of the mutated CFTR protein. However, these first-in–class drugs are not as efficacious as expected due at least in part to two key facts. First, their chemical structures and their combination (VX809+VX770 or VX661+VX770) may have adverse and/or non-CFTR effects. Second, there are multiple defects in CFTR related to biogenesis, stability, channel gating and the thermal instability of mutants such as F508del-CFTR at physiological temperature that must be overcome.

We therefore still need to better understand the abnormalities in CFTR and its pathological variants so that a polypharmacology approach can be developed that optimizes future treatments to restore normal epithelial fluid homeostasis and human health.

Dr. Becq received his doctoral degree in Sciences in 1993 from the University of Aix-Marseille III (France). He then pursued post-doctoral training at McGill University under the supervision of John Hanrahan (1994-1995) and then at the INSERM U270 in Marseille under the supervision of Bernard Verrier (1995-1996). Dr. Becq holds a permanent position at the Université de Poitiers (France) where he is Professor of Physiology. Since 2014 he has also been director of the Laboratoire Signalisation et Transports Ioniques Membranaires (STIM) and team leader of the Ion transport and cystic fibrosis group. He was director of the Institut de Physiologie et Biologie Cellulaires (Université de Poitiers) from 2008 to 2013 and the VP Research (Université de Poitiers) from 2012 to 2016. Since 2016, Dr. Becq has been director of the Ecole Doctorale Biologie-Santé de l'Université de Poitiers. He is a member of The European Respiratory Society, The European Cystic Fibrosis Society, The American Physiological Society, and The French Physiological Society. Dr. Becq has 165 publications in international peer-reviewed scientific journals (h-index: 32) and a portfolio of 10 patents. His field of interest is the pharmacology and function of chloride channels and especially of the epithelial cAMP-regulated and ATP-gated CFTR and its role in the human genetic disease cystic fibrosis. With his research group he is screening small molecules with the aim of identifying new therapeutics for CF patients.

9h30 - 3D cystic fibrosis lung chip: next generation 3D bioprinting of normal and CF lung tissues

Roger C. Levesque, Maxime Bourrelle-Langlois, Irena Kukavica-Ibrulj and Brian Boyle

Institut de Biologie Intégrative et des Systèmes (IBIS), Département de microbiologie, immunologie et infectiologie, Faculté de médecine, Université Laval, Québec

It is now well known that in many cases data acquired through animal models mimicking various human pathologies including Cystic Fibrosis (CF) lung disease is limited. Testing of novel therapeutics in animal models has also been shown to not always be easily translated to humans due to genotypic, phenotypic and major differences in metabolism. In addition, classical cell culture may, in several cases, lack the complexity and tissue-like structure relevant to detailed biological analysis, not necessarily predictive in treatment and providing poor clinical outcomes. This major challenge in several human diseases including CF can now be met using 3D bioprinting, a disruptive technology at the forefront of research in life sciences. 3D bioprinting of human lung tissues was performed using innovative bioinks resulting in maximum cell integrity in the final assembly. In our CF studies, 3D bioprinting of human lung tissues was done using CELLINK's third-generation printer, the Bio X. The alveolar tissue region of the lung were printed using appropriate human lung cell lines and primary cells for the formation of the stroma and the epithelium. To establish the printed lung tissue response to molecules alone, microbial infection and treatment, the classical histological, immune and tissue viability assays were combined with a genomics-based RNA-Seq. The AmpliSeq RNA-Seq profiling of 20,000 genes covering 95% of human transcripts was used. This complementary genomics-based analysis will change the paradigm in 3D bioprinting giving critical information as a panoramic view in gene expression. In agreement with the known data from the normal human lung transcriptome, transcriptomic profiling of individual and assembled cell lines using 3D bioprinting was performed to demonstrate the relevance of such an engineered tissue and genomics-based complementary methods used. Therefore, 3D bioprinted CF tissues offer improved *ex vivo/in vitro* models that could now be used for high-throughput assays in genomics, transcriptomics and proteomics, and leading to massive data acquisition of greater biological significance. Lung constructs with and without *Pseudomonas aeruginosa* as a CF infection model will be treated with Tobramycin, mimicking CF treatment, as well as with *P. aeruginosa* novel quorum sensing inhibitors. Data mining for levels of expression, SNPs, gene organization, and rearrangements will be pursued. The repertoire of expressed genes will be compared to normal and CF tissues in the Lung Expression Atlas. 3D bioprinting of lung tissues using well-differentiated human airway cells will lead to functional near-native microanatomy providing powerful means for *ex vivo* modeling of tissue morphogenesis. A second step will be attempted to functionalize the bio-scaffold in order to provide useful CF tissues constructs including macrophages and with molecular signaling to better reproduce the CF-built lung tissue and environment. Several end-products are expected from our project including the development of a CF human chip used for screening of drug candidates and the lung biological changes associated with CF. Ultimately, this CF-based 3D bioprinting approach can be now be used as a general strategy for the development of other 3D printed lung models for other lung pathologies including COPD, bronchiectasis, bronchitis,

asthma, emphysema, idiopathic pulmonary fibrosis, major lung infectious diseases caused by bacterial and viral pathogens and in lung cancer

9h50 - Tissue-on-chip technologies: microscale engineered models for disease

Christopher Moraes

Department of Chemical Engineering, Department of Biomedical Engineering, Goodman Cancer Research Centre, McGill University, Montreal

Microscale tissue engineered models provide us with an unprecedented capacity for precision, throughput and control in understanding biological development, homeostasis and disease. Using techniques originally developed in the electronics semi-conductor industry, these strategies afford scientists the opportunity to closely observe reconstructed and highly realistic live tissue sections, while they undergo various disease processes in real-time. Such 'on-a-chip' systems may ultimately be used to predict disease progression, stratify patient risk groups, and identify potential therapeutic strategies. More immediately however, the throughput, precision and dimensions of tissues engineered at this length scale provide a remarkable capacity to 'watch' biology happen in unique ways. For example, mechanical forces are now known to play a pivotal role in tissue homeostasis and disease progression, but our technical capacity to watch mechanics evolve in 3D tissues is severely limited. In this talk, I will describe recent and ongoing work in our lab to recreate 3D tissues, construct dynamic 'maps' of tissue mechanics, and leverage this understanding towards developing rational tissue engineering design strategies for the pancreas, a key organ affected by cystic fibrosis.

Notes

10h10 - Probing host-pathogen interactions to understand the persistence of *Pseudomonas aeruginosa* in the CF lung

Kelly Kwong[1], Emmanuel Faure[1], Valerie Waters[3], Peter Jorth[4], Dianne Newman[4], Manon Ruffin[2], Emmanuelle Brochiero[2], Simon Rousseau[1], Dao Nguyen[1]

[1]*Meakins-Christie Laboratories, Research Institute of the McGill University Health Centre, Montreal*
[2]*CR-CHUM, Département de Médecine, Université de Montréal*
[3]*Sick Kids, Division of Infectious Diseases, Toronto*
[4]*California Institute of Technology*

Pseudomonas aeruginosa is a ubiquitous environmental organism. Yet, it also causes chronic and fatal lung infections in most patients with Cystic Fibrosis (CF), where it persists despite active innate immune responses and antibacterial therapy. Although the prevailing view suggests that impaired mucocilliary clearance and a bacterial biofilm lifestyle are the main reasons why the CF lung fails to eradicate *P. aeruginosa*, the mechanisms of bacterial persistence remain incompletely understood. Using experimental and translational approaches that target both pathogen and host, we aim to understand how *P. aeruginosa* evades host defenses and persists in the CF lung. First, we examine how the certain bacterial phenotypes of *P. aeruginosa* promote resistance to neutrophil-mediated antibacterial functions, and demonstrate that these bacterial-host interactions are associated with bacterial persistence in CF children undergoing eradication therapy for early *P. aeruginosa* infections. Second, we establish the presence of intracellular *P. aeruginosa* within human lung epithelial cells, and investigate how this bacterium is internalized and survives in human bronchial epithelial cells. This previously unrecognized lifestyle may plays an important role in *P. aeruginosa* long-term persistence within the host.

12h00 - The complex interplay between bacterial infections, inflammatory response, airway epithelial repair processes and CFTR rescue with Orkambi

Manon Ruffin[1,2], Damien Adam[1,2], Émilie Maillé[1,2], Laura Sognigbé[1,2], Claudia Bilodeau[1,2], Anik Privé[1], Fabiana Valera[1], Martin Desrosiers[1], Lucie Roussel[3], Simon Rousseau[3,] Christelle Coraux[4], Dao Nguyen[4], Emmanuelle Brochiero[1,2]

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[4]*INSERM UMRS903, Université Champagne Ardennes, Reims, France*

Progressive airway damage due to chronic bacterial infections and inflammation remains the first cause of morbidity and mortality in cystic fibrosis (CF) patients. Our previous work revealed that the capability of CF airway epithelia to repair is less efficient than in healthy subjects, most likely due to the basic CFTR defect and bacterial infections. We then discovered that CFTR rescue with correctors enhances epithelial repair in non-pathogenic conditions. However, there is now evidence, including from our laboratory, that CFTR rescue is dampened by infection. Interestingly, our data also indicated that another class of ion channels, i.e. K^+ channels (KvLQT1) plays a key role in the repair of the respiratory epithelium.
Our goals were thus 1) to study the relationship between bacterial infection, inflammatory response, airway epithelial repair and CFTR rescue with corrector/potentiator (Orkambi®) and 2) to identify therapeutical strategies to improve epithelial repair and CFTR rescue, despite the presence of infection.
To achieve our goals, we are using differentiated primary cultures of airway epithelial cells (AEC) collected from non-CF and CF patients, exposed to bacterial exoproducts (*Pseudomonas aeruginosa* or *Staphylococcus aureus*) and treated with the Orkambi combination (VX-809 corrector + VX-770 potentiator) and/or the K^+ channel activator R-L3. The wound healing rates, migration/lamellipodia dynamics, cytoskeletal organization, CFTR-F508del rescue (CFTR maturation and currents) and inflammatory response were then measured.
Our data first indicated that *P. aeruginosa* and *S. aureus* exoproducts significantly impaired airway epithelial repair processes (reduced wound healing rates, altered migration/lamelipodial dynamics and cytoskeleton disorganisation). This deleterious impact of *P. aeruginosa* was prevented by restraining elastase/protease production and interfering with bacterial quorum sensing (QS) with a QS inhibitor (QSI). This strategy, with a QSI, also allowed to counteract the negative impact of *P. aeruginosa* on the Orkambi-induced CFTR rescue in AECs. Our data also indicated that *P. aeruginosa* bacteria, elicited different outcomes on the efficiency of CFTR correctors, as a function of their genotypic and phenotypic characteristics.
Furthermore, one of our recent study demonstrated that Orkambi treatment has a CFTR-dependent anti-inflammatory effect (with a reduction in cytokine induction in response to *P. aeruginosa* exposure in AECs). Our work also showed that CFTR-rescue was associated with improved wound healing rates. Specifically, the Orkambi combination (VX-809+VX-770) elicited

a greater beneficial effect, than the corrector alone, on the repair of airway epithelia from patients homozygous for F508del as well as heterozygous patients (carrying F508del and another class II mutation). The effect of Orkambi was however dampened in the presence of *P. aeruginosa* exproducts, indicating that a complementary approach is required to efficiently restore epithelial integrity. Interestingly, *P. aeruginosa* did not affect KvLQT1 channels, which are widely expressed in airway epithelial tissue, including in progenitors cells. Finally, a combined treatment with Orkambi and a KvLQT1 activator greatly improved the restoration of airway integrity, despite the presence of infection.

Such strategies, targeting *P. aeruginosa* (with QSI) and both CFTR and K^+ channels (with modulators) would deserve further investigation to enhance the efficiency of treatments in cystic fibrosis.

Oral Presentations / Communications orales

13h20 - New antibiotic combination for *Staphylococcus aureus*, its small-colony variant (SCV), and *Pseudomonas aeruginosa* in cystic fibrosis

François Malouin[1], Maxime Lamontagne Boulet[1], Charles Isabelle[1], Isabelle Guay[1], Eric Brouillette[1], Jean-Philippe Langlois[1], Pierre-Étienne Jacques[1], Sébastien Rodrigue[1], Ryszard Brzezinski[1], Pascale B. Beauregard[1], Kamal Bouarab[1], Kumaraswamy Boyapelly[2], Pierre-Luc Boudreault[2], Éric Marsault[2]

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[2]Département de Pharmacologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke

Staphylococcus aureus (SA) and *Pseudomonas aeruginosa* (PA) are the two most frequently isolated bacterial pathogens from lungs of CF patients. PA has been recognized for years for its contribution to the worsening of disease and treatment options include inhaled formulations of tobramycin. Recent evidence show that the antibiotic-resistant form of SA (MRSA) can persist in the CF lung and also contribute to the deterioration of patient health. Moreover, a simultaneous infection by MRSA and PA leads to a worse prognostic. Persistence of MRSA may involve Small Colony Variants (SCVs), which are bacteria that remain hidden in the lungs. The SCV phenotype can arise by a selective pressure provoked by PA or aminoglycoside antibiotics like tobramycin. SCVs represent a phenotype specialized for persistence. SCVs produce more biofilms than normal strains and can persist inside epithelial cells. SCVs can also develop high levels of antibiotic resistance. The ability of SA or MRSA to switch to SCVs, and *vice versa*, maintains a pool of SA or MRSA in the CF lung and increases the chances of co-infections with PA. All three pathogens (MRSA, SCVs, PA) need to be controlled.

We recently discovered a natural product, tomatidine (TO), which kills SCVs and significantly increases the killing of multi-resistant MRSA by tobramycin. We have elucidated the molecular target of TO and analogs against SA using a genomic analysis of *in vitro*-generated TO-resistant strains. The mutations in genes involved in resistance identified the bacterial ATP synthase as the cellular target. Sequence alignments and structural models were performed to understand the specificity of action of TO and analogs.

This allowed developing a target-based assay (ATP synthase assays) to fully support development of TO and analogs by medicinal chemistry. The ATP synthase assay allowed correlation of antibiotic potency with ATP synthase inhibition. The selectivity index of the TO analog FC04-100 (inhibition of ATP production by bacteria *vs*. by mitochondria) is estimated to be $>10^5$.

With this knowledge, we built a pharmacophore model to perform a virtual High Throughput Screening (vHTS), with the goal to identify simpler, more readily diversifiable chemical scaffolds than TO. vHTS of the ZINC15 database of 14 M commercially available compounds identified a non-steroidal TO analog that was indeed validated as an inhibitor of the SA ATP synthase. This important result considerably expands the molecular landscape of the project with a new scaffold more readily diversifiable than TO. The combination of an ATP synthase inhibitor with tobramycin should greatly surpass the benefits of inhaled tobramycin, currently used to control PA only. The combination should be effective against both MRSA, SCVs and PA.

Notes

13h40 - The role of derangements in lipid metabolism in lung inflammation and CFTR dysfunction in cystic fibrosis

DeSanctis J, Garic D, Youssef M, Kanagaratham C, Abu-Arish A, Wojewodka G, Saeed Z, Guilbault C, Shah J, Hajduch M, Hanrahan, J, Radzioch, Danuta*

McGill University and Research Institute of the McGill University Health Centre, Montreal

Cystic fibrosis (CF) patients display abnormalities in lipid metabolism that result in increased levels of arachidonic acid (AA) and decreased levels of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and very long chain ceramides (VLCCs) in blood and in the tissues most affected by the disease. Disruption of chloride and sodium transport and immuno-inflammatory changes in the respiratory tract, pancreas, gastrointestinal tract, and sweat glands are highly correlated with the abnormalities in lipid metabolism and are apparent even before CF lung disease is diagnosed. This implies a strong connection between CFTR dysfunction, lipid dysregulation and the immuno-inflammatory responses in the lung although the exact mechanisms are not yet elucidated.

We have developed a unique mouse model with spontaneous lung disease that resembles some aspects of human CF. We found that the uninfected CFTR k/o mice have epithelial cell hyperplasia, basement membrane thickening, and increased inflammatory cell infiltration into lung tissue when compared to uninfected wild-type (WT) control mice. The CF lung phenotype is age dependent as revealed by MMP-9 immunostaining and histological analysis of the lungs in both uninfected and *Pseudomonas aeruginosa* infected mice. We also found that these CF mice are more susceptible to *P. aeruginosa* infection compared to WT littermate controls.

We discovered that fenretinide normalizes lipid metabolism in mice that have been infected with *P. aeruginosa* CF mice and also in a mouse model of severe allergic asthma. Fenretinide treatment reduces inflammatory cell influx and excessive inflammatory mediator expression in the lungs of both CF and asthmatic mice. It also improves the resolution of inflammation and reduces excessive lipid and protein oxidation in lung tissue. *In vitro* studies of human airway epithelial cells indicate that fenretinide treatment together with zinc (Zn^{2+}) supplementation further improves the modulation of cytoplasmic phospholipases (cPLA2) activity and rebalances lipids in CF cells expressing F508del CFTR.

CFTR potentiators (e.g. ivacaftor/VX-770, which increases channel open probability) and correctors (lumicaftor/VX-809, tezacaftor/VX-661 and GLPG2222), offer hope for individuals with CF with at least one copy of F508del however they do not provide obvious clinical benefit for many patients. The reason for this variability in CFTR modulator efficacy is not known but may be related to the inability of correctors to normalize lipids and the correct immune-inflammatory response. Our current efforts are therefore focused on resolving the lipid imbalance problem and eliminating symptoms of chronic inflammation as they may improve the responsiveness of many patients to CF drugs.

14h00 - CFTR, mucins and neutrophils in cystic fibrosis airway defence

André M. Cantin, Ginette Bilodeau, Alexandre Cloutier

Service de pneumologie, Département de médecine, Faculté de médecine et des sciences de la santé, Université de Sherbrooke

When *cystic fibrosis transmembrane conductance regulator* (CFTR) decreases below 25% of normal, tissues composed of tubular structures in which mucus is secreted are at risk of mucostasis and damage. Mucus solids are composed mostly of mucins, proteins with a structure similar to bottle brushes in which the wire center is the amino acid core (20%) and the bristles are sugar polymers (80%) which define mucus viscosity. The 2 major secreted mucins in the airway are Muc5AC and Muc5B. Secreted mucins, particularly those from cystic fibrosis airways are rich in anionic residues such sialylated and sulphated sugars. The isoelectric point of mucins is very low at 2 or 3, meaning that at physiological pH Muc5AC and Muc5B form negatively charged polymers that may impact on cell transmembrane potential differences. Furthermore, the lack of CFTR decreases bicarbonate ion concentration in CF airway surface liquid. Both transmembrane potential difference and pH may impact on neutrophil function.

Obstruction of the airways by overly viscous mucus in persons with CF leads to chronic mucosal infection, neutrophil influx, neutrophil elastase release and subsequent structural damage known as bronchiectasis. Neutrophils are professional phagocytes and represent the most effective leukocyte in bacterial killing. Although neutrophils are markedly increased in the CF airways, bacteria thrive in their presence. This leads us to conclude that neutrophil bactericidal function must be abnormal in the CF airways. Abnormal neutrophil function reflects either an intrinsic defect associated with the lack of CFTR or an extrinsic defect that stems from the abnormal airway surface environment in the absence of epithelial cell CFTR, or both.

Previous investigators have identified intrinsic defects in CF neutrophils characterized by abnormalities in oxidative burst and degranulation. If an intrinsic neutrophil defect is responsible for infection, then tissues other than those of the respiratory tract should also be infected, a situation not observed in CF. We hypothesized that CF neutrophils isolated from peripheral blood have a normal capacity to kill bacteria, but lose these properties in an CFTR-deficient environment of concentrated mucins and depleted bicarbonate.

Neutrophils isolated from peripheral blood of patient with CF and healthy volunteers were stimulated with either opsonized zymosan or *Burkholderia cepacia* bacteria, and oxidative burst was measured by chemiluminescence. We also examined the neutrophils' capacity to kill *B. cepacia*. We found no difference in the oxidative burst between CF and non-CF neutrophils. Neutrophils from CF peripheral blood also killed *B. cepacia* as effectively as did neutrophils form healthy volunteers. In contrast, exposure of either CF or non-CF neutrophils to purified Muc5AC and Muc5B mucins markedly suppressed the oxidative burst. The oxidative burst was restored by the addition of cationic molecules, suggesting that the negative surface charge of mucins induces neutrophil membrane depolarization that prevents electrons from flowing through the NADPH oxidase complex to reduce oxygen to superoxide. Furthermore incubation of neutrophils in media depleted of bicarbonate also inhibited the oxidative burst. The addition of bicarbonate,

or the addition of fluid from the apical surface of Calu-3 cells in which CFTR was stimulated with dibutyryl cAMP restored the oxidative burst.

We conclude that although some CFTR-related defects in neutrophil function exist, they are relatively modest and do not induce a major bacterial killing defect ex vivo. In contrast, the CFTR-dependent airway surface fluid environment likely plays a critical role in neutrophil function and defines the efficacy of phagocytic oxidant synthesis and bacteria killing. CFTR deficiency creates an environment in which neutrophil oxidant release and bacterial killing are defective in respiratory mucus, akin to a mucosal form of NADPH oxidase deficiency.

14h20 - Identification du gène bactérien aprF comme modulateur de la réponse inflammatoire des cellules épithéliales humaines

Mellissa Gaudet et Simon Rousseau

Les laboratoires Meakins-Christie, IR-CUSM, Montréal

L'infection chronique des voies respiratoires des personnes atteintes de fibrose kystique (FK) par *Pseudomonas aeruginosa* (PA) est d'un grand intérêt en raison de la réponse inflammatoire dommageable induite par cette bactérie. La réponse inflammatoire induite par des souches de PA isolées dans un même poumon a été évaluée afin de mieux comprendre la dynamique d'interaction hôte-pathogène. Huit souches cliniques provenant de quatre patients FK ont été sélectionnées. Les quatre pairs de co-isolats étudiés ont induit des réponses inflammatoires différentielles dans des cellules épithéliales bronchiques humaines et dans un modèle *in vivo* d'infection avec le cnidaire *Hydra magnipapillata*. La parenté clonale des co-isolats a été confirmée à l'aide d'une analyse de typage génomique à multiples locus. Une analyse de polymorphismes nucléotidiques a permis d'identifier aprF comme un gène ayant influençant l'expression d'IL-8 dans les cellules épithéliales. La perte de fonction d'aprF diminue la dégradation de la flagelline ce qui résulte en une augmentation de l'activation du récepteur TLR5 et la synthèse d'IL-8. L'inactivation de TLR5 par la technologie CRISPR-Cas9, prévient cette augmentation d'IL-8 induite par la perte de fonction d'aprF. Ainsi, la perte de fonction d'aprF préviendrait l'exportation de la protéase alcaline responsable de la dégradation de la flagelline, causant une augmentation de cette dernière dans le milieu extracellulaire. Cette présence accrue de flagelline crée une suractivation de la voie TLR5 chez l'hôte, entraînant une réponse inflammatoire accrue. En conclusion, en utilisant une caractérisation de mutants naturels de PA retrouvés chez les personnes atteintes de FK, nous avons identifié un système bactérien important pour la modulation de la réponse inflammatoire via la dégradation de la flagelline.

Notes

14h40 - Contribution of the Janus-faced chaperones in peripheral protein quality control of CFTR

Miklos Bagdany,[1] Tsukasa Okiyoneda[1], Guido Veit,[1] Ryosuke Fukuda,[1] Imad Baaklini,[2] Jay Singh,[3] Haijin Xu,[1] Pirjo M. Apaja,[1] Ariel Roldan,[1] William Balch,[3] Jason C. Young,[2] and Gergely L. Lukacs,[1,2]

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Molecular chaperones are pivotal in folding and degradation of the cellular proteome at the endoplasmic reticulum (ER) and other subcellular locations, but their role in the protein homeostasis (proteostasis) of membrane proteins at the cell surface is incompletely understood (1). We have previously identified CHIP and RFFL, an Hsc70-dependent and chaperone-independent E3 ubiquitin ligases, respectively, that can recognize and eliminate partially unfolded ∆F508-CFTR, the most common CF mutation, from the PM for lysosomal degradation (2,3). This process can limit the efficacy of CFTR folding correctors. Intriguingly, the conformational stabilization of near-native and metastable membrane proteins with disease relevance by molecular chaperones remains unknown at the cell surface. Here we show that the thermally-induced unfolding at 37°C and concomitant functional inactivation of the temperature rescued ΔF508-CFTR can be partially suppressed by the constitutive activity of Hsc70 and Hsp90 chaperone/co-chaperone complexes at the PM and post-ER compartments in human bronchial epithelial cells (CFBE) and at the single molecule level after reconstitution the channel into phospholipid bilayer. Conformational remodeling of the mutant was confirmed by kinetic and thermodynamic remodeling of the ΔF508-CFTR gating energetic toward its wild-type counterpart. Thus, molecular chaperones are not only critical in facilitating misfolded membrane proteins degradation, but can simultaneously contribute to their functional maintenance at the cell surface proteome as well. This is exemplified by the chaperone activity that can reshape the conformational energetic of the ΔF508-CFTR final fold, a phenomenon with possible implications in the regulation of metastable ABC-transporters and other PM proteins activity in health and diseases.

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15h00 - Can combinations of correctors of F508del-CFTR trafficking provide an effective CF therapy?

Graeme W. Carlile[1,2], Elizabeth Matthes[1,3], John W. Hanrahan[1,2] & David Y. Thomas[1,2]

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There have been many advances in the detection and care of patients that have led to remarkable improvements in survival of patients, but almost 30 years after the discovery of the CFTR gene we do not have an effective therapy that effectively corrects the basic trafficking defect of F508del-CFTR.
Despite intensive research and development efforts the currently available therapies do not give high levels of correction and much of the mutant protein is retained in the endoplasmic reticulum (ER). In principle the required level of correction should be the same as for CFTR heterozygotes (50%) who are asymptomatic. Corrector compounds that have been identified in extensive cell base screens of chemical libraries and they fall into two classes, pharmacological chaperones and proteostasis modulators.
So far, the most effective compounds are pharmacological chaperones that bind to the mutant F508delCFTR protein and stabilize its folding and thus avoid its retention in the ER. However, even the most potent compounds, such as Vertex VX-809, do not correct all the ER-retained protein. We have identified new pharmacological chaperones that are additive in their correction abilities, with more that 30% of the mutant F508delCFTR protein corrected. These compounds bind to different sites on the CFTR molecule.
We have also identified a chemically heterogeneous group of proteostasis modulators that correct F508delCFTR trafficking but somewhat less efficiently, and act on pathways that determine protein quality control. These compounds include known drugs such as inhibitors of phosphodiesterases , kinases and cyclo-oxygenases and point to new strategies for developing more potent, clinically effective and cost-effective F508delCFTR drugs.

Supported by grants from the Canadian Institutes of Health Research and Cystic Fibrosis Canada

15h50 - CFTR Correction by Lumacaftor-Ivacaftor Effects on Stimulated Interleukin-8 in Cystic Fibrosis Bronchial Epithelial Cells

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Cystic Fibrosis (CF) is characterized by neutrophilic airway inflammation. Interleukin (IL)-8 is the primary neutrophilic chemoattractant produced by bronchial epithelial cells. Decreased CFTR function due to mutant CFTR has been thought to be the cause for excessive IL-8 production by CF cells. Recently there has been the commercial availability of CFTR correctors (Lumacaftor-Ivacaftor) for patients homozygous for the delF508 CFTR mutation. We hypothesized that correction with Lumacaftor-Ivacaftor would reduce stimulated IL-8 production in primary CF bronchial epithelial cells from delF508 homozygous patients. Our preliminary results with primary cells did not support our hypothesis. In this project we sought to further examine the effect of lumacaftor-ivacaftor in isogenic cell lines overexpressing either widltype or delF508 CFTR.
Methods: The CFBE41o- cell line overexpressing delF508 or wildtype CFTR, and the parental line were a kind gift from D. Gruenert. Cells were grown at the air-liquid interface conditions for 7 days. Cells were pre-treated with Lumacaftor-Ivacaftor (1/0.1 µM), dexamethasone (100 nM), or respective diluents for 18 hours prior to stimulation. At the stated concentrations, Lumacaftor-Ivacaftor augmented CFTR function (short-circuit current) in cells grown at the air-liquid interface. After treatment, cells were stimulated for 24 hours with either flagellin (Invivogen) or IL-1β (BD Biosciences) at the basolateral surface. Following stimulation, basolateral cell supernatants were analyzed for IL-8 levels (ELISA). Alternatively, cell lysates were analyzed for expression of mature CFTR band C (Western Blot; prior to stimulation) or for IL-8 mRNA expression (qPCR; 8 hours of stimulation). Results of these experiments will be presented at the meeting.

Funded by: Cystic Fibrosis Foundation, Kreible Foundation, Verona Pharmaceuticals

16h10 - Precision medicine for cystic fibrosis (CF): Validation by analysis of variance and implications for prospective assays

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[3]*Department of Physiology, McGill University, Montreal*

The variable clinical benefit and high cost of CF drugs provide compelling medical and economic arguments for precision medicine approaches in CF. The rationale will be further strengthened when new medications become available as it may be possible to choose the best drugs for individual patients. A premise of precision medicine is that the variation in clinical benefit provided to different patients by a CF drug results from variable correction of mutated CFTR at the cellular level. Another premise is that correction can be assayed reliably enough *in vitro* to prospectively determine which drug an individual should receive. At present there is little support for either of these assumptions. We examined the functional rescue of F508del-CFTR in primary bronchial epithelial cells from 22 patients homozygous for this mutation. Using the "gold standard" short-circuit current assay of CFTR-dependent secretion across well-differentiated bronchial epithelial cells, we determined the relative contributions of CFTR modulators (VX-809+potentiator) and patient-patient variability to the rescue of CFTR functional expression. We found that drug treatment accounts for most (51%) of the variability in F508del CFTR rescue as expected, and differences between patients contribute another 19% and are also a highly significant source of variation ($p<0.001$). This inter-patient variation supports the application of precision medicine approaches in CF. However, repeated analyses of cryopreserved cells from selected patients under identical conditions (31-49 times over 8-14 months) revealed intra-patient variation in drug responses that was also highly significant (14% of total variance, $p<0.001$). While these results validate the development of precision medicine for CF in theory, they also show that the feasibility of prospective testing will depend critically on the efficacy of the drugs and statistical properties of the *in vitro* assay.

Supported by CF Canada, CIHR, CFI and McGill Faculty of Medicine

Notes

16h30 - The YKL-40 project: what we learn from since 2014

Bouvet, G. F.[1], Coriati A.[1], Bulka O.[1], Sognigbé L[1], Ménard A.[1], Beaudoin N.[12], Massé C.[1], Lavoie-Pilote A.M.[1], Chou Y.-C.[2], Letendre V.[2], Brindle-Tessier A.[2], Ducruet T.[3], Jouquan A.[1] and Berthiaume Y[1].

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Using a system biology approach, we found that the chitinase-3-like-1 gene (CHI3L1) is overexpressed in CF epithelial cells. This gene, which codes for the circulating protein YKL-40, is a biomarker of various inflammatory diseases, including diabetes and lung disease. *We hypothesize that YKL-40, released by inflammatory and/or epithelial cells, is involved in the CF-inflammatory response.* Our main objective was to investigate the role of circulating YKL-40 in CF pathophysiology. In a cohort of 186 CF patients, we explore the association between circulating YKL-40 and the evolution of CF disease. First, we studied if specific variants (SNPs) of CHI3L1 or different CF patients' phenotype correlate with the circulating levels of YKL-40 in CF patients**.** Second, we measured YKL-40 concentration overtime (21 months) to determine if it can be used as a biomarker of CF disease evolution. To better understand the biological involvement of YKL-40 in the CF-inflammatory response, we studied the impact of YKL-40 on immune cells since YKL-40 is known to modulate both the inflammatory and the adaptive immune response. Our results show that YKL-40 modulates *(i)* the gene expression profile of CF PBMCs and that *(ii)* Galectin-3 (GAL-3), a molecule involved in the fibrosis response, is a binding partner for YKL-40. Overall these studies show that YKL-40 might play a role in the inflammatory response associated with CF.

16h50 - CFRD: Pathophysiology exploration and current treatment

Rémi Rabasa-Lhoret

IRCM, Université de Montréal

Cystic fibrosis-related diabetes (CFRD) has become one of the most common complications in cystic fibrosis (CF), affecting approximately 10% of children and up to 50% of adults. In this session, we will discuss physiological factors leading to its appearance. CFRD occurrence can be associated with reduced growth, delayed puberty, weight loss in adults and reduced pulmonary function. It is believed that high glucose and/or low insulin could favour pulmonary exacerbation and weight loss. Screening test and early treatment are essential to allow early treatment. The exact course of progression from normal glucose tolerance to impaired glucose tolerance and finally to CFRD is not linear and glucose tolerance can fluctuate a lot before established CFRD. Among the possible factors responsible for glucose intolerance appearance, we can note CFTR mutation, chronic inflammation, pancreas destruction, type 2 diabetes modifier genes and possibly insulin resistance. CFRD frequently requires insulin treatment, which is the only option that has shown positive effect on weight and/or lung function. However, insulin injections add a significant constraint to the already complex disease management. In this perspective, ongoing studies are focusing on alternative therapies including lifestyle and oral hypoglycemic agents. Specific CF therapies such as CFTR correctors and potentiators could also improve insulin secretion and glucose tolerance. Among key unanswered questions are when to initiate treatment and for which reason (prevention of diabetes complications vs. lung protection and/or weight maintenance)?

17h10 - Clinical impact of hyperglycemia in CF patients

Adèle Coriati

St. Michael's Hospital, Toronto

Glucose abnormality, the most common secondary complication in CF, is primarily caused by a defect in insulin secretion. Accelerated clinical deterioration (loss of weight and lung function) is observed before diagnosis of CFRD. A number of risk factors associated to CFRD development have been identified and characterized including pancreatic insufficiency, delF508 mutation, lung infection and sex. The standard method used to diagnose and study the pathophysiology of CFRD is the 2 hour oral glucose tolerance test (OGTT). Based on the intermediate glucose values during the OGTT, patients are classified as normal, indeterminate, intolerant or CFRD. The newly established glucose tolerance group named indeterminate (INDET: 60-min OGTT > 11.0 mmol/L but 2h-OGTT < 7.8 mmol/L) is at high risk of developing CFRD. It was recently shown that the INDET group display lowered lung function comparable to the newly diagnosed CFRD group. The 60-min OGTT glucose value has been identified as a good marker of disease severity. In fact, an association has been reported between high blood sugar at 60-min of the OGTT and decreased lung function in a pediatric CF population. A later study showed that, in the adult CF population, a negative association exist between blood glucose levels at 60-min of the OGTT and lung function. Moreover, a positive correlation is present between insulin at 60-min of the OGTT and body mass index (BMI). In both the airways of the general and CF populations, detectable levels of glucose is found in the mucus when blood glucose is above 8 mmol/L, which is a favourable condition for bacterial growth. One emerging microorganism in CF known as *Stenotrophomonas Maltophilia* was identified as a disease severity marker in dysglycemic patients. Heightened inflammation is observed in CF patients and even more in dysglycemic CF patients. Numerous inflammatory markers such as the YKL-40 protein are increased in the systemic circulation of CFRD patients and associated to worse lung function. High variability in OGTT results is frequently observed in CF patients over the years. This occurrence can be partly explained by the mild variation in insulin sensitivity. It is crucial to elucidate the important pathophysiological mechanisms involved in order to better predict the occurrence of clinical deterioration before CFRD onset.

17h30 - Current and emerging CFRD screening methods

Valérie Boudreau

IRCM, Université de Montréal

Because of its high prevalence and possible adverse consequences on CF health (e.g. weight loss and/or reduced pulmonary function), it is currently advised to screen for CFRD annually by the age of 10 years. The currently recommended screening test following an expert consensus is the 2-hour oral glucose tolerance test (OGTT). Thresholds to establish glucose intolerance for this test are based on risk of diabetes retinopathy in patients with type 2 diabetes. As OGTT is a test requiring time and efforts from both healthcare teams and patients, many research groups have been studying alternatives to the OGTT. Unfortunately, fasting blood glucose and glycated hemoglobin (a biomarker of mean glucose over 3 months) lack in sensitivity in patients with CF. New technologies like continuous glucose monitoring systems are promising because they provide real life conditions data for a hole week or more. However, diagnostic thresholds with such device are debated. Several questions remain about whether the threshold for diabetes diagnosis should be based on diabetes complications or on possibly more relevant CF complications such as the risk of weight loss and/or lung function reduction. In addition, many patients express their reluctance to the current screening test. The OGTT is associated with some side effects (e.g. nausea), it takes time and requires a prolonged fasting period, not to mention the fear of being diabetic for many patients. This presentation focuses on current and emerging CFRD screening methods.

Notes

Posters / Affiches

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1 – Print-OMICS: next generation 3D bioprinting of normal and cystic fibrosis human lung tissues
Maxime Bourrelle-Langlois

2 – Impact of *Pseudomonas aeruginosa* and *Staphylococcus aureus co*-colonization in a murine lung infection model
Guillaume Millette

3 – The dual phosphodiesterase 3/4 inhibitor, RPL554, stimulates rare class III and IV CFTR mutants
Mark Turner

4 – Les canaux potassiques comme cibles thérapeutiques dans la réparation de l'épithélium respiratoire fibrose kystique humain en présence d'exoproduits de *Pseudomonas aeruginosa*
Laura Sognigbé

5 – L'activation des canaux potassiques KvLQT1 améliore la régénération de l'épithélium des voies aériennes en fibrose kystique
Damien Adam

6 – SLC26A9 is prematurely degraded with the cystic fibrosis transmembrane regulator (CFTR) mutant F508del-CFTR.
Yukiko Sato

7 – Oxidant stress increases susceptibility of cystic fibrosis human bronchial epithelial cells to air pollutant particulate matter
Victor Dumitru

8 – Mechanism of the antibiotic action of the combination tomatidine and aminoglycoside against *Staphylococcus aureus* virulent and persistent phenotypes
Jean-Philippe Langlois

9 – Intracellular *Pseudomonas aeruginosa* persistence in cystic fibrosis airway epithelial cells
Emmanuel Faure

10 – Tomatidine and FC04-100 as lead antibiotic molecules against *Staphylococcus aureus* and its small colony variant
Charles Isabelle

11 – Identification of *Pseudomonas aeruginosa* genetic variants in chronic lung infections of cystic fibrosis patients and their effect on lung disease severity
Jannik Donner

12 – Interleukin-6 trans-signalling in pulmonary exacerbations in cystic fibrosis
John Lin

13 – Microrheology of mucin granules in CF and non-CF human bronchial epithelial cells
Olga Ponomarchuk

14 – The Primary Airway Cell Biobank (PACB), an international resource for CF research
Julie Goepp

15 – Role of PDZ domain binding in CFTR aggregation at the cell surface
Asmahan Abu-Arish

16 – Exploring the role of intracellular *Pseudomonas* in the establishment of chronic CF lung infections
Lisa Hennemann

17 – SENBIOTAR: Sensitizing *Pseudomonas aeruginosa* biofilms to antibiotics and reducing virulence through novel target inhibition in cystic fibrosis lungs
Irena Kukavica-Ibrulj

18 – Genomic diversity of *Pseudomonas aeruginosa* in the environment and in cystic fibrosis
Jean-Guillaume Emond-Rheault

19 – *Pseudomonas aeruginosa*-induced intracellular infection promotes inflammation and neutrophil adhesion on bronchial epithelial cells
Perrine Bortolotti

20 – The main mechanism associated with progression of glucose intolerance in older patients with cystic fibrosis is insulin resistance and not reduced insulin secretion capacity
Johann Colomba

21 – *Pseudomonas aeruginosa* evasion of neutrophil phagocytosis and bacterial clearance in early cystic fibrosis lung infection
Kelly Kwong

Posters / Affiches

1 – Print-OMICS: next generation 3D bioprinting of normal and cystic fibrosis human lung tissues

Maxime Bourrelle-Langlois, Iréna Kukavica-Ibrulj, Brian Boyle and Roger C. Lévesque

Institut de Biologie Intégrative et des Systèmes (IBIS), Département de microbiologie, immunologie et infectiologie, Faculté de médecine, Université Laval, Québec

3D bioprinting has emerged as a promising new technology for standardized and reproducible construction of stratified, multi- and pluricellular *ex vivo* tissue models. Bioprinting allows accurate dispensing of the desired human cells in the appropriate bioink ultimately mimicking the *in vivo* environment. The resulting 3D complex architecture and cellular heterogeneity offer near *in vivo* conditions for cell-cell and cell-extracellular matrix (ECM) interactions which promote proper cell and tissue differentiation. Therefore, bioprinted tissues offer improved *ex vivo/in vitro* models that could now be used for high-throughput assays in genomics, transcriptomics and proteomics, and leading to massive data acquisition of greater biological significance. In several cases data acquired through animal testing have shown to be non-transferable to humans due to genotypic and phenotypic differences. In addition, classical cell culture lacks the complexity and tissue-like structure relevant to detailed biological analysis, not necessarily predictive in treatment and providing poor clinical outcomes. In our CF studies, 3D bioprinting of human lung tissues will be performed using CELLINK's third-generation printer, the Bio X. The alveolar tissue region of the lung will be printed using appropriate human lung cell lines and primary cells for the formation of the stroma and epithelium. In agreement with the human lung transcriptome, transcriptomic profiling of individual and assembled cell lines will be performed to demonstrate the relevance of such an engineered tissue. Moreover, uninfected and infected lung constructs with typical bacterial strains will be treated with Tobramycin, mimicking CF therapy, as well as non-toxic novel quorum sensing inhibitors. To establish the printed lung tissue response to molecules alone, infection and treatment, the classical histological, immune and tissue viability assays will be combined with a genomics-based RNA-Seq. AmpliSeq measures the expression levels of over 20,000 genes covering 95% of human transcripts giving an intimate knowledge of expressed genes. The repertoire of differentially expressed genes will be compared to normal and CF tissues in the lung Expression Atlas. Furthermore, data mining for levels of expression, SNPs, gene organization and rearrangements will be pursued. Ultimately, 3D bioprinting lung tissues using human airway cell lines and primary cells will lead to functional near-native microanatomy providing powerful means for *ex vivo* modeling of tissue morphogenesis and organogenesis.

2 - Impact of *Pseudomonas aeruginosa* and *Staphylococcus aureus co*-colonization in a murine lung infection model

Millette G., Fugère A., Brouillette E., Cantin A., Malouin F.

Biologie, Sciences, Université de Sherbrooke, Sherbrooke

Staphylococcus aureus (SA) and *P. aeruginosa* (PA) are the two most frequent 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 seen by each individual pathogen infections, 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 cell 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 (n=7). While some SA-PA pairs behaved as prototypical strains and affected SA growth, some pairs did not display such antagonism. Overall, the co-isolated SA-PA pairs showed various degrees of antagonism or even indifference *in vitro*. 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 non-antagonistic co-isolates in a murine lung infection model, a significant increase in SA colonization was observed when lungs were co-infected with PA. Remarkably, an antagonistic PA *in vitro* increased SA colonization even more *in vivo*. Upon compiling all the *in vivo* data, the following relation was established; the more PA colonizes the murine lungs, the more SA colonization is enhanced. Thus, regardless if PA is antagonistic or not *in vitro*, it promotes SA colonization *in vivo*; this could explain the deleterious effect of PA-SA co-infection on CF patient health.

Posters / Affiches

3 – The dual phosphodiesterase 3/4 inhibitor, RPL554, stimulates rare class III and IV CFTR mutants

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Cyclic nucleotide phosphodiesterases (PDEs) are a large family of enzymes responsible for breaking down the cyclic nucleotides cAMP and cGMP. PDE inhibitors can be used to elevate intracellular cAMP levels and thus promote cAMP-dependent downstream signalling processes, including the cAMP/PKA-dependent stimulation of CFTR. The PDE family consists of 11 different members in humans (PDE1-11), most of which have multiple isoforms that are in distinct subcellular compartments and expressed in different tissues. Thus, specific PDEs regulate cyclic nucleotide-dependent processes in different cell types. We, and others, have identified that, in human airway epithelia, PDE4D is the predominant enzyme that terminates cAMP-dependent stimulation of CFTR in human airway epithelia. We found that the dual PDE3/4 inhibitor RPL554 (Verona Pharma plc) stimulates wild-type CFTR and the CF-causing Class IV mutant R117H CFTR in primary human bronchial epithelial cell cultures (HBEC), indicating it may have therapeutic potential for CF patients with this mutation. We also assessed whether RPL554 might serve as a potential therapeutic for other CFTR mutants such as Class III and Class IV mutants that have defects in channel gating or conductance, respectively. We studied Fischer Rat Thyroid cells (FRT) made to stably express heterologous wild-type human CFTR, R347P, T338I, R334W, S549R or G551D. Cells were grown as polarized monolayers and CFTR-dependent ion transport was quantified by measuring short-circuit current (I_{sc}) in Ussing Chambers. RPL554 significantly enhanced forskolin-stimulated I_{sc} by 7.9 ± 0.7 µA cm^{-2} and 2.9 ± 0.3 µA cm^{-2} in cells that expressed T338I and R334W CFTR respectively; demonstrating that RPL554 positively regulates these CFTR mutants. Although we observed neither forskolin nor RPL554 to have any significant effect on S549R or G551D CFTR activity, when cells expressing these mutants were pretreated with VX809 (1 µM) and VX770 (100 nM) for 24 h, RPL554 did enhance forskolin-stimulated, CFTR-dependent I_{sc} activity by 21.5 ± 4.3 µA cm^{-2} and 40.2 ± 8.4 µA cm^{-2} in S549R and G551D-expressing cells respectively; indicating that functional expression of these mutants is positively regulated by RPL554. Together, these data indicate that RPL554 can stimulate Class III and Class IV mutants in addition to R117H CFTR and implicate it as an anti-CF drug that can be administered alone or in combination with Orkambi to many patients who possess different CFTR mutations.

Posters / Affiches

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4 – Les canaux potassiques comme cibles thérapeutiques dans la réparation de l'épithélium respiratoire fibrose kystique humain en présence d'exoproduits de *Pseudomonas aeruginosa*

Laura Sognigbé[1], Bilodeau C[1], Maillée E[1], Merjaneh M[1], Nguyen D[2], Coraux C[3], Adam D[1], Brochiero E[1]

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Introduction: En fibrose kystique (FK), due à des mutations du canal CFTR, les infections chroniques à *Pseudomonas aeruginosa* (*P. a.*) des voies aériennes (VA) entraînent leur destruction progressive. De plus, nos travaux ont démontré que l'épithélium FK présente un retard de réparation, due au défaut de CFTR et à l'infection mais que l'activation des canaux potassiques KvLQT1 stimule la réparation de l'épithelium des VA. Notre but était donc d'étudier le rôle de KvLQT1 dans la réparation épitheliale, en présence ou non d'infection.

Méthodes: La vitesse de réparation a été évaluée en vidéomicroscopie dans des modèles de culture primaire de cellules des VA (hAEC) FK et non-FK en 2D et 3D suite aux traitements avec un activateur de KvLQT1 (R-L3) et/ou des modulateurs de CFTR; en présence ou non d'exoproduits de *P. a.*

Résultats: Nous avons d'abord confirmé que les canaux KvLQT1 sont exprimés dans les hAEC non-FK et FK et que la présence d'exoproduits de *P.a* n'affecte pas leur niveau d'expression. Dans des cultures hAEC non-FK, l'activation de KvLQT1 améliore significativement la vitesse de réparation; cet effet est renversé par l'inhibiteur du KvLQT1. Le R-L3 améliore également la réparation épithéliale FK, malgré la présence d'infection. Un effet synergique est observé avec des co-traitements au RL-3 et modulateurs de CFTR.

Discussion/Conclusion: Nos données mettent en évidence le rôle bénéfique de l'activation des canaux KvLQT1 dans la réparation de l'épithélium des VA non-FK et FK, malgré la présence d'infection. Des traitements combinés avec des modulateurs des canaux CFTR et K^+ pourraient ainsi être une stratégie intéressante pour favoriser la réparation de l'épithélium respiratoire FK.

Financements: Association Vaincre La Mucoviscidose, Respiratory Health Network of the Fonds de Recherche du Québec en Santé (FRQS) and the Canadian Institutes of Health Research (CIHR).

5 – L'activation des canaux potassiques KvLQT1 améliore la régénération de l'épithélium des voies aériennes en fibrose kystique

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Objectifs: Le déclin respiratoire lié au dommage progressif de l'épithélium des voies aériennes est la principale cause de mortalité chez les patients atteints de fibrose kystique (FK), due aux mutations du canal CFTR. Nous avons montré que les processus de régénération sont retardés en FK et conduisent à la restauration d'un épithélium anormal. Notre but était donc de développer des stratégies favorisant la régénération de l'épithélium des voies aériennes FK en ciblant les canaux potassiques KvLQT1.

Méthodes: La cinétique de régénération été évaluée non seulement *in vitro* sur des cultures de cellules primaires épithéliales humaines non-FK et FK différenciées en interface air-liquide (IAL, quantification des cellules ciliées par un immunomarquage de la tubuline-βIV) mais également dans le modèle de xénogreffe bronchique humanisée dans la souris *nude* (analyses histologiques). La régénération a été étudiée en présence de traitements pharmacologiques (inhibiteur de KvLQT1: clofilium; activateur: R-L3).

Résultats: Le traitement chronique par le R-L3, tant dans les cultures en IAL que dans le modèle de xénogreffe, favorise la régénération d'un épithélium différencié (accélération de la différenciation des cellules ciliées) et fonctionnel (localisation membranaire de CFTR). Ces phénomènes sont inhibés par le traitement au clofilium, indiquant un rôle de ce canal dans la différenciation et régénération épithéliale.

Discussion/Conclusion: Nos résultats démontrent que l'activation de KvLQT1 a des effets pro-régénérateurs et anti-remodelant. Ce canal pourrait donc être identifié comme cible thérapeutique intéressante pour favoriser les processus de régénération de l'épithélium des voies aériennes FK.

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6 – SLC26A9 is prematurely degraded with the cystic fibrosis transmembrane regulator (CFTR) mutant F508del-CFTR.

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Currently available drugs for Cystic Fibrosis (CF) that correct F508del-CFTR misfolding provide only modest clinical benefit, therefore other anion channels such as SLC26A9 are being explored as potential therapeutic targets. SLC26A9 is constitutively active however its channel function is modulated by CFTR and its conductance is significantly reduced in cells that express F508del-CFTR. SLC26A9 has been hypothesized to interact with the regulatory (R) domain of WT-CFTR through its Sulphate Transporter AntiSigma factor antagonist (STAS) domain, however the interaction site is not known. It is also uncertain whether a similar interaction with F508del-CFTR causes retention of SLC26A9 in the endoplasmic reticulum and leads to premature degradation by the proteasome. BHK cells overexpressing wild-type (WT) or F508del-CFTR and parental BHK cells devoid of CFTR were transiently transfected with SLC26A9 cDNA. Total SLC26A9 protein expression was assessed by immunoblotting cell lysates and its expression at the plasma membrane was assessed by cell surface biotinylation assays. The amount of SLC26A9 protein in whole cell lysates was reduced in cells co-expressing F508del-CFTR when compared with those expressing WT-CFTR. SLC26A9 surface expression was also reduced by 4-fold in F508del-CFTR cells compared to either WT-CFTR cells or control parental BHK cells that lack CFTR. This strongly suggests that F508del-CFTR has a dominant-negative effect on SLC26A9 expression. In addition, SLC26A9 expression in whole cell lysates was 1.5-fold higher in WT-CFTR expressing cells relative to parental BHK cells, and this difference was further enhanced by cAMP/PKA stimulation. Partial correction of F508del-CFTR trafficking by incubation at low temperature or by pre-treatment with the corrector VX-809 elevated both total and cell surface expression of SLC26A9. Expression of SLC26A9 in F508del-CFTR cells was also rescued when cells were co-transfected with WT- CFTR cDNA. Preliminary data indicate that this rescue could be mimicked by co-expression of only the R domain of CFTR. Finally, we also found that inhibiting the proteasome pathway increased SLC26A9 whole cell expression in parental BHK cells, as observed for F508del-CFTR. These results suggest that plasma membrane expression of SLC26A9 depends on the trafficking and surface expression of CFTR, and support the notion that CFTR and SLC26A9 physically interact through the phosphorylated R domain of CFTR. The interaction of SLC26A9 with F508del-CFTR apparently increases ER-associated degradation of SLC26A9 in CF cells. Understanding the mechanism of this interaction will be important for the future development of SLC26A9 as a therapeutic target. The goal is to use high throughput screening to identify compounds that disrupt this interaction, thereby increasing SLC26A9 delivery to the plasma membrane where it may function as an alternative Cl^- pathway in CF patients.

7 – Oxidant stress increases susceptibility of cystic fibrosis human bronchial epithelial cells to air pollutant particulate matter

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Introduction: Cystic fibrosis (CF) is a disease in which mutations of the gene coding for the ion channel cystic fibrosis transmembrane conductance regulator (CFTR) cause subsequent deficiencies in chloride secretion, mucus hydration, and mucociliary clearance in the lung. Exposure to particulate matter (PM) from air pollution is associated with exacerbations of symptoms in CF patients. However, the impact of PM on CFTR expression and function, along with its mechanisms of action are poorly understood. 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. It is also known that oxidative stress decreases CFTR expression. An oxidant stressor is predicted to exacerbate the effects of PM exposure with a greater effect on susceptible CF cells.

Methods: We used both polarized, immortalized CF bronchial epithelial and primary human bronchial epithelial cell lines. We used standard reference material 1648 for overnight PM exposure. Our conditions of exposure included vehicle, 300µg/cm^2 PM alone, 1mM H_2O_2,10mM H_2O_2, PM combined with 1mM H_2O_2, and PM combined with 10mM H_2O_2. We measured expression of CFTR at the level of whole cell protein using Western blot and at the transcriptional level using qPCR. We measured function of CFTR using Ussing chambers with a chloride gradient where we compared changes in forskolin-stimulated short circuit current (I_{sc}).

Results and conclusions: Combining PM and H_2O_2 exposures resulted in decreased forskolin-stimulated ΔI_{sc} compared to either exposure alone or to vehicle control. In drug-corrected CF cells, these same combinations resulted in traces comparable to epithelial membranes that are no longer intact indicating that CF cells are more susceptible compared to non-CF cells. Western blotting showed that non-CF cells have no change in CFTR protein expression with any exposure. However, CF cells had decreases in protein expression once again indicating that CF cells are more susceptible. Preliminary results from ongoing qPCR experiments show a decrease in CFTR mRNA expression in CF cells when exposed to PM. Increased negative effects on CFTR caused by PM in the presence of oxidative stress is relevant for susceptible populations such as those with CF.

8 – Mechanism of the antibiotic action of the combination tomatidine and aminoglycoside against *Staphylococcus aureus* virulent and persistent phenotypes

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Amongst all the pathogens recovered from cystic fibrosis (CF) patients, *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) are the two most prevalent. SA and PA co-infections are frequent and associated with a worse outcome for the patients, compared to patients infected with one pathogen or the other. Those patients are hospitalized more frequently, have a decreased pulmonary function and have an increased mortality and morbidity rate. During lung infections, SA can adopt two distinct phenotypes, a prototypical (WT) and that of a small colony variant (SCV). The first phenotype is the virulent form of the pathogen, while the second is the persistent phenotype. The SCVs are characterized by a deficient electron transport chain (ETC), which occurs by mutations. In addition, the presence of an ETC inhibitor, like PA's quorum sensing metabolite 4-hydroxy-2-heptylquinoline N-oxide (HQNO), will also provoke emergence of the SCV phenotype.
Currently, CF patients are treated with aminoglycoside in prophylaxis. This antibiotic is efficient against PA, but is not optimal to control SA population. In fact, SCVs are resistant to aminoglycosides due to their deficient ETC. Furthermore, aminoglycoside treatment selects the SCV phenotype and promotes persistence of SA. There is a need for an efficient way to control both PA, SA and SCVs to reduce the bacterial burden of CF patients. We have shown that a new antibiotic molecule, tomatidine, a steroidal alkaloid, is highly efficient against SCVs and also against the normal SA phenotype when in combination to an aminoglycoside.
Recently, our lab uncovered the molecular target of tomatidine, the subunit c of the ATP synthase, encoded by the *atpE* gene. We obtained several resistant SCV strains which were mutants in *atpE*. These mutants had however a significantly lower ATP production, compared to the SCV parental strain. Therefore, the mechanism of action of the tomatidine is, for the most part, known, but the mechanism of action of the synergistic effect between tomatidine and gentamicin against prototypical strains is yet unidentified. This project aims at elucidating the mechanism of action of the synergy between tomatidine and aminoglycosides. To do so, the different phenotypes emerging from the mutations associated with resistance to tomatidine and to the combinaison of tomatidine and an aminoglycoside, such as membrane hydrophobicity and membrane potential, will be evaluated. Since one of our hypothesis is that tomatidine facilitates the uptake of gentamicin, an aminoglycoside assay will be performed. Furthermore, The implication of the overproduction of ROS in the killing of SCVs will be verified. Thus, we seek a better understanding of the mechanism of action of tomatidine with or without the addition of aminoglycosides to develop an efficient therapy against PA and both the WT and SCV phenotypes of SA.

9 – Intracellular *Pseudomonas aeruginosa* persistence in Cystic Fibrosis airway epithelial cells

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Rationale: Cystic Fibrosis (CF) lung disease is characterized by chronic *Pseudomonas aeruginosa* infections in airways. Why *P. aeruginosa* persists despite antimicrobial and activation of immune responses remains incompletely understood. To date, the prevailing view suggests that impaired muco-cilliary clearance and biofilm lifestyle are the primary mechanisms contributing to the persistence in CF lung. Although *P. aeruginosa* is an extracellular pathogen, previous *in vitro* studies have reported that it can be internalized in several different human epithelial cells. This led us to hypothesize that *P. aeruginosa* is internalized and persists in airway epithelial cells, contributing to *P. aeruginosa's* evasion from extracellular antimicrobial therapies and host defense.

Methods: We have developed an *in vitro* model of long-term intracellular persistence of *P. aeruginosa* in human airway epithelial cells, using several cell lines (BEAS-2B, CFBEwt and CFBEΔF508), and have tested different *P. aeruginosa* strains (PAO1 lab strain and isogenic mutants, CHA clinical isolate). Epithelial cells are grown as immersed monolayers, or polarized at the air-liquid interface, and incubated with *P. aeruginosa* at MOI 1 during 4 hours. For the following 5 days, epithelial cells are treated with tobramycin to maintain the extracellular culture milieu sterile. Intracellular bacterial burden was measured at different time points by plate counting of CFU. We assessed cell death by LDH assay. Confocal microscopy was used to localize and count intracellular *P. aeruginosa*. Finally, we use MiPACT (Microbial identification after Passive CLARITY Technique), a technique that allows direct visualization of *P. aeruginosa,* using targeted antibody or RNA probe, in "cleared" lung explant tissues from CF patients, creating a three-dimensional biogeographical map of the lung tissue and the infecting pathogens.

Results: We showed that *P. aeruginosa* persists and replicates in BEAS-2B and CFBE cells over 5 days without causing significant host cell cytotoxicity in polarized and immersed cells. Approximately 5 to 10% of the initial bacterial inoculum of PAO1 strain is internalized by epithelial cells. Moreover, bacterial persistence is increased ~ 2-fold in CFBEΔF508 cells compared to CFBEwt. The proportion of infected cell is similar in CFBEwt and CFBEΔF508 cells (about 5%), but the intracellular bacterial count is increased in CFBEΔF508 cells compared to CFBEwt (average of 5.8 vs 2.5 bacteria per infected cell respectively). Intracellular bacterial persistence is increased with a non-flagellated *P.aeruginosa fliC* mutant as well as in the absence of TLR5 signaling (BEAS-2B TLR5 KO), and *P.aeruginosa* co-localizes with LAMP-1, a specific lysosomal pattern. Notably, treatment with CFTR targeted therapies decreases the intracellular bacterial burden in CFBEΔF508 cells. Finally, using MiPACT, we showed a positive intracellular staining of *P. aeruginosa* in airway epithelial cells from CF lung explants.

Conclusion: Our *in vitro* model suggests that *P. aeruginosa* is internalized and persists more readily in CF than non-CF airway epithelial cell. The possibility that *P. aeruginosa* may have an intracellular niche within the CF lung highlights a potentially new mechanism of bacterial persistence in CF lung disease.

10 – Tomatidine and FC04-100 as lead antibiotic molecules against *Staphylococcus aureus* and its small colony variant

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Staphylococcus aureus (SA) and *Pseudomonas aeruginosa* (PA) are the most prevalent lung pathogens 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. Tomatidine (TO), a steroidal alkaloid, is a potent bactericidal agent against SCVs, although it lacks antibacterial activity against the normal phenotype of SA. However, FC04-100, a TO derivative, also possesses activity against non-SCV strains and prevents high-level resistance development in prototypic strains and SCVs. Also, TO and FC04-100 produce a significant synergic antibiotic effect with aminoglycosides against prototypical strains. Since CF patients are often subjected to inhaled aminoglycoside (tobramycin) therapy to control PA infections, a combination treatment with steroidal alkaloids such as TO or FC04-100 appears attractive to tackle all three pathogens: PA (with the aminoglycoside), prototypical SA (with the synergy of the aminoglycoside and TO) and its SCV phenotype (with TO).Furthermore, using genomic analysis of *in vitro*-generated TO/FC04-100-resistant SA strains to identify mutations in genes involved in resistance, we identified the bacterial ATP synthase as the cellular target. An ATP synthesis assay allowed the observation of a correlation between antibiotic potency and ATP synthase inhibition.
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 ATP synthase 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 structure–activity relationship (SAR), the MIC of TO derivatives against Δ*hemB* were determined by a microdilution method in 96-well plates and then used in the ATP synthase assay. The IC50 of TO and FC04-100 for the Δ*hemB* membrane vesicles were 18.5±1.9 µg/mL and 18.9±3.6 µg/mL, respectively. The selectivity index (inhibition of ATP production by bacterial ATP synthase versus that of mitochondria) is estimated to be $>10^5$ for FC04-100. Overall, we showed that FC04-100 is a possible candidate for the developement a new antibiotic against pathogens that colonize CF lungs (both PA, SA and SCVs), especially when used in combination with aminoglycosides.

Notes

11 – Identification of *Pseudomonas aeruginosa* genetic variants in chronic lung infections of cystic fibrosis patients and their effect on lung disease severity

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Nearly 60% of adult CF patients are chronically infected by *Pseudomonas aeruginosa* (*Pae*) in their lifetime. Lung disease upon chronic infection is mainly caused by direct virulence of the infecting bacterial pathogens, and the presence of an excessive and injurious inflammatory response. Over time, progressive lung damage impairs its function to the point where lung transplantation becomes necessary. Interestingly, the rate of progression in CF lung disease is highly heterogeneous between patients. Even within the same patient, individual lungs can show significant regional variation in tissue damage and disease severity, which cannot be attributed to host genetics or environmental factors alone. Recent work indicates that regional disease heterogeneity could be due to the microevolution of *Pae* in the CF lung, as region-specific clonal *Pae* populations can differ markedly in phenotype, virulence, antibiotic resistance and immunogenicity.
Until now, the genetic variation of *Pae* in chronic lung infections has mostly been studied by whole-genome sequencing of single *Pae* isolates isolated from sputum samples. However, such approaches are limited to the analysis of only few *Pae* clones per patient and most likely overlook the extensive genetic heterogeneity present. Moreover, single genetic variants identified in *Pae* CF sputum cannot be directly linked to lung disease severity at the histopathological level because it is unknown where in the lung the *Pae* clones originate from.
We hypothesize that highly virulent *Pae* variants are present in severely damaged tissues, but not in mildly diseased ones, and that these cause more severe lung pathology due to increased cytotoxicity or inflammation.
In order to study the genetic diversity of *Pae* in chronic CF lung infection and to assess the effect of *Pae* variant populations on lung damage severity, we have collected tissue and mucus samples from whole lung explants obtained from CF patients with end-stage lung disease undergoing lung transplantation. The regional severity of lung disease was assessed based on clinical radiographic chest imaging and gross anatomical appearance of the lung explants. We performed tissue sampling in regions containing areas of mild, moderate or severe disease. To identify genetic variants in regional *Pae* populations, we designed two Ion AmpliSeq probe panels for massively parallel PCR amplification of 209 *Pae* target genes known to evolve during chronic CF lung infection or to be involved in the pathogenesis of *Pae*. To date, we have validated the IonAmpliSeq panels by analyzing total genomic DNA extracted from *Pae*-infected CF sputum and

of clinical *Pae* isolates. Moreover, spike-in samples containing different absolute quantities of *Pae* genomic DNA (equivalent to 0.2 or 2% *Pae* DNA to human DNA) and at different proportion of two *Pae* strains (PAO1, PACS2) were analyzed with the Ion AmpliSeq technology. Known gene variants of the PACS2 strain (absent in PAO1) were accurately detected the expected frequency in the spike-in samples.
We are currently analyzing lung tissues and mucus DNA samples collected from CF lung explants to identify *Pae* genetic variants associated with regions of severe disease. The most promising candidate variants will be validated in cell culture-based pathogenicity assays.
The Ion AmpliSeq technique provides a novel, quick and cost-efficient approach to analyze genetic variants potentially associated with *Pae* pathogenicity and survival in the CF lung. The identification of *Pae* gene products contributing to host tissue damage will be a first step on the way to develop novel treatment strategies for chronic *Pae* infections in CF and to increase the patients' well-being.

12 – Interleukin-6 trans-signalling in pulmonary exacerbations in cystic fibrosis

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Introduction: Loss-of-function cystic fibrosis transmembrane conductance regulator (CFTR) mutations increase levels of IL-6, a multifunctional cytokine. IL-6 has been previously been described as both a pro-inflammatory and an anti-inflammatory cytokine. There are two signalling pathways for IL-6: classic signalling, and trans-signalling. In classic signalling, IL-6 binds to IL-6 receptor alpha (IL-6Rα) and gp130, activating the JAK-STAT3 pathway. In trans-signalling, IL-6 binds to soluble IL-6Rα (sIL-6Rα), which then binds to gp130 and activates the JAK-STAT3 pathway. Classic signalling is important for host defense in mice models and ciliated epithelial cell differentiation. Conversely, trans signalling is correlated with immunopathological inflammation. In cystic fibrosis (CF), IL-6 levels correlate with pulmonary exacerbations (PE), which reduce lung function. We hypothesized that in PE, IL-6 trans-signalling might contribute to reduced lung function and we sought to determine in vitro the underlying signalling pathways that may lead to immunopathological inflammation.

Methods: To determine if CF bronchial epithelial cells were more reactive to IL-6 compared to normal controls, we compared phosphorylation of STAT3 (pSTAT3) of these cells to normal bronchial epithelial cells via western blot. Additionally, we compared pSTAT3 by IL-6 and IL-6 + sIL-6Rα in CF and normal cells. We also sought to determine if there was a difference between IL-6 and IL-6 + sIL-6Rα on the regulation of downstream inflammatory genes via qPCR.

Results: CF cells were more responsive to IL-6 compared to normal controls. Furthermore, IL-6 + sIL-6Rα induced higher levels of pSTAT3 compared to IL-6 alone in both CF and normal cells. Finally, IL-6 + sIL-6Rα induced higher levels of ICAM-1 mRNA in CF cells, and, along with TNF-α, synergistically induced higher levels of ICAM-1 mRNA compared to stimulation of the cytokines separately.

Conclusions: IL-6 trans-signalling may play an important role in PEs in CF, as the upregulation of the ICAM-1 gene may help the adhesion of incoming pro-inflammatory leukocytes. If uncontrolled, this may lead to damage of lung tissue, ultimately reducing lung function. More importantly, further research on the synergy between IL-6 trans-signalling and various other pro-inflammatory cytokines may lead to better insight on the complexity of inflammation in other disease models, and ultimately, result in novel approaches to treating inflammation.

13 – Microrheology of mucin granules in CF and non-CF human bronchial epithelial cells

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Mucus is a complex biological material that lines the luminal surfaces of respiratory, gastrointestinal and other tissues. At macro scale bulk mucus behaves as a viscoelastic gel displaying properties of both a fluid (viscosity) and a solid (elasticity). However, at microscale mucus consists of microscopic domains (pores) between entangled mucin fibers that are filled with low viscosity fluid resulting in vastly different local micro-rheological properties. Normal airway mucus has bulk viscosity (macroviscosity) in the range of 12-15 $x10^3$ cP while in CF 14-110 $x10^3$ cP. Mucus rheological properties at micro- and nano-length scales (10-100 nm) is similar to that of water (~1 cP) but, as opposed to macroviscosity measurements, only slightly higher in CF (~3 cP, Lai et al *Adv Drug Deliv Rev* 2009). Microrheological characterization of mucus genesis is important to better understand the observed differences in terms of mucus barrier properties at length scales relevant to pathogens, toxins, and foreign particles between CF and non-CF. In this study, we developed a membrane-permeant molecular viscometer (molecular rotor) suitable for fluorescence life time imaging microscopy (FLIM) and applied it to CF and non-CF human bronchial epithelial cells (HBEC). Our goal was to first investigate microviscosity of mucins stored in the lumen of mucin granules prior to their secretion. Validation study with agarose and sucrose confirmed that our molecular viscometer is sensitive to nano-scale viscosity of its surrounding environment. The average mucin granule microviscosity was 26 ±5.2 cP in non-CF but it was elevated to 34 ±5.8 cP in CF HBEC. Interestingly cytoplasm microviscosity was similar in non-CF and CF cells, 53 ±11 cP and 54 ±9.8 cP, respectively. Our study demonstrates that microviscosity of mucin granule lumen is much higher than expected for simple liquid phase of a hydrogel, especially for CF cells. This may suggest more complex colloidal structure of mucin granule lumen.

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14 – The Primary Airway Cell Biobank (PACB), an international resource for CF research

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The mission of the CFTRc is to accelerate development of effective therapies and ultimately a cure for CF by providing a platform for basic and translational research. The CFTRc is a virtual centre comprising ~30 researchers who study the physiology and pathophysiology of CF, the biochemistry, cell biology and ion channel biophysics of CFTR, and disease-related aspects of immunology, microbiology, respirology and gastrointestinal physiology. The CFTRc offers unique infrastructure and expertise and is open to all members.

The Primary Airway Cell Biobank (PACB) is one of three platforms hosted by the CFTRc. It was renovated and equipped with funding from the CFI and operating costs are supported by CF Canada, the McGill Faculty of Medicine, and by partial cost recovery from users. It is run as a not-for-profit facility and provides CF investigators in academia and industry with standardized, high-quality primary airway cells isolated from individuals with CF undergoing lung transplantation, non-CF donors, and people with various other diseases including asthma and COPD. Epithelial cell lines are convenient but they lack the genetic variation that exists within human populations and they do not differentiate into cells that resemble airway epithelium in vivo. The PACB isolates epithelial cells and many other useful cell types along with samples of sputum and extracellular matrix. Primary bronchial epithelial cells are expanded and cryopreserved at first passage and all preparations undergo extensive quality control to check for viral contamination and assess CFTR functional expression, responsiveness to correctors, histological differentiation. Highly differentiated primary bronchial epithelial cells are considered to be “the gold standard” when studying disease pathogenesis and developing new therapeutics, and are essential for many studies of airway physiology/pathophysiology, cell biology, inflammation, mucosal immunity. The PACB has highly trained personnel dedicated to this effort and routinely prepares high quality HBE cells for a fraction of the cost of commercial suppliers while also providing training and telephone support to users. The cells have been used successfully to develop at least two lead series of CF drugs and to carry out many fundamental studies of CF epithelial biology related to mucus clearance, epithelial ion transport and airway host-defense. We encourage everyone to contact us and take advantage of this resource.

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https://mcgill.ca/cftrc/platforms/primary-airway-cell-biobank-pacb

15 – Role of PDZ domain binding in CFTR aggregation at the cell surface

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The cystic fibrosis transmembrane conductance regulator (CFTR) interacts with many proteins such as the scaffold protein sodium-hydrogen exchanger regulatory factor 1 (NHERF1). NHERF1 binding to a PDZ domain binding motif at the C-terminus of CFTR facilitates the recycling of CFTR to the plasma membrane from endosomes. Interaction with PDZ domain proteins also inhibits CFTR lateral mobility at the plasma membrane, reducing its apparent diffusion coefficient by ~5-fold in studies using image correlation spectroscopy (ICS) and fluorescence recovery after photobleaching (FRAP), and almost immobilizing quantum dot-labeled CFTR in single particle tracking experiments. Recently we have also shown there are at least two populations of CFTR molecules on the plasma membrane of primary human bronchial epithelial cells (pHBE) under control conditions. Some channels are diffusely distributed while others produce punctate fluorescence due to their aggregation into clusters that are too small to be resolved by conventional optical microscopy. k-space ICS also revealed the presence of two dynamical populations, one displaying small spatial scale transport dynamics and confinement and another having larger spatial scale dynamics due to movement both outside and within microdomains. Dependence of the confined population on cholesterol suggested that it is in nanoscale lipid rafts. Finally, exposure to cell stressors such as viruses and cigarette smoke increased CFTR recruitment into clusters and their fusion into large ceramide-rich platforms. In this study we examined whether interactions with PDZ domain proteins such as NHERF1 are required for this CFTR clustering and platform formation. To test this, a CFTR mutant lacking the C terminus (CFTR-ΔDTRL) was prepared in an adenoviral vector for comparison with adenoviral wt-CFTR. NHERF1 co-immunoprecipitated efficiently with exogenously expressed wt-CFTR but was not detected in CFTR-ΔDTRL pulldowns. When pHBEs were transduced with GFP-tagged CFTR-ΔDTRL, its distribution on the plasma membrane after several days was indistinguishable from that of wt-CFTR. No differences were observed in cluster density, degree of aggregation, or dynamical behavior. Under stress-inducing conditions, wt-CFTR and CFTR-ΔDTRL both formed large platforms and had quantitatively similar aggregation and dynamics. These results indicate that PDZ domain-CFTR interactions play little role in CFTR clustering and platform formation under these conditions, unlike maneuvers that alter membrane cholesterol or ceramide, which strongly affected both wt-CFTR and CFTR-ΔDTRL. Ongoing studies of pHBE cells that overexpress both NHERF1 and CFTR should reveal if endogenous NHERF1 levels are too low to regulate heterologous CFTR. Regardless, it is already clear that interaction with PDZ domain proteins is not required for CFTR aggregation in rafts and ceramide-rich platforms.
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16 – Exploring the role of intracellular *Pseudomonas* in the establishment of chronic CF lung infections

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In order to prevent chronic *P. aeruginosa* (PA) infections, early antibiotic eradication therapy is the standard of care for initial PA infections. While these treatment protocols successfully eradicate PA in most patients, 10-30% of patients have sputum that remain positive for PA and eventually develop chronic infections.
Preliminary studies from our group suggest that intracellular PA can be found in airway epithelial cells (AECs) in CF lung explant tissues, indicating that an intracellular reservoir of PA may exist *in vivo*. We hypothesize that intracellular PA persisting in AECs may be a potential reservoir for PA, contributing to the development of chronic CF lung infections.
Our first aim is to investigate the PA bacterial factors involved in internalization and intracellular persistence within AEC. In order to examine this, we infected AECs with various PA strains at MOI 1 for 4h followed by tobramycin treatment to kill extracellular bacteria. AECs are then lysed to quantify intracellular bacteria at different time points. PA internalization is measured at 4h after infection, and intracellular persistence is measured at 24h and 120h after infection. Using this assay, we plan to screen a collection of genetically engineered PA mutants such as mucoidy or loss of the type III secretion system (T3SS), which have been associated with chronic PA infections in CF.
Preliminary results suggest that *lasR* mutants are more readily internalized than their respective wildtype parental strains. Furthermore, mutations in the T3SS genes *popB* and *pscN* increase intracellular survival. Together, these results suggest that mutations associated with chronic CF infection may promote an intracellular PA lifestyle. Additional mutants will be tested to gain a more complete understanding of the complex PA-host cell interactions.
Our second aim is to examine whether PA clinical isolates that persisted in young CF patients undergoing eradication therapy at the time of initial PA identification show greater internalization and intracellular survival in AECs compared to PA isolates from patients that successfully eradicated their initial PA infection. We will assess this by infecting AECs with a collection of PA clinical isolates collected prior to inhaled tobramycin therapy as part of an eradication study of early PA infections in young CF patients. Early PA infection was defined as first-time PA infections or re-emergence of PA after being culture-negative for at least 12 months. After receiving inhaled tobramycin over 28 days, the PA infection was considered persistent if the repeat sputum culture remained PA positive, and eradicated if the culture was negative.

In conclusion, our goal is to elucidate the clinical significance of intracellular PA in the persistence of PA infection in CF patients and to gain insights into the complex interactions between intracellular PA and its host cells.

17 – SENBIOTAR: Sensitizing *Pseudomonas aeruginosa* biofilms to antibiotics and reducing virulence through novel target inhibition in Cystic Fibrosis lungs

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The opportunistic pathogen *Pseudomonas aeruginosa* is a leading cause of long-term chronic lung infections in cystic fibrosis (CF) individuals. Despite intensive antimicrobial therapy these infections are a principal cause of morbidity and mortality. *P. aeruginosa* antimicrobial resistance is increasing in CF lungs and in many cases, the chronic infection is caused by more virulent isolates. An additional factor to complicate therapy as a multifactorial problem now well-defined but difficult to eradicate is represented by the bacterial transition from the planktonic to the biofilm mode of growth and enhancing resistance to multiple antibiotics.
The traditional therapeutic approaches targeting bacterial killing often induce strong selective pressures resulting in the rapid emergence of antimicrobial resistance. An alternative approach is to inhibit virulence gene expression rather than aiming for bacterial lethality. In the opportunistic human pathogen *P. aeruginosa* found in lungs of CF individuals, virulence is co-ordinately controlled through quorum sensing (QS), a global cell-to-cell communication system employing diffusible signal molecules. The aim of the SENBIOTAR project is to optimize hit compounds and peptide nucleic acids (PNAs) specifically targeting Pseudomonas Quinolone Signal (PQS) biosynthesis and/or PQS signal transduction. These small molecules have been shown to not only render *P. aeruginosa* avirulent but also to sensitize biofilms to the action of antibiotics.
The project brings together world experts in chemical synthesis, formulation, *in vitro* evaluation of QS inhibition, analysis of biofilm production and *in vivo* efficacy studies in animal models of chronic lung infection. QS small molecule inhibitors (QSI) were designed by structure-function modeling and optimized for *in vitro* activity inhibition of *P. aeruginosa*. Physiochemical properties of QSI were optimized for solubility and limited to low toxicity. After confirming the low cytotoxicity levels in human lung cell lines (A549), the efficacy of the QSI was assessed using the chronic lung infection model in rats.
Several hit compounds demonstrated excellent potency *in vitro* including reduction in chromophore production such as pyocyanin, in AQ signals and in biofilm production. A family of QS inhibitors showed synergy in their activity when used in combination with antibiotics and/or PNAs. Very promising *in vivo* results were obtained for the less toxic compounds in combination with Tobramycin, after optimal solubility was achieved.

The lead compounds QS inhibitors developed by the SENBIOTAR international team could have significant potential for the treatment of CF individuals but also in other extremely difficult to treat opportunistic *P. aeruginosa* infections found in hospital and intensive care units dealing with wounds, bloodstream and various medical-device associated infections.

18 – Genomic diversity of *Pseudomonas aeruginosa* in the environment and in cystic fibrosis

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Cystic Fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) leading to failure in epithelial ion transport. CF individuals are mainly affected in lung and the digestive system. A key marker in CF disease is mucus hyperproduction found in CF airways and biofilm production caused by recurrent bacterial infections.

Eventually, nearly all CF patients become infected by one or many bacterial species, of which *Pseudomonas aeruginosa* is one the most prevalent. *P. aeruginosa* is ubiquitous in the environment (water, soil). As a well-adapted opportunistic pathogen it causes severe and often fatal infections among immunocompromised, burn and CF individuals. Its capacity to colonize plants, insects and other animals, humans and at a high level CF individuals suggest that this capacity originates from its genetic diversity and an exquisite repertoire of more than 500 transcriptional genes.

One of the paradigms in *P. aeruginosa* CF pathogenicity in lung infections is the challenge of defining bacterial diversity and a clear cut reservoir of isolates linked to CF. By using such a strategy one could orient -omics and machine learning based approaches in the quest for key *P. aeruginosa* genes implicated in chronic CF lung infections.

In this study, we sequenced 629 *P. aeruginosa* isolates coming from 19 clinical (including patients with cystic fibrosis, burns, wounds, pneumonia, COPD and cancer) and 7 environmental sources (including plants, rivers, soil, animals and oil sands) to improve the overall dataset sampling of *P. aeruginosa* genetic diversity. In order to have a complete picture of maximum genetic diversity of *P. aeruginosa*, we added to this dataset 682 high-quality genome assemblies (<100 scaffolds) from NCBI to build a final dataset of 1,311 genome assemblies. We selected 50 *P. aeruginosa* genomes representing maximal diversity for PacBio sequencing based on the global phylogeny using 55,664 SNPs and 448 core genes. We also included in the phylogeny complete genomes as a single circular chromosome from the RefSeq database and avoided redundancy of known similar genomes. The resulting phylogenetic tree was imported in R, converted to a dendogram and was separated into 80 hierarchical clusters (empirically determined). We chose to sequence one *P. aeruginosa* genome per cluster not in RefSeq and developed a panel for optimal genome diversity. The 50 genomes sequenced with Illumina and PacBio were re-assembled using Unicycler and aiming for a single circular chromosome in the final assembly.

This data set of genomes is a first step in building a panel representing optimal genetic diversity. Further analysis will include additional genomes and data will be further refined in transcriptome profiling and compared to all known *P. aeruginosa* CF strains. This global integrative genomic profiling may pinpoint key *P. aeruginosa* genes critical in CF lung infections used in machine learning to predict virulence, antibiotic resistance, epidemiology, and CF lung disease.

19 – *Pseudomonas aeruginosa*-induced intracellular infection promotes inflammation and neutrophil adhesion on bronchial epithelial cells

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Rationale. Pseudomonas aeruginosa (Pa) is the main pathogen involved in CF lung disease in adults. *Pa* persists in the airways despite the host immune response and concomitant antibiotic treatments. Persistent *Pa* infection triggers a dysregulated proinflammatory host response with enhanced recruitment of polymorphonuclear cells which is responsible for tissue damage and airways remodeling, leading to the impairment of lung function and mortality. *Pa* persistence mechanisms in CF lung is mainly attributed to biofilm formation, but the question of an intracellular epithelial reservoir has recently risen. Whether persistent intracellular *Pa* alters the epithelial inflammatory response remains unknown.

Methods. Beas2B, A549, CFBEΔF508 and CFBE WT cells were exposed to *Pa* (PaO1 or clinical CF strain CHA) at various multiplicity of infection. After 4 hours, cells were treated with tobramycin, killing extracellular bacteria and keeping supernatant sterile. Intracellular bacterial count was performed at 24h, 72h and 120h by serial dilution of cell lysates on agar plates. Epithelial response was assessed by IL-8 and ICAM-1 gene expression. Additionally, IL-8 protein production was measured by ELISA. Membrane-bound ICAM-1 expression was assessed by flow cytometry and immunofluorescence on cell lines, and by immunohistochemistry on native lung tissue from CF patients. An in vitro neutrophil adhesion assay using calcein AM-labeled primary human neutrophils was used to assess the percentage of neutrophils binding on an epithelial cell layer by fluorescence spectrophotometry and by direct visualization using fluorescence microscopy.

Results. Intracellular *Pa* infection is associated with a pro-inflammatory immune response characterized by increased IL-8 and ICAM-1 production at both transcriptional and post-transcriptional levels. The proinflammatory answer persists after 120 hours infection. In addition, neutrophil binding is increased on Beas2b cells infected with intracellular *Pa* at 24hours of infection. Accordingly, ICAM-1 is highly expressed on epithelial cells from lung of CF patient with persistent intracellular *Pa* infection.

Conclusion. Persistent intracellular *Pa* alters the epithelial inflammatory response by enhancing IL-8 and ICAM-1 expression and promotes neutrophil adhesion on the airway epithelial cells.

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20 – The main mechanism associated with progression of glucose intolerance in older patients with cystic fibrosis is insulin resistance and not reduced insulin secretion capacity

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Background: Aging cystic fibrosis (CF) patients are at high risk of developing glucose intolerance which can lead to CF-related diabetes (CFRD). Hyperglycemia in CF is associated to lower body weight and pulmonary function. Decrease in insulin secretion over time is the main hypothesis to explain this increasing prevalence but mechanisms are still not well elucidated.

Objective: Assess evolution of glucose tolerance and insulin secretion/sensitivity in aging CF patients.

Methods: Prospective observational analysis of baseline versus last follow-up data from the Montreal Cystic Fibrosis Cohort recording. Oral glucose tolerance test (OGTT; with glucose and insulin measurements every 30 minutes for 2-hour), pulmonary function test (FEV1) and anthropometric measurements were performed. Data from adults CF patients (n=46) who were followed-up for at least 4 years were included.

Results: After a mean of 9.9 ± 2.6 years follow-up, an increase of body weight of 2.6 ± 6.5 kg ($P = 0.01$) and a decrease in pulmonary function, -10% FEV ($P \leq 0.001$) were observed. Normal glucose tolerant patients decreased from 52.2 % at baseline to 28.3 % at follow-up. Accordingly, OGTT glucose values and area under the curve (AUC) for glycaemia all increased ($P \leq 0.017$) but no significant change in insulin secretion (individual measurements & OGTT-AUC) were observed. Stumvoll index analysis revealed a significant decrease in insulin sensitivity ($P = 0.033$) over time.

Conclusion: In older CF patients the progression of impaired glucose tolerance is occurring with stable insulin secretion but reduced insulin sensitivity.

Key words: Cystic Fibrosis, diabetes, CFRD, glycaemia, insulin secretion.

21 – *Pseudomonas aeruginosa* evasion of neutrophil phagocytosis and bacterial clearance in early cystic fibrosis lung infection

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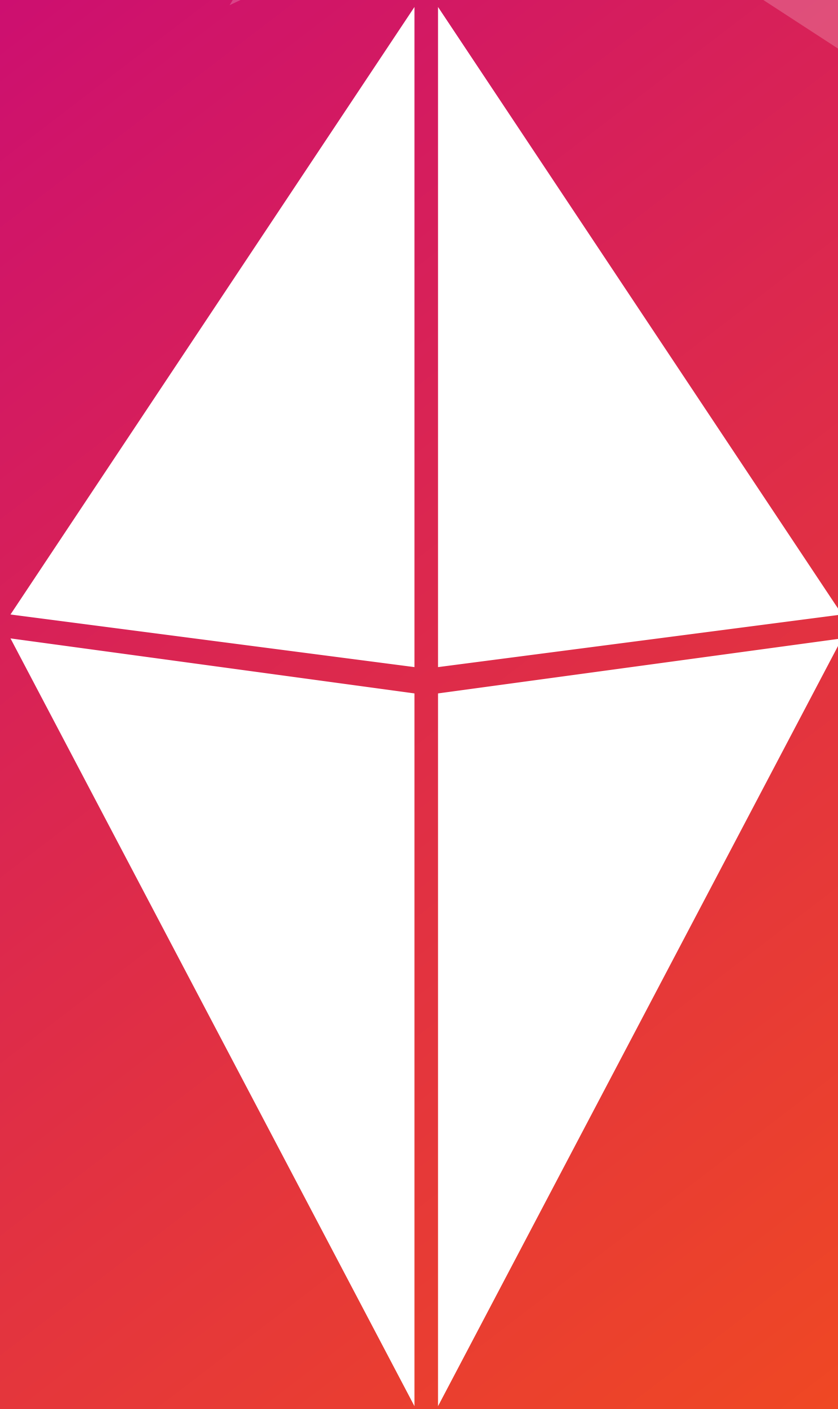
Pseudomonas aeruginosa (PA) is the predominant pathogen that causes chronic lung infection in adult patients with cystic fibrosis (CF). Current antibiotic treatment such as inhaled tobramycin can eradicate PA only during early infection stages. In two major prospective studies of CF children with new onset of PA infections, 28 to 38% patients failed tobramycin eradication therapy and the reason for eradication failure remains unclear. Failure to clear PA by innate host defenses during early stages of infection leads to persistent PA infections in the CF airways, which results in progressive lung tissue damage. Since the adequate recruitment and function of neutrophils (PMNs) is a key step for successful PA eradication, we hypothesized that PA isolates that are not eradicated after inhaled tobramycin elicit impaired PMN antibacterial responses, compared to PA isolates that are successfully cleared.

In this study, we used clinical PA isolates from the Sick Kids PA eradication clinical study where patients with early PA infection either succeeded (eradicated isolates) or failed (persistent isolates) eradication therapy with inhaled tobramycin. We compared *in vitro* PMN phagocytosis and intracellular bacterial killing by neutrophil-like cells (differentiated HL-60) in response to eradicated vs persistent clinical PA isolates. We observed a lower phagocytosis and intracellular bacterial killing of persistent PA isolates compared to eradicated PA isolates. This suggests that persistent PA isolates exploit strain specific bacterial factors to evade PMN phagocytosis and intracellular bacterial killing. Subsequently, we assessed the interaction of PMNs and specific bacterial factors (type 4 pilus, Psl, flagellum and mucoid). We found that type 4 pilus mediated twitching motility has modest association with PMN phagocytosis, but not flagellum mediated swimming motility. Of all the clinical PA isolates, a greater proportion of persistent PA isolates are mucoid compared to eradicated PA isolates, and mucoid PA isolates from both groups elicited reduced PMN intracellular bacterial killing. In addition, a preliminary analysis of representative subset of PA isolates showed that persistent PA isolates produce increased Psl, an exopolysaccharide, compared to eradicated PA isolates. These results highlight the potential role of PA strain specific bacterial factors and their interaction with PMNs as a mechanism that is associated with PA eradication outcomes in CF patients. Future experiments will be involved with dissecting the role of Psl and its *in vivo* relevance in persistent vs eradicated PA isolates.



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À l'an prochain !

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