Fibrose kystique Québec

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2e Colloque scientifique sur la fibrose kystique

« Infection et inflammation en fibrose kystique »

Jeudi 7 mai 2015, *hotel Gouverneur Place Dupuis, Montréal*

avec la collaboration de

Cystic Fibrosis Translational Research Center

CFTRc

Centre de Recherche Translationnelle sur la Fibrose Kystique

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2e trimestre 2015

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La magnifique collaboration du Centre de recherche translationnelle sur la fibrose kystique de l'Université McGill (CFTRc) et de Dr Cantin du CHUS l'an dernier nous a permis de commémorer le 25e anniversaire de la découverte du gène CFTR responsable de la maladie. Ce fut un grand succès!

Cette année, nous avions envie de collaborer à nouveau avec cette formidable équipe et cela nous a mené à proposer aujourd'hui ce colloque sur les infections et les inflammations en fibrose kystique. Un grand merci au comité responsable de la programmation :

- Emmanuelle Brochiero Ph.D. (CHUM),
- André Cantin M.D. (CHUS),
- Annick Michelle Guyot Ph.D. (McGill),
- John W. Hanrahan Ph.D. (McGill),
- Dao Nguyen M.D. (McGill),
- Renaud Robert Ph.D. (McGill).

Ce colloque vous propose de prendre connaissance des plus récentes conclusions en matière diagnostic des infections, de physiopathologie de la maladie, d'espérance de vie, avec l'espoir de mise au point de thérapies spécifiques.

Notre fondation travaille d'arrache-pied pour soutenir chaque année le travail de nos chercheurs et cliniciens. Nous sommes ravis que certains d'entre eux aient pu se libérer aujourd'hui pour partager avec vous quelques éléments de leurs travaux.

La contribution des chercheurs, cliniciens, médecins et étudiants combinée au généreux soutien d'entreprises et du public font une grande différence dans la recherche d'une solution et dans l'avancement de la cause. Il y a ainsi de l'espoir pour un remède ou un traitement au cours de notre vie.

Merci à chacun d'entre vous d'avoir répondu positivement à notre invitation de participer à ce colloque aujourd'hui. Il me fait grand plaisir de voir réunie la grande famille des chercheurs, cliniciens, médecins et professionnels de la santé liés à cette cause qui nous unit.

Je profite de cette occasion pour remercier chaleureusement **Vertex** pour son engagement à titre de commanditaire principal et inconditionnel. Merci à **Merck** pour son soutien indéfectible, année après année, merci à la Faculté de médecine de l'**Université McGill** et merci à tous les exposants et autres partenaires de l'événement. Tous ensembles, vous permettez à cette journée d'être non seulement possible mais de faire du colloque annuel un rendez-vous incontournable pour tous les professionnels engagés à trouver des solutions pour la fibrose kystique.

Le président-directeur général,

Mark Bordeleau

Horaire / *Schedule*

8h00	Inscription et petit-déjeuner continental / *Registration and continental breakfast*
9h00	Mot de bienvenue, avec **Mark Bordeleau**, pdg de FK Québec
9h05	Animation: **Renaud Robert**, Ph.D. CFTRc McGill University
9h10	"*Staphylococcus aureus* Small-Colony Variants; virulence and impact for the CF lung" with **François Malouin**, Ph.D., Université de Sherbrooke
9h50	"*Pseudomonas aeruginosa* adaptation to the CF lung, and its impact of airway inflammation" with **Dao Nguyen**, M.D., M.Sc., McGill University
10h30	Pause-café & communications affichées
11h00	"The Evolution of Survival in CF: Pediatrics to Geriatrics" with **Anne Stephenson**, M.D., Ph.D., Director of the Canadian CF Registry
11h40	« Exploitation clinique de données génomiques produites par le consortium international Pseudomonas: le projet 1000 génomes » avec **Roger C. Lévesque**, Ph.D., Univ. Laval
12h20	Dîner parmi les exposants et communications affichées
13h25	Témoignage de **Dominique Paré**, bénéficiaire du Ivacaftor (Kalydeco)
13h45	Communications orales (2 x 20 min.) • **Valérie Boudreau**, nutritionniste, IRCM: Abnormal glucose tolerance in cystic fibrosis patients: What is the contribution of insulin resistance? • **Raquel Farias**, M.D., Ph.D. student, McGill University: IL-33, an Inflammatory Mediator in Cystic Fibrosis Lung Disease
14h25	« Stratégies thérapeutiques pour la correction de CFTR et l'amélioration de la réparation épithéliale dans les voies aériennes fibrose kystique en présence d'infection » avec **Emmanuelle Brochiero**, Ph.D., CHUM
15h05	"Decoding the host-pathogen interactions in the CF lung" avec **Simon Rousseau**, Ph.D., Université McGill
15h45	Pause offerte par PTC Therapeutics et communications affichées
16h15	"Polymicrobial perspectives on airway infections in cystic fibrosis" with **Michael G. Surette**, Ph.D., McMaster University
16h55	Récapitulation et vin d'honneur offert par Traffick Therapeutics

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François Malouin, Ph.D,

Staphylococcus aureus Small-Colony Variants: virulence and impact for the CF lung

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 an inhaled formulation 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, which kills SCVs and significantly increases the killing of multi-resistant MRSA by tobramycin.

François Malouin is professor (microbiology) at the *Département de biologie* at the *Faculté des sciences* of *Université de Sherbrooke* since 2000. He is a microbiologist that has 25+ years of academic and industrial experience in drug discovery for use in humans and food-producing animals. He was recently appointed to the *Comité d'experts scientifiques sur la résistance aux antibiotiques de l'Institut national de santé publique du Québec* (2011-2015), a committee that is providing recommendations to the provincial government on the matter of antibiotic resistance. Prof Malouin obtained his bachelor degree (microbiology) at U. de Sherbrooke (1982), a M.Sc. degree in microbiology and immunology at U. de Montréal (1985), a doctoral degree (Ph.D.) in Medical Sciences (Medical Microbiology) at U. of Calgary (1988) and did postdoctoral training in the anti-infective research group at Lilly Research Laboratories (Eli Lilly & Co., Indianapolis, USA) from 1988 to 1990. Prof Malouin was also assistant professor of microbiology at the *Faculté de médecine* of *U. Laval* where he had a Fellowship from the Medical Research Council of Canada (1990-1994). He was then recruited by biotechnology companies, first Microcide Pharmaceuticals, Inc., then a sister company, Iconix Pharmaceuticals, Inc. (California, USA) from 1994 to 2000, where he was associate director of technology development for the discovery of new therapeutic targets and antibiotics. He was also the co-founder of Ulysses Pharmaceuticals (Sherbrooke, QC). Currently at U. de Sherbrooke, his research projects aim at exploiting virulence genes for the development of new antibiotics and vaccines for application in human and animal health.

Dao Nguyen, M.D.

Pseudomonas aeruginosa adaptation to the CF lung and its impact of airway inflammation

Cystic fibrosis lung disease is characterized by chronic airway infections with the opportunistic pathogen *Pseudomonas aeruginosa* and severe neutrophilic pulmonary inflammation. *P. aeruginosa* undergoes extensive genetic adaptation to the CF lung environment, and adaptive mutations in the quorum sensing regulator gene *lasR* arise very commonly. Mutations in *lasR* alter host-pathogen relationships and induce exaggerated host inflammatory responses in respiratory epithelial cells in vitro and in vivo. Furthermore, CF patients infected with *lasR* mutants have increased plasma IL-8, a marker of inflammation. These findings suggest that bacterial adaptive changes may worsen pulmonary inflammation and contribute directly to the pathogenesis and progression of chronic lung disease in CF patients.

Dao Nguyen has received her MD.CM (1997) and MSc Epidemiology (2004) degree from McGill University, completed her specialty clinical training at Tufts (Boston) and McGill University, and a post-doctoral research training at the University of Washington (Seattle) in Microbiology. She is an Assistant professor in the Department of Medicine at McGill University since 2009, and a clinician-scientist in the Division of Respiratory Medicine at the McGill University Health Centre. Her lab is focused on the molecular microbiology of *Pseudomonas aeruginosa*, biofilms and the molecular mechanisms of antibiotic tolerance, as well as host-pathogen interactions in pulmonary infections.

Anne Stephenson, M.D., Ph.D.

The Evolution of Cystic Fibrosis: Pediatrics to Geriatrics

Understanding the factors that increase the risk of death in CF is vital to allow clinicians to intensify medical therapy, consider new therapies, or refer for transplantation as the patient's clinical condition dictates. Two pivotal Canadian studies, published over 15 years ago, provided clinically useful information on predictors of survival in CF; however, diagnosis and management of CF have rapidly improved over the last 2 decades and the previously established predictors are no longer applicable in the current era of CF care. The objectives of our study were to calculate survival estimates for the Canadian CF population over the last 30 years and to identify clinical and geographic factors associated with survival in CF using contemporary Canadian CF registry data. Survival estimates were calculated for the entire cohort from 1990 to 2012. These included age at death as well as death rate. Median age of survival for a 5 year moving window was estimated using Cox proportional hazards models. There were 5,787 individuals with cystic fibrosis followed in the registry between 1990 and 2012. Canadian median survival age has increased over time going from 31.9 years (95% confidence intervals [CI] 28.3-35.2) in 1990 to 49.7 years (95% CI 46.1-52.2) in 2012. Individuals with a BMI less than 18 kg/m2 were 1.8 times more likely to die compared to normal weight individuals. Other significant predictors of death included CF-related diabetes (CFRD), have more than 1 pulmonary exacerbation per year, and being infected with B. cepacia complex. This work highlights the fact that survival in Canadians with CF continues to increase and identified key factors that are associated with increased risk of death in a contemporary group of individuals with CF. Further work is required to explore the reasons for the improved survival in Canada and the development of a predictive model to estimate the risk of death in the current era of CF care would be useful for clinicians.

Dr. Stephenson attended medical school and internal medicine training at the University of Toronto. She completed her training in Respiratory Medicine at the University of British Columbia in Vancouver in 2000 and has been working at St. Michael's Hospital in the Division of Respirology since 2001. In 2010 she successfully completed her PhD in Clinical Epidemiology at the University of Toronto. In addition to her clinical work as a CF Respirologist, Dr. Stephenson is a Clinician Scientist at St. Michael's Hospital doing epidemiological research in CF. At Cystic Fibrosis Canada, she is the Director of the Canadian CF Registry which records important clinical data on all Canadians with CF across the country.

Roger C. Lévesque, Ph.D.

The International Pseudomonas Consortium: the 1000 Plus genomes project

The International Pseudomonas Consortium (IPC) 1000 Plus genomes project is producing an extensive collection of genomes from a single species for studying genome evolution, comparative genomics of antibiotic resistance and virulence genes by cataloguing SNPs, indels and genome rearrangements. Given the potential link between environmental strains and evolution towards infection by *P. aeruginosa* in animals and in humans, we need to better understand the role of environmental strains in genome evolution, how patients become infected and identify prognostic markers for better evidence-based decisions on patient care. We are sequencing the genomes of more than 500 *P. aeruginosa* isolates from Cystic Fibrosis (CF) patients and 500 strains from other human and animal infections, and environmental strains. The objective of the IPC is to sequence a minimum of 1000 *P. aeruginosa* genomes, link the data to pseudomonas.com, potentially integrate the information with the Canadian CF registry and develop a user-friendly pipeline to study these genomes. We created the International Pseudomonas Consortium Database (IPCD) as an open source web application designed to not only store data for the *P. aeruginosa* collection but also provide access to each isolate's phenotypic and genomic data. IPCD is available at the address http://ipcd.ibis.ulaval.ca/. IPCD combines basic information including date of isolation, geographical origin, clinical details, and phenotypic data and sequencing information. The IPCD's general architecture was developed for easy web access and relies on a Mysql 5 database to store data. We have selected 1000 *P. aeruginosa* isolates in order to represent maximum genomic diversity by comprehensively sampling geographic origin, VNTR/AT typing, host, *in vitro* phenotype and *in vivo* behaviours. High quality draft genome sequencing is performed using Illumina MiSeq and combining 48 genomes per run with 300 bps paired-end libraries for a median coverage of 40 X, followed by genome assembly using an integrated pipeline. Priority genomes will be selected and sequenced using PACBIO RS technology to generate complete genome assemblies for annotation. A pipeline being implemented at Pseudomonas.com will be used for a standardized high throughput comparative genomics annotation and easy access to data for clinical applications. Clinical exploitation of genomics data will be supportive to molecular epidemiology performed for surveillance and outbreak investigation in CF and has the potential for future genotypic antimicrobial susceptibility testing for *P. aeruginosa*, as well as the identification of novel therapeutic targets and prognostic markers.

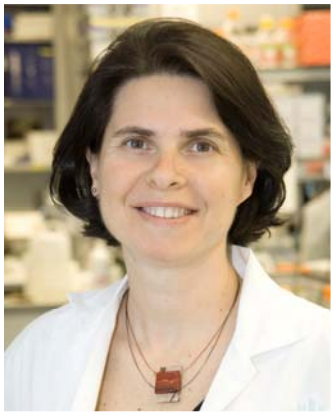
📖 Dr. Levesque is the founder and director (2009) of the *Integrative and systems biology Institute* (IBIS) at Université Laval. He is full professor in the Département de microbiologie-infectiologie et d'immunologie, Faculté de médecine, Université Laval. He obtained a Ph.D. from Laval in microbiology in 1981. After completing postdoctoral training at Harvard University and at Cold Spring Harbor in 1984, he was awarded several FRSQ scholarships, an FRSQ Scholar of Exceptional Merit and recently the Robbie Award from Cystic Fibrosis Canada. He has participated in several CHIR grant committees including scientific officer on the CIHR genomics committee. He received an investigator award from the American Society for Microbiology, was president of the Canadian Society for Microbiologists and a member of the Canadian Bacterial Disease Network Centre of Excellence. His current research is funded by Cystic Fibrosis Canada, CIHR, NSERC, FRQNT, FRQS, Génome Québec and Genome Canada. His current interests are the development of genomics tools in clinical microbiology. Clinical exploitation of genomics data will be supportive to molecular epidemiology performed for surveillance and outbreak investigation and has the potential for future genotypic antimicrobial susceptibility testing, as well as the identification of novel therapeutic targets and prognostic markers.

He is funded by Cystic Fibrosis Canada for the clinical exploitation of genomics data produced by the Pseudomonas International Consortium: the 1000 plus genomes project. He is the director of the Pseudomonas consortium involving 52 scientists from around the world; his laboratory hosts the The International Pseudo Consortium Database at http://ipcd.ibis.ulaval.ca/ and is currently completing at IBIS the 1000 plus genomes sequencing, assemblies and annotation.

Emmanuelle Brochiero, Ph.D

Therapeutic strategies for CFTR rescue and epithelial repair improvement in infected cystic fibrosis airways

/ Stratégies thérapeutiques pour la correction de CFTR et l'amélioration de la réparation épithéliale dans les voies aériennes fibrose kystique en présence d'infection



Respiratory failure due to airway epithelial damage and remodeling remains the main cause of morbidity and mortality in CF patients. Several years ago, we thus decided to devote our efforts to better understand the mechanisms controlling epithelial repair and identify some strategies to promote airway regeneration.

Our analyses of airway tissues from CF patients revealed extended areas of epithelial damage. Epithelial injury is a deleterious component of the CF pathology, since it may further impair the defense against pathogens, thus creating a vicious circle of infections and injuries. Although repair processes are engaged after damage to restore the epithelial integrity, we demonstrated that repair processes of the CF epithelia are less efficient than in healthy tissues, even in pathogen free-conditions. In fact, we have shown that the function of ion channels, especially CFTR and K^+ channels, is crucial in airway repair. Thus, the basic CFTR defect in CF could be responsible, at least in part, for the inefficient epithelia repair in CF. Interestingly, we then discovered that CFTR rescue, with CFTR correctors, enhanced the repair capacity of airway monolayers, thus highlighting a new, unsuspected function of CFTR correctors.

Bacterial infection, particularly by *Pseudomonas aeruginosa*, is another deleterious component of the CF lung disease. In addition to its involvement in the development of airway damage, this pathogen also affected the repair capacity of airway epithelia. Furthermore, we recently discovered that the presence of exoproducts from *Pseudomonas aeruginosa* impaired CFTR maturation and functional rescue by CFTR correctors. We also showed that part of the beneficial effect of CFTR correctors on airway epithelial repair was prevented by *Pseudomonas aeruginosa*. Of interest for CF therapeutics, we recently identified some of the factors that may be responsible for the deleterious effect of infection. We also noted that some combined treatments with CFTR correctors and potentiator allowed a significant level of improvement in airway repair despite the presence of infection.

Après avoir obtenu son doctorat à l'Université de Nice, en France en 1995, Dre Emmanuelle Brochiero a entrepris une première formation post-doctorale à l'Université de Montréal, sous la direction du Dr Jean-Yves Lapointe (1996-2000). Elle a ensuite réalisé un second stage post-doctoral dans le laboratoire du Dr Yves Berthiaume au CRCHUM en 2001-2002. Depuis mars 2002, Dre Emmanuelle Brochiero est chercheure au CRCHUM et elle est professeure agrégée au Département de médecine de l'Université de Montréal. Elle a mis en place un programme de recherche visant à explorer le rôle des canaux ioniques dans la physiopathologie de maladies pulmonaires, en particulier la fibrose kystique, le syndrome de détresse respiratoire et le cancer du poumon. Son laboratoire étudie notamment le rôle des canaux ioniques et l'impact des composantes infectieuses et inflammatoires sur les processus de réparation épithéliale. Son but est de développer de nouvelles stratégies thérapeutiques visant à promouvoir la régénération épithéliale en fibrose kystique et après des lésions pulmonaires aigues. Au cours des dernières années, Dre Brochiero a reçu des subventions de plusieurs organismes, dont Cystic fibrosis Canada, les IRSC et le NSERC. Elle a siégé sur plusieurs comités scientifiques d'évaluation, dont celui de Cystic Fibrosis Canada. Dr Brochiero dirige également la banque de tissus respiratoires du CHUM ainsi que le regroupement fibrose kystique du réseau en santé respiratoire.

Simon Rousseau, Ph.D.

Decoding the host-pathogen interactions in the CF lung /

Décoder les interactions hôte-pathogène dans le poumon FK

One of the hallmarks of the chronic infection found in Cystic Fibrosis lungs is the presence of Pseudomonas aeruginosa. P. aeruginosa adapts to the lung environment as the chronic infection progresses. One of the adaption observed is a mucoid phenotype. *P. aeruginosa* mucoidity is associated with worse CF lung disease and decreased survival. As CF lung disease results from chronic infection leading to airway inflammation, we determined whether the switch to a mucoid phenotype by *P. aeruginosa* has an impact on the inflammatory response of airway epithelial cells. In an investigation of 51 CF patients seen at the Montreal Chest institute, we observed higher inflammation, more frequent pulmonary exacerbations and worst lung function in patients colonized with mucoid P. aeruginosa. Moreover, we found that the mucoid phenotype of P. aeruginosa activated airway epithelial cells via dimerization of TLR2 to TLR6 in addition to that of TLR5, which occurs in both non-mucoid and mucoid strains.

Dr Simon Rousseau obtained his Ph.D. in cellular and molecular biology in 2000 from the Université Laval in Québec city. During his Ph.D. he studied the intracellular signaling pathways activated by the *Vascular Endothelial Growth factor* (VEGF) leading to endothelial cell migration, under the supervision of Professor Jacques Huot. He then joined the group of Professor Sir Philip Cohen at the MRC Protein Phosphorylation Unit in Dundee, Scotland, a world re-known scientific unit in the field of signal transduction Funded by two consecutives fellowships award from the CIHR, Dr Rousseau investigated the signaling pathways involved in pro-inflammatory cytokine production by macrophages. In January 2008, he was recruited as an assistant professor at McGill University and joined the Meakins-Christie Laboratories as a research director to investigate signal transduction mechanisms driving pulmonary inflammation. A major focus of his recent work has been host-pathogen interactions in Cystic Fibrosis lungs. From 2012 to 2014, he led the strategic grouping on Cystic Fibrosis of the Québec Respiratory Health Network. He received the 2010-2011 Robbie most promising new research award from Cystic Fibrosis Canada and the 2012 Bhagirath Singh Early Career Award in Infection and Immunity from CIHR. His research is currently funded by NSERC, Cystic Fibrosis Canada and CIHR and he his a Chercheur Boursier Junior 2 of the FRQS. To date his work focused on the role of Mitogen-Activated Protein Kinases (MAPKs) in orchestrating cellular functions has resulted in more than 40 manuscripts that have been cited over 3500 times.

Michael G. Surette, Ph.D.

Polymicrobial Perspectives on Airway Infections in Cystic Fibrosis

In cystic fibrosis the chronic colonization of the lower airways by bacterial pathogens is the leading cause of morbidity and mortality. It is now well established that the lower airways in CF patients are colonized by a more complex polymicrobial community comprised primarily of bacteria found in the upper respiratory tract along with the traditional pathogens associated with CF airway disease. Considering these infections from a polymicrobial perspective offers new insights into disease and alternative treatment strategies. We have shown this community also includes pathogens missed by conventional CF microbiology and organisms that can enhance the pathogenicity of *P. aeruginosa* in animal models. These observations now guide disease management in some patients and are being applied to other respiratory diseases. Considering respiratory infections as polymicrobial diseases offers new avenues to for treatment and possible new directions for developing new therapies. The CF airways have been the focus of molecular microbiome studies, however, there remain many challenges that must be overcome if microbiome profiling is going to inform clinical practice.

Dr. Surette is currently Professor and Canada Research Chair Interdisciplinary Microbiome Research at McMaster University. He is also co-director of the McMaster Genomics Facility and chair of the Research Subcommittee of Cystic Fibrosis Canada. His research is addresses polymicrobial infections and bacterial pathogenesis, the human microbiome in health and disease, and culturing the microbiome. Specific projects are focused on cystic fibrosis, asthma, pneumonia, sepsis, ulcerative colitis, and irritable bowel syndrome. His lab is currently supported by grants from the Canadian Institutes for Health Research, Cystic Fibrosis Canada, Crohn's and Colitis Foundation of Canada and the Canadian Foundation of Innovation.

Communications scientifiques / Oral Presentations

1. **Abnormal glucose tolerance in cystic fibrosis patients: What is the contribution of insulin resistance?**

Valérie Boudreau[1,3], Adèle Coriati[1,3], Imane Hammana[1,3], Sophie Ziai[1,3], Yves Berthiaume[1,2,3], Rémi Rabasa-Lhoret[1,2,3]

[1]Université de Montréal
[2]Cystic Fibrosis Clinic of the Centre hospitalier de l'Université de Montréal
[3]Institut de recherches cliniques de Montréal

Background and aims: Insulin secretion defect plays a key role in the deterioration of glucose tolerance in patients with cystic fibrosis (CF). However, the role of insulin resistance remains unclear. The aim of this study is to investigate the respective roles of impaired insulin secretion and insulin resistance in the progression of glucose tolerance in patients with CF.

Hypothesis: Insulin secretion and/or insulin resistance are important factors associated with the alteration of glucose tolerance in adult patients with CF.

Methods: A total of 152 patients (≥ 18 yrs) without known diabetes from the Montréal CF cohort underwent a 2-h oral glucose tolerance test (OGTT) at baseline and after a 21,19 ± 5,47 months follow-up. Pulmonary function and anthropometric measurements were also collected at each visit. Based on their OGTT results, patients were then stratified in glucose tolerance groups: normal glucose tolerance, impaired glucose tolerance or CF-related diabetes. Their transition from a glucose tolerance status to another was studied.

Results: At follow up, 55.8% of the patients kept a similar glucose tolerance (A), 14.3% improved their glucose tolerance (B), and 30.0% deteriorated their glucose tolerance (C). Between all 3 groups, patients had similar age, body mass index, glycated haemoglobin values, exocrine pancreatic insufficiency and inflammation levels at baseline. Genetic profile was different between the 3 groups (P= 0.024). Insulin secretion capacity and resistance were statistically different between groups. For instance, insulin sensitivity was lower in group B when compared to group A (P =0.029). Insulin secretion capacity and insulin sensitivity did not differ at follow-up for patients in group A. Moreover, patients in group B ameliorated their insulin sensitivity at follow-up (P =0.003). Insulin secretion capacity did not change at follow-up for patients in group C (P =0.312), however insulin sensitivity as well as pulmonary function deteriorated (P <0.001 and P=0.001, respectively).

Conclusion: In the context of a reduced insulin secretion, worsening of insulin sensitivity could have an impact on the deterioration of glucose tolerance in patients with CF.

2. **IL-33, an Inflammatory Mediator in Cystic Fibrosis Lung Disease**

Raquel Farias[1], Simon Rousseau[2]

[1]Université McGill
[2]McGill University

During Cystic Fibrosis (CF), chronic infection with Pseudomonas aeruginosa (Psa) leads to an increase in pro-inflammatory mediators with subsequent epithelial injury. Tissue damage is further potentiated during acute exacerbations. In this context, inflammation is sustained through damage-associated molecular patterns (DAMPs). These endogenous molecules are released following cell death and act upon pattern recognition receptors (PRRs) on immune cells. IL-33, a member of the IL-1 family of cytokines, is a recently discovered DAMP. In addition to its transcriptional regulatory properties, IL-33 is a very potent neutrophil chemoattractant and a key amplifier of innate immunity. Our central hypothesis is that decreasing IL-33 levels will reduce inflammation in CF.

Our first aim is to determine whether the expression of IL-33 is increased in CF airway epithelial cells following an acute infection with Pseudomonas aeruginosa. Secondly, we will study the signaling

pathways regulating IL-33 expression in the CF airway epithelium. Finally, we will determine the role of IL-33 as a mediator of inflammation in CF.

My data shows that CFTRΔF508 airway epithelial cells but not their wild type counterpart responded to the presence of Pseudomonas aeruginosa through up-regulation of IL-33 mRNA, as measured by RT-qPCR. Signaling through toll-like receptors is in part responsible for the increased IL-33 expression since exposure of CFTRΔF508 airway epithelial cells to the TLR2 and 5 agonists PAM3CSK4 and Flagellin, respectively, significantly increased IL-33 mRNA. Furthermore, experiments using inhibitors of specific kinases downstream TLRs show that TAK1, Tpl2, MEK1/2 and IKKβ modulate IL-33 expression in response to P. aeruginosa. The increase in IL-33 mRNA is followed by an increase in intracellular protein, as assessed by immunoblotting. Interestingly, P. aeruginosa increases IL-33 in the cytoplasm of both, CF and non CF airway epithelial cells. However, IL-33 is not released in our model of acute infection. Finally, to assess the role of intracellular IL-33 as a transcriptional regulator, airway epithelial cells stably expressing an NFκB luciferase reporter were transfected with full-length IL-33 and with two IL-33 mutants lacking a consensus chromatin-binding motif (CBM). Transfection with both CBM mutants attenuated NFκB transactivation in response to Flagellin. In line with the latter result, transfection of IL-33 constructs significantly decreased IL-8 mRNA.

In conclusion, P. aeruginosa increases IL-33 expression in CF airway epithelial cells. The TAK1-IKKβ-Tpl2-MEK1/2 signaling pathway modulates IL-33 expression in response to bacterial infection. This is followed by an increase in intracytoplasmic IL-33. Intracellular IL-33 dampens NFκB transactivation in response to TLR5 signaling, resulting in a decrease of pro-inflammatory cytokine production. Future experiments will aim to identify specific intracellular IL-33 binding partners and potential postranslational modifications occurring in airway epithelium.

Communications affichées / *Poster Session*

1. **Staphylococcus aureus inhibits IL-8 responses induced by Pseudomonas aeruginosa in airway epithelial cells**

Samuel M. Chekabab[1], Richard Silverman[1], Yishan Luo[1], Shantelle Lafayette[1], Simon Rousseau[1], Dao Nguyen[1]

[1]McGill

Pseudomonas aeruginosa (PA) and Staphylococcus aureus (SA) are major respiratory pathogens and can concurrently colonize the airways of patients with chronic obstructive diseases, such as cystic fibrosis. Airway epithelial cell signalling is critical to the activation of innate immune responses. In the setting of polymicrobial colonization or infection of the respiratory tract, how epithelial cells integrate different bacterial stimuli remains unknown. Our study examined the inflammatory responses to PA and SA co-stimulations. Immortalised airway epithelial cells (Beas-2B) exposed to bacteria-free filtrates from PA (PAF) induced a robust production of the neutrophil chemoattractant IL-8 while bacteria-free filtrates from SA (SAF) had a minimal effect. Surprisingly, co-stimulation with PAF+SAF demonstrated that SAF strongly inhibited the PAF-driven IL-8 production, showing that SAF has potent anti-inflammatory effects. Similarly SAF decreased IL-8 production induced by the TLR1/TLR2 ligand Pam3CysSK4 but not the TLR5 ligand flagellin in Beas-2B cells. Moreover, SAF greatly impaired TLR1/TLR2-induced NF-κB activation, but not p38 MAPK. We observed this SAF-dependent anti-inflammatory activity in several SA clinical strains, as well as in the CF epithelial cell line CFBE41o-. These findings show a novel direct anti-inflammatory effect of SA on airway epithelial cells, highlighting its potential to modulate inflammatory responses in the setting of polymicrobial infections.

2. **Characterization of airway submucosal gland epithelial cell cultures at the CFTRc**

Julie Goepp[1,2], Junwei Huang[1,2], Elizabeth Matthes[1,2], David Y Thomas[1,3], Renaud Robert[1,2], John Hanrahan[1,2,4]

[1]CF Translational Research Centre (CFTRc), McGill Univ.
[2]Dept. Physiology, McGill Univ.
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Surface airway epithelial cells have been studied extensively in primary culture however less is known regarding submucosal gland cells, which secrete a large fraction of the fluid, mucus and antimicrobials that protect the surface of proximal airways. To investigate submucosal gland cultures and compare them with primary cultures of surface epithelium and model cell lines such as Calu-3, gland cells were isolated from bronchial submucosal glands by multiple cycles of enzymatic digestion, amplified in collagen-coated plastic flasks, enriched by differential trypsinization as described by Merten et al. (2003). Gland cells, which proliferated rapidly on plastic compared to bronchial surface epithelial cells, were plated on inserts in serum-free medium and induced to differentiate at the air-liquid interface into serous or mucous phenotypes using methods adapted from Widdicombe et al. (2010). Electron microscopy of the serous cultures revealed two cell layers, tight junctions, and predominantly electron-dense granules. Serous cells did not have cilia and did not produce mucus, in contrast to cells with a mucous phenotype which expressed mainly electron-lucent granules and were covered by a conspicuous mucus layer. When serous monolayers were mounted in Ussing chambers in the presence of an apical-to-basolateral chloride gradient and the basolateral membrane was permeabilized using nystatin, forskolin stimulated a (reversed) short-circuit current of 11 μA.cm-2 which was abolished by apical GlyH-101 (100μM) and inhibited ~40% by apical CFTRinh-172 (10μM). Forskolin stimulated net bicarbonate secretion of 0.5 μEquiv.cm-2.h-1 across unpermeabilized cells under open circuit and pH-stat conditions. With a basolateral-to-apical chloride gradient, forskolin stimulated short-circuit currents of 13.3 μA.cm-2 which were partially inhibited by sequential additions of bumetanide (50 μM) and DIDS (200 μM) to the basolateral side. The results suggest that gland cells can be differentiated to mucous or serous phenotypes and that NKCC1 and NBCe1 both contribute to anion secretion by serous cells under short-circuit current.

Support: CIHR and CF Canada

3. **Characterizing the role for CFTR in intestinal infection**

Travis Ackroyd[1], Barbara Mindt[1], Mitra Yousefi[1], Daisy Ji[1], Eugene Kang[1], Lei Zhu[1], Jie Liao.[1], Bruce Vallance[2], Jorg Fritz[1], John Hanrahan[1], Samantha Gruenheid[1]

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Cystic fibrosis (CF) is an autosomal recessive disease that affects approximately 1 in 3000 Caucasians. Caused by a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), human CF pathology is characterized by thick, viscous secretions and bacterial overgrowth in the lung. ΔF508 CF mice (Cftrtm1Eur), which have a targeted insertion ("knock-in") of the most common human disease-associated mutation, display little lung pathology. However, a strong phenotype is observed in the gastrointestinal tract of these mice including intestinal obstruction, inflammation, and bacterial overgrowth.

Here we show that CF mice are highly susceptible to infection with Citrobacter rodentium, a murine intestinal pathogen. C. rodentium exhibits atypical localization to the jejunum, cecum, and proximal colon during peak infection in CF mice, as opposed to the distal colon in WT mice. In these tissues, bacterial loads are higher compared to WT mice, and C. rodentium is tightly associated with the epithelial surface. Following infection, CF mice have an altered immune response compared to WT mice. Notably, there is a significant influx of neutrophils, eosinophils, and monocytes as well as increased inflammatory cytokine expression in CF mice. Furthermore, gut-corrected CFTR mutant mice, which express a wild-type human CFTR transgene exclusively in intestinal epithelial cells to correct the intestinal defect, are also more susceptible to C. rodentium infection. This provides

evidence that C. rodentium susceptibility in CF mice is related to loss of CFTR function in cells other than intestinal epithelial cells.

Further examination is required to determine the contributing roles of immune and environmental factors in the observed pathology. The development and characterization of this infection model may provide insights related to CF associated diseases.

4. **Membrane Protein Complex ExbB4–ExbD1–TonB1 from Escherichia coli Demonstrates Conformational Plasticity**

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Iron acquisition at the outer membrane (OM) of Gram-negative bacteria is powered by the proton motive force of the cytoplasmic membrane (CM), harnessed by the CM-embedded complex of ExbB, ExbD and TonB. Its stoichiometry, ensemble structural features and mechanism of action are unknown. By panning combinatorial phage libraries, periplasmic regions of dimerization between ExbD and TonB were predicted. Using overexpression of full-length exbB–exbD-His and tonB-S-tag, we purified detergent-solubilized complexes of ExbB–ExbD–TonB from Escherichia coli. Protein-detergent complexes of ~230 kDa with a hydrodynamic radius of ~6.0 nm were similar to previously purified ExbB4–ExbD2 complexes. Significantly, they differed in electronegativity by native agarose gel electrophoresis. Stoichiometry was determined to be ExbB4–ExbD1–TonB1. Single particle electron microscopy agrees with this stoichiometry. Two-dimensional averaging supported the phage display predictions, showing two forms of ExbD–TonB periplasmic heterodimerization: extensive and distal. Three-dimensional (3D) particle classification showed three representative conformations of ExbB4–ExbD1–TonB1. Based on our structural data, we propose a model in which ExbD shuttles a proton across the CM via an ExbB inter-protein rearrangement. Proton translocation would be coupled to ExbD-mediated collapse of extended TonB in complex with ligand-loaded receptors in the OM, followed by repositioning of TonB through extensive dimerization with ExbD. Here we present the first report for purification of the ExbB–ExbD–TonB complex, molar ratios within the complex (4:1:1), and structural biology that provides insights into 3D organization.

5. **Tyrosine phosphorylation regulation of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel**

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The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) channel is activated by phosphorylation and gated by ATP. It is generally assumed that PKA-mediated phosphorylation is required for maximal stimulation because exposure to PKA causes robust activation and the consensus sequences for phosphorylation by PKA are highly conserved among species. In a recent study we demonstrated that the muscarinic agonist carbachol stimulates CFTR through multiple signaling pathways, and about half the response was mediated by tyrosine kinases (Billet et al, 2013). In order to explore this non-canonical regulation of CFTR further we have used the patch clamp technique and inside membrane patches from BHK cells that stably express CFTR-wt or a mutant lacking consensus PKA sites (15SA-CFTR).

Exogenous application of the active tyrosine kinase p60c-Src or the proline-rich tyrosine kinase Pyk2 increased CFTR activity to levels that were similar to those elicited by PKA. The mean ratio of currents measured before addition of 1 mM AMP-PNP (a non-hydrolyzable analog of ATP causing

the lock of activated CFTR) to those measured after adding AMP-PNP was 0.49 ± 0.04 (n=5) for p60-cSrc stimulated patches and 0.49 ± 0.02 (n=3) for Pyk2 stimulated patches vs 0.54 ± 0.04 (n=5) for patches stimulated by PKA. In addition, channels were spontaneously active when CFTR was coexpressed with v-Src or the kinase domain of Pyk2, and further stimulated by the tyrosine phosphatase inhibitor dephostatin. Exogenous Src strongly activated the 15SA-CFTR variant which lacks 15 potential PKA sites and has a negligible response to PKA.

We conclude that tyrosine kinases can activate CFTR and that phosphotyrosine is a potent, PKA-independent stimulus of channel activity. This has major implications for the molecular mechanism of regulation by the R domain and may suggest new therapeutic strategies for the treatment of CF and other diseases that involve abnormal CFTR function.

Supported by the McGill University Health Centre Research Institute, NIH (NIDDK) and the Canadian Institutes of Health Research (CIHR).

6. **New opportunities for progress in CF research: the Cystic Fibrosis Translational Research Center (CFTRc)**

Renaud Robert[1], Aurélie Cleret-Buhot[1], Julie Goepp[1], Stevo Radinovic[1], Elizabeth Matthes[1], Carmen Lampron[1], Annick Guyot[1], David Y Thomas[1], John Hanrahan[1]

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The goal of the CFTRc is to find a cure for cystic fibrosis by providing a platform for basic CF research and the development of therapies targeting the basic defect that underlies cystic fibrosis and other protein trafficking diseases. The CFTRc regroups more than 30 members who interact in physiology and pathophysiology, biochemistry, cell biology, ion channel biophysics, immunology, bacteriology, respirology and gastrointestinal physiology. The CFTRc offers unique infrastructure and network to give the opportunity to our members to make a breakthrough in their field. Here we present three state-of-the-art facilities of the CFTRc.

The Primary Cell Culture Core Facility, the first of its kind in Canada, is dedicated to supply for CF research across Canada, standardized, high-quality primary epithelial cells representing genetic variability. This non-for-profit facility provides to researchers, highly differentiated primary epithelial cells that are essential for studies of airway physiology/pathophysiology, cell biology, inflammation, mucosal immunity, and for translational research to develop new therapeutics. This platform involves the establishment of a procurement network through Canada, involving several physicians and surgical teams, following ethical requirements.

The CFTRc Imaging Facility has 4 state-of-the-art light microscopes to answer several questions about CF research. These microscopes will allow the researchers to generate high quality images and quantitative data for high level publications, elevating research projects to the next level. Live cell imaging, confocal imaging, DIC, FCS to study ligand binding in solution and in cells, TIRF, quantitative imaging of protein and vesicle dynamics, measurement of intra and extracellular pH, Ca2+, are among techniques that could be applied at the CFTRc.

The scientific aim of the HTS Facility is to facilitate the development of chemical biology programs in academic labs by supporting high throughput screens of various natures through shared infrastructure, materials and expertise. We aim to create an open and interactive environment to stimulate both drug discovery and basic research from different fields and institutions and to provide a training ground for graduate students and post-doctoral fellows. We offer complete and customized services for both screening and non-screening projects, and provide an environment for our customers to make a breakthrough in their field. We strive to establish an enduring relationship with our customers and collaborators in addressing their research needs and expertise.

In conclusion, to accomplish its mission, the CFTRc is (1) enhancing CF research by offering unique infrastructure, state-of-the-art equipment, and network resources that accelerate breakthroughs in the field, (2) providing a translational research platform for the development of therapies that target the basic defect underlying CF and other protein trafficking diseases, (3) developing a trans-Canada lung

tissue procurement network and isolate and distribute cells to researchers across the country. This poster will present the center and its main facilities.

7. **The impact of Pseudomonas aeruginosa lasR mutants on airway inflammation in chronic lung infections**

Shantelle Lafayette[1,2], Daniel Houle[2], Trevor Beaudoin[1,2], Lucas Hoffman[3], Jane Burns[3], Ajai Dandekar[3], Nicole Smiley[3], Josephine Chandler[3], James Zlosnik[4], David Speert[4], Joanie Bernier[5], Elias Matouk[1,5], Emmanuelle Brochiero[6], Simon Rousseau[1], Dao Nguyen[1,2,5]

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Nearly 80% of adult cystic fibrosis (CF) patients develop chronic Pseudomonas aeruginosa (Pa) airway infections. This progressive and often fatal lung disease is characterized by severe neutrophilic inflammation. Null mutations in lasR, which encodes a quorum sensing regulator that activates the expression of several acute virulence factors, frequently arise in Pa strains infecting CF airways; up to 30% of chronically-infected patients harbor lasR null mutants. While attenuated in acute virulence, lasR null mutants are paradoxically associated with decreased lung function and worst prognosis in CF patients. We hypothesize that phenotypic changes occurring in lasR null mutants may alter inflammatory responses in CF airways thereby affecting the progression of chronic lung disease.

Our study focused on two fully sequenced clonally-related Pa isolates taken from a single patient: the late (L) isolate, isolated 7.5 years after the early (E) isolate, had accumulated 68 mutations including a lasR null mutation. In vitro stimulation of human airway epithelial cells (AEC) with diffusible planktonic and biofilm products from the E and L isolates revealed that the L isolate elicited a significantly more robust IL-6 and IL-8 inflammatory response. The increased IL-6 and IL-8 levels from AECs stimulated with the L isolate were due to the inability of this strain to produce the LasR-regulated secreted protease LasB, which is capable of degrading both cytokines. Furthermore, we found that Pa strains that produce less LasB stimulate increased neutrophil recruitment in vitro. Studies using a murine model of chronic Pa airway infection to characterize the in vivo inflammatory response to Pa strains harboring lasR mutations are ongoing.

Understanding how loss of function mutations in lasR, modulate host inflammatory responses and promote bacteria persistence can provide us insights into the pathogenesis of chronic infections, a paradigm distinct from acute virulence.

8. **Toward Single-Cell Genomic Analysis with Single-Molecule Sensitivity**

François Michaud[1], Daniel Berard[1], Sabrina Leslie[1]

[1]Université McGill

Linearly extending long DNA molecules in sub-50 nm nanochannels for genomic analysis, while retaining their structural integrity, is a major technological challenge. We employ ``Convex Lens-induced Confinement'' (CLiC) microscopy to gently load DNA into nanogrooves from above, overcoming the limitations of side-loading techniques used in direct-bonded nanofluidic devices. In the CLiC technique, the curved surface of a convex lens is used to deform a flexible coverslip above a glass substrate, creating a nanoscale gap that can be tuned during an experiment to load and confine molecules into nanoscale features embedded in the bottom substrate. Since DNA molecules are loaded into the embedded nanotopography from above, CLiC eliminates the need for the high pressures or electric fields required to load DNA into direct-bonded nanofluidic devices. To demonstrate the versatility of CLiC, we confine DNA to a variety of nanostructures (linear, circular, gridded patterns of nano grooves), demonstrating DNA nanochannel-based stretching and

denaturation mapping (Berard et al, PNAS 2014). We have successfully extended DNA in as small as 27-nm channels, achieving high stretching (90 percent) that is in good agreement with Odijk deflection theory and demonstrating mapping of genomic features using denaturation analysis. Additionally, we have recently demonstrated single-fluorophore resolution and resolved nick-labeled DNA in these devices, establishing compatibility with a suite of DNA mapping methods in the biotechnology sector.

9. **McGill Life Sciences Complex, Advanced BioImaging Facility (ABIF)**

Erika Wee[1,2,3], Cleret-Buhot A.[1,2,3], Brown C.M.[1,2,3]

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The ABIF provides the research community at McGill University and the Montreal scientific community with access to advanced imaging equipment for cellular and tissue imaging, as well as technical support from our facility staff with over 35 years of combined microscopy experience. The facility aims to support researchers on all aspects of their research projects from sample preparation to publication. The facility offers thirteen microscope platforms for such things as: confocal microscopy, laser micro-dissection, total internal reflection fluorescence (TIRF) microscopy, live cell imaging, spectral imaging, FRET, FRAP, photo-activation, fluorescence correlation spectroscopy (FCS), high content/throughput automated imaging (HCS), automated image processing assays (cell cycle, mitotic index, translocation), image correlation spectroscopy and advanced image processing and analysis (Imaris, MetaMorph, ImagePro, AutoQuant). Basic and advanced training is available and users have ongoing support for all of the equipment and analysis workstation in our facility. The facility also provides advice on specific applications such as sample preparation, fluorescent dyes, fluorescent proteins, live cell imaging, cameras, appropriate microscopy platforms for given applications, and image processing and analysis.

10. **Canaux K+ en tant que cibles thérapeutiques dans la réparation et la régénération de l'épithélium respiratoire humain fibrose kystique**

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

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

Objectifs : Notre but était d'étudier le rôle d'un canal K+ voltage-dépendant, le canal KvLQT1, dans la réparation/régénération de l'épithélium des voies aériennes et d'identifier les composés activateurs de ce canal, en combinaison ou non avec les correcteurs de CFTR, capables d'améliorer efficacement ces processus dans un contexte exempt ou non d'infection.

Méthodologie : La vitesse de réparation a été évaluée in vitro sur des cultures de cellules épithéliales FK et non-FK non-différenciées en monocouches et de cellules différenciées en interface air-liquide (IAL). La cinétique de régénération été évaluée non seulement in vitro sur des cultures différenciées en IAL (quantification des cellules ciliées par un immunomarquage de la tubuline-β et localisation du

canal CFTR observée par microscopie confocale) mais également dans le modèle de xénogreffe bronchique humanisée dans la souris nude (analyses histologiques). Les cellules sont soumises à des traitements pharmacologiques (inhibiteurs et activateurs des canaux KvLQT1, en absence ou en présence des correcteurs de CFTR) et exposées ou non à des exo-produits sécrétés de P. aeruginosa (PsaDM).

Résultats : Nous avons tout d'abord observé que l'expression totale de la protéine canal KvLQT1 n'est pas modifiée suite à une exposition des cellules non-FK et FK à des exo-produits sécrétés de P. aeruginosa (PsaDM). De plus, l'activation du canal KvLQT1 accélère la vitesse de réparation alors que son inhibition induit un ralentissement de la réparation de monocouches indifférenciées de cellules primaires épithéliales non-FK en présence ou non d'infection. Dans les cultures FK, la correction et potentialisation du CFTR (avec le Vx809 et le Vx770) améliore la réparation, mais cet effet est altéré en présence d'infection. De façon intéressante, l'activation du KvLQT1 permet de stimuler la réparation épithéliale malgré la présence d'exoproduits bactériens. Nous montrons également le rôle du canal KvLQT1 dans la réparation de cultures de cellules différenciées non-FK cultivées en IAL. Le traitement par le R-L3 tout au long de la culture en IAL accélère également la ciliogenèse alors que le traitement par le clofilium l'inhibe, indiquant un rôle de ce canal dans la différentiation et régénération épithéliale. En effet, les résultats obtenus avec notre modèle de xénogreffe montrent que l'exposition des greffons à l'inhibiteur de KvLQT1 ralentit la régénération.

Conclusions : Nos résultats montrent que la fonction du canal KvLQT1 est cruciale pour la réparation et la régénération des épithélia des voies aériennes. De façon intéressante, nous montrons pour la première fois que l'activation de ce canal permet de favoriser la réparation des lésions de l'épithélium non-FK et FK, et ceci malgré la présence d'infection. Nos expériences montrent également que ce canal joue un rôle important dans la régénération et la fonctionnalité épithéliale, et notamment sa capacité à former des cellules ciliées.

Nos travaux sont supportés par Cystic Fibrosis Canada, l'association française Vaincre la mucoviscidose et le RSR de FRQS pour la biobanque du CRCHUM.

11. The Role of SMPD1 in the Innate Immune System's Response to Respiratory Bacterial Infections

Elyse MacFadden-Murphy[1], Simon Rousseau[2]

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Sphingomyelin phosphodiesterase (SMPD1) is responsible for converting sphingomyelin to ceramide. Niemann-Pick disease (NPD) types A and B are rare autosomal recessive diseases that are characterized by having decreased SMPD1 activity and thus have a lack of ceramide. Cystic Fibrosis (CF) is a hereditary disease where by chronic respiratory bacterial infections leads to the morbidity and mortality of this disease, and increased ceramide levels in the lungs. Ceramides are potent activators of p38α MAPK. These two fatal diseases involve genetic mutations causing imbalances in ceramide levels and metabolism. As a result patients have increased susceptibility to respiratory bacterial infections. Bacterial infections and the subsequent inflammation involve an increase in phosphorylation of p38α MAPK. Upon bacterial infection, epithelial cells send distress signals by secreting cytokines, which are involved in neutrophil migration to the site of infections. The overall goal of this project is to explore SMPD1's role in defense against bacterial infection via activation of p38α MAPK by stimulation with P. aeruginosa diffusible material (PsaDM), an important infective agent of CF airways. To examine this, several methods are performed in human airway epithelial cells (AEC) exposed to bacterial stimuli. Firstly, immunoblots are used to visualize and quantify the phosphorylation of p38α MAPK activation. Secondly, real-time quantitative PCR is performed to measure messenger RNA levels to evaluate SMPD1 expression and the expression of pro-inflammatory cytokines. Furthermore, I have generated stable AECs expressing a short-hairpin RNA (shRNA) against SMPD1. The data shows that when SMPD1 is knocked down, there is an elevation of p38α activation at the basal level, an overall elevation of cytokines produced, and greater number of neutrophils recruited. Understanding

the role played by SMPD1 in host defense mechanisms of the lungs is crucial to develop novel therapies aimed at improving the quality of life of patients suffering from CF and NPD.

Acknowledgements: Dr. Simon Rousseau's Lab, CIHR, MUHC.

12. **Impact of correctors and potentiator combinations on CF airway epithelial repair under infectious conditions**

Claudia Bilodeau[1,2], Damien Adam[1,2], Manon Ruffin[1,2], Nguyen Thu Ngan Trinh[1,2], Émilie Maillé[1,2], Simon Rousseau[3], Emmanuelle Brochiero[1,2]

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Cystic fibrosis (CF) caused by CFTR mutations is characterized by bacterial colonization and chronic inflammation leading to a progressive damage of the airway epithelium. Our previous studies highlighted a delayed wound repair in CF airways that could be due, at least in part, to the basic defect of CFTR. Accordingly, improved repair rates were measured after CFTR correction with VRT-325. However, our data also indicated that CFTR correction and repair improvement were compromised in presence of Pseudomonas aeriginosa diffusible material (PsaDM). Because the use of CFTR corrector in combination with CFTR potentiator has been shown to elicit a better restoration of F508-CFTR function, we then decided to test the impact of this combination on epithelial repair under non-infectious and infectious conditions.

For that purpose, we evaluated the efficiency of the CFTR correctors (VRT-325, Vx-809) and potentiator (Vx-770) on the early phases of epithelial repair, by performing wound healing experiments on different cell models as well as on human primary airway epithelial cells, in the absence or presence of PsaDM.

We first confirmed that Vx-770 slightly increased the repair rates of non-CF NuLi-1 cell monolayers and as well as non-CF primary airway epithelial cell monolayers in the absence of infection. CFTR correction, with VRT-325 or Vx-809, significantly improved the repair capacity of the CuFi-4 cell line carrying ΔF508/G551D mutations, whereas VRT-325 only was able to enhance the wound healing rates of CuFi-1 (carrying ΔF508/ΔF508mutations). Interestingly, a higher increase in CuFi-4 and CuFi-1 repair rates were observed with the combination of a corrector (VRT-325 or Vx-809) and a potentiator (Vx-770) compared to a treatment with a corrector alone. Similarly, the epithelial repair capacity of CF human primary airway cells was enhanced after rescue with Vx-809 or VRT-325, but this improvement was further marked after a combined treatment with corrector and potentiator. In the presence of infection (PsaDM), the beneficial effect of VRT-325 or Vx809 on human primary airway epithelial repair was severely impaired, but a significant improvement in wound healing rates was observed with the combination of corrector/potentiator, even in presence of PsaDM.

Altogether, our data first demonstrated that the presence of infectious products affected the ability of CFTR correctors to improve CF airway epithelial repair. However, CFTR functional rescue with CFTR corrector in combination with potentiator allowed a better restoration of repair rates, even in the presence of infection.

Project supported by Cystic Fibrosis Canada, Vaincre la Mucoviscidose (VLM) and Biobank of CRCHUM and RHN of FRQS.

13. **Impact d'exoproduits de Pseudomonas aeruginosa sur la fonction du canal CFTR dans les cellules épithéliales des voies aériennes**

Émilie Maillé[1], Nguyen Thu Ngan Trinh[1,2], Claudia Bilodeau[1,2], Manon Ruffin[1,2], Marie-Claude Quintal[3], Martin Y Desrosiers[1], Simon Rousseau[4], Emmanuelle Brochiero[1,2]

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La présence d'infections bactériennes à P.aeruginosa (PA) dans les voies aériennes est une caractéristique de plusieurs pathologies pulmonaires telles que les maladies pulmonaires obstructives chroniques (MPOC) et la Fibrose Kystique (FK). Cet aspect de la pathologie a été grandement étudié, mais son rôle sur la fonction du canal CFTR, n'est pas clairement défini. Nous avons donc évalué l'impact d'exoproduits de PA (PSADM), sur l'expression et la fonction de CFTR dans les cellules épithéliales des voies aériennes non Fibrose Kystique ainsi que leurs impact sur la correction du CFTR muté delta-F508 par le VRT-325 dans les cellules épithéliales des voies aériennes Fibrose Kystique.

Tout d'abord, nous avons pu constater que la présence de PSADM diminue le courant et l'expression de CFTR dans les cellules épithéliales bronchiques non-FK (CFBE wt) via une augmentation de la vitesse de dégradation et une diminution de la synthèse de la protéine. Dans les cellules épithéliales bronchiques FK (CFBE delta-F508), le PSADM réduit de façon importante la maturation du CFTR induite par le correcteur VRT-325. Nous avons observé le même effet délétère du PSADM sur la fonction du canal CFTR dans les cellules primaires non-FK ainsi que sur la correction de la fonction du CFTR delta-F508 par le VRT-325 dans les cellules primaires FK. Afin d'identifier plus finement les exoproduits de PA entrainant cet effet, nous avons étudié l'impact de filtrat provenant de souches mutantes de PA et nous avons pu constater que les exoproduits sous le contrôle du quorum sensing LasR semblent grandement impliqués dans la perte de correction et de maturation du CFTR delta-F508 dans les CFBE.

En conclusion, la présence de PA affecte la fonction et la correction du canal CFTR, suggérant que l'efficacité des correcteurs de CFTR pourrait être limitée par la présence d'agents infectieux chez les patients FK.

Supporté par : CFC, FRSQ-RSR, Banque de tissus respiratoire CRCHUM-RSR

14. The downregulation of CFTR due to cigarette smoke is not likely mediated by the aryl hydrocarbon receptor pathway nor the MEK/ERK1/2 pathway

Hilalion Ahn[1], Renaud Robert[1], Elizabeth Matthes[1], John Hanrahan[1]

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel expressed in lung epithelial cell membranes and is essential for maintaining normal airway homeostasis. Cigarette smoke exposure significantly reduces the expression and function of CFTR; however little is known about the mechanism by which CFTR is internalized. It has recently been reported that cigarette smoke activates both the aryl hydrocarbon receptor (AhR) and the MEK/ERK1/2 MAPK pathway, leading to upregulation of inflammatory cells, increased mucus production, and ultimately the disorder of lung epithelium homeostasis. Given this, we hypothesize that the AhR pathway and the MEK/ERK1/2 MAPK pathway mediates the downregulation of CFTR that is induced by cigarette smoke in airway epithelial cells.

To test this hypothesis, we measured short-circuit current across monolayers of cystic fibrosis bronchial epithelial cells (CFBE41o-) that had been complemented with wild-type CFTR. Independent treatment with aryl hydrocarbon antagonists or MEK/ERK1/2 MAPK inhibitors failed to block the decrease of CFTR activity induced by cigarette smoke extract. These results suggest that the AhR pathway nor the MEK/ERK1/2 MAPK pathway are likely to play a role in downregulating CFTR channels during acute cigarette smoke exposure.

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