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MEAKINS-CHRISTIE LABORATORIES - MONTREAL - MUHC-RI - MCGILL

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Abu-Arish, Asmahan
Category: Research Associate (Poster 1)
Supervisor: John Hanrahan

Cigarette Smoke-Induced Aggregation of CFTR into Ceramide Platforms is ROS-Dependent

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The cystic fibrosis transmembrane conductance regulator (CFTR) anion channel is required for cAMP-stimulated fluid secretion across airway epithelia and enables inhaled substances to be removed from the lung by mucociliary clearance. Airway epithelia are exposed to many environmental contaminants during normal breathing including cigarette smoke. To examine the acute effects of cigarette smoke on the behavior of CFTR at the airway surface, we examined the distribution, aggregation state, and mobility of fluorescently-tagged CFTR (GFP-CFTR) on primary human bronchial epithelial (pHBE) cells that had been exposed to cigarette smoke extract (CSE). We showed previously that some CFTR occurs in sub-resolution clusters which are homogeneously distributed on the surface of pHBEs under control conditions and obtained evidence for two dynamically-distinct populations. One CFTR population had small spatial scale transport dynamics and confinement that was dependent on membrane cholesterol, consistent with localization within lipid rafts. The other population had larger spatial scale dynamics indicating lateral mobility both outside and within rafts. Here we report that acute exposure to CSE causes a significant redistribution of CFTR between these two populations, which is mediated by recruitment into nano-scale clusters and also fusion of the clusters into large platforms (0.5-5 μm).

Acute CSE exposure caused the appearance of conspicuous platforms along with 3.5-fold increases in CFTR aggregation and confinement and a 2-fold increase in total surface expression. Since cigarette smoke contains oxidants and can potentially stimulate the intracellular production of reactive oxygen species (ROS), we examined the role of ROS in the CSE-induced changes in CFTR distribution, aggregation state, and mobility by pre-treating cells with the antioxidant N-acetylcysteine (NAC). NAC prevented platform formation and abrogated increases in CFTR aggregation and confinement, suggesting that CSE effects on surface CFTR are strongly dependent on ROS, most likely through stimulation of membrane ceramide production by acid sphingomylinase (aSMase) and formation of ceramide platforms.

To further examine the role of ROS in the response of CFTR to CSE, cells were loaded with a newly developed, cell-permeant ROS indicator and studied by confocal microscopy. A gradual increase in probe fluorescence requiring ~1 h was detected during CSE exposure indicating a slow increase in ROS concentration. Pre-treatment with NAC prevented this increase in fluorescence, confirming that the probe provides a measure of intracellular ROS concentration. By contrast, acute H_2O_2 addition to the bath solution caused immediate, step-like increase in probe fluorescence as predicted since cell membranes are highly permeable to H_2O_2. Together these results imply that the slow accumulation of ROS observed during CSE exposure reflects intracellular production by NADPH oxidases and/or mitochondria.

In summary, acute CSE exposure induces ROS production and the aggregation and confinement of CFTR within ceramide platforms. This transient increase in surface expression may enhance the secretory response that rids the epithelial surface of noxious substances.

Funding: Cystic Fibrosis Canada (CFC), Cystic Fibrosis Foundation (CFF) & NSERC

Aintabi, Daniel
Category: MSc Student (Poster 19)
Supervisor: Larry Lands

Physical Activity Patterns and Exercise Capacity in Adults with Cystic Fibrosis (CF)

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Introduction: Forced expiratory volume in 1 second (FEV_1) is the main prognostic factor used in the CF population to assess lung function. Patients with CF are associated with greater airflow limitation in comparison to healthy individuals of similar height and age (FEV_1%predicted). Lung function is a significant predictor of exercise capacity in the CF population. Performing more habitual physical activity can significantly attenuate the rate of decline in FEV_1 in CF patients. Time spent performing moderate-vigorous physical activity has been shown to have a positive correlation with exercise capacity in CF. While physical activity patterns are well documented for CF children, few studies have assessed these patterns in CF adults. This study aimed to assess physical activity patterns and its impact on exercise performance in CF adults. **Methods:** As part of an international prospective study to increase physical activity through motivation using a 6-month training program, patients with CF were recruited from the McGill University Health Centre (MUHC). Inclusion criteria consisted of participants having an FEV_1 ≥35%predicted and performing ≤4 hours/week of vigorous physical activity. Physical activity was subjectively assessed using The Habitual Activity Estimation Scale (HAES) (time spent "somewhat active" and "very active") and the 7-day Physical Activity Recall (7D-PAR) ("moderate", "hard", "very hard" and "strength" physical activity). Skinfold measurement was used to assess body composition. FEV_1 and forced vital capacity (FVC) were obtained using spirometry. Incremental cycle ergometry following the Godfrey protocol was used to assess peak oxygen capacity (VO_2peak) and maximal workload (Wmax). **Results:** Baseline data of 2 female and 4 male CF adults (age= 36.67±15.36) was collected. Participants had a mean weight of 66.01±11.92kg, height of 171.2±10.3cm, and BMI of 22.5±3.3. Mean fat free mass (FFM) and %body fat were 53.23±9.92kg and 19.26±5.18% respectively, all within normal values. Mean FEV_1%predicted was 68.04±18.8 and FVC%predicted was 91.08±20.26. A mean of 41.82hrs/week of habitual physical activity was observed using the HAES questionnaire, 0.32hrs/week of which was spent as very active and 41.5hrs/week as somewhat active. A mean of 18.5hrs/week of physical activity was observed using the 7D-PAR questionnaire (15.86hrs "moderate", 1.98hrs "hard", 0.40hrs "very hard" and 0.26hrs "strength"). While different mean values were obtained, a positive correlation was identified between the reported total amount of physical activity using the HAES and 7D-PAR questionnaires ($r=0.84$, $p<0.05$). Participants achieved a mean VO_2peak%predicted of 88±15.7 and Wmax%predicted of 97.5±28.1. Total time spent performing physical activity using the HAES and 7D-PAR questionnaires both strongly correlated with VO_2peak%predicted ($r=0.96$ $p<0.005$, $r=0.92$ $p<0.05$). Total time spent performing more than moderate physical activity for the 7D-PAR questionnaire also significantly correlated to VO_2peak%predicted ($r=0.95$ $p<0.005$). No significant correlations with VO_2%predicted were seen for FEV_1%predicted, %body fat or FFM.**Conclusion:** Using the HAES and 7D-PAR questionnaires, this study provides baseline data on physical activity patterns in CF adults while validating the use of these two questionnaires. The results of this study suggest that increasing physical activity in CF adults may improve their exercise capacity.

Funding: Cystic Fibrosis Canada

Al-Habeeb, Fatmah
Category: MSc Student (Poster 16)
Supervisor: Carolyn Baglole

The Role of HuR in myofibroblast differentiation: implications for pulmonary fibrosis.

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Introduction: Idiopathic pulmonary fibrosis (IPF) is an under-diagnosed lung disease characterized by progressive lung scarring with median survival of 3-5 years from initial diagnosis. Although the etiology of IPF is unclear, excessive extracellular matrix (ECM) deposition is a key event in disease pathogenesis. ECM in the form of collagen and fibronectin are excessively deposited due to increased differentiation of fibroblasts into the α-SMA expressing myofibroblasts. The pathways leading to this lung scarring are poorly understood but likely involve the pro-fibrotic cytokine transforming growth factor (TGF)-β1. We predict that TGF-β1 acts via Human Antigen R (HuR), an RNA binding protein whose principle function is to promote protein translation. HuR is localized in the nucleus under normal conditions, but upon translocation from the nucleus to the cytoplasm, HuR may promote the translation of pro-fibrotic ECM mRNAs (including TGF-β1) into protein. The role of HuR in promoting the differentiation of fibroblasts to myofibroblasts in association with ECM deposition is completely unknown.

Hypothesis: HuR promotes the differentiation of lung fibroblasts to myofibroblasts, which increases ECM proteins that stiffen the lungs.

Methods: Lung tissue was obtained from individuals undergoing lung resection surgery at McMaster University. Following derivation, normal human lung fibroblasts (HLFs) were treated with TGF-β1 (1ng/ml, 5 ng/ml or 10 ng/ml) from 6 -72 hours. Total HuR expression as well as the expression of α-smooth muscle actin (α-SMA; a marker of myofibroblasts) was assessed by Western Blot. Immunofluorescence (IF) was performed to assess HuR localization in response to TGF-β1 and evaluated using the Airyscan Immunofluorescence microscope (LSM880). HLFs were treated with different doses of TGF-β1 (see above) for 6, 24 or 48 hours to evaluate HuR translocation. Hoechst 33342 was used to stain the nucleus (blue) while HuR was stained with Alexa Fluor 555 (red). Actinomycin D (Act D; a transcription inhibitor that activates HuR translocation) was used as a positive control and untreated cells as negative control.

Results: Exposure of HLFs to TGF-β1 increased the total protein levels of HuR in a time-dependent manner, peaking 24 hours post-treatment; this was followed by gradual decrease by 72 hours. There was greatest expression in HuR levels with 10 ng/ml of TGF-β1. Moreover, α-SMA expression was also strongly induced by TGF-β1 within 24h after treatment, reaching statistically-significant results at 48 hours and 72 hours post-treatment. Our results also showed that TGF-β1 increases the HuR cytoplasmic translocation with 10 ng/ml at 24 and 48 hours, being statistically-significant at 48 hours post-treatment.

Conclusions: TGF-β1 induces the differentiation of fibroblasts to myofibroblasts. Our preliminary data indicate that HuR expression and translocation are increased in response to TGF-β1. This increase in HuR is associated with increase in myofibroblasts differentiation assessed by increased levels of α-SMA. Thus, TGF-β1 may be a driving factor in the production of pro-fibrotic factors in IPF.

Funding: Boehringer Ingelheim ltd., - King Saud University, Riyadh, Saudi Arabia.

Aloufi, Noof
Category: MSc Student (Poster 21)
Supervisor: Carolyn Baglole

Human antigen R (HuR) regulates Cigarette Smoke-induced inflammation: Implications in COPD

Noof Aloufi, Parameswaran Nai, David H. Eidelman and Carolyn J. Baglole

Background: Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the world. It is an incurable respiratory disorder characterized by progressive, irreversible airflow limitation. COPD pathogenesis is associated with a chronic inflammatory response initiated by inhalation of cigarette smoke. Cigarette smoke may contribute to lung inflammation via human antigen R (HuR). HuR is a ubiquitously-expressed RNA-binding protein that regulates mRNA stability, which promote the translation of proteins, many of which are associated with inflammation. HuR expression and cytoplasmic localization are elevated in lung cancer, a feature that correlates with inflammatory protein expression. The expression and localization of HuR in COPD or its molecular regulation by cigarette smoke is not known. Therefore, I hypothesize that cytoplasmic translocation of HuR contributes to regulate inflammatory protein production in response to cigarette smoke typical of COPD.

Objective and Aims: The objectives of our study is to evaluate the role of HuR in COPD by evaluating the expression and localization of HuR in human lung tissue and structural cells from Normal (never-smokers without COPD), Smokers (without COPD) and COPD subjects, and to evaluate if the expression and the cytoplasmic localization of HuR is induced by cigarette smoke. Then, we assess if HuR regulates cigarettes smoke-induced inflammatory proteins production.

Methods: Multiplex Immunohistochemistry (mIHC) was performed on human lung tissue obtained from Normal, Smoker and COPD subjects and comparison of total HuR protein as well as nuclear and cytoplasmic levels will be quantified. HuR protein was evaluated by western blot in human lung fibroblasts derived from same subject groups exposed to control media or cigarette smoke extract (CSE), an *in vitro* surrogate for cigarette smoke exposure. Nuclear and cytoplasmic protein were fractionated and the levels of HuR assessed by western blot. The inflammatory protein COX-2 and IL-8 expression were evaluated by western blot and ELISA, respectively, after treating siControl or siHuR transfected cells with media or 2% CSE for 8h and 24h.

Results: There was more abundant cytoplasmic localization of HuR in lung tissue from Smoker and COPD subjects whereas in Normal subjects, HuR was predominantly localized in the nucleus. There was also more cytoplasmic HuR in macrophages in Smoker and COPD subjects. While there was slight increase in total HuR protein in Normal and COPD human lung fibroblasts after exposure to 2% CSE for 4 hours, there was an increase in cytoplasmic HuR. The expression of COX-2 and IL-8 were significantly increased after treating siHuR transfected cells (to decrease HuR protein levels) with 2% CSE for 24 hours.

Significance: Understanding the role of HuR in regulating cigarette smoke-induced inflammation could provide the basis for development of new target therapy for diseases such as COPD.

Alsowayan, Waleed
Category: Clinical Fellow (Poster 11)
Supervisor: Jean Bourbeau

Practice patterns and care gaps according to COPD physician diagnosis in primary care

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Background In Canada, the rate and accuracy of COPD, asthma and asthma COPD overlap detection in primary care is unknown. There is indication-based framework for inhaled medication selection but we don't know how often primary care deviate from guideline-indicated treatment.
Objectives- The objective of this population-based longitudinal study was to evaluate the undiagnosed and diagnosed COPD, asthma and asthma COPD overlap, the use of the spirometry and the management in primary care in Canada.

Methods This study was embedded in the Canadian Cohort Obstructive Lung Disease (CanCOLD) study, an ongoing multi-center study involving subjects with COPD sampled from the general population, e.i., 1,556 individuals with 832 COPD diagnosed using post-bronchodilator spirometry according to GOLD criteria.. Diagnosis subsets were divided as followed: i) COPD or asthma not diagnosed by physician; ii) physician diagnosed COPD; iii) physician diagnosed asthma and; iv) physician diagnosed asthma COPD overlap (ACO). Sub cohort analysis was done for subjects who completed an exacerbation evaluation for at least one year (tel. every 3 months).

Results The study sample included 700 COPD subjects. Sixty percent (60%) of the subjects reported not having a physician diagnosis of COPD and/or asthma, 20% having a physician diagnosed asthma, 10% a physician diagnosed COPD and ACO. Spirometry was done in only 30% of undiagnosed subjects and 70-90% in those reporting a physician diagnosed COPD and/or asthma. Subjects with increased symptom burden (GOLD B 57% AND D 78%) and increased risk of exacerbations (GOLD C 43% and D 78%) are more likely to have a physician diagnosis. When subjects were receiving inhaled medications, ICS alone was administered in 25-30% of any GOLD ABCD and mostly in physician Dx asthma or ACO. ICS-LABA and triple Rx was administered in 60% GOLD D, 40% GOLD AB and 30% GOLD C and most of the subjects with physician Dx asthma, COPD or ACO . However still 67% didn't receive any respiratory medication (50% GOLD B, 73% GOLD C and 31% DOLD D).

Conclusion The study has identified care gaps in primary care having at least 60% of the subjects with undiagnosed COPD. Spirometry is clearly under-utilized. And significant proportion of subjects (67%) didn't receive inhalation therapy.

The CanCOLD study is currently funded by the Canadian Respiratory Research Network (CRRN); industry partners: Astra Zeneca Canada Ltd; Boehringer Ingelheim Canada Ltd; GlaxoSmithKline Canada Ltd; Novartis. Previous funding partners are the CIHR (CIHR/ Rx&D Collaborative Research Program Operating Grants- 93326); the Respiratory Health Network of the FRSQ.

Balassy, Zsombor
Category: MSc Student (Oral Presentation)
Supervisor: Anne-Marie Lauzon

Molecular Mechanics of the Myosin-Actin Interaction in the Latch-State

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Background: Compared to the better known striated muscle, smooth muscle is unique in its ability to maintain force efficiently and for long periods of time. This property, called the latch-state, has been observed experimentally at the whole muscle level and it consists of force maintenance at low energy (ATP) consumption and low myosin activation (phosphorylation) levels. This property has been observed predominantly in tonic (e.g. blood vessels) compared to phasic (e.g. gut) smooth muscle. Multiple theories have been proposed to explain the underlying molecular mechanism of the latch-state, however, none has been verified at the molecular level. With the use of the in vitro motility assay and a microfluidic device, I intend to elucidate this phenomenon at the level of the contractile proteins, myosin and actin, by attempting to create latch-bridges (myosin strongly bond to actin).

Hypothesis: I hypothesize that if smooth muscle myosin gets dephosphorylated while attached to actin filaments, it will remain attached and will increase the load on the actin, thus decreasing the actin filament velocity in the in vitro motility assay.

Methods: To test this hypothesis, I used the in vitro motility assay in which fluorescently labeled actin filaments are propelled by a lawn of myosin molecules on the surface of a coverslip. To induce dephosphorylation, I developed a microfluidic chamber to inject myosin light chain phosphatase (MLCP) efficiently but without creating bulk flow on the motility surface. I mixed phasic muscle myosin with skeletal muscle myosin, the latter being a faster myosin. Because skeletal muscle myosin is not affected by MLCP, I expect to observe a transient decrease in velocity, due to the load induced by the latch-bridges (attached, dephosphorylated myosin), that will resume after their detachment.

Results: Preliminary data show the following trend: prior to injection, a 50-50 mixture of skeletal and phasic smooth muscle myosin produced a motility with an average velocity of 1.70±0.07 μm/s. Dephosphorylation of smooth muscle myosin took place in 50 seconds after injection, as was independently measured. During this time, the average velocity decreased to 1.3±0.1 μm/s. After all the smooth muscle was dephosphorylated (>50 seconds after injection), the remaining mixture of weakly binding unphosphorylated myosin and fully functional skeletal myosin produced an increased velocity of 2.05±0.09 μm/s.

Discussion: The resulting velocities of the myosin mixtures were as expected to demonstrate the presence of latch-bridges. That is, the strongly binding smooth muscle myosin (latch-bridges), that presumably resulted from their dephosphorylation, mixed with skeletal muscle myosin slowed down motility. The fully dephosphorylated smooth muscle myosin, known to only bind weakly to actin and which cannot undergo a powerstroke, resulted in higher velocity, dominated by the skeletal muscle myosin velocity.
More measurements are underway to complete this data set. Measurements will also be performed with tonic muscle myosin because its slower kinetics might increase the chances of it getting dephosphorylated while attached and thus, increasing its chances of forming latch-bridges.

Funding: NSERC, FRQS

Barrecheguren, Miriam
Category: Postdoctoral Fellow (Poster 10)
Supervisor: Jean Bourbeau

Inflammatory patterns in COPD: preliminary results from the CanCOLD population

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Introduction: In the past years there has been a growing interest in the identification of COPD phenotypes that can help to tailor pharmacological treatment. New complementary tests such as sputum cell count, blood eosinophils and FeNO have been used as markers for treatment with Inhaled corticosteroids (ICS), although the utility in daily clinical practice is not clear yet.

Method: Prospective study embedded in the Canadian Cohort Obstructive Lung Disease (CanCOLD) aiming to characterize a COPD population based on inflammatory features. We included healthy individuals, individuals at risk of COPD (active smokers) and COPD patients (GOLD1 and GOLD2+). Demographic and clinical characteristics, blood eosinophils, lung function, CT scan, symptoms and quality of life questionnaires were collected at baseline. FeNO, sputum induction, IgE and prick-test were added to the CanCOLD visits.

Results: To date, 82 individuals have completed the study visit (13 healthy controls, 18 at risk, 26 COPD GOLD 1 and 25 COPD GOLD 2+). There were no statistically significant differences in demographics (age and sex) between groups although the % of women tended to be higher in the healthy group. Mean FeNO was 28.1 (SD=16.2) ppb in healthy individuals, 23.5 (10.7) ppb in smokers, 24.8 (11.8) ppb in GOLD1 and 25.0 (13.8) ppb in GOLD2+. Up to 33 (28%) of individuals had high levels of FeNO (≥30 ppb) and 25% had blood eosinophils ≥3%, without differences between groups.
Among the whole cohort, a sputum sample was obtained in 61 (75%) individuals although only 37 (45%) had good quality and provide a cell count. 3 samples (5%) had a sputum eosinophil count ≥3% (1 healthy control, 1 COPD GOLD 1 and 1 COPD GOLD 2+, 9% of the total COPD patients). There was no correlation between sputum eosinophils and blood eosinophils (r=0.19), but we observed a moderate correlation between FeNO and sputum eosinophils (r=0.48, p=0.02).

Conclusions: sputum induction has a moderate rate of success. No differences were observed in inflammatory patterns between our groups and only 2 COPD patients (9%) showed sputum eosinophilia, although conclusions can not be made yet due to the small sample size.

Funding: The Canadian Cohort Obstructive Lung Disease (CanCOLD) study is currently funded by the Canadian Respiratory Research Network (CRRN); industry partners: Astra Zeneca Canada Ltd; Boehringer Ingelheim Canada Ltd; GlaxoSmithKline Canada Ltd; Novartis. Researchers at RI-MUHC Montreal and Icapture Centre Vancouver lead the project.

Biem, Henry Jason
Category: Clinical Fellow (Poster 4)
Supervisor: Ron Dandurand

Reproducibility of the Main Pulmonary Artery to Ascending Aorta Ratio (PA:A) in Real-World, Non-Research Chest CT Scans

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BACKGROUND: Recent studies have shown research CT scan PA:A ratios >1 to correlate with pulmonary hypertension (PH) and be predictive of acute exacerbations of COPD (AECOPD). This predictive ability has yet to be demonstrated using non-research CT scans at the community level. Before determining if the PA:A of real-world CT scans could be used to predict AECOPD in community practice, we felt it important to measure the reproducibility of the PA:A as determined by non-radiologists in the community setting.

METHODS: From a database of 1040 clinical scans, 33 scans from 6 subjects (4 COPD, 1 asthma, 1 PH) were selected to measure the PA:A. A medical student (Rater 1) and a mid-career gynecologist (Rater 2) performed 3-4 blinded randomized determinations of the PA:A, and a general radiologist completed 1 determination. Bland-Altman analysis (BAA), intraclass correlation coefficient (ICC) and Cohen's kappa (K) were determined between successive and final determinations (Table 1).

RESULTS: In this feasibility study using real-world CT scans, we found the PA:A had very good intra- and inter-rater reliability in the range of published studies using research CT scans (ICCs ranging from 0.70 to 0.95, and from 0.80 to 0.88, respectively). The non-radiologist raters improved with experience and achieved results similar to the radiologist after 3-4 determinations (Ks ≥0.89).

CONCLUSION: Our results demonstrate community CT scan PA:A is reproducible suggesting the PA:A from real-world CT scans may be predictive of AECOPD. Confirmation of this notion awaits further study.

Table 1. Bland-Altman Analysis, Intraclass Correlation Coefficients and Cohen's Kappa of Real-World CT Scan PA:A Ratio

	Bias (CI)	Lower Limit of Agreement (CI)	Upper Limit of Agreement (CI)	Intraclass Correlation Coefficient	Cohen's κ
Rater 1					
Determination 1 vs. 2	0.056 (0.028, 0.084)	-0.099 (-0.147, -0.051)	0.210 (0.162, 0.258)	0.70 (0.34, 0.87)	0.43
Determination 2 vs. 3	-0.001 (-0.015, 0.014)	-0.082 (-0.107, -0.057)	0.081 (0.055, 0.106)	0.93 (0.86, 0.97)	0.87
Determination 3 vs. 4	-0.010 (-0.023, 0.003)	-0.080 (-0.101, -0.058)	0.060 (0.038, 0.081)	0.95 (0.90, 0.97)	1.00
Rater 2					
Determination 1 vs. 2	0.015 (-0.008, 0.037)	-0.109 (-0.147, -0.07)	0.139 (0.100, 0.177)	0.85 (0.72, 0.92)	0.82
Determination 2 vs. 3	-0.005 (-0.021, 0.012)	-0.096 (-0.125, -0.068)	0.087 (0.014, 0.115)	0.91 (0.83, 0.96)	0.82
Non-Radiologists					
Final Determinations	-0.033 (-0.052, -0.015)	-0.134 (-0.165, -0.103)	0.067 (0.035, 0.099)	0.86 (0.61, 094)	0.89
Radiologist vs.					
Rater 1	0.007 (-0.014, 0.028)	-0.108 (-0.133, -0.072)	0.123 (0.087,0.159)	0.88 (0.78, 0.94)	0.89
Rater 2	-0.026 (-0.051, -0.001)	-0.164 (-0.207, -0.121)	0.112 (0.059, 0.155)	0.80 (063, 0.90)	1.00

Bortolotti, Perrine
Category: Postdoctoral Fellow (Poster 32)
Supervisor: Carolyn Baglole, Simon Rousseau

Activation of the aryl hydrocarbon receptor by *Pseudomonas aeruginosa* kynurenine pathway modulates the immune response in lung epithelial cells

Perrine Bortolotti, Meakins-Christie Laboratories, RI-MUHC, McGill University
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Project rationale*: Pseudomonas aeruginosa (Pa)* is a Gram-negative bacterium frequently involved in health care-associated and chronic pneumonia with poor clinical outcome. *Pa* catabolizes tryptophan through the kynurenine pathway and can secrete high levels of kynurenine and kynurenic acid (µM to mM). Interestingly, these metabolites are known ligands for the aryl hydrocarbon receptor (AhR), a receptor/transcription factor that is highly expressed in lung epithelial cells. Previous work has shown a critical role for the AhR in the airway epithelial cells in regulating the innate and adaptive immune response to pathogens. Thus, modulation of the AhR pathway could interfere with the host response to *Pa*-induced respiratory infections.
Objective: Our study aims to determine whether *Pa*-derived kynurenines modulate AhR activity in airway epithelial cells.
Methods: A549 cells were stimulated with synthetic kynurenine and kynurenic acid for 1, 3 and 6 hours to assess their ability to activate the AhR. Then, A549 were exposed to sterile diffusible material from filtrate of overnight culture of *Pa* for 3 hours, or infected for 2, 4, 6, 8 and 24 hours with live bacteria. *Pa* and related mutant strains that are either unable to produce kynurenines or overproduce them, were used to study the ability of bacterial kynurenines to activate the AhR. AhR activation was quantified by assessment of *Cyp1a1* gene expression by real-time quantitative PCR. Concomitantly, the expression of IL-8 mRNA was assessed. These results were compared to A549 AhR knock-out cells (A549-AhR^{ko}).
Results: Both kynurenine and kynurenic acid activated the AhR in A549 cells. Diffusible material collected from kynurenine-producing strains also leads to the activation of the AhR, suggesting that secreted bacterial metabolites from the kynurenine pathway act as AhR ligands. In addition, the AhR was strongly activated after infection for 24 hours with strains producing kynurenines, with a concomitant decrease in IL-8 expression. Conversely, in A549-AhR^{ko}, IL-8 expression increased during infection with strains producing kynurenines. These results suggest a modulatory role for the AhR in lung epithelial cells during *Pa* Infection. Interestingly, *Pa* strain with a non-functional kynurenine pathway could also activate the AhR, but at a lower level, arguing for additional (currently undefined) ligands involved in activation of the AhR by *Pa*. Activation of the AhR was undetectable in shorter infections and was not related to cell death, suggesting the involvement of specific host-pathogen interactions occurring during prolonged cells infection.
Conclusion: Bacterial kynurenines of *Pa* can activate the AhR in lung epithelial cells, together with other mechanisms/ligands involving specific host-pathogen interactions during prolonged *Pa* infection. The activation of the AhR by the bacteria leads to a modulation of IL-8 expression. Taken together, these results strongly support the involvement of the AhR in the regulation of the host immune response in *Pa*-induced airway infection. The underlying mechanisms involved in the activation of the AhR by *Pa* and their consequences on the pathophysiology of the infectious process are under investigation.

Funding : Meakins-Christie Laboratories collaborative research award

Caron, Melissa
Category: PhD Student (Oral Presentation)
Supervisor: Kevin Schwartzman, Russell Steele

Assessment of the Use and Performance of Pulmonary Function Tests as Outcome Measures for Systemic Sclerosis Interstitial Lung Disease Progression: A Systematic Review

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Rationale: Systemic sclerosis (SSc) is a progressive autoimmune disease involving a complex interplay of vasculopathy, inflammation and fibrosis. Interstitial lung disease (SSc-ILD) is the leading cause of morbidity and mortality in SSc and is estimated to occur in over 50% of patients. As such, SSc patients are continuously monitored for SSc-ILD presence and progression using pulmonary function tests (PFTs). In recent years, forced vital capacity (FVC) has become the preferred surrogate marker for SSc-ILD progression, despite the apparent paucity of any conclusive evidence favouring it over other PFT measures. To better understand the current preference for FVC, we performed a systematic review of the literature aiming to outline the historical use and validation of PFTs as surrogate markers for SSc-ILD progression.
Methods: Only studies published in English or in French were considered. All original research (including abstracts and clinical trial registrations) were eligible for inclusion. No restrictions were placed on study design. All included studies either used at least one PFT measure as a longitudinal outcome for SSc-ILD progression (*i.e.,* outcome studies) and/or reported at least one classical measure of validity for the association between PFTs and high-resolution computed tomography (HRCT) findings (*i.e.,* validation studies). A minimum of 20 SSc patients in the study was also required. The MEDLINE, Embase, and Web of Science databases were searched to identify all potentially relevant studies. In addition, ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform were also searched for clinical trial registrations.
Results: A total of 3,258 records were retrieved. Following de-duplication and a two-stage screening process performed by two independent reviewers, 212 studies were selected for inclusion in the systematic review. One-hundred and seventy of these studies qualified as outcome studies, while 50 were listed as validation studies. (Eight records satisfied the inclusion criteria for both outcome and validation studies.) The outcome studies revealed that diffusion capacity for carbon monoxide (DLCO) was cumulatively the most commonly used PFT for most of the study period, only to be surpassed by FVC in 2009. A clear primary PFT endpoint was specified in 68 studies. DLCO percent predicted comprised only a small proportion of primary PFT endpoints overall (8.8%), while FVC percent predicted was the preferred outcome of interest in 75.0% of these studies. Finally, among the 50 studies with a reported measure of validity, only five had the clear objective of validating the different PFT measures against HRCT in SSc-ILD. Two of these studies concluded that DLCO was the best measure of SSc-ILD extent, while the remaining three studies did not conclude in favour of any PFT measure.
Conclusions: Available evidence does not overwhelmingly favour one PFT measure as the best surrogate marker for SSc-ILD, rendering it challenging to support the current preference for FVC. Indeed, the perceived superiority of FVC is not reflected in rigorous PFT validation studies. While FVC has the potential to be a viable surrogate marker for SSc-ILD, it would be ill-advised at this stage to discount other potentially interesting PFT measures, such as DLCO.

Casgrain, Pierre-André
Category: Research Assistant (Poster 2)
Supervisor: Dao Nguyen

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

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

Funding: CIHR and Cystic Fibrosis Canada

Dembele, Marieme
Category: PhD Student (Poster 33)
Supervisor: Bruce Mazer

Intravenous immunoglobulin G (IVIg) concurrently promotes Th2 and regulatory cells development while abrogating Th1 andTh17 immunity

Marieme Dembele, Shao Tao, Di Xue, Gabriel N. Kaufman, Laura Mendonca, Maziar Divangahi, Bruce D. Mazer

Rationale: Intravenous Immunoglobulin (IVIg) is a plasma-derived product containing polyclonal IgG antibodies pooled from thousands of human donors. It is used to treat immune deficiencies, and it is extremely effective in treating a wide variety of inflammatory and autoimmune disorders. We aimed to further elucidate the mechanisms by which IVIg modulates the balance between Th17, Th2, Th1 and regulatory immune cells.
Hypothesis: High-dose IVIg abrogates a mixed Th1/Th17/Th2 allergic response by rendering Antigen Presenting Cells (APCs) tolerogenic, and by inducing T regulatory cells and promoting Th2 immunity.
Aim 1: To characterize *in-vivo* the effect of IVIg treatment in granulocytes, dendritic cells and interstitial macrophages (IMs) sub-populations and cytokine production in
an antigen driven model of allergic airway disease. **Aim 2:** To determine, *in-vitro,* the ability of of tolerogenic DCs to modulate Th2, Th1 and Th17 responses.

Methods: Using an OVA-driven murine model of allergic airway disease exhibiting mixed a Th17/Th1/Th2 response, we performed cellular phenotyping of lung homogenates using flow cytometry. In addition, we assessed cytokine production by intracellular staining and ELISA, and performed in-vitro DC:T cells co-culture studies.

Results: IVIg treatment reduced lung inflammation and diminished IL-17A and IFNg production as measured in the BAL. In contrast, the frequency and absolute number of eosinophils and basophils was increased, while IgE production was abrogated in the IVIg group. This was accompanied by a significant increase in IL-5 concentration in the BAL. Furthermore, the frequency of Foxp3+IL10+ cells within CD4+ T cells and absolute number of IL-10 secreting dendritic cells was augmented in the lung. We also observed an increase in IL-10 production and CD206 expression in interstitial macrophages (IMs).Using an *in vitro* DC: T cell co-culture system, IVIg-treated DC inhibited IL-17A and IFNg production by T cells and increased IL-13 production.

Conclusion: IVIg modulates Th2/Th17/Treg balance by favoring Treg and Th2 cells while inhibiting Th17 and Th1 cells. Furthermore, IVIg renders both dendritic cells and macrophages tolerogenic by enhancing IL-10 production in these latter subsets of cells.

Funding: CIHR

Dintakurti, Aparna
Category: MSc Student (Oral Presentation)
Supervisor: Larry Lands

Macrophage Differentiation and Responsiveness to Corticosteroids in Severe Neutrophilic Asthma

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Rationale: Severe neutrophilic asthma is a heterogeneous, chronic airway inflammatory disease, in which affected individuals show diminished responsiveness to inhaled corticosteroids (CS), the current gold standard of treatment. Unfortunately, there is no effective alternative therapy. The underlying pathogenesis of severe neutrophilic asthma includes persistent airway inflammation and an abnormal lung bacterial composition. At the center of these phenomena are alveolar macrophages, which in addition to being a major source of pro-inflammatory cytokines and eliciting important anti-bacterial properties show diminished responsiveness to CS in severe asthmatics. Macrophages are generally classified into two phenotypes: the pro-inflammatory "M1" or the anti-inflammatory "M2". Interestingly, there is certain degree of plasticity between these two phenotypes. We hypothesized that diminished responsiveness to inhaled corticosteroids in severe neutrophilic asthma results from a predisposed macrophage differentiation to the pro-inflammatory M1 phenotype.

Methods: Studies were completed using macrophages grown from blood monocytes (monocyte-derived macrophages [MDM], M0 phenotype) as previously described by us {Br J Pharmacol 2015; 172: 4757- 71} from healthy individuals and severe neutrophilic asthmatics. After maturation, MDM were differentiated to the M1 phenotype using Interferon (IFN)-γ and Lipopolysaccharide (LPS) and to the M2 phenotype using Interleukin (IL)-4 for 18 hours, following which whole transcriptome analysis was conducted using RNA-sequencing.

Results: Preliminary transcriptome analysis of healthy M0, M1 and M2 MDM from two individuals revealed that 1515 genes were uniquely upregulated in the M1 phenotype with a fold change of 2 or greater. These genes were modulated specifically and uniformly, with minimal differences between the two donors. Hierarchical cluster analysis showed that the M1 phenotype is very distinct from the resting M0 phenotype. In contrast, only 166 genes were uniquely upregulated in the M2 phenotype with a fold change of 2 or greater. These genes showed variability in the magnitude of modulation between the two donors and hierarchical cluster analysis indicated that the M2 phenotype is not very distinct from the M0 phenotype. Thus, in healthy MDM, the M1 differentiation drivers (IFN-γ + LPS) modulate genes in a singular manner despite individual differences, while the M2 driver (IL-4) modulates genes heterogeneously. Transcriptome analysis of severe neutrophilic asthmatic M0, M1 and M2 MDM will follow.

Conclusion: Our study is the first to take a comprehensive approach to characterizing macrophage differentiation in humans suffering from severe neutrophilic asthma, taking into consideration the heterogeneity seen both within macrophage phenotypes and between severe asthma endotypes. We expect our project to provide a greater understanding of the M1 and M2 phenotypes and their plasticity, as well as set a premise for exploring macrophage differentiation as an alternate targeted therapy for severe neutrophilic asthma.

Supported by Marcel and Rolande Gosselin Foundation

Dintakurti, Aparna
Category: MSc Student (Poster 28)
Supervisor: Larry Lands

The Macrophage-like Cell Lines THP-1 and U937 are Differentially Inclined Towards M1 and M2 Phenotypes

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Rationale
Differentiation of human macrophages, the central innate immunity cells, yields diverse phenotypes, including the antibacterial and inflammatory "M1" or inflammation- resolving "M2" phenotype. Macrophage differentiation can be studied using primary cells or "macrophage-like" tumor cell lines THP-1 and U937. The latter are frequently used to investigate macrophage inflammatory responses. There is insufficient appreciation about the ability of these cell lines to differentiate to M1 or M2 phenotypes. This study aimed to address this knowledge gap.

Methods
Macrophages were grown from blood monocytes (monocyte-derived macrophages [MDM], M0 phenotype) as previously described by us {Br J Pharmacol 2015; 172: 4757- 71}. The M0 phenotype was induced in THP-1 and U937 cells by exposure to phorbol ester for 72 hours. At M0, basal and stimulated (bacterial and inflammatory stimuli {Br J Pharmacol 2015; 172: 4757-71}) production of Interleukin (IL)-8 and Tumor Necrosis Factor (TNF)-α (both by ELISA) was assessed. Subsequently, M0 MDM and cell lines were differentiated for 18 hours exposed to either the M1 (Interferon [IFN]-γ ± Lipopolysaccharide [LPS]) or M2 (IL-4) stimuli. M1 / M2 differentiation was gauged by upregulation of M1 genes CXCL11, TNF-α, and CXCL10, or the M2 gene CD209, quantified by qPCR (relative expression) or digital droplet PCR (absolute quantification).

Results
Compared with M0 MDM, both basal and stimulated IL-8 was markedly higher in the cell lines. Stimulated U937 cells produced significantly ($p < 0.05$) more IL-8 than THP-1 cells. Qualitatively, the IL-8 response pattern was comparable between M0 MDM and the cell lines for most tested inflammatory stimuli. TNF-α production was not readily measurable in M0 MDM, precluding further comparisons. In M0 MDM and cell lines, expression of M1 genes was comparably low and was upregulated significantly ($p < 0.05$; all comparisons vs. M0) by IFN-γ + LPS, with the highest magnitude in THP-1 cells ($p <$
0.05 vs. MDM and U937). Interestingly at M0, expression of the M2 gene CD209 tended ($p = 0.057$ vs. MDM and THP-1) to be higher in U937 cells. IL-4 upregulated the M2 gene CD209 in MDM and the cell lines, with the highest magnitude in U937 cells.

Conclusions
At M0, the inflammatory profiles of THP-1 and U937 cells qualitatively resemble M0 MDM. M1 differentiation appears to be more robust in THP-1 cells. U937 cells show propensity towards M2 phenotype, both under resting and stimulated conditions. Studies of inflammatory profiles at M1 and M2 will ensue.

Funding: Supported by Marcel and Rolande Gosselin Foundation

Donovan, Adamo
Category: MSc Student (Oral Presentation)
Supervisor: Benjamin McDonald Smith

Diaphragm muscle density and function in chronic obstructive pulmonary disease

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Background: Studies of diaphragm muscle morphology and function in chronic obstructive pulmonary disease (COPD) have yielded conflicting results.

Objectives: To investigate in vivo diaphragm muscle structure and function in COPD.

Methods: Smokers 50-79 years old with and without spirometry-defined COPD underwent full- lung computed tomography (CT) at suspended maximal inspiration, pulmonary function testing according to European Respiratory Society standards, and symptom-limited incremental cardiopulmonary exercise testing on a cycle ergometer with a gastro-esophageal balloon catheter. Diaphragm muscle density and volume were measured by mapping the left hemi-diaphragm, and related to measures of trans-diaphragmatic pressure (Pdi), COPD assessment test (CAT) score, and breathlessness unpleasantness ratings at peak exercise.
Regression techniques adjusted for age, gender, height, and post-bronchodilator forced expired volume in one second percent predicted (FEV1pp).

Results: Fourteen participants completed the study to date. Independent of age, body size, and FEV1pp, lower diaphragm muscle density was associated with lower Pdi during resting inspiratory capacity maneuver ($p=0.02$), higher CAT score ($p=0.002$), and breathlessness unpleasantness at peak exercise ($p<0.0001$). Diaphragm muscle volume was associated with Pdi ($p=0.006$), but not CAT score or breathlessness at peak exercise ($p>0.05$). Results were similar with adjustment for diaphragm dome height.

Conclusion: Among smokers with COPD, diaphragm muscle density assessed in vivo is associated with reduced diaphragm function, impaired health status, and breathlessness- limited exercise that is independent of spirometric disease severity.

Funding: 2016/2017 Studentship & Fellowship RI-MUHC Award

Donovan, Adamo
Category: MSc Student (Poster 22)
Supervisor: Benjamin McDonald Smith

Diaphragm morphology assessed by computed tomography in the Canadian Cohort of Obstructive Lung Disease (CanCOLD)

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Rationale: Chronic obstructive pulmonary disease (COPD) is associated with limb muscle atrophy and dysfunction, but studies of diaphragm morphology and function have yielded conflicting results. We assessed diaphragm morphology in vivo using computed tomography (CT), and hypothesized that diaphragm volume would be associated with COPD severity, inspiratory capacity, respiratory health status, and exercise capacity.

Methods: CanCOLD is an on-going, population-based, longitudinal study of over 1,400 participants. Baseline assessment included standard pulmonary function testing, COPD Assessment Test™ (CAT) questionnaire, thoracic CT, and symptom-limited incremental cardiopulmonary exercise testing on a cycle ergometer. Presence of COPD was defined by a forced expired volume in one second (FEV1)-to-forced vital capacity ratio less than 0.7, and severity categorized according to the Global Initiative for Obstructive Lung Disease (GOLD) strata of percent predicted FEV1. Left hemi-diaphragm morphology was assessed by mapping the diaphragm area on contiguous axial CT images (two-rater intra-class correlation coefficient: 0.92). Three-dimensional reconstruction of mapped areas yielded measures of hemi-diaphragm volume in the range of -150 to 150 Hounsfield units. Associations between diaphragm volume, percent predicted FEV1, inspiratory capacity, CAT score, peak oxygen uptake per kg body weight (peak VO2), and peak work rate were assessed using regression to adjust for age, gender, height, body mass index, smoking status, and pack-years, and expressed per standard deviation difference in diaphragm volume of the sample.

Results: (Mean values ± SD are provided) Left hemi-diaphragm morphology was assessed in 234 CanCOLD participants to date (age: 65±9 years; 61% male; 24% never smokers; 53% former smokers; 23% current smokers; pack-years: 23±24). The proportion of participants with COPD was 45% (66% mild, 34% moderate-to-severe), and mean left hemi-diaphragm volume was 73±23 cm3. A 1-SD decrement in left hemi-diaphragm volume was associated with lower percent predicted FEV1 (-192ml; 95%CI: -243 to -141ml; $p<0.001$), lower inspiratory capacity (-316 mL; 95%CI: -363 to -269 mL; $p<0.001$), higher CAT score (+1.2; 95%CI: +0.53 to +1.9; $p=0.07$), lower peak VO2 (-2 ml/min/kg; 95%CI: -2.7 to -1.4 ml/kg/min; $p<0.001$), and lower peak work rate (12 W; 95%CI: -16 to -8.6 W; $p<0.001$). Associations between diaphragm volume and co-variates are summarized in Table 1.

Conclusion: Diaphragm volume assessed in vivo by CT is associated with airflow obstruction, and lower inspiratory capacity, respiratory health status, and exercise capacity.

Funding: 2016/2017 Studentship & Fellowship RI-MUHC Award

Downey, Jeffrey
Category: PhD Student (Poster 34)
Supervisor: Maziar Divangahi

Cyclophilin D Protects Against Influenza A Virus Infection by Preventing Pulmonary Damage

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Introduction: Influenza A Virus (IAV) infection remains a chief pulmonary pathogen with limited available therapy. Immunity to IAV is multifaceted, requiring both antiviral defense mechanisms to clear the pathogen, as well as immune tolerance mechanisms to limit tissue damage. While antiviral strategies have been well characterized in immunity to IAV, our understanding of host tolerance remains limited. In this vein, we recently showed that mice lacking the mitochondrial protein Cyclophilin D (*CypD*$^{-/-}$) are highly susceptible to another major pulmonary pathogen *Mycobacterium tuberculosis*. Interestingly, this susceptibility was not due to any differences in bacterial load over the course of infection, but, rather, to increased T-cell-mediated immunopathology in the lungs of CypD-deficient mice. Thus, we hypothesized that *CypD*$^{-/-}$ mice are more susceptible to IAV infection, due to aberrant inflammatory responses and enhanced tissue damage.

Methods: Wild Type (WT) and *CypD*$^{-/-}$ mice were infected intranasally with a sublethal (50pfu) or lethal (90pfu) dose of IAV Puerto Rico/8/34 (PR8) and survival and weight loss were assessed. Innate and adaptive inflammatory responses were characterized via flow cytometry and cytokines by ELISA. Lung damage was measured by levels of fluorescent dye in the lungs and plasma of infected mice, as well as by measurements of pulmonary wet/dry ratios. Lung function was assessed by methacholine challenge, followed by FlexiVent.

Results: *CypD*$^{-/-}$ mice were susceptible to IAV infection, despite comparable antiviral cytokine responses and viral clearance. Instead, susceptibility was associated with a marked increase in pulmonary damage and a breakdown of the pulmonary epithelial-endothelial barrier. Upon infection, *CypD*$^{-/-}$ mice exhibited enhanced inflammatory cell influx and antigen-specific CD8+ T-cell responses. Current study is focused on understanding the mechanisms by which CypD prevents lung tissue damage in response to IAV and the relative contribution of hematopoietic versus structural cells in mediating this phenotype, via the generation of chimeric mice.

Conclusion: IAV has devastating consequences with few therapeutic options. Thus, a better understanding of the balance between immunity and immunopathology is required. In the current study, we identify CypD as an unexpected mediator of immunity to IAV by regulating pulmonary inflammatory responses and preserving pulmonary integrity and function.

Jeffrey Downey – Funding from the Department of Pathology and the RI-MUHC (Studentship)
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Dr. Maziar Divangahi—Foundation Grant from the CIHR

Dumitru, Victor
Category: MSc Student (Poster 15)
Supervisor: John Hanrahan

Impact Of Urban Particulate Matter On Normal And Cystic Fibrosis Primary Human Bronchial Epithelial Cells

Victor Dumitru, John W. Hanrahan

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

Farias, Raquel
Category: Postdoctoral Fellow (Oral Presentation)
Supervisor: Jean Bourbeau

Innovations in treating COPD exacerbations: Action Plans using new technology

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Rationale: COPD exacerbations are the first cause of preventable hospital admissions in Canada. Prompt treatment decreases recovery time and hospitalizations. Effective self-management of exacerbations can be achieved with written Action Plans and case management. Communication technology has proven beneficial as a support tool for the treatment of chronic respiratory diseases and might increase patients' adherence to COPD Action Plans. The objective of this study is to determine whether the use of a phone tele-system increases Action Plan adherence during pulmonary exacerbations in a real life practice of a COPD clinic.

Methods: Forty patients from the COPD clinic at the Montreal Chest Institute were enrolled in the study. Patients received regular automated phone calls and could initiate contact with the tele-system at any time. Action Plan adherence was defined as patients taking their rescue medication and/or contacting healthcare professionals within 3 days of exacerbation onset. The tele-system issued alarms during exacerbations, in which case patients received follow-up calls from both, their nurses and the tele-system. Detailed data from patients' behaviours during exacerbations were recorded on monthly telephone evaluation by a third party, the research assistant.

Results: Thirty three patients (12 M/21F; 69±6.9 years) completed the one-year study. Ten patients (30.3%) were GOLD2; 14 patients (42.4%) were GOLD3 and 7 patients (21.2%) were GOLD4. A total of 93 exacerbations were reported. On average, patients had 2.5 ± 1.8 exacerbation episodes in one year, with 12% of the patients experiencing one exacerbation, 43% two exacerbations, 33% three or more exacerbations and 4 patients (12%) with no exacerbations. During these episodes, 60% of the patients contacted their nurse case manager by phone and only 25% by tele-system. Action Plan adherence was observed for 70% of the patients although only 22% was done through the tele-system. Fifty three percent of patients experiencing an exacerbation modified medication by themselves. Among these patients, the median time to rescue bronchodilator was 0 days; to antibiotic 2 days and to prednisone 2.5 days. Finally, at the end of the intervention, patients significantly increased their self-efficacy in managing COPD exacerbations.

Conclusions: Patients enrolled in this study had increased Action Plan adherence than what has been previously reported in the literature. However, patients still had a tendency to call their nurse case manager directly and it may take longer to change patient's behaviour towards the tele-system. An additional benefit of the tele-system implementation is increased self-efficacy to manage COPD exacerbations.

Study funded by GlaxoSmithKline as an investigator initiated award (ClinicalTrials.gov identifier: NCT02275078)

Farias, Raquel
Category: Postdoctoral Fellow (Poster 9)
Supervisor: Jean Bourbeau

The Quebec Respiratory Health Education Network (QRHEN): integrating a model of self-management education in COPD primary care

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Background: The most recent guidelines in respiratory health recommend the implementation of self-management education programs with written Action Plans for the prevention of COPD exacerbations. Such programs need to be studied in primary care clinics with COPD patients with mild to moderate disease.

Objective: To evaluate the impact of COPD self-management education with coaching of respiratory therapists on both, patient-related outcomes and physician practice changes in primary care.

Methods: COPD patients were recruited from six Family Medicine Clinics (FMCs) in Quebec. Patients participated in three follow-up visits in which they learned COPD self-management strategies which included treatment adherence, inhaler techniques, smoking cessation and the use of a written COPD Action Plan for exacerbations. Patient self-management skills and COPD knowledge were assessed at each visit. Practice of primary care physicians was assessed based on changes in prescriptions for COPD medication, immunizations and written Action Plans.

Results: Fifty four patients completed the follow-up visits. The number of yearly unscheduled visits to the physician went from 40 at baseline to 17 one year after the educational intervention (p=0.033). Emergency room visits and hospitalizations were infrequent with no significant changes after the intervention. Health-related quality of life, treatment adherence, inhaler technique and COPD knowledge improved significantly. The program resulted in significant increases in prescriptions for bronchodilators with/without inhaled corticosteroid, flu immunizations and written COPD Action Plans by primary care physicians.

Conclusion: The COPD self-management educational intervention in FMCs was successful in reducing unscheduled visits to the clinic and in improving patients' quality of life, self-management skills and knowledge. The program had a positive impact in COPD-related practices by primary care physicians in the FMCs.

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Faure, Emmanuel
Category: Postdoctoral Fellow (Poster 13)
Supervisor: Simon Rousseau, Dao Nguyen

Intracellular persistence of *Pseudomonas aeruginosa* in lung epithelial cells

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Pseudomonas aeruginosa is mainly considered as an extracellular opportunistic pathogen, responsible for chronic lung infections by persisting extracellularly in a biofilm lifestyle. However, although P. aeruginosa owns suitable weapons for intracellular lifestyle, no study assessed the possibility of an intracellular persistence in airway. We hypothesized that P. aeruginosa may persist in epithelial cells and that intracellular persistence may contribute to pathogenicity.

To this end, using bronchial epithelial cells, a long-term persistence model was performed. BEAS-2B (B2B) and CFBE cells were infected for 4 hours with PAO1 and related mutant strains. Thereafter, Tobramycin, known to have poor intracellular diffusion, was added in the culture medium to remove extracellular bacteria. Cells were incubated for 120 hours. Intracellular and extracellular bacterial burden and cytotoxicity were assessed after 2 hours, 24 hours and 120 hours, by serial dilutions and LDH release respectively. At each time point, Western Blot analysis and quantitative PCR expression were performed to assess host immune response. Concomitantly, gene expression of intracellular bacteria and their motility were assessed. Finally, B2B-CRISPR for targeted genes were generated to identify critical intracellular host pathways involved in response to pathogen.

Here we show that P. aeruginosa can persist intracellularly in B2B and CFBE during 5 days, inducing low cytotoxicity. Moreover, we demonstrate that P. aeruginosa has low replication intracellularly. We show that TLR2, TLR4 and TLR5 are involved in the detection of intracellular bacteria. Finally, we show that epithelial cells with mutation for Cystic Fibrosis (CFBEΔF508) showed increased susceptibility to intracellular persistence compared to CFBE.
Taking together, our data suggest that P. aeruginosa can persist intracellularly into airway epithelial cells in vitro.

Funding: Meakins-Christies Laboratory Collaborative Research Award, Lille University Research Award, French Infectious Disease Society Research Award

Gaudet, Mellissa
Category: MSc Student (Poster 30)
Supervisor: Simon Rousseau

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

Mellissa Gaudet**,** J. Bérubé, R. Levesque, D. Nguyen, S. Rousseau

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

An extensive screen of over 150 clinical isolates of *P. aeruginosa* from different CF patients was initiated. Thirteen isolates from six different CF patients were observed to induce differential inflammatory responses. More specifically, isolates found colonizing the same patient showed differential IL-8 expressions in lung epithelial cells.

A comparative single-nucleotide polymorphism (SNP) analysis was conducted for each co-isolated strain. This investigation provided 948 discordant genes between the thirteen isolates and 58 of these corresponding genes were found to be common in at least two patients. The following six genes were the most frequently mutated: fiuA, alkA, aprF, chpA, fusA1 and gyrB. Of these genes, we focused on aprF because it forms the transmembrane protein subunit of the type I secretion system (TISS) which secrets the alkaline protease aprA [4]. AprA is a known *P. aeruginosa* virulence factor implicated in host innate immune evasion through the cleavage of monomeric flagellin [5].

To assess the implication of mutations in the aprF gene on host responses, a *P. aeruginosa* transposon mutant of this gene will be characterized to determine if its disruption could be responsible for the heightened IL-8 mRNA induction. To check the possible protease interactions that underline the flagellin cleavage and immune evasion, functional assays will be performed using several PA01 mutants and recombinant proteins of aprA, aprI and flagellin type B. These assays include the evaluation of protease activity by flagellin cleavage of the *P. aeruginosa* clinical isolates that have mutations in aprF. The inflammatory response profile of the clinical isolates carrying aprF mutants will be investigated using a TLR5 CRISPR knockout BEAS-2B cell line. This cell line will determine whether the IL-8 inflammatory response induced by these clinical isolates are TLR5 dependent.

Understanding the role of aprF mutations in *P. aeruginosa* clinical isolates may allow the identification of potential gene targets to attenuate virulence factors in *P. aeruginosa.* Ultimately leading to improved personalized management and treatment of CF patients who are infected with *P. aeruginosa.*

1. Canada, C.F., *Canadian CF Registry 2013.* Canadian CF Registry 2013, 2013.
2. Rada, B. and T.L. Leto, *Pyocyanin effects on respiratory epithelium: relevance in Pseudomonas aeruginosa airway infections.* Trends in Microbiology, 2013. **21**(2): p. 73-81.
3. Shanks, K.K., W. Guang, and K.C. Kim…, *Interleukin-8 production by human airway epithelial cells in response to Pseudomonas aeruginosa clinical isolates expressing type a or type b flagellins.* Clinical and Vaccine …, 2010.
4. Bleves, S., et al., *Protein secretion systems in Pseudomonas aeruginosa: A wealth of pathogenic weapons.* International Journal of Medical Microbiology, 2010. **300**(8): p. 534-543.
5. Bardoel, B.W., et al., *Pseudomonas Evades Immune Recognition of Flagellin in Both Mammals and Plants.* PLoS Pathogens, 2011. **7**(8).

Guerrina, Necola
Category: PhD Student (Oral Presentation)
Supervisor: Carolyn Baglole

Reduced AhR expression upregulates pathogenic autophagy and drives the development of cigarette smoke-induced emphysema.

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Background: Chronic Obstructive Pulmonary Disease (COPD) is a prevalent and complex respiratory disorder primarily caused by cigarette smoke (CS). However, only 15% of smokers develop COPD, suggesting that genetic factors contribute to disease burden. One component of COPD is emphysema, characterized by permanent alveolar destruction due partially to dysregulation of cell death programs such as apoptosis and autophagy. Previously, we have shown that AhR protects against CS-induced apoptosis *in vitro* but the role of AhR in regulating autophagy is unknown. We therefore hypothesized that AhR protects against the development of CS-induced emphysema via alterations in cell death mechanisms in the mouse lungs, such that low AhR levels in humans may predispose to the development of emphysema in susceptible individuals.

Methods: AhR expressing ($AhR^{+/-}$) and deficient ($AhR^{-/-}$) mice were exposed to air or CS daily for up to 4 months, and lung function was subsequently assessed by flexiVent™ (SCIREQ®). Lung tissue was harvested to assess airspace enlargement via morphometric analysis and 3D lung micro-CT scans (Mediso nanoScan SPECT/CT/PET). In addition, $AhR^{+/-}$ and $AhR^{-/-}$ mouse lung fibroblasts (MLFs), A549-AhR^{KO} and WT cells and MLE-12 cells whereby AhR was knocked-down via siRNA were treated with cigarette smoke extract (CSE) or media. Autophagy markers were assessed by qRT-PCR, western blot, transmission electron microscopy, and imaging flow cytometry (Amnis®). To elucidate the mechanism through which AhR regulates autophagy, LC3BII, a marker of autophagosomes, was assessed in MLFs containing a mutation in either the AhR nuclear localization signal or the DNA binding domain. Systemic AhR mRNA levels were assessed by ddPCR in peripheral blood samples from human subjects with and without COPD in CanCOLD (Canadian Cohort of Obstructive Lung Disease).

Results: $AhR^{-/-}$ mice exhibited a significant increase in airspace enlargement relative to $AhR^{+/-}$ mice after a 4-month CS-exposure. There was increased expression of LC3BII in the lungs of $AhR^{-/-}$ mice. LC3BII expression and presence of autophagosomes were also higher in CSE-treated $AhR^{-/-}$ cells. Regulation of CSE-induced autophagy required AhR nuclear localization and DNA binding. Yet, the AhR did not transcriptionally-regulate key autophagy genes (GABARAPL1, Atg7, Beclin1, LC3B, p62). Finally, we show that systemic AhR expression was significantly lower in COPD subjects with emphysema relative to smokers without COPD.

Significance: These results suggest a role for AhR in protecting against cigarette smoke-induced emphysema by regulating autophagy, and could provide the basis for the development of new therapeutic agents or biomarkers for COPD.

Hamed, Rola
Category: MSc Student (Poster 26)
Supervisor: Larry Lands

Differential regulation of stimulated Interleukin-8 and Eotaxin 3 by Interleukin-17a

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Rationale: Severe asthmatics form a heterogeneous population that can be classified into different clusters based on the numbers of neutrophils and eosinophils found in their sputum (Zaihra et al. 2016, BMC pulmonary medicine). Therefore, different patient groups have been characterized, notably a group marked by high levels of neutrophils, and another marked by high levels of eosinophils. Some severe asthmatics are characterized by high levels of both neutrophils and eosinophils. Interleukin (IL)-8 is a major neutrophil chemoattractant is and Eotaxin 3 is a major eosinophil chemoattractant, both present in high levels in severe asthmatic airways. In addition, severe asthmatics are characterized by high levels of IL-17a, a modulator of inflammation. Studies have described a tentative potentiation of IL-8 by IL-17a whereas potentiation of Eotaxin 3 has not been explored. This study evaluated the potentiating effect of IL-17a on cytokine stimulated IL-8 and Eotaxin 3 expression.

Methods: The airway epithelial cell line BEAS-2B was cultured in BEGM medium. Cells were pretreated with IL-17a for 2 hours (10ng/mL) followed by stimulation with one of four different inflammatory cytokines: IL-1□□ Tumor necrosis factor (TNF-a, IL-4, or IL-13 (all at 10ng/mL). All stimulations were done in basal medium (no growth factors or retinoic acid). After 1 hour the stimuli were removed, and cells were further cultured for 5 and 23 hours. IL-8 and Eotaxin 3 mRNA levels were measured by qPCR at both latter time points. Additionally, supernatant IL-8 protein at 24 hours was measured by ELISA. In addition, mRNA kinetics of IL-1b and TNF-a stimulated IL-8, and IL-4 stimulated Eotaxin 3, were studied, with and without IL-17a pre-treatment, during 0 to 5 hours post-stimulus removal.

Results: IL-1Band TNFa significantly upregulated IL-8 mRNA at 5 hours post-stimulation (8 and 10 fold respectively; $p<0.05$, both comparisons); Eotaxin 3 mRNA was not affected by the aforementioned stimuli. IL-4 and IL-13 significantly upregulated Eotaxin 3 mRNA (25 and 16 fold respectively; $p<0.05$, both comparisons), without affecting IL-8 mRNA. IL-17a significantly potentiated the stimulated IL-8 mRNA ($p<0.05$, both IL-1b and TNF-a stimulation, with higher potentiation of the TNF-□ stimulated IL-8). In contrast, IL-4 and IL-13 stimulated Eotaxin 3 was not potentiated by IL-17a. IL-17a also potentiated IL-8 mRNA and protein at 24 hours, but not Eotaxin 3. Time points before 5 hours post-stimulation did not show potentiation of IL-8 or Eotaxin 3 mRNA by IL-17a.

Conclusions: IL-17a potentiates stimulated IL-8, while having no effect on stimulated Eotaxin 3. Our further studies will determine whether these differential IL-17a effects are due to differences in modulation of mRNA stability of IL-8 (known regulation by mRNA stability) and Eotaxin 3 (no known regulation).

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Ho-Wo-Cheong, Dennis
Category: Clinical Fellow (Poster 6)
Supervisor: Marta Kaminska

Inflammatory Markers Are Associated With Obstructive Sleep Apnea (osa) In Parkinson's Disease (pd)

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Introduction: Inflammatory cytokines have been linked with adverse consequences of OSA. Brain-derived neurotrophic factor (BDNF) has been implicated in learning and memory. Our previous work indicates that OSA affects PD non-motor symptoms including cognition. We therefore hypothesized that OSA- associated alterations in these biomarkers would relate to non-motor symptoms in PD.

Methods: Demographic data, medical history and non-motor symptoms, including unified Parkinson's disease rating scale, part 1 (UPDRS1, higher scores worse), and Montreal Cognitive Assessment (MoCA, higher scores better) were assessed in patients with idiopathic PD. Patients underwent overnight polysomnography with venous blood sampling the following morning. Apnea-hypopnea index (AHI) and oxygen desaturation index (ODI) were calculated. Those with OSA (AHI ≥15/h) were offered CPAP. Patients were re-evaluated after 6 months regarding symptoms and biomarkers. Serum interleukin 1 Beta (IL-1b), IL-6, IL-8, tumor necrosis factor alpha (TNFα), C-reactive protein (CRP) and BDNF were quantified by Miliplex magnetic enzyme linked immunosorbent assay.

Results: Baseline blood samples were available for 65 patients with PD. Baseline IL-6 levels correlated with both AHI (r=0.28, p=0.034) and ODI (r=0.4, p=0.002). CRP also correlated with ODI (r=0.25, p=0.057). BDNF correlated with better sleep time and quality (total sleep time r=0.28, p=0.031; sleep efficiency r=0.25, p=0.054; wake after sleep onset r=-0.26, p=0.047). BDNF was also associated with higher MoCA (r=0.25, p=0.067).
Follow-up samples were available for 40 patients, including with CPAP-treated OSA, and 10 with untreated OSA. There was no significant change from baseline in biomarkers in any group. However, among those without OSA, but not others, increasing inflammatory markers correlated with deterioration in UPDRS1 and MoCA (Table). In those with OSA on CPAP, increasing BDNF correlated (trend) with improving UPDRS1.

Conclusion: We found that inflammatory markers were associated with OSA severity in PD patients. Increase in inflammatory markers over time was associated with worsening non-motor symptoms, but only in those without baseline OSA. BDNF levels were associated with better sleep architecture and cognition at baseline, and among treated OSA patients, increasing BDNF correlated with improved non-motor symptoms.
These results suggest that OSA may promote an inflammatory state in PD, which might exacerbate non-motor symptoms. Furthermore, we speculate that better sleep quality might foster BDNF production and thereby a

protective effect on cognition. Further work is required to elucidate the role of sleep, and of OSA and its treatment on these putative mechanisms in PD.

	Without OSA N=15		CPAP-treated OSA N=15		OSA not on CPAP N=10	
	ΔMOCA	ΔUPDRS1	ΔMOCA	ΔUPDRS1	ΔMOCA	ΔUPDRS1
ΔIL-6	r=-0.61, p=0.04	r=0.45, p=0.007	r=0.24, p=0.4	r=0.25, p=0.4	r=-0.25, p=0.5	r=-0.50, p=0.2
ΔTNFα	r=-0.77, p=0.004	r=0.44, p=0.008	r=0.03, p=0.9	r=-0.1, p=0.9	r=0.04, p=0.9	r=0.19, p=0.6
ΔCRP	r=-0.21, p=0.5	r=0.58, p=0.05	r=-0.21, p=0.4	r=-0.18, p=0.5	r=-0.16, p=0.7	r=-0.19, p=0.6
BDNF	r=0.34, p=0.3	r=-0.08, p=0.8	r=0.19, p=0.5	r=-0.44, p=0.10	r=-0.38, p=0.4	r=0.31, p=0.4

Ijiri, Naoki
Category: Postdoctoral Fellow (Poster 35)
Supervisor: James Martin

LncRNA NEAT1 promotes IL-8 expression in fibroblasts derived from COPD patients and in response to cigarette smoking

Naoki Ijiri, MD, PhD, Alice Panariti, PhD, Andrea Mogas, MSc, Parameswaran Nair, MD, PhD, Qutayba Hamid, MD, PhD, James G Martin, MD, PhD, and Carolyn J Baglole, PhD

Introduction Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation and abnormal inflammatory response of the lung. The most important risk factor of COPD is cigarette smoke known to increase the expression of pro-inflammatory cytokines such as IL-8. Long non-coding RNA (LncRNA) is most commonly defined as transcripts greater than 200 nucleotides without protein coding functions. Dysregulation of lncRNAs participates in oxidative stress, inflammation, and apoptosis. The expression of 84 lncRNAs was first analyzed by lncRNA array in fibroblasts derived from smoker with and without COPD as well as from non-smoker exposed to 2% cigarette smoke extract (CSE). NEAT1 was higher expressed in COPD and in presence of CSE compared to non-smoker fibroblasts. NEAT1 (nuclear paraspeckle assembly transcript1) exists in two isoforms, 3.7 kb NEAT1-1 and 23 kb NEAT1-2. The sequence of NEAT1-2 includes NEAT1-1. Upon viral infection, NEAT1 is highly induced in cells. Thus, increasing NEAT1 relocates SFPQ protein from the IL-8 promoter to the paraspeckles. Consequently, IL-8 transcription is activated (1).

Materials and methods
Cell culture: Fibroblasts were isolated from lung parenchyma in cancer-free regions. They were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1% Glutamax, 1% mixture of antibiotic and antimycotic, and 0.1% gentamicin 50mg/ml at 37 °C and incubated in humidified 5% CO2 atmosphere. **CSE treatment:** One cigarette (3R4F) was bubbled into10 ml of serum-free MEM and then filtered through a 0.45-µm pore filter to remove bacteria and large particles. Cells were exposed to 2% CSE which was diluted in serum-free MEM. **RNA extraction and analysis:** Total RNA was extracted using the NucleoSpin RNA. 100ng of RNA were converted to cDNA using iScript RT Supermix and analyzed by RT-qPCR. The relative mRNA expression of target genes in each sample was quantified and normalized to the GAPDH based on the comparative Ct method. **IL-8 ELISA:** We used ELISA kit for IL-8. **siRNA treatment:** Non-smoker fibroblasts were grown in MEM containing 10% FBS and transfected with small interfering RNA (siRNA) against NEAT1, NEAT1-2, SFPQ before exposing them to 2% CSE. AllStars Negative Control was used as a negative control. **DMTU treatment:** Cells were pre-treated with 30 mM DMTU for 1 hour and then coincubation with 2% CSE. Cells were collected for RNA and for proteins extraction respectively after 6 and 24 hours of exposure to 2%CSE.

Results NEAT1 RNA was significantly higher expressed in non-smoker fibroblasts with 2% CSE and COPD fibroblasts compared to non-smoker fibroblasts. IL-8 mRNA and protein levels were also significantly higher expressed in non-smoker fibroblasts with CSE and COPD fibroblasts compared to non-smoker fibroblasts. The knockdown of NEAT1 or SFPQ suppressed the increase of IL-8 in non-smoker fibroblasts with 2% CSE. DMTU as an antioxidant suppressed the effect of CSE in NEAT1 and IL-8 expression.

Conclusion NEAT1 and SFPQ regulate IL-8 expression in non smoker fibroblasts exposed to cigarette smoke. Oxidative stress is responsible of the increase of NEAT1 after CSE treatment.

(1) *Imamura et al., Mol Cell 53, 393-406 (2014)*

Jang, Joyce
Category: MSc Student (Poster 20)
Supervisor: James Martin and Matt Kinsella

Role of the Cystic Fibrosis Transmembrane Regulator in CF Airway Remodelling

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Introduction Cystic Fibrosis (CF) is a disease that causes dysfunction of epithelial tissues, in particular in the airways. The resulting airway remodeling includes thickening of the airway smooth muscle (ASM) layer that may contribute to this dysfunction. Increased ASM mass may exaggerate the narrowing of the airways and account for airway hyperresponsiveness, a phenomenon commonly observed in CF. We aimed to study the proliferative activity of CF ASM to understand whether the loss of the Cystic Fibrosis Transmembrane Conductance Regulator affects the tendency for ASM proliferation to occur. Further we explored the role of glycolysis in ASM proliferation as it has been implicated in the proliferation of cells in cancer. Established proliferative pathways linked to ASMC were also studied.

Methods ASM cells were harvested from CF lungs removed at the time of transplantation. Control ASM cells were obtained from airway tissues from lung transplant donors with no history of airway diseases. Cells were cultured in DMEM/10% fetal bovine serum (FBS) and Penicillin, Streptomycin and Amphotericin B but were serum deprived in 0.1% FBS for 24 hours prior to experimentation. Proliferation was assessed by cell counts and incorporation of bromodeoxyuridine (BrdU) using flow cytometry. Influence of epithelial cells on proliferation was assessed through a co-culture model using the epithelial cell line, BEAS-2B. ASM cells were further incubated in 0.1% FBS for 24 hours before RNA or protein extraction for qPCR or Western blot. Metabolic activity was assessed using Seahorse Bioscience assay. Cells were incubated with 10μM of 3PO, a PFK-FB3 inhibitor, for 24 hours to assess the role PFK-FB3 in proliferation. To assess CFTR activity, cell were treated with either 1uM of Lumacaftor or Orkambi (1 μM Lumacaftor or 250ng Ivacaftor) during the 48h of starvation.

Results CF ASM cells proliferated more rapidly than control cells as assessed by increased BrdU incorporation and cell counts. Cell cycle analysis suggested that CF ASM cells were not accelerated through the S to G2/M-phase transition. CF ASM proliferation was not affected by co-culture with BEAS-2B cells in contrast to control cells, which showed enhanced proliferation. There was no significant difference in glycolytic rate between CF and control ASM cells. There was no difference in expression of glycolytic regulators, Hexokinase and Phosphofructokinas-1. PFK-FB3 expression was only greater in CF cells at the mRNA level. No difference in mitochondrial content was found through TOM20 staining. This was reflected by the lack of significant differences in oxidative respiration. 3PO reduced proliferative rates of CF ASM cells and slowed the S to G2/M-phase transition. No differences in the expression of proliferative factors linked with ASM proliferation was detected. Orkambi significantly reduced the proliferation of CF ASM cells.

Conclusion CF ASMC are intrinsically more proliferative than control cells, which is not mediated by PFK-FB3. The restoration of both proper CFTR expression and activity can reduce the proliferation of CF ASMC. Further efforts need to be addressed for the elucidation of the increase of CF cell proliferation.

Funding: CF Canada

Kaufmann, Eva
Category: Postdoctoral Fellow (Poster 36)
Supervisor: Maziar Divangahi

BCG reprogramming of hematopoietic stem cells generates protective innate immunity against tuberculosis

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The dogma that adaptive immunity is the only arm of the immune response with memory capacity has been recently challenged by several studies demonstrating evidence for memory-like innate immune training. In this context it has been shown that the TB vaccine BCG – which has already been used for nearly a century, but whose protective mechanism still remains elusive – induces epigenetic changes in human peripheral blood monocytes leading to enhanced unspecific protection against reinfection with related but also unrelated pathogens. However, with regard to the nature of monocyte/macrophage differentiation and their relatively short lifespan, the underlying mechanisms and location for generating such innate memory responses *in vivo* remained unknown.

The progenitors of all cells of the blood system including monocytes/macrophages, are hematopoietic stem cells (HSCs). HSC are – in contrast to monocytes/macrophages – long-lived cells, self-renewing and mainly reside in the bone marrow (BM). We therefore hypothesize that access of BCG to this critical site is mandatory for generating a unique set of educated monocytes/macrophages that are protective against TB.
Using murine models, we show that BCG accesses the bone marrow (BM) following intravenous (iv), but not subcutaneous (sc) vaccination. The presence of BCG in the BM induces local HSC expansion and enhanced myelopoiesis at the expense of lymphopoiesis. Importantly, HSC reprogramming led to the generation of epigenetically-modified macrophages that provided significantly better protection against virulent *M. tuberculosis* infection than naïve macrophages. By using parabiotic and chimeric mice as well as adoptive transfer approaches, we demonstrate that education of the monocyte/macrophage lineage via BCG-induced HSC reprogramming is sustainable *in vivo*.

Our results indicate that targeting the HSC compartment provides a novel approach for vaccine development.

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Khadadah, Sulaiman
Category: Clinical Fellow (Poster 8)
Supervisor: John Kimoff

Does Scoring of Autonomic Hypopneas Improve Clinical Decision Making in Obstructive Sleep Apnea?

Sulaiman Khadadah, Philippe Lachapelle, Sushmita Pamidi, Marta Kaminska, Allen E. Olha, Andrea Benedetti, R John Kimoff

RATIONALE
We previously reported (AJRCCM 193;2016:A5980) that scoring of autonomic hypopneas (AnH, defined as events with >30% reduction in airflow associated with ≥6 bpm heart rate increase) improved the diagnostic sensitivity of type 3 home studies compared to conventional scoring by identifying hypopneas that would otherwise not be captured. We now investigate the impact of clinical decision-making based on type 3 studies with versus without AnH scoring compared to PSG for diagnosis and treatment of obstructive sleep apnea (OSA).

METHODS
We prospectively recruited 69 sleep clinic patients in whom a sleep study for OSA was requested. Type 3 monitoring was conducted in-laboratory simultaneously (3SIM) with full PSG. AASM 2013 criteria were used with and without AnH to score respiratory events. We report here on a preliminary clinical decision-making analysis in 20 patients. Sleep experts (n=3) were presented on separate occasions with standard clinical data and one of 3 sleep studies (3SIM without AnH, then 3SIM with AnH, then PSG). The sequence of patients was randomized. The experts were required to decide based on the information presented whether: (Question 1, Q1) further diagnostic testing for OSA was necessary; and (Q2) if they recommended OSA treatment.

RESULTS
Subjects (n=20, 45% female) were of mean (±SD) age=39±12 y, Epworth score=9±5 and BMI=27±4 kg/m2 with a range of OSA severity (PSG AHI =21±17/h, RDI=30±19, ODI= 8±9/h, Arousal Hypopnea Index=11±10/h). 3SIM AHI with AnH scoring (21±40/h) more closely approximated PSG AHI compared with 3SIM AHI without AnH (11±10/h). The inclusion of AnH improved the 3SIM diagnostic sensitivity and area under the ROC curve for a PSG AHI threshold value ≥15/h (Table). Inter-expert reliability measured as percent agreement was 85% for PSG, 60% for 3SIM with AnH and 70% for 3SIM without AnH. Analysis of Q1 demonstrated that AnH scoring was associated with a relative reduction of 51±34% for additional diagnostic testing compared to conventional scoring. For Q2, considering PSG-based decisions regarding CPAP or any OSA treatment as the “gold standard”, agreement for 3SIM-based decisions with inclusion of AnH scoring was considerably better than when AnH was not used (Table).

CONCLUSIONS
In this preliminary clinical decision analysis, AnH scoring led to a reduction in inconclusive Type 3 studies and thus a reduction in subsequent PSG requests. Agreement for clinical decisions regarding OSA treatment based on type 3 studies compared with those based on PSG was substantially improved by the incorporation of AnH scoring.

Table

	Diagnostic accuracy		Clinical decision analysis ǂ			
	PSG AHI Cut-Off > 15		OSA Treatment Recommendation ◊		CPAP Recommendation	
n	11/20		15/20		13/20	
3SIM Scoring Method	Without AnH	AnH included	Without AnH	AnH included	Without AnH	AnH included
Sens (%)	36.4	100.0	31.9 ± 7.6	72.2 ± 14.0	33.8 ± 7.8	63.9 ± 4.0
Spe (%)	100.0	66.7	100.0 ± 0.0	93.3 ± 11.5	91.7 ± 14.4	93.3 ± 11.5
PPV (%)	100.0	78.6	100.0 ± 0.0	96.7 ± 5.8	100.0 ± 0.0	96.7 ± 5.8
NPV (%)	53.3	100.0	34.8 ± 9.4	57.2 ± 15.1	39.0 ± 15.9	51.1 ± 15.8
AUC	0.68	0.83	0.66 ± 0.04	0.83 ± 0.12	0.67 ± 0.04	0.79 ± 0.08

ǂ Mean values for 3 experts

◊ Either CPAP, positional therapy or mandibular advancement

Abbreviations: Sens: Sensitivity; Spe: Specificity; PPV: Positive Predictive value; NPV: Negative Predictive Value: AUC: Area under the Receiver Operating Characteristic (ROC) curve

Khan, Nargis
Category: Postdoctoral Fellow (Poster 5)
Supervisor: Maziar Divangahi, Irah King

The impact of the microbiota in shaping host defense against tuberculosis

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Mycobacterium tuberculosis (Mtb) remains one of the most successful pathogens in human history with ~1.6 million people dying from active TB each year and >2 billion asymptomatic carriers of latent *Mtb* worldwide. Although anti-TB drugs are effective in controlling *Mtb* growth, prolonged antibiotic (Abx) treatment remains a significant risk factor of disease reactivation as well as reinfection. Therefore, it is important to understand why our defense system is unable to generate permanent immunity to *Mtb* despite prolonged Abx therapy. Intestine contains highest density of commensal microbes, known as microbiota which play a significant role in immunological process in the body. Short-term (7-10 days) alteration of the microbiota by Abx treatment can profoundly affect systemic immune responses. However, the prolong (~6 months) effects of anti-TB Abx (rifampicin or isoniazid and pyrazinamide) on gut microbiota and immunity to *Mtb* is surprisingly still unknown. Here we aimed to study how anti-TB Abx modulate microbial-immune crosstalk in the gut and prevent the generation of permanent immunity to *Mtb*. Our studies revealed that *Mtb* treated with rifampicin or isoniazid and pyrazinamide prior or post *Mtb* infection altered gut microbiota including decrease in Firmicutes and expansion of Bacteroidetes phyla. Interestingly, mice treated with isoniazid and pyrazinamide prior to *Mtb* infection showed higher *Mtb* burden in the lungs and spleens. Collectively, these data indicate that despite controlling *Mtb* growth, anti-TB Abx cause gut microbial dysbiosis that may promote the susceptibility of host towards *Mtb* infection. Studies are now underway to link microbial dysbiosis to pulmonary immune responses and delineate the molecular mechanisms by which the microbiota affect protective mechanism against TB.

Kim, Dusik
Category: Postdoctoral Fellow (Poster 37)
Supervisor: John Hanrahan

Role of pendrin (SLC26A4) in secretion by Calu-3 and nasal epithelial cells

Junwei Huang,[*] Dusik Kim[*], Arnaud Billet, Jiajie Shan, Yishan Luo, Saul Frenkiel and John W. Hanrahan

Anion secretion by airway epithelial cells is essential for mucociliary clearance however the efflux mechanisms at the apical membrane remain uncertain. Here we examine the role of the anion exchanger pendrin (SLC26A4) in the Calu-3 cell line and in well differentiated primary nasal epithelial cells. Pendrin was expressed at low levels in Calu-3 cells, and stable shRNA knockdown of pendrin transcripts in Calu-3 did not affect basal or forskolin-stimulated secretion of fluid or HCO_3^-, HCO_3^- transport under pH stat conditions, or net HCO_3^- flux across basolaterally permeabilized monolayers. Alterations in pH_i induced by varying external Cl^- or HCO_3^- concentration were unaffected by pendrin shRNA but abolished by CFTR shRNA. The cytokine IL-4 caused a modest increase in pendrin expression in Calu-3 cells without affecting fluid or HCO_3^- secretion. By contrast, IL-4 caused a remarkable elevation of pendrin expression in primary nasal cells and increased apical anion exchange ~4-fold. After upregulation of pendrin in nasal primary cells by IL-4, specific knockdown by si-RNA targeting pendrin strongly inhibited forskolin-stimulated secretion, suggesting that pendrin enhances CFTR activation by cAMP. Enhanced CFTR activation was confirmed in transfected BHK cells co-expressing pendrin. We conclude that Calu-3 cells contain very low levels of pendrin and it has little role in HCO_3^- secretion, however when upregulated by IL-4 in primary nasal cells it mediates apical Cl^-/HCO_3^- exchange and strongly enhances cAMP-stimulated anion secretion that is mediated by CFTR.

Funding: CF Canada and the Canadian Institutes of Health Research

Kwong, Kelly
Category: MSc Student (Poster 29)
Supervisor: Dao Nguyen

Pseudomonas aeruginosa evasion of neutrophil phagocytosis and bacterial clearance in CF children during early infection

Kelly Kwong, Valerie Waters, Dao Nguyen

Rational

Pseudomonas aeruginosa (*Pa*) is the predominant pathogen causing chronic lung disease in adult patients with cystic fibrosis (CF). Failure to clear *Pa* by innate host defenses during early 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 is a key step for successful *Pa* eradication, we hypothesized that *Pa* isolates that are not eradicated at the time of early infection and persist in CF patients elicit dysregulated neutrophils responses, leading to impaired bacterial clearance.

Methods

In this study, we compared *in vitro* neutrophil phagocytosis and intracellular bacterial killing in response to eradicated vs persistent *Pa* clinical isolates from the Sick Kids *Pa* eradication clinical study. Subsequently, we explored bacterial factors that modulate neutrophil responses, such as type IV pilus mediated twitching motility, Psl exopolysaccharide production, flagellum mediated swimming motility and alginate overproduction (mucoidy).

Results

We observed a higher phagocytic uptake and more rapid intracellular killing of *Pa* from the eradicated group compared to those from the persistent group. This suggests that persistent *Pa* isolates exploit bacterial strain specific mechanisms to evade neutrophil phagocytosis and intracellular killing. Among all *Pa* isolates, bacterial twitching motility was significantly but only moderately correlated with neutrophil phagocytosis. In addition, mucoid and Psl expressing *Pa* isolates were more frequent in the persistent group compared to the eradicated group, and both mucoidy and Psl expression impair neutrophil function.

Conclusion

These results highlight the potential role in specific *Pa* and neutrophil interactions that contribute to bacterial eradication in CF patients. Future experiments will dissect the mechanism of bacterial persistence and evasion of neutrophil functions associated with bacterial eradication failure in young CF patients. This work will provide an extensive characterization of the host-pathogen responses that distinguish eradicated from persistent *Pa* isolates. Most importantly, this will also provide insights on what causes CF children to fail eradication during early *Pa* infections by looking at specific *Pa* bacterial factors that are associated with eradication failure.

Funding: CIHR, CFC, Meakins

Liang, Feng
Category: Research Associate (Poster 3)
Supervisor: Basil Petrof

Evidence for Ventilator-Induced Diaphragmatic Dysfunction In A Newborn Lamb Model Of Surfactant Deficiency

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Rationale: Conventional controlled mechanical ventilation (CMV) has been demonstrated to induce oxidative stress, increased cytokine expression, activation of proteolysis, and muscle weakness in previously healthy diaphragms, a condition referred to as ventilator-induced diaphragmatic dysfunction (VIDD). These prior studies have been performed in adult subjects or in previously healthy animals, whereas very little is known about the occurrence of VIDD during the neonatal period or in the setting of surfactant deficiency associated with prematurity.

Methods: Newborn male lambs (1-4 days old) delivered at term were subjected to repeated intrapulmonary saline lavage in order to induce severe surfactant deficiency ($PaO_2/FiO_2 < 100$). The anaesthetized and paralyzed animals were then ventilated for 5-6 hours using lung-protective CMV (n=4), with the goal of maintaining SaO_2 at 88-95% and arterial pCO_2 at 45-60 mmHg. Non-ventilated lambs in the same age range served as controls (CON; n= 4). Expression levels of genes related to inflammation, the ubiquitin-proteasome system, autophagy, and oxidative stress were assessed by qRT-PCR and/or western blotting.

Results: As compared to CON, CMV was associated with significant ($p<0.05$) increases in protein carbonylation in newborn lamb diaphragms, consistent with augmented oxidative stress. In the CMV group, significantly increased mRNA levels of IL-6 (6-fold), the autophagy-related GABARAPL1 (2.5 fold), and the metabolic stress sensor SIRT1 (2-fold) were observed ($p<0.05$). At the protein level, LC3B-I and II were decreased after CMV, which in combination with elevated GABARAPL1 transcript levels suggests enhanced autophagy activation in the diaphragm. In contrast, transcript levels of E3 ubiquitin ligases Atrogin and MuRF1, as well as myosin heavy chain isoforms, were unchanged.

Conclusions: In newborn lambs with severe acute surfactant deficiency, short-term CMV using a lung-protective strategy induces signs of VIDD.

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Martins, Dorival
Category: Postdoctoral Fellow (Oral Presentation)
Supervisor: Dao Nguyen

Superoxide dismutases modulate membrane permeability and multidrug tolerance in stationary phase *Pseudomonas aeruginosa*

Dorival Martins, Geoffre Mckay, Gowthami SampathKumar, Malika Khakimova, Cajeta Neubauer, Dianne Newman and Dao Nguyen

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

Methods: We compared antibiotic susceptibility of wild-type (WT) *Pa* to its isogenic SOD-null mutant (*sodAB*), which has no measurable SOD activity and ~3-fold higher superoxide levels than WT. To assess multidrug tolerance, the strains were grown to stationary-phase where a high proportion of the population becomes tolerant to bactericidal antibiotics, challenged with two different classes of clinically relevant antipseudomonal bactericidal drugs (quinolones and carbapenems) and their survival was determined through plating and colony forming units (CFU) counts after drug exposure. Prior to the antibiotic challenge, the SOD activity of individual samples was measured to determine its correlation with antibiotic tolerance. To assess whether SODs modulate antibiotic tolerance by preserving membrane structure and thus reducing drug uptake, we measured membrane permeability using ethidium bromide uptake and β-lactamase leakage methods, and carbapenem uptake using fluorescent labeled meropenem. Fatty acid composition of the membranes was assayed by gas chromatography coupled to mass spectrometry (GC/MS).

Results: The *sodAB* mutant had 2- to 3-$\log_{10}$ CFU lower survival to quinolone and carbapenem challenge than WT, and we found a strong positive correlation between antibiotic tolerance and SOD activity. Thus, SOD activity contributes to multidrug tolerance in *Pa*. Ethidium bromide uptake and β-lactamase leakage are 3-fold higher in the *sodAB* mutant, suggesting a high overall and outer membrane permeability, respectively. The *sodAB* mutant displays 2-fold higher meropenem uptake than WT, suggesting that SODs modulate drug tolerance by restricting drug uptake. The increased membrane permeability in *sodAB* was associated with 2-fold lower levels of cyclopropane fatty acids (CFAs), which contribute to tolerance to multiple stressor in stationary-phase *Pa*. Deletion of the gene encoding CFA synthase resulted in full depletion of CFA levels, 2-fold increased membrane permeability and 3- to 4-$\log_{10}$ CFU lower tolerance to quinolones and carbapenems. Therefore, CFA levels modulation by SODs is modulates drug uptake and tolerance in stationary phase *Pa.*

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

Funding: Cystic Fibrosis Canada (CFC), Canadian Institutes of Health Research (CIHR), Burroughs Wellcome Fund

Mendonca, Laura
Category: MSc Student (Poster 23)
Supervisor: Maziar Divangahi

Cracking the Macrophage Code in Immunity to TB: Ontogeny & Metabolism

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Rationale. *Mtb* is a parasite of the intracellular milieu of pulmonary macrophages (Mϕ), where it not only survives but replicates in a naturally hostile environment. Bone marrow-derived (BMD) monocytes are the main source of tissue Mϕ during infection. However, a recent fate-mapping study has demonstrated that under steady state conditions, tissue Mϕ from the brain, spleen, peritoneal cavity, and lung are all of prenatal origin and do not require BMDMϕ for self-renewal. As the functional role of residential alveolar Mϕ (AMϕ) versus recruited BMDMϕ during *Mtb* infection has not been studied yet, we aim to delineate the contribution of AMϕ and recruited Mϕ to the regulation of pulmonary host defense.

Methods. Bone marrow was collected from mice and differentiated into Mϕ using L929 media for 6 days prior to 24hr polarization into M1 (to mimic recruited Mϕ) or M2 Mϕ (to mimic anti-inflammatory AMϕ). Primary AMϕ were harvested from the BAL of naive mice. M1 and M2 Mϕ and naïve AMϕ were then infected with H37Rv. After 48h of infection, culture supernatants, RNA and Mϕ were collected for ELISA, RT-qPCR and CFU assays respectively. Additionally, Mϕ were subjected to Seahorse assays to measure basal levels of glycolysis and oxidative phosphorylation (OXPHOS) after H37Ra infection.

Results. Our data indicate that following H37Rv infection, M1 Mϕ are able to produce IL-6 and control the bacterial load. This was associated with an increase in glycolysis and decrease in OXPHOS under similar infection conditions. On the other hand, AMϕ were unable to produce IL-6 and showed an inability to control the H37Rv infection. Additionally, these Mϕ did not increase glycolysis, which may be related to uncontrolled bacterial replication. We therefore hypothesize that metabolism is the overall link between Mϕ function and host protection.

Milner, Siobhan
Category: MSc Student (Poster 17)
Supervisor: Tania Janaudis-Ferreira

Rate of, and barriers and enablers to Pulmonary Rehabilitation Referral in COPD: A Scoping Review.

Siobhan Milner, Cecile Beaurepaire, Jill Boruff, Saeed Alghamdi, Sara Ahmed, Tania Janaudis-Ferreira

RATIONALE Despite the fact that pulmonary rehabilitation (PR) is an evidence-based treatment recommended for people with chronic obstructive pulmonary disease (COPD), referral to the service seems to be low. Systematic reviews on patient barriers and enablers to PR uptake exist but no such knowledge synthesis specifically focusing on healthcare professional barriers and enablers to referral is available.
Collating the evidence on referral rates and perceived barriers and enablers may help provide knowledge needed to implement measures to improve referral rates and health care professionals' perceptions of PR. The purpose of this scoping review was to determine 1) the rate of referral of patients with COPD to PR by health care professionals and 2) the barriers and enablers to referral perceived by healthcare professionals.

METHODS We conducted a systematic scoping review of the published literature, theses and conference abstracts. We searched health science databases including CINAHL, EMBASE, OVID Medline and ProQuest Global. Two investigators screened the titles and abstracts of 229 non-duplicate articles. The included abstracts then underwent review for full text inclusion by two separate investigators. One investigator extracted the data from the final included studies using a standardized form, and one other investigator then verified the data extraction. We included articles that reported referral rates and/or healthcare provider perceived barriers and/or enablers to PR referral. The research articles included were varied in design, including prospective, retrospective and cross-sectional studies. Articles were excluded if they were not focused on COPD, were not primary research, did not present rates of referral in terms of a fraction or percentage, or did not present barriers or enablers to referral that were healthcare professional perceived.

RESULTS Twenty eight observational studies reported on referral rates of eligible COPD patients which ranged from 1.6% to 85%, and seven intervention studies that looked at the effects of different interventions to improve COPD care (including referral) reported a range of 1.7% - 56.3% referral pre-intervention, compared to 7.8% - 70.7% post-intervention. All intervention studies reported an improvement in referral rates post-intervention. Nine studies reported healthcare professional perceived barriers to referral. The two most common barriers were low knowledge of what PR is and its benefits (or even a disbelief in the benefits) and low knowledge of the referral process, followed by a lack of knowledge about eligibility criteria, referral not being part of the clinical workflow or a lack of reminders to refer, and the perceived or actual difficulty of gaining a behaviour change in patients. Six studies reported healthcare professional perceived enablers to referral. The most common enabler was training or experience in PR, followed by a streamlined referral process and awareness events or reminders that helped to make PR referral a part of their daily workflow.

CONCLUSION The rate of referral to PR is suboptimal, but there are some commonly reported barriers and enablers that may help with the creation of actionable changes. Namely, healthcare professionals need more knowledge of what PR is, the evidence behind it, who is eligible, and how to refer.

Moarbes, Vanessa
Category: PhD Student (Poster 38)
Supervisor: Elizabeth Fixman

Effect of STAT6 on IL-33 mediated macrophage polarization in a murine allergy model

Vanessa Moarbes, Hedi Zhao, Haya Aldossary, Jichuan Shan, Véronique Gaudreault St-Laurent, Katherine Restori, Elizabeth Fixman

Background: Asthma and allergy rank among the costliest of all chronic diseases and are steadily increasing in both severity and occurrence. An extensive literature demonstrates that adaptive $CD4^+$ Th2 cells and, more recently, type 2 innate lymphoid cells (ILC2s) orchestrate the immune-pathologic Th2 responses in the lung in asthma and some respiratory viral infections. Data from asthmatic patients and murine asthma models demonstrate that the STAT6 transcription factor regulates several responses in the allergic lung contributing to poor lung function. Recent interest has focused on innate type cytokines, including IL-33 and TSLP, which promote Th2 immunity and are new targets for drug development in asthma. In addition to inducing cytokine production from a number of innate cells in the lung, IL-33-induced production of IL-4 or IL-13, has been linked to autocrine/paracrine dependent responses in macrophages (Mφs) and eosinophils. Recent data from Kurowska-Stolarska et al demonstrated that in mice, IL-33 delivery to the lungs induces chemokine production, eosinophilic inflammation, and Mφ differentiation into type 2 alternatively activated Mφs (AAM) – all in an IL-13-dependent manner.
Data from the Fixman lab demonstrate that a cell penetrating peptide-based STAT6 inhibitor (STAT6-IP) diminishes maladaptive inflammatory Th2 responses and airway hyperresponsiveness in murine models of asthma and respiratory syncytial virus infection upon delivery to the lungs. The overall objective of these experiments is to examine the role of STAT6 in acute IL-33-induced airway eosinophilic inflammation and AAM polarization. We hypothesize that these IL-33-induced responses will be reduced in STAT6 knockout (KO) mice and in wild-type mice treated with STAT6-IP.

Methods: Balb/c mice, treated daily with STAT6-IP (or negative control, STAT6-CP) or STAT6 KO mice on a Balb/c background were used for all experiments. Mice were treated with IL-33 or IL-33+OVA daily for 3 days and sacrificed after a 3-day rest. Lungs were harvested in order to assess Mφ phenotype by multiparametric flow cytometry. Levels of mRNA encoding IL-13 as well as markers of Mφ differentiation were quantified by qPCR. BAL fluid inflammatory cell counts were quantified. The right lung was minced, plated in media, and re-stimulated ex vivo with saline or IL-33. Supernatants were harvested 48h later for cytokine analysis.

Results: Our data demonstrate that IL-33 delivery to the lungs of wild-type mice increased AAM polarization and eosinophilic influx. Levels of mRNA encoding markers of AAM polarization, including IL-13, were also increased. Lung cells from IL-33-treated mice cultured ex vivo with IL-33 produced large amounts of IL-13. All of these responses were reduced in mice treated with STAT6-IP and in IL-33-treated STAT6 KO mice.

Significance: Altogether, these data suggest that the ability of IL-33 to promote type 2 inflammatory responses in the lungs, likely before the onset of adaptive immunity, is dependent upon STAT6, and possibly IL-13 production. Future experiments will clarify the interaction between IL-33, IL-13, and STAT6 in AAM polarization and these inflammatory responses. Ultimately, knowledge acquired from this project will provide mechanistic information helpful in guiding development of STAT6-IP as a promising human therapeutic.

Funding: DoD (US Department of Defense)

Mostafavi, Yousof
Category: MSc Student (Poster 25)
Supervisor: Jean Bourbeau

Asthma-COPD Overlap (ACO) in the CanCOLD Cohort: Prevalence, Attributes, and Natural History

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Rational: Defining Asthma-COPD Overlap (ACO) has been challenging, with various definitions being proposed. The aim of this study was to identify unique clinical and radiological features, and prognosis of individuals with ACO, when
compared to those with COPD alone.

Methods: The study is embedded in the Canadian Cohort Obstructive Lung Disease (CanCOLD) study. Six definitions of ACO were studied, based on definitions reported across our review of the literature. Subjects who did not meet any of the six definitions were classified as a non-ACO group with COPD.

Results: 1552 subjects were enrolled; of whom 715 had COPD from post bronchodilator GOLD criteria were included in the present analysis. Four hundred nineteen (58.6%) have completed their first follow-up visit and were included in the longitudinal analysis. The prevalence of ACO varied from 24.9% to 4.6%. ACO definitions were stable when including atopy and physician diagnosis of asthma but all were very unstable when including reversibility post bronchodilator. All ACO subjects, especially those who were younger had more co-morbidities and worse health status. ACO subjects had worse lung function (airway obstruction and increased hyperinflation at rest) (Table 1). Chest CT Scan showed a lower score of emphysema for definition 4 and 6, i.e., atopy and physician diagnosis of asthma, and ≥ 12% and ≥ 200ml reversibility post Short-acting beta agonist (SABA) and atopy and physician diagnosis of asthma. In a multivariable regression, adjusted for age, sex, race, current smoking, and respiratory medications taken, subjects in group 4, i.e., atopy and physician diagnosis of asthma had increased comorbidities (OR 1.42, 95% CI: 1.30 – 1.55) and lower emphysema score (OR 0.49).

Conclusion: This study suggests that the prevalence of ACO varies significantly based on the definition used. Bronchodilator reversibility was found to lack specificity and was an attribute that was not consistent at follow-up. Further studies, phenotyping ACO vs non-ACO COPD with inflammatory, and studying the possibility of a unique genotype are needed.

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Naderi, Nafiseh (Nadia)
Category: MSc Student (Poster 14)
Supervisor: Jean Bourbeau

Long-term azithromycin therapy to reduce acute exacerbations in patients with severe chronic obstructive pulmonary disease.

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Rationale: Randomized clinical trials have reported that azithromycin, taken daily for 1 year, decreased exacerbations of chronic obstructive pulmonary disease (COPD)

Objectives: The aim of this study was to evaluate the effectiveness of long-term azithromycin in reducing exacerbations in patients with severe COPD on optimal therapy, in a real-life practice.

Methods: We conducted a retrospective observational study of patients with severe COPD who were prescribed azithromycin (250 mg, at least 3 times weekly for at least 6 months). The control group included patients with severe COPD not exposed to azithromycin. The primary endpoint was yearly exacerbations requiring a change in treatment or health service use. Data were extracted from clinical chart review.

Main Results: There were 126 cases and 69 controls included in the study. Patients had severe airflow obstruction, most had emphysema and one-third bronchiectasis on CT scan. A predominant feature in the case group was the colonization of the respiratory tract with Pseudomonas aeruginosa. The mean number of exacerbations per patient per year in the case group was 3.2± 2.1 before initiation of azithromycin, and 2.3 ± 1.6 during the following year on therapy ($p<0.001$). Patients in the control group had 1.7±1.3 and 2.5±1.7 exacerbations during the first and second year of follow-up respectively ($p<0.001$). The change in exacerbations from pre to post differed between groups ($p<0.001$). Furthermore, patients in the case group had significant decrease in emergency visits and hospital admissions. The reduction in the number of exacerbations and proportion of patients having ≥2 exacerbations extended to the second year of azithromycin treatment.

Conclusion: These data from real life practice showed that long-term azithromycin reduces the number of exacerbations in patients with severe COPD, and benefits persist beyond one year. Desirable effects are more likely to outweigh the risks and adverse events in patients colonized with Pseudomonas aeruginosa.

Panariti, Alice
Category: Postdoctoral Fellow (Poster 7)
Supervisor: James Martin

Changes of contractile phenotype in response to pro-proliferative stimuli in airway smooth muscle cells

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Research Institute of the McGill University Health Centre, Meakins-Christie Laboratories, McGill university

Introduction: The contractile and proliferative properties of the airway smooth muscle cells (ASMCs) are of central importance to the induction of airway hyperresponsiveness in asthma. The co-transcription factor myocardin controls the expression of contractile genes by competing with the pro-proliferative factor Elk1 for the binding to the serum response factor (SRF). At present it is poorly understood whether the proliferative and contractile phenotypes of ASMC are mutually exclusive phenotypes as previously proposed (Halayko AJ et al. 1996) or linked events that occur simultaneously in the same cell. Based on this we aimed to investigate the contractile properties of ASMCs in response to pro-proliferative stimuli.

Methods: ASMCs from healthy donors were grown for 48 hours in DMEM with increasing concentration of fetal bovine serum (FBS, from 0 to 10%) or in presence of the pro-proliferative factor heparin-binding epidermal growth factor (HB-EGF; 100 ng/ml). Proliferation was assessed by bromodeoxyuridine (BrdU) incorporation and the expression of proliferative and contractile markers was analyzed by real time PCR and Western Blot. As an index of the initiation of cross-bridge cycling we analyzed the response of the cell to FBS to histamine (0,3 μM) induced calcium release by Fura-2 ratiometric imaging.

Results: As expected, the number of ASMCs positive for BrdU increased with increasing concentrations of FBS reaching the maximum value at 10% FBS (55.6±5.3 vs 6.1±1.1, 10% vs 0%) and in response to HB-EGF (25.7±2.8 vs 12.4±2.3, 100ng/ml vs 0ng/ml). In accordance with cellular proliferation, Elk1 mRNA increased following both treatments. However we found that myocardin increased as the cells became more proliferative in response to FBS (20 times more at 10% than 0% FBS) but not to HB-EGF, while SRF was not affected in either condition. α-actin transcript level significantly decreased in response to FBS while calponin and myosin heavy chain increased at protein level ($p<0.05$). No changes in calcium release were found in response to pro-proliferative stimuli.

Conclusions: We found that although in a proliferative status ASMCs overexpressed contractile protein, including myocardin. Understanding the function of this overexpression as well as the analysis of contractile properties in proliferative status will be important in elucidating the dynamics of phenotypic and functional switching in ASMCs in airway diseases.

Pareek, Swati
Category: MSc Student (Poster 31)
Supervisor: Carolyn Baglole

RelB Regulation of Cigarette Smoke-Induced Pulmonary Inflammation

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Rationale: The lung is continuously exposed to the environment and subject to injury by irritant inhalants. RelB is a member of the non-canonical NF-κB pathway that may control lung inflammation and cell death caused by cigarette smoke (CS), a risk factor for chronic obstructive pulmonary disease (COPD). We have shown that RelB protects against CS-induced apoptosis and inflammation *in vitro*. Other exposures that damage the lung include lippolysaccaride (LPS) – a gram-negative bacterial component- and chlorine – an oxidative irritant. Whether RelB suppresses inflammation *in vivo* in response to these agents is unknown. Therefore, we hypothesize that RelB protects the lung against environmentally-induced inflammation.

Methods: Wild-type ($RelB^{+/+}$) and RelB-deficient mice ($RelB^{-/-}$) mice were exposed to CS or room air for 3 days using a whole-body exposure system (SCRIEQ®). Lung tissue was harvested and bronchoalveolar lavage (BAL) was collected 24 or 72 hours post- exposure. For the assessment of responses to chlorine and LPS, $RelB^{+/+}$ and $RelB^{-/-}$ mice were intranasally exposed to 100 ppm chlorine for 5 minutes or 10 µg of LPS and sacrificed 24 hours later. Differential cell counts and cytokine levels via multiplex assay were subsequently analyzed.

Results: Air-exposed $RelB^{-/-}$ mice had significantly higher BAL macrophage, neutrophil, and lymphocyte compared to $RelB^{+/+}$ mice. $RelB^{+/+}$ mice exposed to CS had more neutrophils and macrophages. CS-exposed $RelB^{-/-}$ mice had a decrease in inflammatory cells that was largely reflective of decreased neutrophils and lymphocytes. This decrease in lung cellularity was transient, as BAL collected 72 hours post-exposure had more inflammatory cells than BAL collected 24 hours post-exposure. Although chlorine and LPS treatment heightened inflammation in $RelB^{+/+}$ mice, neither exposure altered the level of inflammatory cells in $RelB^{-/-}$ mice. $RelB^{-/-}$ mice had higher BAL IL-4 , IL-6, KC, MCP-1 and TNFα and lower IFN-γ and IL-10 levels at baseline; CS did not significantly alter the level of these cytokines. Interestingly, CS- exposed $RelB^{+/+}$ mice also had lower levels of IFN-γ and IL-10 than air-exposed $RelB^{+/+}$ mice along with higher levels of KC.

Conclusion: Our results support the concept that RelB plays a role in exerting homeostatic control over pulmonary inflammation. It also demonstrates an irritant-specific regulation of environmentally-induced inflammation such that $RelB^{-/-}$ mice exposed to CS have reduced inflammation while inflammation in $RelB^{-/-}$ mice exposed to chlorine or LPS remains unchanged. Understanding how RelB regulates CS-induced inflammation may potentiate the discovery of new therapeutic strategies for many of the inflammatory diseases caused by CS.

Funding: CIHR

Parmar, Robin
Category: Clinical Fellow (Poster 12)
Supervisor: Nicole Ezer, Andreea Morogan

Metalloptysis after bilobectomy for lung cancer: Case report and literature review

Robin Parmar , Nicole Ezer, Andreea Morogan
McGill University

A 73-year-old female who underwent a right upper and middle lobectomy for stage 1 adenocarcinoma of the lung in May 2014 was referred to our respiratory service two years later for expectoration of surgical clips. She presented with a 5-day history of cough, increased sputum production, chills and sore throat. While these symptoms were resolving without treatment, she began experiencing metalloptysis and expectorated 30 surgical clips. In this context, a bronchoscopy and a CT chest with contrast were performed. The bronchoscopy demonstrated a normal surgical stump, no evidence of dehiscence, and absence of surgical clips noted in the airways. Imaging revealed air bubbles at the level of the surgical stump.

Metalloptysis is an uncommonly described phenomenon meaning expectoration of surgical clips. Its management is uncertain in the literature. It has most commonly been described with thoracic surgery involving a bovine pericardium staple line in the lung, abnormal lung parenchyma or pulmonary infection. It has, however, been reported in one patient without evidence of underlying infectious process or lung parenchymal abnormalities. The majority reported late metallotypsis, whereas 2 cases occured quite early in the postoperative course (in 30 days).

This review has guided us to consider that the management of metalloptysis should include adequate imaging (CT chest) and a bronchoscopy. The aim is to eliminate a source of infection or other parenchymal abnormalities. Patients should be re-assured that expectoration of clips will not necessarily affect the surgical stump. Furthermore, the instances of metalloptysis may be understated in the literature.

Pernet, Erwan
Category: Postdoctoral Fellow (Oral Presentation)
Supervisor: Maziar Divangahi

Leukotriene B_4 prevents IAV-induced immunopathology via regulating macrophages in situ proliferation

Erwan Pernet, Jeffrey Downey, Isabelle Meunier and Maziar Divangahi

Rationale. Despite the worldwide application of vaccination and other antiviral interventions, influenza A virus (IAV) infection remains a serious threat to humans. The success of IAV infection is linked to its ability to cause lower airways infections, which activates alveolar macrophages and often leads to a cytokine storm, pneumonia and respiratory failure. At this stage, uncontrolled host inflammatory response is the major cause of death. Therefore, understanding the mechanisms of host tolerance during IAV infection is critical in preventing IAV-induced immunopathology and mortality. Although the role of cytokines has been extensively studied in immunity to IAV infection, little is known about the function of eicosanoids. We have recently demonstrated that cyclooxygenase-derived PGE_2 plays a deleterious role in protection against IAV infection by inhibiting antiviral type I interferon (IFN). Herein, we aimed to investigate the potential contribution of 5-lipoxygenase-derived leukotriene B_4 (LTB_4) in IAV-immunity using the BLT1R (*Blt1R*$^{-/-}$) deficient mice.

Methods. Based on our established model of influenza viral infection, WT and *Blt1R*$^{-/-}$ mice were infected with influenza virus (PR8 strain) at a lethal (MOI~90) or sublethal (MOI~50) dose to analyze protection/morbidity/mortality and the kinetic of the immune response. At different days p.i., lungs were harvested and cytokines (e.g. type I IFNs), viral titres and innate cellular responses were determined by B16 cell reporter assay, ELISA, plaque assay and flow cytometry respectively. To address the role of the LTB_4 signaling in macrophages, WT and *Blt1R*$^{-/-}$ bone marrow-derived macrophages (BMDM) were generated and levels of type I IFNs and cytokines were determined after IAV infection.

Results. Our data indicate that despite the intact antiviral immunity as assessed by similar pulmonary IAV titers, *Blt1R*$^{-/-}$ mice were more susceptible to IAV infection. The increased susceptibility of IAV-infected *Blt1R*$^{-/-}$ mice was coupled with higher frequency and number of Ly6C^{hi} monocytes/macrophages in the lungs, resulting in enhanced immunopathology and lung damage. Interestingly, higher numbers of Ly6C^{hi} cells were not due to increased recruitment, but to dysregulated *in situ* Ly6C^{hi} cells proliferation in IAV-infected *Blt1R*$^{-/-}$ mice. We also observed decreased production of IFN-α resulting in decreased pulmonary bioactive type I IFN levels, further identified as critical regulator of Ly6C^{hi} cells proliferation. Mechanistically, we demonstrated in BMDM that LTB_4 enhanced STAT1 activation, key protein in the signaling pathway downstream of type I IFN receptor. Remarkably, a single dose of LTB_4 administration (100ng, i.n.) after 5 days of infection protected WT mice from lethal IAV infection.

Conclusions. Collectively, these findings identified the protective mechanisms of LTB_4 during IAV infection by regulating Ly6C^{hi} monocytes/macrophages *in situ* proliferation and limiting IAV-induced immunopathology. Our work may pave the way for novel anti-influenza treatments using stable bioactive lipids.

Richard and Edith Strauss postdoctoral fellowship
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Sato, Yukiko
Category: PhD Student (Oral Presentation)
Supervisor: John Hanrahan and David Thomas

SLC26A9 is prematurely degraded along with misfolded F508del-CFTR

Yukiko Sato, Renaud Robert, David Y. Thomas and John W. Hanrahan

A key channel involved in airway anion secretion is the cystic fibrosis transmembrane conductance regulator (CFTR), which is defective in cystic fibrosis (CF) patients. The most common mutation, deletion of phenylalanine at position 508 (F508del-CFTR), causes retention of the mutant in the endoplasmic reticulum (ER) and currently available drugs for CF provides only modest clinical benefit, therefore other anion channels such as SLC26A9 (A9) are being explored as potential therapeutic targets. A9 is a constitutively active channel which interacts with the regulatory (R) domain of wild-type CFTR (WT) through its STAS domain, therefore we examined whether a similar interaction with F508del-CFTR might lead to retention of A9 in the endoplasmic reticulum and premature degradation by the proteasome. BHK cells overexpressing WT or F508del-CFTR were transfected with A9 and protein expression was assessed by immunoblotting cell lysates and by use of a cell surface biotinylation assay. The amount of A9 protein in whole cell lysates as well as the surface expression was reduced in cells that co-express F508del-CFTR when compared with those expressing WT-CFTR. In addition, A9 expression in whole cell lysates was increased by 1.5 fold in WT-CFTR expressing cells compared to parental BHK cells, and this difference was further enhanced by cAMP/PKA stimulation. Partial correction of F508del-CFTR trafficking elevated both total and cell surface expression of A9. Expression of A9 in F508del-CFTR cells was also restored by transfecting cells with WT- CFTR cDNA. Finally, we also found that inhibiting the proteasome pathway increased A9 whole cell expression, similarly to CFTR. These results indicate that A9 surface expression depends on the trafficking and surface expression of CFTR, and support the notion that CFTR and A9 physically interact via the phosphorylated R domain of CFTR. This interaction probably causes A9 to be degraded prematurely in CF cells that express F508del-CFTR. Fully understanding the mechanism of the interaction between CFTR and A9 will be important for future investigations into the potential use of A9 as a therapeutic target in CF treatment.

Funding: FQRS, GRASP Award

Torabi, Bahar
Category: MSc Student (Poster 27)
Supervisor: Bruce Mazer

Salivary IgG4 Increases in Milk Oral Immunotherapy

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Rationale: Cow's milk allergy (CMA) is a frequent cause of severe allergic reactions and anaphylaxis in children. Oral immunotherapy (OIT) has shown promising results with immunological changes occurring during desensitization. Our team at the Research Institute of McGill University Health Centre has demonstrated an increase in serum IgG4 during the escalation and maintenance phases of milk OIT. Salivary IgG4 in OIT has not been studied to date. We assessed the changes in salivary IgG4 during milk OIT, as a potential non-invasive biomarker of desensitization.

Methods: We performed an interim analysis at baseline (prior to the start of treatment) and at the end of escalation phase (200ml dose) of the milk OIT protocol in 9 subjects who successfully completed the escalation phase. Milk protein component (α-lactalbumin, β-lactoglobulin, casein) -specific salivary IgG4 were evaluated at baseline and 200ml.

Results: Salivary IgG4 significantly increased from baseline to 200ml for all three milk proteins. The mean salivary IgG4 concentration at baseline was 33.9 ng/ml (SEM 33.9ng/ml), 34.8 ng/ml (SEM 33.6ng/ml), and 159.7 ng/ml (SEM 69.4 ng/ml) for α-lactalbumin, β-lactoglobulin, and casein respectively. The mean salivary IgG4 concentration at the 200ml dose of escalation phase was 538.1 ng/ml (SEM 288.1 ng/ml), 1163 ng/ml (SEM 660.2 ng/ml), and 1498 ng/ml (SEM 702 ng/ml) for α-lactalbumin, β-lactoglobulin, and casein respectively.

Conclusion: Successful escalation phase of milk OIT in IgE-mediated CMA in children is associated with a significant increase in salivary milk protein-specific IgG4. This suggests it could be used as a potential non-invasive biomarker of desensitization in OIT in children.

Funding: The Richard and Edith Strauss Clinical Fellowship
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GET-FACTS
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Traboulsi, Hussein
Category: Postdoctoral Fellow (Poster 39)
Supervisor: Carolyn Baglole, Benjamin Smith

FGF10 haploinsufficiency is associated with smaller airways increasing susceptibility to COPD

Hussein Traboulsi, Pr, Carolyn Baglole, Dr, Benjamin smith

Background: Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation and represents the third leading cause of death globally and in North America. Cigarette smoking is the main risk factor for COPD, but only 10%–20% of all heavy cigarette smokers develop COPD. Although the number of smokers have declined the past 5 decades, the incidence of COPD has not decreased. These observations show that together with smoking other factors seems to be of importance for the development of such disease. Understanding factors that contribute to COPD pathogenesis, beside tobacco smoke, are therefore urgently needed. Genetic studies in humans and in animal models suggest that genes associated with lung developmental processes are implicated in COPD. Lung is a brunched organ and its development requires branching morphogenesis, a process that is dependent on cellular communication between the mesenchyme and the lung epithelium.

It was recently demonstrated (by Dr Benjamin smith) using computed tomography (CT) that proximal airway anatomy variants were present in 26% of a population-based sample of humans. Interestingly, one of these variants is an absence of the right-medial basal airway, which was observed in 6.1% of subjects. This absence of right-medial basal airway was associated with a 100% higher COPD prevalence among smokers when compared with standard anatomy. Therefore, this suggest that this airway anatomy could be a COPD risk factor and easily identifiable by CT. Furthermore, they identified associations of genetic variants in fibroblast growth factor 10

FGF10 is expressed in the mesenchyme and its signalling is mediated by the receptor FGFR2 and it is required for the development of many branched organs including lungs, thyroid, pituitary, lacrimal and salivary glands. Clinically, mutations in FGF10 are associated with hypoplasia of the Lacrimal and Salivary Gland (ALSG) syndrome. Interestingly, recent study showed that ALSG patients has higher COPD prevalence compared with sibling controls. Fgf10 heterozygote mice (Fgf10+/-) exhibit reduced Fgf10 expression and are phenotypically like the human ALSG syndrome. These observations give rise to the question whether FGF10 haploinsufficiency is associated with smaller airways increasing susceptibility to COPD.

Objective: Determine if Fgf10+/- mice develops airway anatomy and if they have more susceptibility to chronic cigarette smoke-induced lung injury and COPD.

Results: By direct visual assessment of CT images using Osirix image-analysis software, we observed that the Fgf10-heterozygotes mice (Fgf10+/-) have smaller airways comparing with the control mice. Moreover, using flexivent, we showed that Fgf10+/- mice has more airway resistance at baseline and during Methacholine-induced bronchoconstriction.

Future direction: Our perspectives is to analyse the lung of these mice histologically by analysis of Hematoxylin and eosin stained sections. Next, we would like to expose control and heterozygotes mice (Fgf10+/-) to cigarette smoke in a SCIREQ inExpose Exposure System for 4-6 months to mimic chronic exposure. Mice will be sacrificed at 4 and 6 months to determine the onset of airspace enlargement. This will allow evaluation if Fgf10 deficiency accelerates a COPD-like phenotype.

Conclusion: our data show for the first time that the Fgf10 deficiency lead to airway anatomy, as observed by the reduced of airway size.

Wald, Joshua
Category: Clinical Fellow (Oral Presentation)
Supervisor: Jean Bourbeau

Undiagnosed COPD leads to a significant symptom burden, with important differences between men and women

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Worldwide, almost 70% of patients with COPD are undiagnosed. A better understanding of the symptom burden associated with undiagnosed COPD and the differences in this burden between men and women is necessary to understand the impact of COPD and to aid in the development of better targeted case-finding interventions. The objectives of our study were i) to determine the symptom burden and functional limitations in patients with undiagnosed COPD and compare with healthy control; ii) to compare the symptom burden between men and women.

Methods: We used data from CanCOLD, a prospective population-based longitudinal cohort study that recruited non-institutionalized adults through random digit dialling (land line) from nine Canadian urban city sites. Patients with undiagnosed COPD, as defined by FEV1/FVC post bronchodilator <0.7 who had never been given a physician diagnosis of COPD, chronic bronchitis, or emphysema, were compared to matched healthy, never smokers without COPD.

Results: A total of 839 patients were included: 519 patients with undiagnosed COPD (191 women, 37%) and 320 healthy controls (170 women, 53%). The mean percent predicted FEV1 in undiagnosed COPD was 86.6% (+/-17.6) and was lower in women (85.2% +/-18.4) than men (90.4% +/-17.4).
Patients with undiagnosed COPD had more respiratory symptoms, lower exercise tolerance, and lower quality of life than healthy controls. The mean CAT score did not differ between undiagnosed COPD and controls but, for those with undiagnosed COPD, the CAT was higher for women than men. Women with undiagnosed COPD had a lower percent-predicted FEV1, more symptoms, and lower quality of life than men, but had an equivalent exercise capacity, as measured by percent predicted peak VO2.

	Controls Total n=320	Undiagnosed COPD Total n=519	P-value	Undiagnosed COPD (men) n=328	Undiagnosed COPD (women) n=191	P-value
Age (years)	66.3±9.8	67.6±10.3	0.086	67.8 ± 10.4	67.2 ± 10.0	0.374
Male gender, n (%)	150 (46.9)	328 (63.2)	<0.001	-	-	-
FEV1 % predicted	101.8 ± 17.1	86.6 ± 17.6	<0.001	88.8 ± 17.3	82.8 ± 17.5	<0.001
Chronic respiratory symptoms (cough, phlegm, wheeze)	66 (20.6)	208 (40.1)	<0.001	125 (38.1)	83 (43.5)	0.231
MRC score	1.3 ± 0.6	1.4 ± 0.7	<0.001	1.3 ± 0.6	1.6 ± 0.7	<0.001
SGRQ total score	8.1 ± 9.9	11.6 ± 12.5	0.011	9.8 ± 10.4	14.8 ± 14.8	<0.001
CAT Score	5.5 ± 4.3	6.2 ± 5.4	0.249	5.5 ± 4.5	7.5 ± 6.4	0.003
Peak Work rate (Watts)	106.0 ± 57.4	84.0 ± 32.3	<0.001	73.0 ± 21.8	103.6 ± 38.3	<0.001
6MWD	520.8 ± 112.9	491.1 ± 105.3	0.003	502.0 ± 112.1	471.5 ± 89.0	0.004*

Conclusion: Patients with undiagnosed COPD have more respiratory symptoms, a lower quality of life, and lower exercise capacity then matched healthy controls suggesting that some of these patients could benefit from diagnosis and treatment of their COPD. Women make up a smaller proportion of undiagnosed COPD than men, but have a higher symptom burden and lower health status. These differences should be taken into account when considering case-finding interventions.

Funding: The Canadian Cohort Obstructive Lung Disease (CanCOLD) study is currently funded by the Canadian Respiratory Research Network (CRRN); industry partners: Astra Zeneca Canada Ltd; Boehringer Ingelheim Canada Ltd; GlaxoSmithKline Canada Ltd; Novartis. Researchers at RI-MUHC Montreal and Icapture Centre Vancouver lead the project.

Waskiw-Ford, Marcus
Category: MSc Student (Poster 18)
Supervisor: Dennis Jensen

Effect of inhaled nebulized furosemide on breathlessness during exercise in the presence of external thoracic restriction: a dose-response study

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1 – McGill University; 2 – McGill University Health Center

Introduction: Breathlessness on exertion dominates the clinical presentation of adults with restrictive lung disorders (RLDs). Alleviating breathlessness is identified as one of the primary goals in the management of RLDs. With the exception of supplemental oxygen and pulmonary rehabilitation, very few therapies exist for the management of exertional breathlessness in RLDs. Inhalation of nebulized furosemide has been shown to alleviate breathlessness provoked experimentally in health and resulting from exercise in chronic obstructive pulmonary disease. Relief of breathlessness following inhalation of nebulized furosemide is thought to result from stimulation of slowly adapting pulmonary stretch receptors mimicking greater tidal volume (V_T) expansion. It follows that nebulized furosemide represents a promising pharmacotherapy for relief of exertional breathlessness in RLDs. Nevertheless, no study has examined the effect of nebulized furosemide on exertional breathlessness in RLDs. Similarly, it is unclear whether relief of exertional breathlessness following inhalation of nebulized furosemide is dose-dependent. We hypothesized that, compared to placebo, inhalation of 40 mg and 120 mg of nebulized furosemide would relieve breathlessness in a dose-dependent manner during exercise with external thoracic restriction sufficient to mimic the negative consequences of a mild RLD on exercise endurance, breathlessness, breathing pattern, dynamic operating lung volumes, inspiratory neural drive and contractile respiratory muscle function during exercise (Mendonca et al., *J. Appl. Physiol.* 116: 570-581, 2014; Kotrach et al., *J. Appl. Physiol.* 118: 1406-1414, 2015).
Methods: Twenty-four healthy men inhaled nebulized furosemide (40 mg and 120 mg) or 0.9% saline (placebo) in a randomized, double-blind, placebo-controlled, crossover study. Following inhalation, participants completed a symptom-limited constant-load cycle endurance exercise test at 80% of peak power output in the setting of external thoracic restriction *via* chest wall strapping to reduce slow vital capacity by ~20% of its unrestricted control value recorded during a screening visit. Detailed assessments of breathlessness, ventilation, breathing pattern, the behaviour of dynamic operating lung volumes, and cardiometabolic function were assessed at rest and during exercise. Diuresis was assessed via post-dose quantification of cumulative urine output.
Results: Compared with placebo, neither 40 mg nor 120 mg of inhaled furosemide had a statistically significant effect on Borg 0-10 scale intensity ratings of breathlessness during exercise. Similarly, neither dose of nebulized furosemide had an effect on cardiometabolic, ventilatory, breathing pattern and dynamic operating lung volume responses to exercise compared with placebo. Compared with placebo, a dose-dependent effect of nebulized furosemide on cumulative urine output was observed. No other side effects were reported.
Conclusions: Inhalation of nebulized furosemide does not alleviate breathlessness during exercise in the setting of abnormal restrictive constraints on V_T expansion in healthy men. Our results do not support the use of nebulized furosemide to alleviate physical activity-related breathlessness in adults with mild RLDs, specifically those arising from abnormalities of the chest wall.

Funding: NSERC

Weinstock, Andrew
Category: Clinical Fellow (Oral Presentation)
Supervisor: Anne Gonzalez

An audit of CT chest reports and their potential impact on the workup of patients with suspected lung cancer

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Rationale Lung cancer stage at disease presentation is correlated with survival, and is a key determinant of treatment. Despite the importance of stage in guiding appropriate management, significant quality gaps exist in the diagnostic evaluation of lung cancer patients. Patients with suspected lung cancer typically undergo chest CT scanning. Physicians heavily rely on the initial CT chest to guide decisions as to the next diagnostic step(s) in the workup of patients with suspected lung cancer. We sought to determine how frequently CT reports provide guideline-based recommendations with regards to additional imaging studies and/or potential invasive diagnostic procedures in patients with suspected lung cancer.

Methods This was a retrospective study. The records of patients referred to the institution's Rapid Investigation Clinic for suspected lung cancer between January 1st 2015 and June 30th 2016 were reviewed. Patients in whom a pathologic diagnosis of lung cancer was established, and for whom both CT scan images and reports were available, were included. Patients' age, gender, lung cancer subtype and stage at initial presentation were recorded. We examined the type of additional imaging studies recommended in radiologists' reports, and whether an invasive diagnostic procedure was suggested. We determined whether these recommendations were concordant with current guidelines for lung cancer diagnosis and staging, based on suspected disease stage.

Results One hundred and fourty-six patients are included in the analysis. Mean patient age was 69 years and 57% were male. The majority of patients (95%) were diagnosed with NSCLC, predominantly adenocarcinoma: of these 37% had early stage disease (stages I & II), and 63% were late stage (III & IV). Additional imaging studies were recommended in 16% of CT reports. PET scan was suggested in only 6% of CT reports. Overall, only 12% of CT reports had guideline-concordant recommendations for additional imaging studies. Potential invasive diagnostic procedures were suggested in one fifth of CT reports. However, only 58% of these recommendations were in keeping with current guidelines. In particular, TTNA or conventional bronchoscopy was suggested in 32% of recommendations despite advanced stage disease.

Conclusion Guideline-concordant recommendations for investigation of suspected lung cancer are rarely available on CT reports. This is true with respect to both imaging studies and invasive diagnostic procedures. Incorporation of more evidence-based suggestions for investigation of patients with suspected lung cancer may reduce quality gaps in lung cancer diagnosis and staging.

Wong, Francis
Category: MSc Student (Poster 24)
Supervisor: John Hanrahan

Cigarette Smoke-Induced Downregulation of CFTR Is Dependent on Lemur Tyrosine Kinase 2 (LMTK2)

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Chloride flux through the cystic fibrosis transmembrane conductance regulator (CFTR) channel drives cAMP stimulated fluid secretion and is required for the efficient mucociliary clearance of inhaled substances from the airway surface. CFTR dysfunction may result from loss-of-function mutations as in CF, or it may be acquired when airways are exposed to air pollutants such as cigarette smoke (1). Clinical studies have shown that CFTR activity is reduced in the airways of smokers, and this may contribute to mucus plugging, infection and inflammation in COPD. The molecular mechanism by which cigarette smoke reduces CFTR activity remains obscure. Previous work has identified lemur tyrosine kinase 2 (LMTK2) as an important regulator of CFTR. LMTK2 activity at the apical membrane of airway epithelia leads to phosphorylation of Ser^{737} on the regulatory domain of CFTR, a well-known inhibitory site that reduces CFTR function and cell surface expression (2). Therefore, we hypothesized that cigarette smoke reduces CFTR activity through activation of LMTK2.
To examine the role of LMTK2 in regulating CFTR, a bronchial epithelial cell line expressing WT-CFTR (CFBE4lo-) was transduced with lentiviral shRNA targeting the LMTK2 gene, or with an empty control vector (PLKO.1). The expression of LMTK2 and CFTR was characterized in both cell lines at the mRNA and protein levels. The shRNA knock down cell line had reduced (by 62.5%) LMTK2 expression as expected while CFTR mRNA levels were unchanged and CFTR protein was increased. The effect of cigarette smoke extract (CSE) on forskolin-stimulated short-circuit current (Isc) was compared using control and LMTK2-deficient cell lines.
Forskolin stimulation caused a robust stimulation of the Isc across control (PLKO.1) cells following 1 h exposure to 25% CSE, however the total CFTR-mediated current during stimulation was reduced when assayed as the $CFTR_{inh}172$ sensitive current, consistent with previous work by us and others that cigarette smoke inhibits CFTR function. By contrast, $CFTR_{inh}172$-sensitive current was not reduced significantly in the LMTK2 knock down cell line. The results suggest that CFTR downregulation by CSE depends on LMTK2, consistent with a mechanism involving phosphorylation of S737 on the R domain of CFTR.

1. Cantin AM, Hanrahan JW, Bilodeau G, Ellis L, Dupuis A, Liao J, Zielenski J, Durie P 2006. Cystic Fibrosis Transmembrane Conductance Regulator function is suppressed in cigarette smokers. *Am. J. Respir. Crit. Care Med.* 173:1139-44.
2. Luz S, Cihil KM, Brautigan DL, Amaral MD, Farinha CM, Swiatecka-Urban A. 2014. LMTK2-mediated phosphorylation regulates CFTR endocytosis in human airway epithelial cells. *J. Biol. Chem.* 289:15080-93.

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