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Respiratory Research Day

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ABSTRACT BOOK

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Presenter (Alphabetical)	Abstract Title	Poster # or Oral	Group
Mira Abou Rjeili	Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease (COPD) and Heart Failure (HF)	47	E
Eisha Ahmed	Computational modeling to predict role of Plexin B2 in germinal center B cell responses	Oral	
Ahmad Al-Dobyany	Persistence of Sleep-Disordered Breathing from Pregnancy to the Postpartum Period	34	D
Fatmah Alhabeeb	The Role of HuR in myofibroblast differentiation: implications for pulmonary fibrosis	3	A
Noof Aloufi	Human antigen R (HuR) controls cigarette smoke-induced inflammatory mediator production: implications for COPD	13	B
Zsombor Balassy	Reproducing the Latch-State at the Molecular Level with the Use of Regulatory Proteins	4	A
Salyan Bhattarai	Altered Phenotype of Bone-Marrow Derived Macrophages in Duchenne Muscular Dystrophy Mice	Oral	
Michael Chen	Heparin-binding EGF-like growth factor modulates the bidirectional activation of CD4+ T cells and dendritic cells independently of the epidermal growth factor receptor	11	A
Julia Chronopoulos	Understanding the immune response to IAV infection during pregnancy	5	A
Maxime Cormier	Disseminated Mycobacterium marinum and Mycobacterium genavense infections in a kidney transplant recipient – a fishy situation	35	D
Maxime Cormier	Excess Supplemental Oxygen: More than Just Hot Air - An Audit of Oxygen Therapy in a Tertiary Care Centre	Oral	
Jannik Donner	Identification of Pseudomonas aeruginosa genetic variants in chronic lung infections of cystic fibrosis patients and their effect on lung disease severity	23	C
Jeffrey Downey	Mitochondrial Cyclophilin D as a novel mediator of host tolerance to Influenza A Virus infection	Oral	
Mikaela Eng	Effect of Amino Acid Restriction on Macrophage Metabolic Programs and Function	6	A
Raquel Farias	Innovations in treating COPD exacerbations: a phone interactive tele-system to improve Action Plan adherence	40	D
Marilena Gentile	Circulating Natural Killer Cells dominate the innate lymphoid response during the invasive stage of enteric helminth infection	14	B
Lisa Hennemann	Exploring the role of intracellular Pseudomonas in the establishment of chronic CF lung infections	7	A
Joyce H. Jang	Modulation of Airway Smooth Muscle Phenotype through Physical Contact with CD4+ T-cells	8	A
Vahid Reza Javan	Feasibility of home portable monitoring (PM) for diagnosis of sleep-disordered breathing (SDB) in adolescents and adults with neuromuscular disorders (NMD)	42	E
Eva Kaufmann	Education of Stem Cells by BCG: An Innovative Approach in TB Vaccine Development	27	C
Sulaiman Khadadah	Positive predictive value of the SAMSPAP (Sleep Apnea in Multiple Sclerosis Positive Airway Pressure) trial eligibility criteria for sleep apnea diagnosis.	39	D
Nargis Khan	Mitochondrial Cyclophilin-D Regulates T Cell-Mediated Disease Tolerance to Tuberculosis	24	C
Suurya Krishnan	Chronic obstructive pulmonary disease categorization using cardiovascular risk: A way towards more personalized care	43	E
Kelly Kwong	Pseudomonas aeruginosa evasion of neutrophil phagocytosis and bacterial clearance in early cystic fibrosis lung infection	15	B
Lydia Labrie	Dual Action Mechanism of STAT6-Inhibitory peptide in Airway Hyperresponsiveness	Oral	
Jean-Philippe Leduc-Gaudet	Role of Autophagy in Sepsis-Induced Skeletal Muscle Dysfunction	16	B
Qian Li	A new model to study tissue-resident and bone marrow-derived macrophage responses to diaphragm muscle injury	17	B
John Lin	Interleukin-6 Trans Signalling in Pulmonary Exacerbations in Cystic Fibrosis	Oral	
Amar Mainra	Physiological mechanisms of exertional dyspnea relief following bariatric surgery for severe obesity	Oral	

Presenter (Alphabetical)	Abstract Title	Poster # or Oral	Group
Dorival Martins	Superoxide dismutase activity confers (p)ppGpp-mediated antibiotic tolerance to stationary phase Pseudomonas aeruginosa by modulating membrane permeability and drug uptake	25	C
Daniela Massierer	Relationship of simple measures of physical function and muscle strength to exacerbation, hospitalization and mortality in COPD: a systematic review	50	E
Alexandre Meli	Bcl6 curtails type 2 responses by limiting IL-10 production by Th2 cells	Oral	
Lynn Meng	Association Between Obstructive Sleep Apnea and Motor Symptoms in Parkinson's Disease Patients	45	E
Vanessa Moarbes	Inhibitory activity of STAT6-IP on IL-33-mediated-IL-13 production and macrophage polarization in an innate murine allergy model	19	B
Yousof Mostafavi	Investigating Fractional Exhaled Nitric Oxide (FeNO) in Chronic Obstructive Pulmonary Disease (COPD) and Asthma-COPD Overlap (ACO): A Scoping Review	38	D
Nafiseh Naderi	From clinic to in vitro: mechanism of action of azithromycin for prevention of COPD exacerbation	2 and Oral	A
Erwan Pernet	Leukotriene B4 – Type I Interferon axis regulates macrophage-mediated host tolerance to influenza infection	28	C
Laïla Samy ***	An Unusual Oscillating Expiratory Flow Volume Loop	33	D
Laïla Samy ***	Domestic and Non-Occupational Exposures in Developing Countries: A Case of Mixed Desert and Hut Lung Syndrome	36	D
Laïla Samy & Adamo Donovan	Inspiratory Pleural Pressures and Left Ventricular Structure in COPD	32	D
Wissam Shalish	Extubation readiness in extremely preterm infants: spontaneous breathing tests under the spotlight	Oral	
Wissam Shalish	Age of first extubation attempt and respiratory morbidities in extremely preterm infants	49	E
Shao Tao	The Effect of Fenretinide on Regulatory B Cell Development and Semaphorin 4C in Allergic Asthma Model	18	B
Mariaenrica Tine'	Blood eosinophilia does not worsen clinical outcomes nor reflects tissue eosinophils in COPD	37	D
Hussein Traboulsi	FGF10 haploinsufficiency is associated with smaller airways lumen: implications for COPD susceptibility	26	C
Lauren Tracey	Physiological and perceptual responses to exercise according to the locus of symptom limitation in patients with COPD	46	E
Joshua Trivlidis	Implications of HuR in Idiopathic Pulmonary Fibrosis	9	A
Diya Wang	Automatic Respiratory Gating for Contrast-enhanced ultrasound Parametric Functional Imaging using Machine Learning	48	E
Yujia Wang	Factors affecting patients' response to anti-IL5 monoclonal antibodies treatment in severe eosinophilic asthma	31	D
Alexander Willms ***	8 and 16 Weeks of Chronic Tobacco Smoke Exposure Negatively Impacts Morphological Characteristics of Motor Axons and Neuromuscular Junctions in the Diaphragm of Mice	10	A
Hualin Zhang	CD109 is a cell-extrinsic regulator of cutaneous IL-17 producing γδT cells	20	B
Jingxuan Betty Zhang ***	Lung Cancer in the Inuit Region of Nunavik, Québec: Descriptive Epidemiology of Patients Diagnosed between 2005 and 2016	44	E

Please join the RI-MUHC Technology Platforms at the following posters:

Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease (COPD) and Heart Failure (HF)

Mira Abou Rjeili[1,2], Raquel Farias[2], Yousof Mostafavi[2], Benjamin M. Smith[1,2,3], Michel White[4], Jean Bourbeau[1,2,3]
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Background and rationale: Chronic Obstructive Pulmonary Disease (COPD) and Heart failure (HF) are two highly prevalent conditions that significantly impact patients, families and the health care system. Although commonly studied independently, these diseases are often concomitant. However, because both diseases have similar clinical presentations and share the main symptom, dyspnea, the presence of comorbid HF and COPD is often overlooked. This needs to be studied if we want to promote a proactive approach in clinical practice. Therefore, a comprehensive evaluation is required in all COPD and HF patients to exclude comorbid HF and COPD, respectively. With this respect, identifying biomarkers and biosignatures of concomitant COPD and HF could be an interesting option to refer patients for a more detailed workup.

Methods: In this observational study, we will determine the prevalence of comorbid COPD and HF in stable patients from specialized pulmonary and cardiac clinical settings (Montreal Chest Institute and Montreal Heart Institute, respectively). Each patient will undergo a detailed cardiopulmonary evaluation and clinical and laboratory tests to establish the diagnosis of cardiac or pulmonary comorbidities. Additionally, levels of the inflammatory biomarkers including pro-hormone N-terminal brain natriuretic peptide (NT-proBNP), interleukin (IL)-6, IL-8, C-reactive protein (CRP), fibrinogen, and surfactant protein D (SP-D) will be quantified from serum of patients. Measures from inflammatory and clinical biomarkers will be used to build statistical models to identify biosignatures that will help guide an early diagnosis and treatment of concomitant COPD and HF. Finally, data on medication and their indications for these patients will be collected from hospital administrative databases and electronic medical records.

Expected results: We expect to have a proportion of around 20% COPD patients with undiagnosed ventricular dysfunction and of around 20% of HF patients with undiagnosed COPD. We expect patients with concomitant COPD and HF to have greater levels inflammatory biomarkers than subjects without either co-morbidity. Additionally, we expect to characterize novel biosignatures to detect COPD and HF patients at risk for cardiac or respiratory co morbidities, respectively. Finally, we expect that chronic administration of bronchodilators will be sub-optimal in HF patients with COPD and that ACEi, ARBs and β-blockers will be under prescribed in COPD patients with HF.

Conclusion: This study will help characterize relevant biosignatures of concomitant COPD and HF that can be used by clinicians for an early identification and treatment of these diseases.

Computational modeling to predict role of Plexin B2 in germinal center B cell responses

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Germinal centers (GCs) in secondary lymphoid organs are the primary sites of B cell maturation, a process regulated by antigen stimulation, co-stimulatory signals, and extracellular cytokines. Dynamic actin cytoskeleton reorganization is critical to these GC reactions, which control the organization of surface receptors, facilitate immune synapse formation, and drive cell migration. Despite their importance in mounting effective humoral immune responses, mediators of GC B cell cytoskeletal dynamics – and their mechanism of action – remain poorly defined. Plexin B2, a transmembrane protein involved in axon guidance and cell migration in other cell types, is selectively upregulated on T-dependent germinal center B cells. However, the role for Plexin B2 in GC B cell responses remain unknown. Here, we present a dynamical model of the gene network downstream of Plexin B2 to predict the molecular mechanisms by which PlexinB2 signaling can affect GC B cell development. Using a bottom-up approach, we construct a preliminary gene regulatory network downstream of Plexin B2. This is used to generate a dynamic model to simulate the kinetics of the associated gene network and study how Plexin B2 regulates GC B cell responses through cytoskeleton reorganization. Finally, we highlight how such computational models can be used in hypothesis generation for downstream wet lab experiments.

Persistence of Sleep-Disordered Breathing from Pregnancy to the Postpartum Period

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Introduction/Rationale: Sleep-disordered breathing (SDB) increases in prevalence over the course of pregnancy. Although the mechanisms are not well understood, gestational weight gain, hormonal influences and fluid shift may play a role. Importantly, maternal SDB is associated with adverse maternal and fetal outcomes, including gestational diabetes, preeclampsia, and delivery of small-for-gestational age infants. However, there is very limited data on whether SDB improves or resolves postpartum with reversal of pregnancy-related physiological changes, and to what extent persistent SDB post-partum may impact on longer-term maternal health outcomes.

Methods: Participants from 3 ongoing pregnancy studies assessing outcomes related to: gestational diabetes**,** oral appliance devices during pregnancy**,** and the risk of delivery of small-for-gestational age infants**,** were included
Pregnant women with SDB (apnea-hypopnea index; AHI >5) diagnosed in the second to third trimester using full PSG (Level 2, Chicago Criteria) were included. Included participants underwent postpartum sleep studies a minimum of 3 months after delivery. Either level 2 or level 3 (without EEG) studies were performed based on participant convenience.
All pregnancy and postpartum sleep studies (even if level 2) were re-scored as level 3 studies using AASM criteria (1A; 3% desaturations) to allow for comparisons. Autonomic hypopneas were scored as previously described by our group[4] (using an increase in oximetry-derived heart rate ≥ 6 bpm as a surrogate marker for EEG arousal).
Participants that underwent both level 2 studies in pregnancy and postpartum (n=20) were also scored using PSG AASM 1A criteria, including obstructive hypopnea events associated with microarousal

Results: 40 participants had level 2 sleep studies during pregnancy. In the postpartum period , 20 participants had level 2 studies and 20 participants had level 3 studies. If we rescored and treated all pregnancy and postpartum studies as **level 3** and included apneas and obstructive hypopneas associated with 3% desaturations only (level III, AASM 1A), 18/40 (45%) have persistent SDB in the postpartum period (AHI≥5). When we included apneas, desaturation-related obstructive hypopneas and autonomic hypopneas[4], 36/40 (90%) have persistent SDB postpartum. Among the 20 participants with level 2 studies in pregnancy and postpartum, only 1 participant had resolution of SDB postpartum (AHI<5). 12/40 (30%) had a 50% reduction in AHI. 15/40 (37.5%) had a < 50% reduction in AHI. 13/40 (32.5%) had an *increase* in AHI

Conclusions: Postpartum SDB at 3-6 months is 46-90% prevalent. The finding that SDB persists in a majority of women studied postpartum stands in contrast to previous limited data. Our findings indicate that further study is warranted to better characterize the natural history of SDB diagnosed during pregnancy with longer follow-up in the postpartum period. Finally, further evaluation of postpartum SDB in relation to its consequences for long-term maternal health, including cardiometabolic outcomes, is needed.

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

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Introduction: Pulmonary fibrosis is an under-diagnosed lung disease characterized by progressive lung scarring. Extracellular matrix (ECM) deposition is a key event in disease pathogenesis. ECM proteins are excessively deposited due to increased differentiation of fibroblasts into the α-smooth muscle actin(α-SMA)-expressing myofibroblasts by transforming growth factor (TGF)-β1. Fibrosis may be accompanied by metabolic reprogramming from aerobic respiration to aerobic glycolysis due to extensive tissue hypoxia, which further enhances fibroblast differentiation via hypoxia inducible factor (HIF-1α). We predict that TGF-β1 promotes fibrosis via human antigen R (HuR), an RNA binding protein that may in turn increase levels of both TGF-β1 and HIF-1α.

Hypothesis: HuR promotes the differentiation of myofibroblasts, which increases ECM proteins and a metabolic shift that stiffen the lungs.

Methods: To explore the role of HuR in TGF-β1-treated human lung fibroblasts (HLFs), we first examined the effect of TGF-β1(5ng/ml) on the expression of HuR and fibrogenic (α-SMA, collagen and fibronectin) and metabolic (HIF-1α, lactate dehydrogenase A (LDH-A), hexokinase) markers. Then, HLFs were transfected with HUR siRNA or Control siRNA, treated with TGF-β1 and fibrotic/metabolic markers assessed. Actinomycin D (ActD)-chase experiments were performed to examine if HuR affects the stability of fibrogenic/metabolic transcripts. Immunofluorescence (IF) was performed to assess HuR localization in HLFs treated with TGF-β1. Seahorse XF96 used to assess metabolic activity in TGF-β1-treated HLFs.

Results: Exposure of HLFs to TGF-β1 increased the total protein levels of HuR. The expression of metabolic and fibrogenic markers were also induced by TGF-β1 treatment. In addition, TGF-β caused the translocation of HuR from the nucleus to the cytoplasm- a feature consistent with HuR activation. siHuR-transfected cells showed a significant reduction in fibrogenic (α-SMA, collagen Ⅰ and fibronectin) and metabolic markers (HIF-1α, LDH-A) in response to TGF-β1 treatment compared to siControl. However, alterations in HuR levels did not affect mRNA stability of the markers. TGFβ1- treated HLF showed increase in acidification rate and oxygen consumption rate as compared to untreated cells.

Conclusions: Our preliminary data show that during fibrotic stimuli, HuR increases the protein levels of key fibrogenic and metabolic markers. Thus, HuR could be a driving factor in the pathogenesis of pulmonary fibrosis

Human antigen R (HuR) controls cigarette smoke-induced inflammatory mediator production: implications for COPD

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Background: Chronic Obstructive Pulmonary Disease (COPD) is an incurable and prevalent respiratory disorder that is characterized by two main features: (1) narrowing and remodeling of small airways and (2) the destruction of the alveoli; both of these features are thought to be the result of chronic inflammation. The inflammatory response in COPD is typified by an increase in inflammatory cells such as macrophages and neutrophils, and proteins such as interleukin-8 (IL-8) and cyclooxygenase-2 (COX-2). COPD is primarily caused by cigarette smoke (CS) and other noxious particles (*e.g*. air pollution). Cigarette smoke may cause lung inflammation via activation of human antigen R (HuR). HuR is a ubiquitously-expressed RNA-binding protein that regulates the stability of mRNA encoding proteins that are associated with inflammation; stabilizing the transcript would indirectly increase expression by facilitating protein translation. To stabilize target mRNA, HuR translocates from the nucleus (where it normally resides) to the cytoplasm. We have now found that cytoplasmic localization of HuR (indicative of activation) is higher in lung tissue from Smoker and COPD subjects compared to Normal (non-smokers) subjects. There is also an increase in cytoplasmic HuR in human lung fibroblasts (HLF) after *in vitro* exposure to 2% cigarette smoke extract (CSE). Contrary to what we expected, we found that the protein and mRNA levels of COX-2 and IL-8 were significantly higher in siHuR-transfected HLF exposed to 2% CSE for 24 hours compared to siCtrl-transfected cells. However, the contribution of HuR to the regulation of inflammatory proteins in the lung is completely unknown.

Hypothesis/Aim: Therefore, we hypothesize that HuR regulates inflammatory response during cigarette smoke exposure. Our aim is to determine the effect of HuR on inflammatory protein production in response to CSE.

Methods: To verify the mechanism by which HuR regulates the production of inflammatory markers in response to CSE (e.g. *Cox-2* and *Il-8*), we will use RNA-binding protein immunoprecipitation followed by qPCR technique (RIP-qPCR). Next, the effect of HuR activation by CSE on Cox-2 and IL-8 expression will be assessed using an HuR inhibitor (CMLD2), which prevents binding of HuR to mRNAs targets.

Preliminary Results: Interestingly, using the RIP-qPCR technique, we found that *Cox-2* and *Il-8* mRNA were enriched in IP-HuR treated cells with 2% CSE for 24h, which means that HuR binds to *Cox-2* and *Il-8* mRNA.

Significance: COPD is a major health problem worldwide with limited therapeutic options and more research is needed to understand the effect of smoking on COPD and its pathogenesis. Therefore, this study is designed to investigate the role of HuR in regulating cigarette smoke-induced inflammation which could provide the basis for the development of novel target therapy for COPD.

Reproducing the Latch-State at the Molecular Level with the Use of Regulatory Proteins

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Smooth muscle (SM) has a unique property, called the latch-state, during which force is maintained for long periods of time at low energy consumption and low myosin activation (phosphorylation) levels. This property has been observed at the whole muscle level. To explain the latch-state, theories were extrapolated to molecular mechanisms, but these were never verified. One such theory states that, during the cross-bridge cycle, if SM myosin gets dephosphorylated while attached to actin, it will remain attached, in a load-bearing mode. Other theories involve the regulatory proteins (e.g. caldesmon (CaD)) for force maintenance to occur. To reproduce the latch-state at the molecular level, we used the in vitro motility assay to measure the velocity (V) of fluorescently labeled actin filaments when propelled by myosin molecules on a coverslip. We also introduced a cutoff velocity below which filaments were considered not moving, allowing us to define a motile fraction (f_{mot}) as the percentage of filaments moving at any one time. To the motility assay, we added a microfluidic chamber in order to inject myosin light chain phosphatase (MLCP) to efficiently dephosphorylate myosin without introducing convective effects. A mixture of SM and skeletal (SK) muscle myosin was used, the latter not being regulated by phosphorylation. The rationale behind this protocol was that if the latch-state occurs, we should observe a transient decrease in either actin filament V or f_{mot}, due to the load induced by the attached, dephosphorylated SM myosin. V or f_{mot} would eventually increase to the level of skeletal muscle myosin after the detachment of the latch bridges. MLCP injection to [SM]=25 µg/ml led to an arrested motility in 100 s. MLCP injection to [SK]=80 µg/ml or to a mixture of [SM]=25 µg/ml/[SK]=80 µg/ml, led to an increase in V but a constant f_{mot}. Conversely, adding CaD to SK alone or to SM + SK, resulted in a motile fraction≈0 from 400 to 800 s, followed by a recovery. We did not observe any suggestion of load-bearing when MLCP was added to actin and myosin only. However, in the presence of Cad, we measured a transient decrease in motile fraction, supporting a load-bearing phase. These results suggest that CaD is an important player for load-bearing. Furthermore, these results suggest that load-bearing is not a property of the type of myosin but can be reproduced given the appropriate concentrations of Cad and MLCP. Funded by NSERC.

Altered Phenotype of Bone-Marrow Derived Macrophages in Duchenne Muscular Dystrophy Mice

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Rationale: Duchenne muscular dystrophy (DMD) is a genetic disease characterized by progressive degeneration of skeletal muscles, including the diaphragm. Monocytes originate from the bone marrow and enter the damaged muscle where they differentiate into macrophages, the major immune cell population that contributes to muscle damage in DMD. Monocytes/macrophages are found to be pro-inflammatory and abnormally persistent in the dystrophic muscle, possibly due in part to cross-talk with damage-associated molecular patterns (DAMPs) released from necrotic muscle fibers. We hypothesized that DAMP release could potentially alter the phenotype of macrophages at the level of hematopoietic precursors in the bone marrow prior to their entry into the dystrophic muscle.

Methods: Cultured bone-marrow derived macrophages (BMDM) from 6-10 weeks old *mdx* mice, a genetically homologous model of DMD, and age-matched wild-type (WT) mice, were studied. Basal expression of genes related to macrophage phenotype (pro- versus anti-inflammatory) was determined by quantitative PCR (qPCR). Macrophage polarization responses were also assessed under the influence of IFNγ ("M1" stimulus) or IL4 ("M2" stimulus). Mitochondrial bioenergetics of *mdx* and WT BMDM were analyzed by Seahorse assay. To determine whether DAMPs could be involved in altering the phenotype of hematopoietic precursors in the bone marrow, we used BMDM from age-matched mdx mice lacking the Toll-like receptor 4 (TLR4) gene (mTLR4 mice). Moreover, to assess the functional BMDM phenotype *in vivo*, we adoptively transferred the bone marrow from *mdx* mice (CD45.2) to congenic WT (CD45.1) mice subjected to acute skeletal muscle injury. We then quantified the adoptively cells within the injured muscles and characterized their expression of pro-inflammatory and anti-inflammatory markers by flow cytometry.

Results: The *mdx* BMDM expressed higher basal levels of the pro-inflammatory markers *iNOS*, *IL6*, *IL12a*, as well as the anti-inflammatory markers *CD206*, *YM1* and *Arg1*, as compared to age-matched WT mice. Whereas polarization responses to IFNγ were comparable, IL4 induced higher levels of *iNOS*, *IL12a*, *TGFβ*, *CD206*, *YM1* and *Arg1* in *mdx* as compared to WT BMDM. Furthermore, Seahorse assays revealed that *mdx* BMDM are less oxidative, as indicated by reduced spare respiratory capacity (SRC). This was accompanied by increased basal expression in *mdx* BMDM of *PKM2* and *PDK1*, genes that regulate glucose metabolism. In contrast, BMDM from mTLR4 mice showed a relative downregulation of all pro- and anti-inflammatory markers. Furthermore, the SRC level was also restored toward WT levels in mTLR4 BMDM. In vivo, we observed that although the percentage of macrophages in adoptively transferred WT and *mdx* bone-marrow cells was comparable, the proportion of recruited exogenous pro-inflammatory macrophages ($iNOS^+CD206^-TGF\beta^-$ and $IL\text{-}1\beta^+CD206^-TGF\beta^-$) in injured muscles was significantly higher in the CD45.1 mice injected with *mdx* bone marrow.

Conclusion: Taken together, these data suggest that the onset of muscle necrosis in DMD releases signals that educate monocyte precursors in the bone marrow prior to their entry into the damaged muscles. These responses are likely mediated in part through DAMPs released from damaged muscle, which may act on monocyte precursors via the TLR4 pathway.

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Heparin-binding EGF-like growth factor modulates the bidirectional activation of CD4+ T cells and dendritic cells independently of the epidermal growth factor receptor

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Background: Allergic asthma results from the improper activation of adaptive immunity. Some of its pathological features have been linked to the epidermal growth factor receptor (EGFR) and its ligands, such as heparin-binding EGF-like growth factor (HB-EGF). Although some EGFR ligands and EGFR have been implicated in immune function, the effect of HB-EGF on the adaptive arm remains unclear. We sought to examine the role of HB-EGF in the function of dendritic cells (DCs) and $CD4^+$ T cells. We hypothesized that HB-EGF promotes the activation of DCs and $CD4^+$ T cells.

Methods: Splenic $CD4^+$ T cells were isolated and purified from ovalbumin (OVA) specific TCR transgenic DO11.10 mice. Mouse bone marrow-derived DCs (BMDCs) were loaded with OVA and co-cultured directly with $CD4^+$ T cells or separated by Transwell inserts. Cells were treated with either HB-EGF neutralizing antibody or afatinib, a covalent EGFR inhibitor, for 24 h. BMDCs were also treated in isolation with recombinant HB-EGF (rHB-EGF) for 24 h. Gene expression was assessed at the mRNA and protein levels by qPCR and flow cytometry. Membrane-bound HB-EGF localization was assessed following BMDC-$CD4^+$ T cell co-culture for 12 h via confocal microscopy.

Results: Both BMDCs and $CD4^+$ T cells express HB-EGF. HB-EGF neutralization but not EGFR inhibition in co-culture reduced cell-surface expression of activation markers CD69 in $CD4^+$ T cells and CD86 and MHC class II in BMDCs. Expression of activation markers was unchanged in co-cultured $CD4^+$ T cells and BMDCs separated by Transwell inserts following treatment with HB-EGF neutralizing antibody. rHB-EGF did not alter BMDC expression of activation markers CD25, CD86, MHC class II, and chemokine (C-C motif) receptor 7; $CD4^+$ T cell polarizing cytokines interleukin (IL) 4, IL-6, IL-12 subunit beta, and interferon-γ; and transcription factors T-bet and GATA3. Membrane-bound HB-EGF co-localized with CD3 and F-actin at DC-$CD4^+$ T cell contact sites.

Conclusions: HB-EGF promotes the activation of $CD4^+$ T cells and BMDCs in a contact-dependent fashion independently of EGFR. Soluble HB-EGF alone is insufficient to activate DCs. HB-EGF is localized at the immunological synapse between DCs and $CD4^+$ T cells. These results suggest that HB-EGF plays a role in the early activation of dendritic cells and $CD4^+$ T cells through interactions at the cell surface.

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Understanding the immune response to IAV infection during pregnancy

Julia Chronopoulos[1,4], James G. Martin[1,4], Maziar Divangahi[2,4], Erwan Pernet[3,4]

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Despite the worldwide application of vaccination and other anti-viral interventions, influenza A virus (IAV) infections remain a persistent threat to human health. In particular, pregnant women have increased susceptibility to IAV infection, which often results in immunopathology, clinically significant worsening of airway function and high mortality rates, as it has been reported during the recent 2009 H1N1 outbreak. This suggests that the particular pulmonary environment of pregnant women is more permissive to IAV to reach the lower airways. Indeed, the success of many highly pathogenic respiratory viruses, such as IAV, is directly linked to their ability to escape innate immunity and to cause lower respiratory tract infections. In this case, viruses infect epithelial cells and activate alveolar macrophages often leading to a "cytokine storm" characterized by massive inflammatory cellular responses leading to pulmonary edema, hypoxia, and respiratory failure. Thus, it can be argued that the virus is not the only threat; but rather, the host's own immune-inflammatory response is jeopardizing survival. The mechanism(s) of how the immune system becomes dysregulated to the point of causing such massive immunopathology during pregnancy is not well understood, but it has been postulated that this might be the consequence of the hormonal changes. Production of estrogen and progesterone has been associated with profound remodeling of the immune system during pregnancy. Their production increases over the course of gestation to peak at the third semester of pregnancy, where pregnant women are more susceptible to IAV infection. Two distinct strategies are involved in the immune response to IAV infection: the host resistance mechanisms to clear the virus and the disease tolerance response to reduce the damages induced by the inflammatory response. Interestingly, pulmonary viral loads are similar between pregnant and non-pregnant mice following IAV infection, suggesting intact host resistance mechanisms. However, IAV-infected pregnant mice have increased production of pro-inflammatory cytokines as well as increased innate cell recruitment into the lungs. These cells, especially inflammatory monocytes, have been previously linked to IAV-induced immunopathology. Therefore, a break in host tolerance rather than host resistance could explain the increased susceptibility of pregnant women to IAV infection. However, the mechanisms leading to such a dysregulated pro-inflammatory response are still unclear. We speculate that the sex hormones will enhance production of inflammatory cytokines and recruitment of inflammatory cells leading to an increase in immunopathology and morbidity in pregnant mice.

Disseminated Mycobacterium marinum and Mycobacterium genavense infections in a kidney transplant recipient – a fishy situation

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Introduction:

M. marinum is a non-tuberculous mycobacteria (NTM) that usually causes a self-resolving skin infection in humans. Disseminated M. marinum infections in humans are exceedingly rare.1 M. genavense is a NTM which classically infects individuals with AIDS. Infection in non-HIV patients is exceedingly rare. 3 This case report describes the first reported case of concurrent disseminated M. marinum and M. genavense infections in a solid-organ transplant recipient, with post-mortem pathology confirmation.

Case Presentation:

A 76 year-old male kidney transplant recipient presented to his dermatologist with cutaneous ulcerated lesions on his forearm which were biopsied. He had recently cleaned a fish tank that had contained dead fish. Two weeks after his biopsy, he presented to our ER with altered mental status and weight loss. A diagnosis of acute kidney injury from his failing kidney transplant was made, and hemodialysis was promptly initiated. His mental status did not improve despite several hemodialysis sessions. A CT of the chest revealed new bilateral nodular densities. The tissue culture from his skin biopsy revealed NTM. He was diagnosed with disseminated NTM infection and he was empirically started on rifampin, IV tigecyclin, IV imipenem-cilastatin, and azithromycin. Despite treatment, his mental status deteriorated, leading to tracheal intubation for airway protection. The patient had multiple episodes of seizures. A brain MRI revealed multiple foci of periventricular and subcortical white matter T2/FLAIR high intensity lesions. Eventually, cultures from the skin biopsies (in upper and lower extremities) and blood cultures came positive for Mycobacterium marinum – confirming the diagnosis of M. marinum disseminated infection. His antibiotics were changed to rifampin and minocycline and his condition improved sufficiently to allow extubation and transfer to the medical ward. The patient then had multiple seizures followed by distributive shock of unknown etiology. The patient eventually passed away. Three months after his death, cultures from bronchoalveolar lavage revealed M. genavense. An autopsy revealed diffuse histiocytic infiltration with acid-fast coccobacilli of lung parenchyma, liver, spleen, transplanted kidney, and mesenteric lymph nodes – confirming the diagnosis of disseminated M. genavense infection.

Discussion/Conclusion

M. marinum is a waterborne NTM that generally causes self-limited granulomatous skin infections in humans – very rarely does it cause disseminated infections. In our patient, M. marinum infection was likely acquired after cleaning a fish tank that had contained dead fish. M. genavense is an ubiquitous NTM that classically infects individuals with HIV/AIDS, although some cases of disseminated infections in SOT recipients have been reported.3 Ours is the first reported case of concomitant M. marinum and M. genavense disseminated infections. Although M. marinum was isolated in blood cultures and skin cultures from 2 different sites in our patient, it was not found in the autopsy skin ulcer specimens, suggesting that the disseminated M. marinum infection had been adequately treated with rifampin and minocycline therapy at the time of death. The long delay (3 months after sample collection) in identifying M. genavense through liquid media culture – and eventually through 16s rRNA PCR – likely contributed to our patient's poor outcomes, as therapy was not adjusted to cover this organism. Our case supports the pressing need to render readily available rapid genetic amplification and sequencing analysis testing to better identify NTM infections in immunocompromised individuals and adjust therapy accordingly

Excess Supplemental Oxygen: More than Just Hot Air - An Audit of Oxygen Therapy in a Tertiary Care Centre

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BACKGROUND : Oxygen is one of the most commonly used drugs in hospital setting, often regardless if the patient is hypoxic or not. Recent evidence shows that unnecessary supplemental oxygen can be harmful in common clinical conditions.

OBJECTIVE: To describe how oxygen therapy is prescribed and monitored for patients in acute medical wards of a teaching hospital in Canada.

DESIGN, PARTICIPANTS AND SETTING: Retrospective, point prevalence study by chart review of adult inpatients admitted on acute care internal medicine, cardiology, respiratory medicine wards, and emergency department at the McGill University Health Centre (MUHC).

RESULTS: 24.0% of inpatients were receiving oxygen therapy for which only half had a valid prescription for oxygen. Out of all patients receiving oxygen therapy, 43.1% were deemed to receive inappropriate or potentially inappropriate therapy. The highest proportion of inpatients on oxygen deemed at risk of iatrogenic hypercapnia was found on the respiratory medicine ward (20.4%) and CCU (21.4%).

CONCLUSIONS: Oxygen therapy is a common and costly treatment administered to acute medical ward and emergency department inpatients. A high proportion were given oxygen without a valid prescription, and many were at risk of potential harms from oxygen therapy. Interventions addressing prescription and monitoring of supplemental oxygen may positively impact quality of care and healthcare expenditures.

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

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Nearly 60% of adult CF patients are chronically infected by *Pseudomonas aeruginosa* (*Pae*) in their lifetime. Lung disease upon chronic infection is mainly caused by direct virulence of the infecting bacterial pathogens, and the presence of an excessive and injurious inflammatory response. Over time, progressive lung damage impairs its function to the point where lung transplantation becomes necessary. Interestingly, the rate of progression in CF lung disease is highly heterogeneous between patients. Even within the same patient, individual lungs can show significant regional variation in tissue damage and disease severity, which cannot be attributed to host genetics or environmental factors alone. Recent work indicates that regional disease heterogeneity could be due to the microevolution of *Pae* in the CF lung, as region-specific clonal *Pae* populations can differ markedly in phenotype, virulence, antibiotic resistance and immunogenicity.
Until now, the genetic variation of *Pae* in chronic lung infections has mostly been studied by whole-genome sequencing of single *Pae* isolates isolated from sputum samples. However, such approaches are limited to the analysis of only few *Pae* clones per patient and most likely overlook the extensive genetic heterogeneity present. Moreover, single genetic variants identified in *Pae* CF sputum cannot be directly linked to lung disease severity at the histopathological level because it is unknown where in the lung the *Pae* clones originate from.

We hypothesize that highly virulent *Pae* variants are present in severely damaged tissues, but not in mildly diseased ones, and that these cause more severe lung pathology due to increased cytotoxicity or inflammation.
In order to study the genetic diversity of *Pae* in chronic CF lung infection and to assess the effect of *Pae* variant populations on lung damage severity, we have collected tissue and mucus samples from whole lung explants obtained from CF patients with end-stage lung disease undergoing lung transplantation. The regional severity of lung disease was assessed based on clinical radiographic chest imaging and gross anatomical appearance of the lung explants. We performed tissue sampling in regions containing areas of mild, moderate or severe disease. To identify genetic variants in regional *Pae* populations, we designed two Ion AmpliSeq probe panels for massively parallel PCR amplification of 209 *Pae* target genes known to evolve during chronic CF lung infection or to be involved in the pathogenesis of *Pae*. To date, we have validated the IonAmpliSeq panels by analyzing total genomic DNA extracted from *Pae*-infected CF sputum and of clinical *Pae* isolates. Moreover, spike-in samples containing different absolute quantities of *Pae* genomic DNA (equivalent to 0.2 or 2% *Pae* DNA to human DNA) and at different proportion of two *Pae* strains (PAO1, PACS2) were analyzed with the Ion AmpliSeq technology. Known gene variants of the PACS2 strain (absent in PAO1) were accurately detected the expected frequency in the spike-in samples.

We are currently analyzing lung tissues and mucus DNA samples collected from CF lung explants to identify *Pae* genetic variants associated with regions of severe disease. The most promising candidate variants will be validated in cell culture-based pathogenicity assays. The Ion AmpliSeq technique provides a novel, quick and cost-efficient approach to analyze genetic variants potentially associated with *Pae* pathogenicity and survival in the CF lung. The identification of *Pae* gene products contributing to host tissue damage will be a first step on the way to develop novel treatment strategies for chronic *Pae* infections in CF and to increase the patients' well-being.

Mitochondrial Cyclophilin D as a novel mediator of host tolerance to Influenza A Virus infection

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Objectives: Influenza A Virus (IAV) remains a significant threat to global health annually. Despite the majority of IAV-induced mortality being caused by a dysregulated inflammatory response, understanding of the factors that mediate host tolerance to IAV is minimal. Interestingly, the mitochondrial protein Cyclophilin D (CypD) modulates both necrosis and inflammatory cytokine production in the lung. Given this link between CypD and pulmonary inflammation, we hypothesized that *CypD*$^{-/-}$ mice are more susceptible to IAV infection, due to aberrant inflammatory responses and enhanced tissue damage.

Methods: Wild Type and *CypD*$^{-/-}$ mice were infected intranasally with varying doses of IAV with survival and weight-loss assessed. Inflammatory responses were characterized via flow cytometry and cytokines by ELISA. Pulmonary epithelial damage was quantified by erythrocyte-influx into the BAL and fluorescent dye leakage from the lung. Recombinant cytokines were delivered intranasally at 5ng/mg of mouse.

Results: We showed that *CypD*$^{-/-}$ mice were more susceptible, despite comparable antiviral cytokine responses and viral clearance, with the susceptibility associated with a marked increase in pulmonary epithelial damage. This damage was due to a lack of IL-22 in CypDdeficient mice, caused by altered NK cell responses. Importantly, administration of IL-22 at 5 days post-infection fully protected the epithelium and abrogated the susceptibility of *CypD*$^{-/-}$ mice to IAV infection.

Conclusions: IAV has devastating consequences with few therapeutic options. Thus, a better understanding of the balance between immunity and immunopathology is required. Herein, we identify CypD as an unexpected mediator of immunity to IAV infection by regulating the NK cell/IL-22 axis to preserve pulmonary integrity and function. Given the lack of effective anti-IAV therapies, targeting this axis may offer a novel immunotherapeutic approach to IAV infection.

Effect of Amino Acid Restriction on Macrophage Metabolic Programs and Function

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Acute lung injury (ALI) is a devastating complication of critical illness. Severe inflammation leads to the breakdown of lung tissue, which can impair the transfer of oxygen and carbon dioxide. Previous work from Dr. Kristof's lab revealed how eukaryotic cells can sense the availability of nutrition as a signal that promotes cell survival or death. We are now particularly interested in how the 'macrophage', an immune cell that resides in the lung to control inflammation and infection, limits the appearance or severity of ALI. Our initial studies indicate that essential amino acids activate unique 'signaling' systems that likely control the function macrophages in the lung. Here, we explore how essential amino acids change the levels of macrophage genes that are involved in inflammation and metabolism. We hypothesize that reduced extracellular availability of essential amino acids, especially arginine, attenuates transcriptional programs in macrophages that are required for tissue repair in ALI. The induction of IL-6 mRNA and protein by LPS and IFN-γ were significantly impaired when cells were exposed to the mTORC1 inhibitor rapamycin, or to media lacking arginine, leucine, and lysine. Amino acid availability also altered selected (*e.g.*, PGC1β, UCP2), but all (*e.g.*, IDH3a) genes involved in mitochondrial function. Interestingly, metabolic profiling indicated that amino acid restriction reduced oxygen consumption rates in macrophages, but only in cells exposed to LPS and IFN-γ. Since critically-ill patients often lack proteins in their diet, a precise understanding of how amino acids change macrophage function might lead to effective nutritional or pharmacological therapies that improve the survival and recovery of patients with ALI.

Innovations in treating COPD exacerbations: a phone interactive tele-system to improve Action Plan adherence

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Rationale: COPD exacerbations are the first cause of preventable hospital admissions in Canada. Prompt treatment of exacerbations with the use of written Action Plans and case management improves recovery and prevents hospitalizations. Communication technologies could be used to 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 and reduces healthcare use in a real life practice of a COPD clinic.

Methods: Initially, forty patients from the COPD clinic at the Montreal Chest Institute were enrolled in the study. Patients received automated phone calls and could initiate contact with the tele-system at any time. The tele-system issued alarms during exacerbations, after which 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. The tele-system use was then extended to cover the caseload of 290 COPD patients. Healthcare utilization before and after tele-system enrollment was assessed through hospital administrative databases.

Results: Thirty three patients (12 M/21F; 69±6.9 years) completed the initial study. A total of 81 exacerbations were reported. Action Plan adherence was observed for 72% of the patients. Patients that adhered to their written Action Plan had significantly reduced their exacerbation recovery time. At the end of the initial study, patients significantly increased their self-efficacy to manage COPD exacerbations. The large-scale implementation of the tele-system was followed by a significant decrease in COPD-related hospitalizations (143 before enrollment vs. 108 after enrollment, p=0.001) and in the number of days spent at the hospital for a COPD diagnosis (1409 before enrollment vs. 1338 after enrollment, p=0.046).

Conclusions: Patients enrolled in our initial study showed increased Action Plan adherence compared to what has been previously reported in the literature. Patients also improved their self-efficacy to manage COPD exacerbations. Large-scale implementation of the tele-system resulted in a significant reduction of COPD-related hospitalizations.

Circulating Natural Killer Cells dominate the innate lymphoid response during the invasive stage of enteric helminth infection

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Intestinal helminths infect over two billion people worldwide and can cause significant morbidity. In many cases, however, individuals fail to develop resistance to these tissue-invasive pathogens indicating that mammalian hosts have evolved unknown strategies to tolerate infection. To investigate the mechanisms underlying host tolerance to helminth infection, we examined the initial tissue invasion stage of *Heligmosomoides polygyrus bakeri* (*Hpb*), a natural parasitic roundworm infection of mice. Unexpectedly, we observed a rapid and robust type 1 inflammatory response characterized by induction of IFNγ, IL-1β, TNFα and Nos2 expression that preceded the expected type 2 immune response. This early type 1 response was associated with a gut-specific expansion of IFNγ-producing Eomesodermin^{+} Natural Killer (NK) cells with no increase in other innate lymphoid cell populations. Parabiosis and confocal microscopy studies indicated that Eomes+ NK cells are recruited from circulation and surround the larvae and associated intestinal vasculature. Although depleting NK cells had no impact on worm burden or parasite fitness, it did increase the incidence of intestinal bleeding, suggesting a role for NK cells in promoting intestinal vascular integrity. Studies are now underway using advanced tissue-clearing techniques to construct three-dimensional images of helminth-infected tissue to examine how NK cells influence the cellular milieu and tissue damage response upon parasite invasion. In summary, our work provides new insight into the cellular dynamics required for protection during intestinal helminth infection and will help inform strategies to maximize host fitness in the context of tissue injury and repair.

Exploring the role of intracellular Pseudomonas in the establishment of chronic CF lung infections

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In order to prevent chronic *P. aeruginosa* (PA) infections, early antibiotic eradication therapy is the standard of care for initial PA infections. While these treatment protocols successfully eradicate PA in most patients, 10-30% of patients have sputum that remain positive for PA and eventually develop chronic infections.

Preliminary studies from our group suggest that intracellular PA can be found in airway epithelial cells (AECs) in CF lung explant tissues, indicating that an intracellular reservoir of PA may exist *in vivo*. We hypothesize that intracellular PA persisting in AECs may be a potential reservoir for PA, contributing to the development of chronic CF lung infections.
Our first aim is to investigate the PA bacterial factors involved in internalization and intracellular persistence within AEC. In order to examine this, we infected AECs with various PA strains at MOI 1 for 4h followed by tobramycin treatment to kill extracellular bacteria. AECs are then lysed to quantify intracellular bacteria at different time points. PA internalization is measured at 4h after infection, and intracellular persistence is measured at 24h and 120h after infection. Using this assay, we plan to screen a collection of genetically engineered PA mutants such as mucoidy or loss of the type III secretion system (T3SS), which have been associated with chronic PA infections in CF.

Preliminary results suggest that *lasR* mutants are more readily internalized than their respective wildtype parental strains. Furthermore, mutations in the T3SS genes *popB* and *pscN* increase intracellular survival. Together, these results suggest that mutations associated with chronic CF infection may promote an intracellular PA lifestyle. Additional mutants will be tested to gain a more complete understanding of the complex PA-host cell interactions.

Our second aim is to examine whether PA clinical isolates that persisted in young CF patients undergoing eradication therapy at the time of initial PA identification show greater internalization and intracellular survival in AECs compared to PA isolates from patients that successfully eradicated their initial PA infection. We will assess this by infecting AECs with a collection of PA clinical isolates collected prior to inhaled tobramycin therapy as part of an eradication study of early PA infections in young CF patients. Early PA infection was defined as first-time PA infections or re-emergence of PA after being culture-negative for at least 12 months. After receiving inhaled tobramycin over 28 days, the PA infection was considered persistent if the repeat sputum culture remained PA positive, and eradicated if the culture was negative.

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

Modulation of Airway Smooth Muscle Phenotype through Physical Contact with CD4+ T-cells

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Background: Remodeling of airway smooth muscle (ASM) is a plausible driver of airway hyperresponsiveness in obstructive airway diseases, such as asthma. Hyperproliferative and/or hypercontractile alterations in the ASM phenotype seem to be features of the remodeling but it is unclear why these alterations take place. CD4+ T-cells are potential mediators of ASM phenotype alterations due to their known interactions with ASM. Th2 cytokines, such as IL-4 and 13, are known to induce airway hyperresponsiveness in mouse models. CD4+ T-cells are also known to physically interact with ASM cells through integrins, MHC-TCR interactions, and membrane nanotubes. In this study, we aim to study the influence that paracrine interactions and physical contact with CD4+ T-cells have on ASM phenotype.

Methods: Human ASM cells were isolated from airway tissues from donors with no history of airway diseases. Human peripheral blood mononuclear cells (PBMCs) were isolated from the blood collected from healthy volunteers after informed consent was given. Jurkat cells, a T lymphocyte cell line, were bought from ATCC. PBMCs and Jurkat cells were activated with PMA and ionomycin for 48 hours and then purified for CD4+ T-cells by magnetic activated cell sorting (MACS) before co-culture. ASM cells were starved in 0.1% FBS in DMEM for 24 hours prior to co-culture. Cells were seeded at a 5:1 ratio (CD4+ T-cells/Jurkat:ASM cells) and cultured for 24 or 48h before harvest. A Transwell™ system was also used to separate the two cell types and study the significance of physical contact in T-cell-ASM interactions. To assess proliferation, the thymidine analogue, bromodeoxyuridine (BrdU), was added 18hours before harvest. Cells were stained using viability dye eflour780, anti-BrdU and anti-CD4 (for T-cell negative selection) antibodies. Incorporation of BrdU was assessed in ASM cells by flow cytometry. mRNA was harvested 24 and 48hours after co-culture and expression of ASM specific contractile genes were measured by qPCR. Cells were loaded with Fura-2 AM to assess calcium responses to histamine by fluorescent imaging.

Results: ASM cells incorporated more BrdU after co-culture with CD4+ T-cells. Transcription of ASM specific contractile genes trended downward after 48hours of co -culture with CD4+ T-cells or Jurkat cells. Peak calcium responses to histamine in ASM cells were also reduced after co-culture with Jurkat cells. These results were not observed in the Transwell™ system.

Conclusions: These results suggest that CD4+ T-cells induce a proliferative phenotype in ASM cells that is dependent on physical contact between the two cell types.No results for Jurkat cells?

Feasibility of home portable monitoring (PM) for diagnosis of sleep-disordered breathing (SDB) in adolescents and adults with neuromuscular disorders (NMD)

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Introduction: Respiratory muscle weakness causing ventilatory impairment is the leading cause of morbidity and mortality in patients with neuromuscular disorders (NMD). Sleep-disordered breathing (SDB) is a first manifestation and early recognition may improve quality of life. A major barrier to diagnosis is the high burden for patients and their caregivers involved in performing sleep study testing in the hospital, which is currently the gold standard for diagnosis. Unattended portable monitoring is performed in the general population, with reported failure rates ranging from 3% to 33%. In this pilot study, our objective is to evaluate the feasibility of the Alice PDx portable monitor (PM) for home diagnosis of SDB in patients with NMD.

Methods: The Study design was cross sectional. Two groups were targeted: adults and adolescents with NMD. In both groups, we recruited patients at risk for hypoventilation or obstructive sleep apnea (OSA): adults with forced vital capacity (FVC) < 50% of predicted or a positive STOP questionnaire, adolescents (age 13-17 years) with FVC < 70% or a positive I'M SLEEPY questionnaire. A research assistant demonstrated the use of PM for the patient and was available by telephone for help if needed. Feasibility was defined based on the adequacy of recordings. A recording was deemed to have failed if no signals were recorded at all or if interpretable signals for at least 4 hours were not available. A recording was deemed suboptimal if signal quality was poor or intermittent, but there were interpretable data. Failed studies were repeated if possible. A failure rate > 25% was defined as poor feasibility.

Results: To date, 28 participants (26 adults, 2 adolescents) completed the study, 17 (60.7%) male, with a mean age for adults of 45 ±17.5 y and for adolescents 15.0 ±1.0 y. The mean FVC% for all participants was 73.5±24.3, the mean capillary PCO_2 was 39.5±5.3; STOP or I'M SLEEPY questionnaires were positive in all candidates except for 3 adults (FVC% 25%, 35%, 46% respectively). Failed recording occurred in 7 cases, including 1 that was thought to result from a device programming error. Three studies were repeated, 2 successfully and 1 failed again due to inadequate pulse oximetry recording. Suboptimal data occurred in 5 additional studies. Hence, the failure rate was 25% on the first study, and 18% if repeated studies were considered. In addition, suboptimal recordings occurred in 5 individuals (18%). Subjects with failed studies did not differ in terms of age, sex, FVC%, PCO2, positive questionnaire, wheelchair use or having assistance at home, from those with a successful study.

Conclusion: Portable sleep monitors may represent a feasible method for assessing sleep-disordered breathing in selected patients with NMD. However, the rate of recording failure and suboptimal data may be higher than in the general population. The results of this study show promise for improved access to diagnosis of SDB in patients otherwise unable to get tested due to remote location or lack of adapted sleep testing facilities.

Education of stem cells by BCG: an innovative approach in TB vaccine development

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Objectives
To date, BCG is still the only available vaccine against TB, but prevents only the disseminated forms of the disease in early childhood. The efficacy of BCG against pulmonary TB in adults ranges from 0-80%. Although control of TB requires T cells to prevent disease progression, clinical trials that tested T cell-targeting vaccines have failed to demonstrate protection against infection with *Mycobacterium tuberculosis* (*Mtb*). We therefore hypothesize that a protective mechanism afforded by BCG in adults is mainly dependent on monocytes/macrophages which are one of the first immune cells to encounter *Mtb* upon infection. However, considering the nature of monocyte/macrophage differentiation and their relatively short lifespan, we hypothesize that BCG must access the bone marrow (BM) for educating their progenitor cells, the hematopoietic stem cells (HSCs) to subsequently generate protective monocytes/macrophages.

Methods
We use a mouse model of TB to investigate the impact of BCG vaccination on HSCs and monocytes/macrophages at both transcriptomic and epigenomic levels. Subsequently, by using chimeric mice, parabiosis, and an adoptive transfer model, we explore the protective capacities of monocytes/macrophages deriving from the educated HSCs during pulmonary *Mtb* infection.

Results
We demonstrated that BCG accesses the BM following intravenous but not subcutaneous vaccination. The presence of BCG in the BM significantly increased the numbers of lineage$^-$c-Kit$^+$Sca1$^+$ (LKS$^+$) HSCs as well as multi-potent progenitors, and led to the generation of epigenetically-modified monocytes/macrophages. By using parabiosis and chimeric mice as well as adoptive transfer approaches, we demonstrate that these educated monocytes/macrophages deriving from the BCG-reprogrammed HSCs provide sustainable protection against *Mtb* infection *in vivo*.

Conclusions
Our findings demonstrate that access of BCG to the BM is critical for generating a unique set of educated monocytes/macrophages that are protective against virulent *Mtb* infection. Targeting the HSC compartment thus provides an innovative approach in vaccine development.

Positive predictive value of the SAMSPAP (Sleep Apnea in Multiple Sclerosis Positive Airway Pressure) trial eligibility criteria for sleep apnea diagnosis.

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Introduction:
A relationship between severe fatigue and obstructive sleep apnea (OSA) in multiple sclerosis (MS) patients has been previously described by our group (Mult. Scler. J. Vol 18; 1159 – 1169). We are currently conducting a randomized, controlled trial (SAMSPAP, NCT01746342) evaluating the effects of PAP treatment of OSA on fatigue and other clinical symptoms in MS patients. The aim of this present analysis was to assess the positive predictive value of our clinical eligibility criteria for this trial to identify OSA in MS patients.

Methods:
Confirmed MS patients on stable immunomodulating medication presenting to our institution's MS clinics were recruited. The clinical eligibility criteria are: severe fatigue (Fatigue Severity Scale (FSS) score ≥ 4), poor subjective sleep quality (Pittsburgh Sleep Quality Index (PSQI) >5), no more than minimal cognitive impairment (Montreal Cognitive Assessment (MoCA) ≥26) and Expanded Disability Status Scale score (EDSS) ≤7. An Apnea-Hypopnea Index (AHI) of ≥ 15 events/h on complete overnight polysomnography (PSG) scored using AASM research (Chicago) criteria defined OSA.

Results:
In the initial screening visit 93 subjects (34% male) were evaluated. 79 (85%) of these subjects met the clinical eligibility criteria and underwent PSG. Reasons for exclusion after screening included (n); withdrawal before completing screening (4); low FSS (5); low MoCA (5); and low PSQI (1). Subjects undergoing PSG were of mean(±SD) age=49±9 y, BMI=29±6 kg/m^2, EDSS=3±2, MoCA=28±1, FSS=6±1, Epworth Sleepiness score (ESS)=9±5 and PSQI=11±4. On PSG they had a total sleep time=5.5±1.1h, AHI=30±22/h, 4% Oxygen Desaturation Index (ODI) =6±10/h and Central apnea index = 1±2/h. 58 of 79 subjects met criteria for OSA (mean AHI = 37±21/h, ODI 6±9, respiratory arousal index =34±20/h). Nine of these 58 subjects surpassed our pre-specified OSA safety threshold for randomization to this 6-month, sham PAP-controlled trial (AHI > 30 with either 4% ODI>15/h (n=8) or ESS ≥ 15 (n=1)). The positive predictive value for OSA of our clinical eligibility criteria was 73% (95% CI 61-83%), and for severe OSA (AHI >30/h) was 40% (95%CI 29-53%).

Conclusion:
The high positive predictive value of our study eligibility criteria indicates that OSA should be considered among ambulatory MS patients presenting with severe fatigue and poor subjective sleep quality. Our ongoing SAMSPAP trial will provide new insights on the efficacy of OSA treatment on severe fatigue in MS patients.

Mitochondrial Cyclophilin-D Regulates T Cell-Mediated Disease Tolerance to Tuberculosis

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M. tuberculosis (*Mtb*) is one of the most ancient human pathogens, yet how our host defense fights *Mtb* remains unclear. Although 1/3 of the world's population is chronically infected with *Mtb*, only 5-10% develops active disease. This indicates that in addition to resistance mechanisms that control bacterial burden, the host has also evolved strategies to tolerate the presence of *Mtb* to limit disease severity. Here we identify mitochondrial cyclophilin D (CypD) as a critical checkpoint of T cell metabolism that controls the expansion of activated T cells. While loss of CypD function in T cells led to enhanced *Mtb*-antigen specific T cell responses, surprisingly, this increased T cell response had no effect on bacterial burden. Rather, mice containing CypD-deficient T cells exhibited significantly compromised disease tolerance and succumbed to *Mtb* infection. This study establishes a mechanistic link between T cell mediated immunity and host tolerance in TB.

Chronic obstructive pulmonary disease categorization using cardiovascular risk: A way towards more personalized care

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Rationale: COPD patients are at an increased risk of morbidity and mortality from cardiovascular (CV) events. Despite this widespread knowledge, CV risk is not routinely assessed in COPD patients and no longitudinal data is available on the effects of high CV risk in COPD outcomes such as quality of life, exacerbations and healthcare utilization. Our objective is to gather epidemiological data on the prevalence of different CV risk categories in COPD subjects with varying disease severities, and longitudinal data to determine its impact on patient outcomes such as CV events and respiratory exacerbations. The integration of CV assessment into current COPD guidelines could lead to more targeted therapies to enable more personalized patient care.

Methodology: Our research will originate from the 'Canadian Cohort Obstructive Lung Disease' (CanCOLD), an ongoing longitudinal study of 1561 participants categorized as non-COPD, at risk and GOLD 1, and GOLD 2+ COPD subjects. Measurements of blood pressure, BMI, age, sex, use of hypertensives, smoking status and presence of diabetes were taken at baseline and used to calculate CV risk using the Framingham risk score (office model). CV events were defined as a diagnosis of angina, myocardial infarction, arrythmia, stroke, coronary artery disease or decompensated heart failure requiring a visit to the emergency department or hospitalization. Data on incidence of CV events was obtained from questionnaires assessing health care utilization at 18 and 36 months after the initial visit and data relating to COPD exacerbations continues to be collected every three months. Kaplan Meier estimators will be used to observe incidence of CV events at 3 years which will be compared to the CV risk score.

Results: Out of the 1561 subjects, 1480 had data to calculate a CV risk score. The prevalence of high CV risk was much lower in the control group at (37.8%) when compared to those at risk (52.2%), GOLD 1 (57.4%), and GOLD 2+ (52.0%), $p<0.001$*. One thousand twenty eight (1028) subjects have completed the 3 year follow up. The incidence of CV events in the control group was 0.5%, the at-risk group was 1.8%, GOLD 1 was 2%, and GOLD 2+ was 4.6%, $p=0.018$*. In the GOLD 2+ group, 13 subjects reported CV events in the 3 year follow up. Three of those subjects (23%) reported a CV event at both the 18 month and 36 month follow up. Furthermore, 9 subjects (69%) of the 13 reported 2 or more COPD exacerbation-like events per year in the same period. Out of all 1028 subjects, 6 reported having 3 or more COPD exacerbations in the preceding year, and all 6 subjects reported a CV event in that year. In COPD subjects, for exacerbations requiring hospitalization or medical services there was an increased relative risk of 1.61 (95% CI 1.16 – 2.23, $p=0.005$*) in the high CV risk group compared to the low CV risk group. No such increased risk 1.17(95% CI 0.77 – 1.77, $p=0.457$) was observed in non-COPD subjects.

Conclusion: Our data shows that the highest incidence of CV events increases with the severity of airflow obstruction. Furthermore, the incidence of pulmonary exacerbations is higher in subjects reporting a CV event. COPD subjects had a greater risk for pulmonary exacerbations if they were in the high CV risk group. The latter did not apply to non-COPD subjects and therefore evaluating CV risk in COPD is necessary. Using this data we will now work to develop a novel COPD CV risk score that will help identify patients that require closer monitoring or other individualized therapeutic care.

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

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

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

Dual Action Mechanism of STAT6-Inhibitory peptide in Airway Hyperresponsiveness

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Respiratory syncytial virus (RSV) infection leads to millions of hospitalizations and many deaths per year. In addition, early childhood RSV infection is associated with development of asthma later in life. Currently, neither antivirals nor vaccines are available for RSV. Novel preventative and therapeutic strategies are therefore urgently needed. We are characterizing mechanisms by which a small protein inhibitor we developed, called STAT6-IP, provides protection in murine models of asthma and RSV infection. This peptide was designed to inhibit a protein modulator of allergic responses called STAT6. As part of these studies we have also used a negative control version of STAT6-IP, called STAT6-CP. Over time, we have noticed that in some experiments STAT6-CP also exhibited inhibitory activity. These observations raised the possibility that STAT6-CP and STAT6-IP might be acting as so-called host defense peptides (HDPs) - small proteins with the ability to modify immune responses. We hypothesize that STAT6-IP, in addition to inhibiting STAT6, also functions as an HDP immune modulator and that STAT6-CP retains activity as an HDP without inhibiting STAT6. The first step of this project was to design a robust model of type-2 immunity. In this regard, we have successfully demonstrated that RSV infection in young mice followed by house dust mite (HDM) challenge leads to an enhanced eosinophilic response in the lung as well as increased IL-13 production in lymph nodes cultured in HDM. Through a better understanding of the mechanisms of action of both STAT6-IP and STAT6-CP, we hope to develop optimized inhibitors with the potential to improve health outcomes in both asthma and respiratory virus infections.

Role of Autophagy in Sepsis-Induced Skeletal Muscle Dysfunction

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Rationale: Sepsis-induced skeletal muscle dysfunction contributes to physical disability, and increased mortality in intensive care unit patients. Autophagy is the catabolic process by which cells degrade their own components. Recent studies indicate that sepsis triggers sustained induction of autophagy in skeletal muscles; however, the impact of autophagy on sepsis-induced contractile and metabolic dysfunctions remains unclear. We evaluated in this study the functional importance of autophagy in sepsis-induced skeletal muscle dysfunction.

Methods: Selective inhibition of autophagy in skeletal muscles was accomplished by cross breeding floxed *Atg7* mice (*Atg7*$^{f/f}$) with those expressing HSA-Cre-ER^{T2}. Cre induction was achieved by feeding Tamoxifen. Control mice were *Atg7*$^{f/f}$-HSA-Cre-ER^{T2} without Tamoxifen feeding. Sepsis was induced for 48 hrs using the cecal ligation and perforation (CLP) procedure. Mice that underwent the sham procedure served as control.

Results: Deletion of *Atg7* in skeletal muscle had a major impact on sepsis-induced loss of body weight and muscle mass as indicated by worsening of body weight loss, more severe sepsis-induced skeletal muscle atrophy and decline in muscle contractility in mice with *Atg7* deletions vs. those with intact *Atg7*. In addition, electron microscopy revealed significantly greater mitochondrial structural abnormalities in skeletal muscles of mice with deleted *Atg7* vs. those with intact *Atg7*. Deletion of *Atg7* resulted in significant impairments in skeletal muscle mitochondrial respiration both in the diaphragm and gastrocnemius muscles. Sepsis did not worsen muscle mitochondrial respiration in Atg7 deleted skeletal muscle. Finally, sepsis triggered significant but similar induction of the expression of ubiquitin ligases (Atrogin-1 and MuRF1) in muscles of mice with intact and deleted *Atg7*.

Conclusion: We conclude that autophagy plays a protective role by preserving muscle mitochondrial quality and function and that autophagy inhibition in skeletal muscles worsens sepsis-induced loss of body weight and muscle atrophy possibly due to exaggerated responses to catabolic stimuli triggered by sepsis.

A new model to study tissue-resident and bone marrow-derived macrophage responses to diaphragm muscle injury

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Background: Diaphragm muscle injury occurs in several diseases and contributes to respiratory insufficiency. Successful muscle regeneration after injury requires both the proliferation and differentiation of myogenic progenitor cells (called satellite cells), which in turn relies upon the appropriate regulation of immune cells. Macrophages in particular have been shown to play a vital role in muscle regeneration by phagocytosis of necrotic muscle, stimulation of myoblast proliferation, and secretion of growth factors that support differentiation into new muscle fibers. Recent research indicates that bone-marrow derived macrophages (BMDM) and tissue-resident macrophages (TRM) may have different developmental origins and functions, depending upon the tissue or organ being studied. In skeletal muscle, very little is known about the relative roles of BMDM versus TRM under basal conditions or following injury. Our objective was to develop an in vivo model to begin to address these questions in the acutely injured diaphragm.

Methods: To distinguish between BMDM and TRM, congenic allelic variant mouse chimeras were generated by transplantation of CD45.2 bone marrow into total body irradiated CD45.1 mice. Unlike traditional irradiation protocols that impair the function of muscle progenitor satellite cells, lead shielding of the diaphragm was employed to protect both satellite cells and TRM from radiation effects. At 60 days post-transplantation, acute diaphragm injury was induced by exposing the abdominal surface of the diaphragm to cardiotoxin (CTX), a myonecrotic agent. Irradiated mice were divided into 4 groups: 1) CTX-negative (sham) without shielding, 2) CTX-positive (injury) without shielding, 3) CTX-negative with shielding, 4) CTX-positive with shielding. The mice were sacrificed at 4 days after sham or CTX injury to identify CD45.1 and CD45.2 immune cells in the bone marrow, blood and diaphragm muscle compartments by flow cytometry.

Results: Diaphragm shielding preserved the myogenic potential of satellite cells isolated from the acutely irradiated diaphragm. In diaphragm-shielded mice, flow cytometry revealed that transplanted bone marrow cells reconstituted over 95% of CD45+ cells in the bone marrow and approximately 90% of CD45+ cells in blood. Bone marrow-derived cells (CD45.2+) made up approximately 90% of the intramuscular macrophages (CD45+,F480+,CD11b+,Ly6G-) at Day 4 following CTX injury.

Conclusion: Our results suggest that diaphragm shielding preserves tissue resident myogenic precursor (satellite) cells and also permits adequate chimerism to distinguish the predominant populations of BMDM versus TRM in the diaphragm. This model will allow future studies of BMDM versus TRM effects on muscle regeneration in the acute and chronically injured diaphragm.

Interleukin-6 Trans Signalling in Pulmonary Exacerbations in Cystic Fibrosis

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

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

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

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

Physiological mechanisms of exertional dyspnea relief following bariatric surgery for severe obesity

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The aim of this study was to elucidate the physiological mechanisms of exertional breathlessness relief following weight loss for severe obesity. To this end, we compared cardiac, metabolic, ventilatory, breathing pattern, operating lung volume and breathlessness intensity responses to symptom-limited incremental cycle exercise testing in 6 adults (3 W) mean±SE aged 44±3 yrs before (PRE) and 3-mo after Roux-en-Y gastric bypass surgery (POST). Compared to PRE, body mass, body mass index and fat mass decreased by an average of 24 kg (129±6 vs.106±6 kg), 9 kg/m^2 (47±2 vs. 38±2 kg/m^2) and 19 kg (62±5 vs. 43±5 kg) at POST, respectively. Peak power output was similar in POST vs. PRE (154±21 vs. 133±11 W). With few exceptions, heart rate, oxygen uptake and ventilation ($\dot{V}_E$) were lower during exercise in POST vs. PRE. Inspiratory capacity (IC) decreased by -0.13±0.11 L from rest to end-exercise in PRE. By contrast, IC increased by +0.21±0.08 L from rest to end-exercise in POST. Inspiratory reserve volume (IRV) was lower at any given $\dot{V}_E$ during exercise in POST vs. PRE (e.g., by 0.19±0.09 L at 55.5 L/min), reflecting the combination of differences in the behavior of dynamic IC and adoption of a relatively deeper and slower breathing pattern during exercise in POST. Breathlessness intensity ratings were lower during exercise at standardized submaximal power outputs ≥ 75 W in POST vs. PRE (e.g., by 1.0±0.3 Borg CR10 scale units at 75 W), whereas breathlessness intensity-$\dot{V}_E$ relationships were superimposed throughout exercise. Breathlessness intensity-IRV relationships were rightward shifted during exercise in POST vs. PRE, such that breathlessness intensity ratings were lower at any given IRV during exercise in POST vs. PRE. In conclusion, relief of exertional breathlessness following bariatric surgery for severe obesity could not be explained by improved dynamic breathing mechanics, but reflected the awareness of reduced $\dot{V}_E$ during exercise.

Superoxide dismutase activity confers (p)ppGpp-mediated antibiotic tolerance to stationary phase Pseudomonas aeruginosa by modulating membrane permeability and drug uptake

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Antibiotic tolerance causes treatment failure and promotes the emergence of genotypic resistant bacteria during chronic infections, such as those caused by the opportunistic lung pathogen *Pseudomonas aeruginosa*. In laboratory stationary phase, *P. aeruginosa* exhibits a slow growing and metabolically quiescent state associated with multidrug tolerance analogous to the metabolic state present in chronic infections. Tolerance requires (p)ppGpp signaling, which mediates the global stress and starvation stringent response (SR), but its downstream effectors that confer tolerance are still largely unknown. Using biochemical activity assays and gene reporters, we have demonstrated that stationary phase cultures of a mutant impaired for the SR (ΔSR) displays 3-fold depressed superoxide dismutase (SOD) activity and 2.5-fold lower expression of the iron co-factored SodB than its parental wild-type, suggesting that the ΔSR mutant superoxide metabolism is ablated. Since antibiotic killing was shown to involve the generation of superoxide and oxidative stress as an off-target toxicity mechanism, we hypothesized that the SOD activity modulation by SR contributes to (p)ppGpp-mediated tolerance. We genetically and chemically complemented the ΔSR mutant using SOD overexpression and treatment with the SOD mimetic Mn^{III}TMPyP, and examined the interventions effect on the mutant's tolerance against the main classes of antipseudomonal antibiotics (aminoglycosides, fluoroquinolones and carbapenems) using killing assays. The SR modulates 20% of the total gene expression in *P. aeruginosa*, but genetic or chemical restoration of SOD activity alone was sufficient to fully rescue multidrug tolerance in stationary phase ΔSR cultures, which are normally 1,000-fold more susceptible to these drugs than the isogenic wild-type. The ΔSR mutant has also ~100-fold lower generation of spontaneous antibiotic resistant mutants, which is restored to wild-type levels using SOD complementation. These data suggest that SODs are pivotal in the SR-mediated tolerance and development of drug resistance. Remarkably, ablation of SOD activity in the ΔSR mutant results in 3-fold increased membrane permeability, which correlates linearly with the survival after antibiotic challenge and is associated with 3-fold increased internalization of the aforementioned drugs. Combined, our results highlight an unprecedented link between SR-mediated multidrug tolerance, SOD activity and membrane permeability in stationary-phase *P. aeruginosa*, and suggest that inhibition of SR and SOD activity may potentiate current antibiotics in the treatment of *P. aeruginosa* chronic infections as well as prevent the generation of drug resistant mutants.

Relationship of simple measures of physical function and muscle strength to exacerbation, hospitalization and mortality in COPD: a systematic review

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Rationale: There has been an increased interest in simple measures of physical function and muscle strength that can be used in all settings to assess individuals with chronic obstructive pulmonary disease (COPD) and predict their prognosis. Our objective was to systematically review studies that have examined the relationship of simple measures of physical function and muscle strength to exacerbation, hospitalization and mortality in individuals with COPD.

Methods: We searched electronic databases (Cochrane, Embase and Medline) and included original studies of any design that examined the relationship of simple performed-based tests or self-reported measures of physical function and muscle strength to exacerbation, hospitalization and mortality in individuals with COPD. We defined "simple" performance-based measures of physical function or muscle strength if: 1) the test does not require a specialized equipment; 2) takes relatively short time to be performed (e.g. less than 10 minutes); 3) can be performed in any setting (e.g. primary care, acute and/or rehabilitation hospital); 4) does not require a professional with specialized expertise. Also, we considered studies that assessed mortality using true observations of all-cause deaths or composite indexes of mortality (BODE or ADO indexes.

Results: A total of 6580 articles were identified and 16 met the inclusion criteria. Nine were prospective cohort studies and 8 were cross-sectional studies. The majority of the studies (n= 13) included performance-based tests. Twelve studies examined the relationship of simple physical function measures and muscle strength test to mortality (6 studies assessed mortality through the BODE or ADO indexes, 5 studies used true observation of mortality and 1 looked at survival probability). Of these 12 studies, 6 studies demonstrated a relationship between mortality and this type of tests. In relation to exacerbation, six articles examined this relationship of simple physical function measures and muscle strength test to exacerbation. Only 2 found a relationship between exacerbation and this type of tests. And finally, only 2 articles examined the relationship of simple physical function measures and muscle strength test to hospitalization. These studies found a relationship between hospitalization and this type of tests. The most commonly used tests were the handgrip strength test (n=5) and 1-min sit-to-stand (n=5). The 1-min sit-to-stand test was strongly associated with mortality (adjusted HR 0.58, 95% CI 0.40–0.85; p=0.004) (per 5 more repetitions). The handgrip strength test was moderately associated with mortality (adjusted HR 0.84, 95% CI 0.72–1.00; p=0.04); with exacerbation risk (risk ratio, 1.05; 95% CI 1.01–1.08) (1-kg decrement in handgrip) and with hospitalization (for each 1-kg decrement in handgrip strength an increment of 6% in the risk for hospital admission).

Conclusions: Simple physical function measures can provide important information about the prognosis of individuals with COPD. It remains unclear whether following a specific rehabilitation intervention, improvements in these physical function measures can translate into improvement in patient prognosis.

Bcl6 curtails type 2 responses by limiting IL-10 production by Th2 cells

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The differentiation of $CD4^+$ T cells into one of many effector lineages is a tightly regulated process that requires a balance between a multitude of transcription factor-dependent signaling pathways. Of particular interest is Bcl6, a transcriptional repressor described as the master regulator of T follicular helper (Tfh) cell differentiation. However, a role for this transcription factor in mediating other aspects of $CD4^+$ T-dependent immune responses has not been detailed. Previous reports describe that deletion of Bcl6 in the $CD4^+$ cell compartment results in the elevated production of the cytokine IL-10, but its source and consequences of its production remain unknown. As IL-10 plays a crucial role in maintaining enteric homeostasis, we hypothesized that Bcl6 inhibits the development of IL-10 producing $CD4^+$ T cells during intestinal helminth infection. Here we demonstrate that Bcl6 deficiency led to increased production of IL-10 by $CD4^+$ T cells in vitro and during infection with the enteric parasite *Heligmosoides polygyrus bakeri (Hpb)*. Specifically, elevated levels of IL-10 derived from $Gata3^+$ Th2 cells. A surplus of IL-10 in this setting resulted in an enhanced Type 2 immune response including increased recruitment of eosinophils, enhanced development of alternatively activated macrophages (AAMs) and increased resistance to infection. Our results indicate that Bcl6 regulates a selective cytokine program in Th2 cells that limits the host response to intestinal infection.

Association Between Obstructive Sleep Apnea and Motor Symptoms in Parkinson's Disease Patients

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Rationale

Obstructive sleep apnea (OSA) is a treatable, common sleep disorder in the general population that is known to affect brain function. Patients with Parkinson's disease (PD) are often affected by sleep disorders, including OSA. The effect of OSA or its treatment on motor dysfunction in PD patients is unknown. We hypothesize that OSA worsens motor function deterioration in PD patients and that, in turn, treatment of OSA improves the rate of decline of motor function in PD patients.

Methods

Data was analysed from a prospective cohort study of idiopathic PD patients from a movement disorders clinic. Patients found to have OSA on polysomnography (OSA+, defined as an apnea-hypopnea index [AHI] of ≥15 events/h, Chicago criteria) were offered treatment using continuous positive airway pressure (CPAP) therapy. A patient was defined as "on CPAP" if average CPAP use was ≥2 h/night (CPAP+) at each follow-up. Motor symptom severity was assessed using the motor section of the Movement Disorder Society Unified Parkinson's Disease Rating Scale (mUPDRS), where a higher value indicates greater dysfunction. Follow-up times were at 3, 6 and 12 months. Change in mUPDRS over time was compared between the OSA/CPAP categories using linear mixed model analyses, adjusted for age, sex, body mass index, levodopa equivalent dose and comorbidities.

Results

A total of 67 individuals were studied, with mean age 64.7 (SD=10.1) years and 61.2% male. At baseline, the mean mUPDRS in the OSA+ group (n=47) was 24.5 (14.0) and in the OSA- group (n=20), it was 16.2 (7.2), p<0.001. There were 20 OSA-CPAP-, 21 OSA+CPAP- and 26 OSA+CPAP+, with mean baseline AHI of 7.3 (4.2), 33.2 (13.3) and 38.0 (21.5), respectively, p<0.001. The mixed model revealed (Figure 1) that mUPDRS increased over the follow-up period in OSA- and OSA+CPAP-, with comparable rates in the two groups (β=0.61 *vs.* 0.33, p=0.32), but that it decreased on average in the OSA+CPAP+ group (β=-0.11, p=0.01 *vs.* OSA-; p=0.12 *vs.* OSA+CPAP-). The mean nightly CPAP use at 12 months was 3.7 (2.1) hours in OSA+CPAP+.

Conclusions

We find that OSA is associated with greater PD motor dysfunction at baseline. We also find that, in patients with PD and OSA, CPAP use was associated with stabilization or possibly improvement of motor function over 12 months. This observational finding opens the way for further research to clarify the role of OSA in PD pathophysiology and symptomatology.

Inhibitory activity of STAT6-IP on IL-33-mediated-IL-13 production and macrophage polarization in an innate murine allergy model

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Rationale: Asthma and allergy rank among the costliest of all chronic diseases. Data from asthmatic patients and murine asthma models demonstrate that the STAT6 transcription factor regulates several responses in the allergic lung. Recent interest has focused on innate type cytokines, IL-33 and TSLP, as promoters of Type 2 immunity and targets for drug development in asthma. IL-33 & TSLP induce autocrine production of IL-4 and/or IL-13, which is associated with STAT6-dependent responses in innate cells, including macrophages (Mφs) and eosinophils. Data from Kurowska-Stolarska et al demonstrate that in mice, IL-33 delivery to the lung induces IL-13-dependent chemokine production, eosinophilic inflammation, and Mφ differentiation into the alternatively activated Mφ (AAM) phenoytpe. We have investigated the ability of a cell penetrating peptide-based STAT6 inhibitor (STAT6-IP) to inhibit acute IL-33-induced inflammatory responses in the lung.

Methods: Wild-type or STAT6 knockout (KO) Balb/c mice were treated intranasally with saline or IL-33, either alone or with STAT6-IP or negative control, STAT6-CP daily for 3 days and sacrificed 72h later. Lung Mφ phenotype and eosinophil infiltration and activation were assessed by flow cytometry. Levels of mRNA encoding IL-13 were quantified by qPCR. Bronchoalveolar lavage fluid (BALF) cytokine & chemokine levels and inflammatory cells were quantified. Lungs were minced and cultured with saline or IL-33 for 48h after which IL-13 was quantified by ELISA.

Results: IL-33 induced a range of inflammatory responses in the lung, including i) AAM polarization; ii) eosinophil recruitment & activation; iii) BALF eosinophil influx; and iv) production of Type 2 cytokines & chemokines. Each of these responses was reduced by STAT6-IP and absent in IL-33-treated STAT6 KO mice. Nevertheless, IL-33 retained the ability to increase lung IL-13 mRNA levels and ex vivo IL-13 production in STAT6 KO mice, albeit to levels significantly lower than those from wild-type mice.

Conclusions: These data demonstrate that the ability of IL-33 to induce acute type 2 airway inflammatory responses is dependent upon STAT6 and suggest that IL-33-induced autocrine/paracrine IL-13 production and STAT6 activation participate in a positive feedback loop to promote IL-33-dependent airway inflammatory responses. STAT6-IP interrupts these maladaptive inflammatory responses, effectively reducing Type 2 inflammation in the airways.

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Investigating Fractional Exhaled Nitric Oxide (FeNO) in Chronic Obstructive Pulmonary Disease (COPD) and Asthma-COPD Overlap (ACO): A Scoping Review

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Introduction: Chronic obstructive pulmonary disease (COPD) is the most common fixed airflow limitation. Individuals may present with the features of both asthma and COPD called asthma-COPD overlap (ACO) syndrome (ACOS) with more severity and worse health-related quality of life than COPD or asthma. One of the promising biomarkers that could be used in clinical practice to differentiate ACO(S) from COPD is fractional exhaled nitric oxide (FeNO). The role of Fractional exhaled nitric oxide (FeNO) in COPD/ACO(S) remains unknown. This scoping review aims to investigate the role of FeNO measurement to differentiate COPD from ACO(S), to anticipate disease severity/progression and treatment response.

Methods: A structured comprehensive literature search was performed in major databases including Medline, EMBASE, CINAHL, Cochrane Library, Web of Science, and BIOSIS from 2005 onwards.

Results: Thirty-eight studies were retrieved. Based on the synthesis of the reviewed literature, six themes emerged. Thirty-four articles covered more than one theme. From which, 24 articles were on modifying factors in FeNO measurement, 18 on FeNO in COPD compared to healthy subjects and 7 compared to ACO(S), 22 on FeNO and disease severity/progression,12 on FeNO and biomarkers, and 8 on FeNO and treatment response.

Conclusion: FeNO measurement cannot be used alone in the clinical settings of COPD patients. Although FeNO level is higher in ACO(S) patients than COPD-only, it is still unclear if there is a FeNO cut-off that can be used to make the diagnosis of ACO(S) and/or to guide therapy with inhaled corticosteroids/glucocorticoids in COPD patients.

From clinic to in vitro: mechanism of action of azithromycin for prevention of COPD exacerbation

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Rationale: Recently, we published a retrospective observational study that was able to show that long-term azithromycin reduces the number of exacerbations in severe COPD patients beyond one year. Beneficial effects of azithromycin were observed in subgroups of smokers/ex-smokers and patients colonized with *P. aeruginosa.*These results led us to develop a translational project to study the mechanisms by which macrolides reduce COPD exacerbations. Beyond their antimicrobial effect, macrolides are known to have anti-inflammatory and immuno-modulatory effects on the host. Therefore, we hypothesized that azithromycin reduces exacerbation frequency and severity by modulating the interaction between airway epithelial cells and *P. aeruginosa* and that this response could be affected by cigarette smoke.

Objectives: To assess the mechanism through which azithromycin modulates host-pathogen interactions in an *in vitro* model of cigarette smoke-exposedairway epithelial cells.
a) Effects of azithromycin on airway epithelial cell expression and release of the inflammatory mediators IL-6 and IL-8 in response to cigarette smoke.
b) Effects of azithromycin on airway epithelial cell inflammation in response to an infection with *P. aeruginosa* in the presence/absence of cigarette smoke.

Methods: In order to study the effect of azithromycin on cigarette smoke-induced inflammation, Beas 2B bronchial epithelial cells were incubated with 5% cigarette smoke extract (CSE) for 3h, 6h and 24h. Expression and release of IL-6 and IL-8 mRNA was analyzed by quantitative real-time PCR (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. Then, airway epithelial cells were pretreated with azithromycin and exposed to 5% CSE. Expression and release of IL-8 and IL-6 were measured by qRT-PCR and ELISA.

Results: We observed a significant increase of IL-6 and IL-8 mRNA following a 24-hour exposure to 5% CSE. Similarly, IL8 secretion was significantly increased after exposure to 5% CSE for 24h. Finally, we observed a trend towards a dose-dependent decrease in the expression of IL6 mRNA when cells were pre-treated with azithromycin and exposed to 5% CSE for 3H.

Conclusion: Treatment with azithromycin resulted in a trend towards a decrease in the expression of inflammatory mediators in beas2b cells exposed to CSE.

Future directions: We will establish the impact of azithromycin on airway epithelial cell inflammation in response to an infection with *P.aeruginosa*. For this purpose, airway epithelial cells will be pretreated with azithromycin, exposed to CSE, and infected with *P.aeruginosa*. Expression of IL-6 and IL-8 will be assessed by qRT-PCR and compared to that of cells infected with *Pseudomonas* but not exposed to CSE.

Leukotriene B4 – Type I Interferon axis regulates macrophage-mediated host tolerance to influenza infection

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Objectives
Despite the worldwide application of vaccination and other antiviral interventions, influenza A virus (IAV) infection remains a persistent threat to humans. Virulent IAV causes lower airways infections, inducing uncontrolled host inflammatory responses leading to morbidity and mortality. Therefore, understanding the mechanisms of immune regulation during IAV infection is critical in preventing IAV-induced immunopathology and compromising host-tolerance. We have previously shown that eicosanoid PGE_2 played an essential role in host-resistance to IAV infection. Herein, we aimed to investigate the potential contribution of leukotriene B_4 (LTB_4) during IAV infection.

Methods
Based on our established model of IAV infection, WT and *Blt1R*$^{-/-}$ (lacking LTB_4 receptor) mice were infected with influenza virus (PR8 strain). At different days p.i., lungs were harvested, and IFN-I/cytokine levels, viral titres and innate cellular responses were determined by B16 cell reporter assay or ELISA, plaque assay and flow cytometry respectively. To address the role of the LTB_4/BLT1R signalling in macrophages, WT and *Blt1R*$^{-/-}$ BMDM were generated and the signalling pathways involved in LTB_4/IFN-I axis was determined after *in vitro* IAV infection.

Results
Our data indicate that despite intact host-resistance, *Blt1R*$^{-/-}$ mice were more susceptible to IAV infection due to enhanced immunopathology and lung damage. This phenotype was coupled with higher frequency and number of pulmonary inflammatory macrophages. Interestingly, this increased number of macrophages was not due to increased recruitment, but rather to decreased IFN-α that led to *in situ* macrophages proliferation. Mechanistically, we demonstrated that LTB_4 enhanced the downstream signalling of type I IFN receptor to regulate IFN-α dependent pulmonary immunopathology.

Conclusions.
Collectively, these findings identified a protective role of LTB_4 during IAV infection independent of host-resistance, but dependent on host-tolerance. Our work may pave the way for novel treatments by using bioactive lipids that limit IAV-induced lung immunopathology.

An Unusual Oscillating Expiratory Flow Volume Loop

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We present a case of a 58 year-old non-smoker female with a history of recurrent pneumonias. On clinical evaluation, she was found to have a low pitch fluttering sound when coughing. Spirometry was suggestive of a restrictive disorder with an FEV_1 of 2.15L (86% of predicted) and an FVC of 2.99 (97% of predicted) and FEV_1/FVC ratio of 72. Flow volume loop was remarkable for reproducible marked expiratory oscillation (*Figure 1*). Computed tomography (CT) of the chest identified significant collapse of the trachea in a crescent shape pattern *(Figure 2)*. In view of these findings, bronchoscopy was performed and confirmed the diagnosis of severe tracheomalacia (TM) (*Figure 3*). Oscillating expiratory flow volume loop, also referred to as « saw-tooth » pattern, is typically described in obstructive sleep apnea, Parkinson disease, neuromuscular disease with bulbar involvement, upper airway stenosis or cough artifact. It has been described in tracheomalacia in only 2.6% of cases but no total vibratory flow occlusion was previously reported. Our case allows to review the etiologies of a saw-tooth pattern and provides the first illustration of a complete vibratory occlusion of the expiratory flow that can be encountered in severe tracheomalacia.

Figure 1

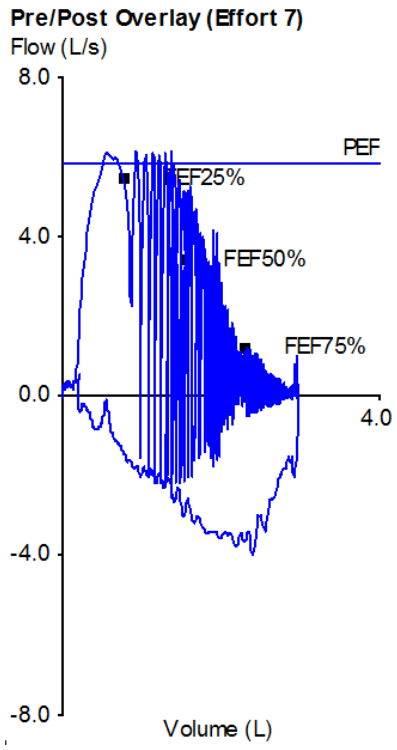


Figure 2

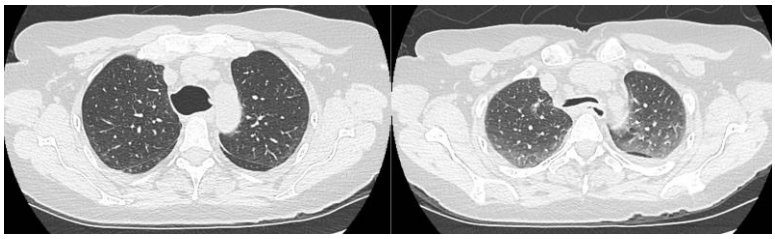
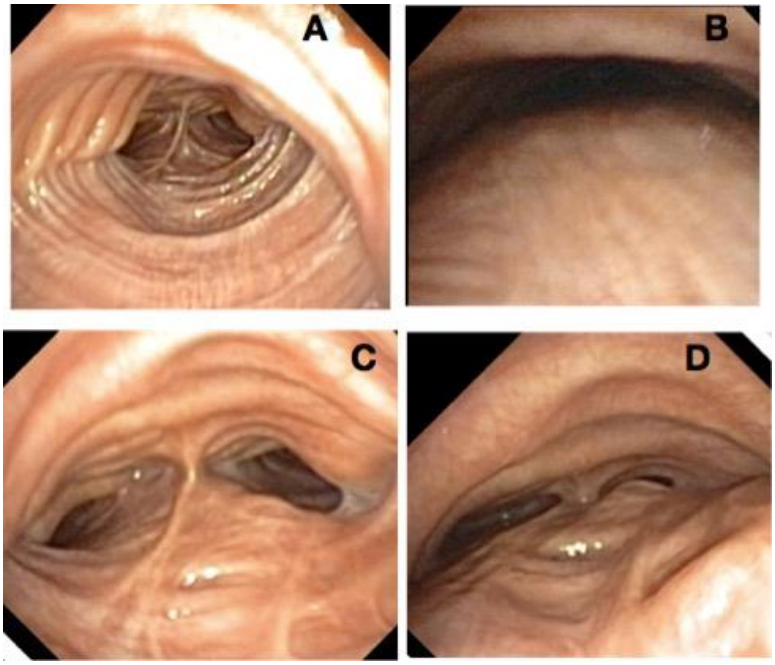


Figure 3



Domestic and Non-Occupational Exposures in Developing Countries: A Case of Mixed Desert and Hut Lung Syndrome

Laïla Samy[1], Richard Fraser[1], Dao Nguyen[1]

[1]McGill

INTRODUCTION

Non-occupational exposure to domestic smoke and environmental dust is highly prevalent in certain regions of the world and may be under recognized in immigrant populations. Since such inhalant exposures may cause domestically acquired particulate associated lung disease and pneumoconiosis, they should be carefully assessed in the evaluation of airway and parenchymal lung diseases. We here present the unusual case of a patient with exposure to both domestic wood smoke and environmental dust exposures, leading to a mixed pattern of pneumoconiosis and bronchial anthracofibrosis.

CASE PRESENTATION

This 75 year-old non smoking women from Afghanistan, presented to our hospital with a two week history of cough productive of white sputum. Computed tomography of the chest was remarkable for innumerable diffuse micronodules involving the mid and lower lung zones, bulky mediastinal and bilateral hilar lymphadenopathy. Pulmonary function testing showed mild airflow obstruction with a FEV1 to FVC ratio of 65% and normal lung volumes. Induced sputums were negative for mycobacterial cultures. Diagnostic flexible bronchoscopy showed multiple bronchial anthracotic plaques. Transbronchial lung biopsy of the right middle lobe revealed the presence of bronchiolocentric accumulation of particle laden macrophages and mild fibrosis suggestive of bronchial anthracofibrosis. The patient lived in rural Afghanistan in an aride region until the age of fifthy and reported decades of domestic wood smoke and farm dust exposure. She was diagnosed with a non-occupational mixed dust pneumoconiosis, also called "Domestically cquired particulate lung disease", attributable to chronic inhalation of biomass smoke and environmental silica-rich dust particles, a combination of “Hut lung” and “Desert lung” syndromes.

DISCUSSION

Although occupational exposure to silica is well known to be associated with activities such as sand-blasting, mining, or stone cutting and grinding, non-occupational exposure to silica-rich dust should also be considered. Desert lung syndrome, a non-occupational mixed dust pneumoconiosis caused by prolonged and intense inhalation exposure to silica-rich dust, was first described in 1952 in Sahara Desert dwellers. Since then, cases have been documented in Saudi Arabia, the Libyan Desert, the Negev Desert, Pakistan and in India (Ladakh an Thar). Radiographic findings are described as a diffuse centrilobular micronodules (<1mm), with a mid to lower lung field distribution, unlike silicosis which is typically upper lobe predominant. The absence of typical silicotic nodules with collagenization confers to the desert lung syndrome a distinct entity described as “simple siliceous pneumoconiosis”. A thorough history of non-occupational environmental exposure is key when faced with patients from developing countries. This includes cooking habits (woodstove, coal) and surrounding landscape (desert, river, mountain, farm).

Inspiratory Pleural Pressures and Left Ventricular Structure in COPD

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RATIONALE Left ventricular (LV) dysfunction is common in chronic obstructive pulmonary disease (COPD), but the mechanisms contributing to this association are incompletely understood. COPD is associated with lower (more negative) inspiratory pleural pressures (P_{insp}) that may *increase* LV wall stress (afterload). We investigated the relationship between P_{insp} at rest and throughout exercise with LV mass assessed by cardiac magnetic resonance (MR).

METHODS We enrolled twenty adult smokers age 45 to 80 years old. P_{insp} was estimated by esophageal manometry at rest and during symptom-limited incremental cycle ergometry. P_{insp} was indexed to minute ventilation (V_E) to account for differences in breathing pattern. Resting LV structure was assessed by cardiac MR (3T). MR-estimated LV mass was indexed to body size using gender-specific allometric scaling terms. Forced expired volume in one second (FEV_1), forced vital capacity (FVC), inspiratory capacity (IC), and blood pressure were measured according to standardized protocols. Emphysema was defined as the percent lung volume below -950 Hounsfield units measured using inspiratory full-lung computed tomography (CT).

RESULTS Sixteen of 20 smokers completed symptom-limited exercise with manometry, cardiac MR, and full-lung CT (mean age: 60±8 years; 56% male; 81% COPD; peak VO_2: 15.2±7.7 ml/kg/min). Resting P_{insp}:V_E was -1.06±0.37 cmH_2O/L/min and LV mass index was 0.76±0.09 $gm/m^{1.88}$. Lower P_{insp} was associated with significantly higher LV mass at rest (8% higher LV mass index per 1-SD lower P_{insp}; 95%CI 4% to 12%; p<0.001), and throughout exercise (Figure; p<0.001). Similar results were obtained after adjusting for age, and hypertension. Predictors of lower P_{insp} were greater airflow obstruction (21% lower P_{insp} per 1-SD lower FEV_1 percent predicted; 95%CI -31% to -10%; p<0.001), and lower IC/VC (33% lower P_{insp} per 1-SD lower IC/VC; 95%CI -51% to -15%; p<0.001). In contrast, greater percent emphysema was associated with higher P_{insp} (22% higher P_{insp} per 1-SD higher percent emphysema; 95%CI 12% to 32%; p<0.001).

CONCLUSION Among smokers with and without COPD, lower P_{insp} was associated with higher LV mass. These findings suggest that P_{insp} in COPD may represent a novel therapeutic target to reduce LV mass.

Extubation readiness in extremely preterm infants: spontaneous breathing tests under the spotlight

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Background: A Spontaneous breathing test (SBT) may be used to determine extubation readiness in extremely preterm (EPT) infants. Current SBT pass/fail definitions use empiric combinations of clinical criteria observed during a brief period of spontaneous breathing on endotracheal continuous positive airway pressure (ET-CPAP), but these definitions have low accuracy.

Objectives: To determine the accuracy of more comprehensive combinations of SBT definitions in predicting successful extubation.

Methods: Infants with birth weight ≤ 1250g deemed ready for extubation and enrolled in the APEX (Automated Prediction of EXtubation readiness) study were included. As part of APEX, infants were switched to 5-min ET-CPAP to acquire cardiorespiratory signals during a period free of mechanical inflations. Throughout ET-CPAP, an investigator or respiratory therapist monitored infants for signs of clinical instability and intervened as per clinical judgment.
Four clinical events were documented: apnea requiring stimulation (A); presence and cumulative duration of bradycardia (B, heart rate < 100bpm); presence and cumulative duration of desaturation (D, SpO_2 < 85%); supplemental O_2 need and maximum amount provided. All infants were extubated thereafter. Infants were divided into success or failure (reintubation within 168h from extubation) and compared using appropriate statistical tests. After dichotomizing continuous variables into 5-sec or 5% bins, an automated algorithm was developed to generate SBT definitions using all possible combinations of the 4 clinical events and compute their diagnostic accuracies.

Results: A total of 202 infants (53 failures) were included. Failures were significantly smaller, less mature and on higher respiratory support prior to extubation (Table 1). 106 infants (52%) had ≥ 1 clinical event during ET-CPAP (Figure 1). Infants who failed extubation had significantly more episodes of A, B, D or ETT-CPAP discontinuation, as well as greater cumulative durations of B, D and amounts of supplemental O_2 (Table 2). A total of 41,602 SBT pass/fail definitions were evaluated (Figure 2), showing overall high sensitivity (i.e. high rates of true SBT success) but very low specificity (i.e. high rates of false SBT success).

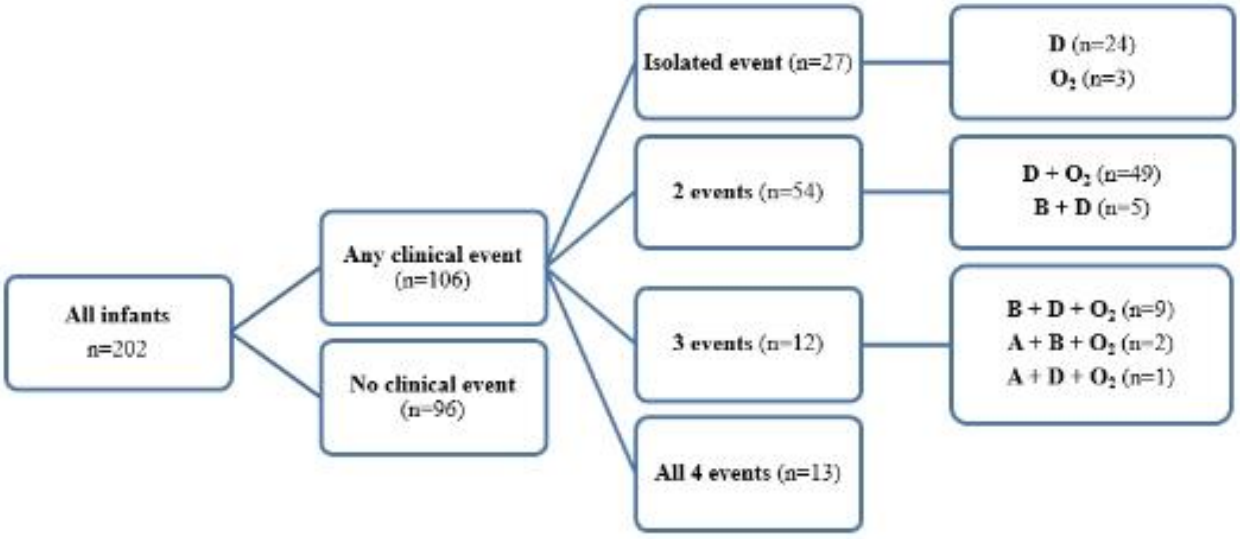
Conclusions: EPT infants commonly show signs of clinical instability during ET-CPAP. Although infants who fail extubation have significantly more clinical events compared to those successfully extubated, the accuracy of SBT definitions using all possible clinical criteria combinations remains low.

Table 1. Characteristics of infants submitted to ET-CPAP

Demographic	Extubation Success (n=149)	Extubation Failure (n=53)	P value
Gestational age, weeks	26.6 [25.1-28]	25.4 [24.7-26.5]	< 0.001
Birth weight, grams	910 [740-1090]	760 [666-883]	< 0.001
Male sex, %	77 (52)	31 (58)	0.393
Antenatal steroids, %	135 (91)	47 (89)	0.687
Surfactant, %	140 (94)	51 (96)	0.731
Caffeine, %	144 (97)	52 (98)	1
Pre-extubation characteristics			
Day of life (DOL) at extubation	5 [3-25]	9 [4-25]	0.358
Corrected age at extubation, weeks	28.6 [27.1-29.7]	27.4 [26.5-28.5]	< 0.001
Weight at extubation, grams	985 [860-1123]	830 [718-950]	< 0.001
Mean airway pressure, cm H_2O	6.8 [6.2-7.8]	7.4 [6.7-9]	0.004
Fraction of inspired oxygen	0.21 [0.21-0.25]	0.26 [0.23-0.28]	< 0.001
pH	7.34 [7.3-7.38]	7.32 [7.29-7.37]	0.146
PCO_2, mm Hg	44 [37-49]	47 [37-55]	0.084
ET-CPAP			
Positive end expiratory pressure, cm H_2O	5 [5 – 6]	5 [5 – 6]	0.294
Starting fraction of inspired oxygen	0.21 [0.21 – 0.25]	0.26 [0.23 – 0.29]	< 0.001
Starting oxygen saturation	95 [92-97]	94 (92-95)	0.061

Values are expressed as medians [IQR] or n (%)

Figure 1. Flow diagram of clinical events during ET-CPAP



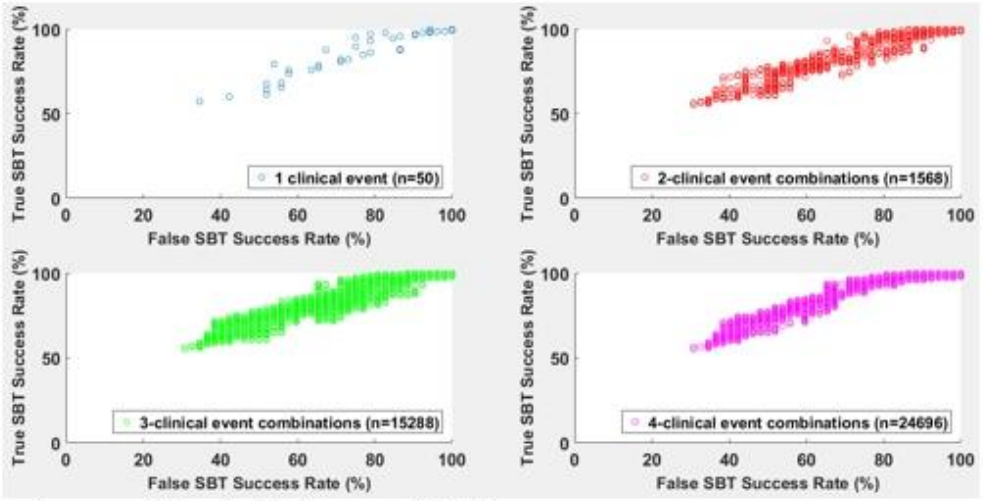
Abbreviations: A – apnea needing stimulation, B – bradycardia (heart rate < 100 beats per min), D - desaturations (oxygen saturation < 85%), O_2 – supplemental oxygen requirements above baseline.

Table 2. Occurrence of clinical events during ET-CPAP and their diagnostic accuracy at predicting successful extubation

Clinical Events	Extubation Success (n=149)	Extubation Failure (n=53)	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Categorical variables						
Apnea needing stimulation	4 (3)	12 (23)[a]	97	23	78	75
Bradycardia	15 (10)	14 (26)[a]	90	26	77	48
Desaturation	66 (44)	35 (66)[a]	56	66	82	35
Need for additional O_2	51 (34)	26 (49)	66	49	78	34
Any clinical event (n=106)	69 (46)	37 (70)[a]	54	70	83	35
2 clinical events (n=54)	42 (28)	12 (23)	72	23	73	22
3 clinical events (n=12)	8 (5)	4 (8)	95	8	74	33
4 clinical events (n=13)	3 (2)	10 (19)[a]	98	19	77	77
Early discontinuation	4 (3)	7 (13)[a]	97	13	76	64
Continuous variables			**Area under the curve**			
Desaturation (sec)[b]	0 [0-35]	10 [0-79][a]	0.6			
Bradycardias (sec)	0 [0-0]	0 [0-4][a]	0.58			
Supplemental O_2 (%)[c]	0 [0-4]	0 [0-15][a]	0.61			

Categorical and continuous variables are expressed as n (%) and median [interquartile range], respectively.
[a] $p < 0.01$
[b] n=146 vs. 53
[c] n=147 vs. 52

Figure 2. Receiver operating characteristics curve of various SBT definitions for predicting successful extubation



Legend: The 4 clinical events consist of: A- apnea needing stimulation; B- cumulative bradycardia duration (thresholds between 0-100 seconds); D- cumulative desaturation duration (thresholds between 0-100 seconds); O_2 - supplemental oxygen requirements (thresholds between 0-30%).

Age of first extubation attempt and respiratory morbidities in extremely preterm infants

Wissam Shalish[1], Lara Kanbar[2], Lajos Kovacs[3], Sanjay Chawla[4], Martin Keszler[5], Smita Roa[1], Karen Brown[6], Doina Precup[7], Robert E Kearney[2], Guilherme M Sant'Anna[1]

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Background: Both prolonged mechanical ventilation (MV) and failed extubation are associated with increased respiratory morbidities/death in extremely preterm (EPT) infants. While early extubation is commonly advocated, it is unclear how age at first extubation and success/failure differentially affect these morbidities.

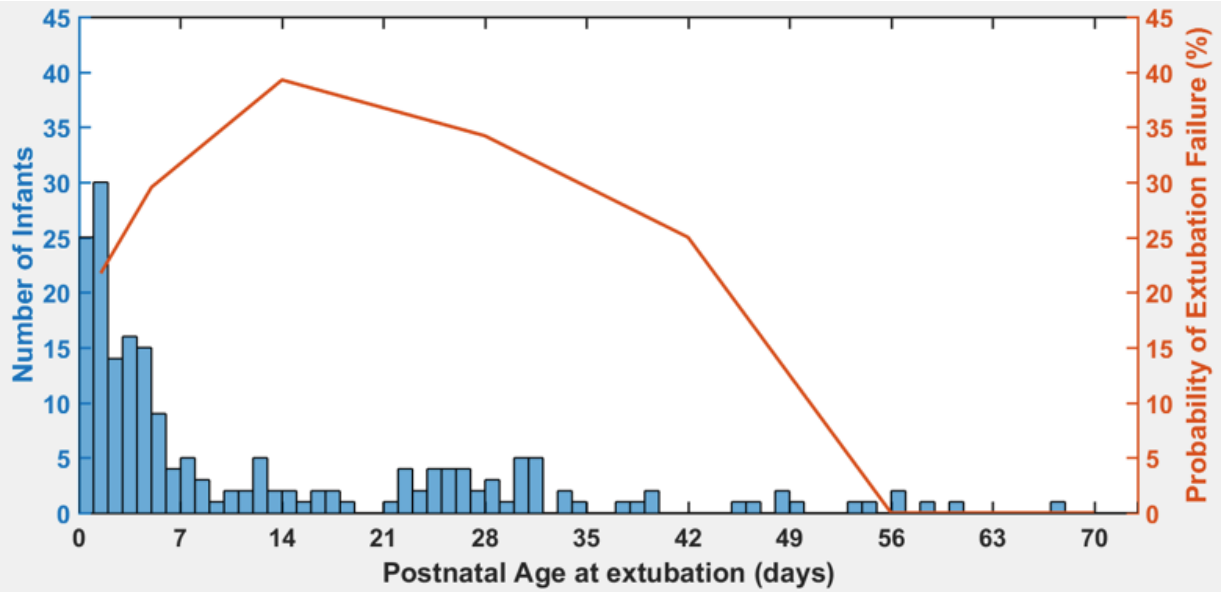
Objectives: Explore the relationship between age at first extubation attempt, extubation outcome, and respiratory morbidities/death in EPT infants.

Methods: Infants with birth weights ≤ 1250g, intubated ≤ 24h of age and enrolled in an ongoing multicenter observational study were included. The frequency distribution of age at first extubation (in days) and the probability of extubation failure (reintubation ≤ 7d) for different age bins were plotted. For the analysis, only infants with a first extubation attempt before 35d were evaluated. Four groups were created based on age at extubation (early=1-7d vs late=8-35d) and outcome of extubation: early success (ES), early failure (EF), late success (LS) and late failure (LF). Patient characteristics were compared between groups using appropriate statistical tests. Multivariate regression analyses were performed to evaluate associations between the groups and death/BPD (bronchopulmonary dysplasia), BPD among survivors and durations of MV, any respiratory support, oxygen therapy and hospitalization. Models were adjusted for gestational age, postnatal steroids, necrotizing enterocolitis and infection.

Results: A total of 196 infants were included (Figure 1): 113 (58%) extubated early, 66 (34%) extubated late and 17 extubated beyond 35d (hence excluded from analysis). Extubation failure was higher amongst infants extubated late, but these infants were significantly smaller, less mature at birth, and had more pre-extubation complications compared to early extubation (Table 1). While ES was associated with significantly less morbidities when compared to all other groups, there were no differences between EF and LS in the unadjusted analysis. After adjustment, EF had significantly greater odds of death/BPD (Figure 2), BPD in survivors, and duration of oxygen therapy compared to infants successfully extubated ≤ 35d (Table 2).

Conclusion: Failure of extubation in the first 7d of life may be an independent risk factor for death/BPD compared to any successful extubation in the first 35d of life. Future prospective research is needed to evaluate the benefits of successful extubation against the costs of failure whilst accounting for the infants' postnatal age and risk profile.

Figure 1. Frequency distribution of DOL at first extubation attempt and the probability of extubation failure



Legend: An additional infant was extubated beyond 70 days of life. The probability of extubation failure (red line, Y2 axis) was computed for the following age bins: days 1-3, 4-7, 8-21, 22-35, 36-48, 49-63 and > 64.

Table 1. Characteristics of the groups

	Early Success (n=85)	Early Failure (n=28)	Late Success (n=42)	Late Failure (n=24)
Demographics				
GA, weeks	27.6 [26.4-29][a]	26.2 [25.8-27.2][b]	25.3 [24.7-26.6][c]	24.7 [24.2-25.3]
BW, grams	1040 [878-1156][a]	855 [760-975][b]	745 [685-880]	690 [605-750]
Male, %	44 (52)	19 (68)	21 (50)	11 (46)
SGA, %	7 (8)	4 (14)	3 (7)	1 (4)
ANS, %	76 (89)	24 (86)	38 (90)	22 (92)
APGAR 5 min	7 [5-8]	8 [6-8][b]	6 [5-7]	6 [5-7]
Pre-Extubation				
PMA, weeks	28 [26.7-29.1][a]	26.7 [26.1-27.4][b]	29 [27.4-29.4][c]	27.5 [27-28.5]
Weight, grams	1010 [858-1100][a]	825 [680-945]	930 [820-1030]	785 [705-950]
DOL	3 [2-5]	3 [2-6][b]	25 [13-29]	23 [14-28]
pH	7.33 [7.29-7.38]	7.36 [7.29-7.38]	7.34 [7.32-7.38][c]	7.3 [7.28-7.33]
PCO_2, mmHg	41 [35-46]	39 [34-45][b]	51 [45-55]	54 [47-59]
MAP, cmH_2O	6.5 [6-7.5]	7.1 [6.2-9.2]	6.9 [6.2-7.8]	7.4 [6.5-8.9]
FiO_2	0.21 [0.21-0.21][a]	0.24 [0.21-0.27]	0.24 [0.21-0.28]	0.26 [0.25-0.33]
NEC, %	0 (0)	0 (0)[b]	3 (7)	2 (8)
Infection, %	1 (1)	0 (0)[b]	8 (19)	8 (33)
PNS, %	1 (1)	0 (0)[b]	21 (50)	13 (54)
Outcomes at Discharge				
MV, days	3 [1-6][a]	22 [8-31]	27 [19-33][c]	37 [33-44]
Any RS, days	43 [30-57][a]	64 [38-82]	71 [60-83][c]	94 [80-103]
O_2, days	15 [1-46][a]	74 [34-101]	51 [23-99][c]	93 [70-106]
LOH, days	72 [52-91][a]	93 [80-114]	103 [92-123]	123 [107-136]
NEC, %	7 (8)	4 (14)	9 (21)	3 (13)
Infection, %	23 (27)	6 (21)	18 (43)	13 (54)
PNS, %	12 (14)[a]	12 (43)	25 (60)	19 (79)
IVH, %	21 (25)	4 (14)	16 (38)	15 (63)
ROP, %	11 (13)[a]	13 (46)	16 (38)[c]	17 (71)
BPD, %	23/82 (28)[a]	21/24 (88)	29 (69)[c]	22/23 (96)
Death/BPD, %	26 (31)[a]	25 (89)	29 (69)[c]	23 (96)

Legend: GA-gestational age; BW-birth weight; SGA-small for gestational age; ANS: antenatal steroids; PMA-postmenstrual age; MAP-mean airway pressure; FiO_2-fraction of inspired oxygen; NEC-necrotizing enterocolitis; PNS-postnatal steroids; MV-mechanical ventilation; RS-respiratory support; LOH-length of hospitalization; IVH-intraventricular hemorrhage; ROP-retinopathy of prematurity; BPD-bronchopulmonary dysplasia.
Results are expressed as n (%) or median [IQR]. [a] p<0.01 – Early success vs. early failure, [b] p<0.01 – Early failure vs. late success and [c] p<0.01 – Late success vs. late failure.

Figure 2. Adjusted odds of death or bronchopulmonary dysplasia

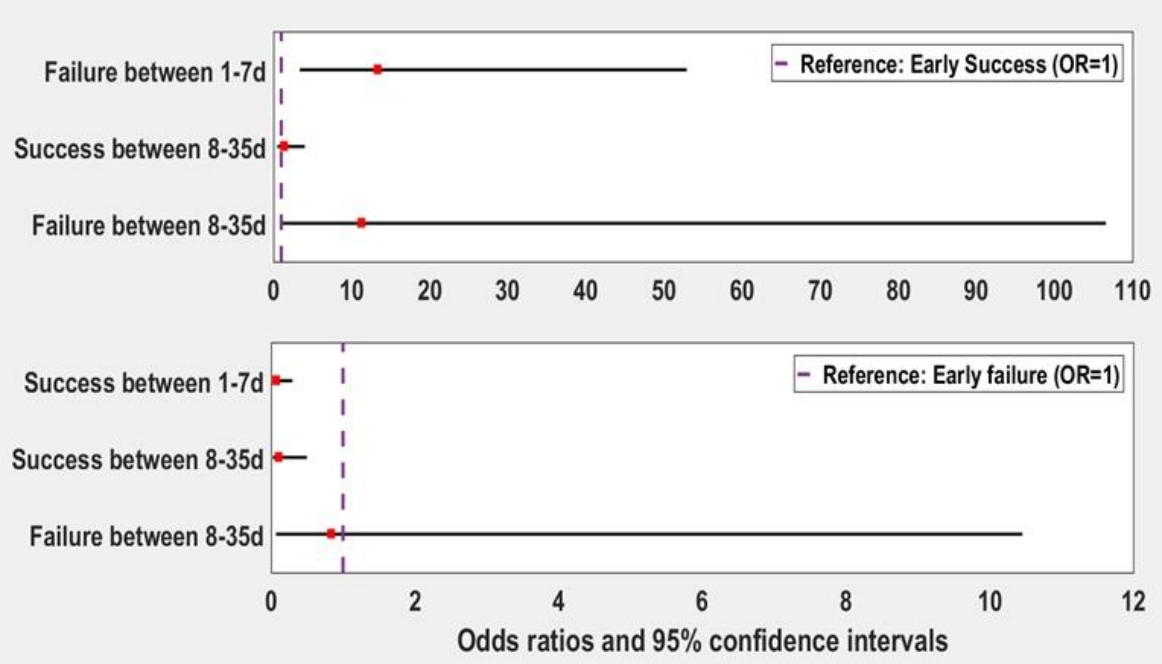


Table 2. Adjusted risk (multivariate linear regression) of secondary outcomes

	Reference: Early Success		Reference: Early Failure	
Duration of mechanical ventilation (days)	EF LS LF	4 (-6.9 – 14.9) 3.2 (-7.5 – 14) 15.7 (2 – 29.4)	ES LS LF	-4 (-14.9 – 6.9) -0.8 (-12.6 – 11.1) 11.7 (-2.4 – 25.8)
Duration of any respiratory support (days)	EF LS LF	2.1 (-11.7 – 15.9) -2.1 (-15.7 – 11.5) 3.3 (-14 – 20.6)	ES LS LF	-2.1 (-15.9 – 11.7) -4.2 (-19.1 – 10.8) 1.2 (-16.6 – 19.1)
Duration of oxygen therapy (days)	EF LS LF	18.9 (2.4 – 35.5) -5 (-21.2 – 11.2) 19.6 (-1.1 – 40.3)	ES LS LF	-18.9 (-35.5 – -2.4) -23.9 (-41.9 – -6.0) 0.7 (-20.7 – 22)
Length of hospitalization (days)	EF LS LF	-2.6 (-17.3 – 12.1) 1.3 (-13.2 – 15.7) 3.8 (-14.5 – 22.2)	ES LS LF	2.6 (-12.1 – 17.3) 3.8 (-12.1 – 19.8) 6.4 (-12.5 – 25.4)

Results are expressed as coefficient estimate (95% CI). Models were adjusted for gestational age, infection, postnatal steroid and necrotizing enterocolitis.

The Effect of Fenretinide on Regulatory B Cell Development and Semaphorin 4C in Allergic Asthma Model

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Allergic asthma is a chronic respiratory disease characterized by robust T_H2 immune responses. In this T_H2-driven inflammation, B cells secrete allergen-specific IgE that leads to hypersensitivity reactions. Independent of antibody secretion, regulatory B cells (Bregs) are a subset of B cells capable of suppressing inflammation through multiple mechanisms, including IL-10 production. Despite their importance in regulating immune responses, no known lineage-marker has been identified. Our laboratory discovered that B cell expression of Semaphorin 4C (S4C) was important for Breg development, and that S4C was downregulated in cells from our patients with either common variable immune deficiency or severe asthma. We have previously demonstrated that Fenretinide (FEN), a strong vitamin A derivative antioxidant, is capable of downregulating inflammation without affecting IgE levels in the blood. Here, we investigate the mechanism of FEN-induced regulatory immune responses using house dust mite mouse model of allergic asthma. We showed that FEN treatment *in vivo* improved lung resistance, increased regulatory B and T cell populations, and decreased IL-4-producing cells. Interestingly, FEN also induced S4C expression and a $Foxp3^+$ plasmablast subset, which has not been previously characterized. Preliminary *in vitro* results showed that primary splenic $CD19^+$ B cells cultured in the presence of FEN increased the $IL\text{-}10^+$ population compared to untreated controls. Importantly, $CD138^+Foxp3^-$ and $CD138^-Foxp3^+$ populations were detected using two different methods: nuclear staining and Foxp3-GFP. Elucidating the specific role of S4C in Breg development at the signaling level and phenotyping the functional role of the $Foxp3^+$ subsets using FEN are currently in progress.

Blood eosinophilia does not worsen clinical outcomes nor reflects tissue eosinophils in COPD

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Background Post hoc and retrospective analysis suggests that blood eosinophils, taken as a reflection of lung eosinophilia, predict worse outcomes in COPD and guide steroids treatment. However these assumptions have not been clearly demonstrated.

Aim To investigate prospectively: 1) Whether blood eosinophils predict outcomes in smokers with and without COPD; 2) whether blood eosinophils reflect lung tissue eosinophils.

Methods 512 smokers (303 with, 209 without COPD) were followed clinically, functionally and with blood eosinophils for at least 5 years. Using blood counts in 5 visits, subjects were categorized as persistently low eosinophils (<150/μL), persistently high eosinophils (≥150/μL) and variable eosinophils (<and≥150/μL). Blood eosinophils in 36 subjects undergoing lung resection were compared with lung tissue eosinophils in central airways, peripheral airways and lung parenchyma.

Results Blood eosinophil number and distribution was similar in smokers with and without COPD. In 5 blood samples 15% of smokers had eosinophils persistently low, 22% persistently high, and 63% variable. No difference was found among COPD subjects with high, low and variable eosinophils in: bronchodilator response (mean±SD:7±11 vs 6±11vs 6±10 %), FEV1 decline (23±38 vs 32±50 vs 34±47ml/year) and disease severity. The number of medically diagnosed exacerbations/year was similar in the three eosinophil groups (1.02±1.69vs0.76±1.66vs0.80±1.54). In COPD subjects a high number of eosinophils was associated with reduced mortality (Kaplan-Meier p=0.001). (Kaplan-Meier p<0.01). There was no difference in the median number of eosinophils/mm^2 in central airways (15, 4-39 vs 25, 6-114), peripheral airways (10, 1-20 vs 0, 0-13) and lung parenchyma (1, 0-3 vs 2, 0-9) in smokers with and without COPD, and tissue eosinophils did not change with disease severity. When all smokers were considered together, the number of eosinophils in lung parenchyma correlated with that in central airways (r=0.65 p=0.002). Blood eosinophils that were similar between smokers with and without COPD (151 vs 119 cells/μL), did not correlate with the number of tissue eosinophils in any of the three compartments.

Conclusions High blood eosinophils do not predict worse clinical outcome in COPD and might be beneficial. Blood eosinophils can't be assumed to reflect lung tissue eosinophilia.

Significance The use of blood eosinophils as a predictor of bad prognosis in COPD and targeted therapies against these cells should be carefully reassessed

FGF10 haploinsufficiency is associated with smaller airways lumen: implications for COPD susceptibility

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Introduction: Chronic obstructive pulmonary disease (COPD) is the third leading cause of death. Despite a 50% decline in cigarette smoking over the past 5 decades, the burden of COPD has not decreased, and one quarter of COPD cases occur among those who have never smoked. These observations suggest that identification of risk factors, beyond smoking, that contribute to COPD are needed. We recently demonstrated- by standard computed tomography (CT)- that there are two central airway branch variations: an accessory central airway branch variant and an absent central airway branch variant. These variations occur in one quarter of a large population and were replicated in two independent cohort (n=3,169 and n=2,746). These common central airway branch variants were associated with up to two-fold higher odds of prevalent COPD, and there was a genetic association between the absent central airway branch variant and fibroblast growth factor 10 (Fgf10). Fgf10 is required for the development of many branched organs, including the lungs, but the role of FGF10 in COPD is not completely known.

Hypothesis: Fgf10 haploinsufficiency is associated with smaller airway lumens, a feature that increase susceptibility to the development of COPD.

Methods: Fgf10 heterozygotes mice (*Fgf10*$^{+/-}$) were obtained from UCSF (Dr. Ophir klein). Airway structure was assessed using µCT, and images were visualized using standard multi-planar reformatting software (Osirix image). Airway function (Newtonian resistance, resistance and elastance) at baseline and after challenge with methacholine (0, 6.25, 12.5, 25 mg/mL) was measured using Flexivent.

Results: *Fgf10*$^{+/-}$ mice have narrowed airways comparing with the control mice. Moreover, we showed that *Fgf10*$^{+/-}$ mice has more airway resistance at baseline and during methacholine-induced bronchoconstriction.

Conclusion: We demonstrated that *Fgf10* haplo-insufficient mice exhibit the similar COPD structure-function phenotype that we previously observed in 2 cohorts (ie. narrowed airways). Variations in airway anatomy, due to genetic factors, could be thus a COPD risk factor and easily identifiable by CT.

Physiological and perceptual responses to exercise according to the locus of symptom limitation in patients with COPD

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We tested the hypothesis that patients with chronic obstructive pulmonary disease (COPD) who stop exercise due to breathlessness (B) vs. leg discomfort (LD) have greater pathophysiological abnormalities in dynamic respiratory mechanics & pulmonary gas exchange efficiency at end-exercise. We compared physiological & perceptual responses at the symptom-limited peak of cycle exercise testing in 41 patients reporting B (30M; mean±SD FEV_1, 48.9±22.9%$_{pred}$) and 20 patients reporting LD (19M; FEV_1, 63.5±22.7%$_{pred}$, $p<0.05$ vs. B) as their main exercise-limiting symptom. Intensity ratings of breathlessness and leg discomfort were significantly higher (5.9±2.1 vs. 3.7±1.5 Borg units, $p<0.0001$) and lower (3.5±2.3 vs. 5.6±2.0 Borg units, $p=0.001$) at end-exercise in B vs. LD, respectively. Patients reporting B vs. LD as their main exercise-limiting symptom exhibited lower levels of cardiorespiratory fitness (VO_{2peak}, 64.8±21.4 vs. 70.3±31.5%$_{pred}$, $p=0.424$), and greater dynamic lung hyperinflation, as evidenced by a lower peak inspiratory reserve volume (0.45±0.42 vs. 0.78±0.37 L, $p=0.004$) and a more dramatic decline in inspiratory capacity from rest to end-exercise (-0.47±0.83 vs. -0.40±0.67 L, $p=0.741$). Measures of pulmonary gas exchange efficiency at end-exercise, including blood O_2 saturation (94.5±18.5 vs. 98.0±9.3%) and the ventilatory equivalent for CO_2 (37.5±7.35 vs. 36.6±6.69), were similar between-groups (both $p<0.05$). Results of this study suggest that impaired dynamic respiratory mechanics is likely the principal pathophysiological factor limiting exercise tolerance in patients with COPD reporting B as their main exercise-limiting symptom. Although additional research is required, these findings have potentially important clinical implications in identifying (or 'phenotyping') patients whose exercise tolerance would be best optimized by improving respiratory mechanics (e.g., bronchodilator therapy) vs. skeletal muscle function (e.g., rehabilitative exercise training).

Implications of HuR in Idiopathic Pulmonary Fibrosis

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Background: Idiopathic Pulmonary Fibrosis (IPF) is a chronic and relentlessly progressive fibrotic lung disease affecting the lower respiratory tract. The precise factors that cause IPF are still unknown. Risk factors such as older age and smoking have been identified, however they do not explain the extensive remodelling and progressive nature of the disease. IPF is characterized by the accumulation of fibrous connective tissue that leads to abnormal lung architecture. IPF pathogenesis is proposed to be driven by the aberrant differentiation of fibroblast into myofibroblasts, which leads to excess extracellular matrix (ECM) deposition resulting in scar tissue formation, stiffening of the lung and compromised lung function. The mechanisms that regulate this uncontrolled differentiation remain poorly understood. Recent studies have demonstrated that fibroblast differentiation into myofibroblasts occur following a metabolic shift to anaerobic glycolysis, which can be caused by hypoxia. Taken together, we believe that a hypoxia-induced metabolic shift to anaerobic glycolysis may contribute to IPF pathogenesis. However, how this hypoxia-induced metabolic shift is regulated at the molecular level is unknown. It has been previously reported that the RNA-binding protein Human Antigen R (HuR), stabilizes Hypoxia Inducible Factor (HIF-1α), a transcription factor known to be involved in the regulation of hypoxic-induced anaerobic glycolysis. Furthermore, in liver fibrosis, HuR targets mRNA that are relevant to the progression of IPF. Therefore, we hypothesize that the HuR may play a role in regulating a hypoxia-induced metabolic shift to anaerobic glycolysis and myofibroblast differentiation.

Aims: This hypothesis will be assessed through the following 3 aims:
1) Assess the effect of hypoxia on HuR expression and subcellular localization**.**
2) Investigate the role of HuR in hypoxia-induced metabolic reprogramming**.**
3) Investigate the role of HuR in hypoxia-induced myofibroblast differentiation.

Methods: To evaluate the effect of hypoxia on HuR, metabolic reprogramming and myofibroblast differentiation, Human Lung Fibroblasts HLF will be incubated either in standard *incubator* (normoxia) or in hypoxia chamber. The optimal conditions of hypoxia (O_2% and duration) will be determined by looking at the appropriate markers for each process. **For aim 1)** HuR mRNA and protein levels will be measured by qPCR and Western Blot respectively. The localization of HuR will be assessed by Subcellular Fractionation followed by Western Blot. **For aim 2)** The effect of hypoxia on metabolic reprogramming will be evaluated by several approaches. First we will measure the level of lactic acid in the cells using Lactate assay kit. Second, the expression of lactate dehydrogenase (LDH) and HIF-1α will be evaluated by qPCR and western blot. Finally, SeaHorse XF96e Bionanalyzer assay will be used to monitor cell metabolism, extracellular acidification rate (a measure for glycolysis) and cellular oxygen consumption. **For aim 3)** First we will evaluate the effect of hypoxia on my myofibroblast differentiation by measuring the expression of α-SMA, collagen and $TGFB_1$ by Western Blot and ELISA assays. Moreover, the scratch assay will be used to measure migration of myofibroblasts. Finally, **for aims 2) and 3)** to investigate the role of HuR in hypoxia-induced metabolic reprogramming and myofibroblast differentiation, HuR expression will be knocked-down using siRNA. The expression of aforementioned markers will then be measured during normoxia/hypoxia and compared to that of cells transfected with non-targeting siRNA.

Expected Results: The expected observations are that HuR will play a role in the metabolic reprogramming and myofibroblast differentiation seen in IPF. Possible outcomes may show that HuR only has appreciable effects solely within the pathway that controls differentiation or solely in the pathway that controls reprogramming.

Significance: In Canada, the incidence rate of IPF is 18.7 per 100,000 and is associated with a poor prognosis. IPF pathogenesis is currently not completely understood and the cause remains to be unknown, further highlighting the importance of additional research in this disease. This project proposes a novel pathological mechanism in fibrosis through the involvement of HuR, which presents the potential for therapeutic intervention. In addition, HuR expression and localization could potentially be used as a biomarker for detection and/or progression of IPF.

Automatic Respiratory Gating for Contrast-enhanced ultrasound Parametric Functional Imaging using Machine Learning

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Multi-parametric functional imaging (PFI) based on dynamic contrast-enhanced-ultrasound (DCEUS) is increasingly used to characterize the hemodynamic features of abdominal tumors. However, its accuracy is limited by out-of-plane severe 3D distortion induced by respiratory motion. This study developed a fully automatic respiratory gating scheme by using principal component analysis to remove distortions and disturbances in free-breathing DCEUS-based PFI, which was validated through *in vitro* and *in vivo* perfusion experiments.

Taking the known and controllable *in vitro* perfusions in a 3D rotary distortion flow model as ground truths, the proposed scheme's accuracy was evaluated and compared with results obtained from non-negative matrix factorization and independent component analysis. Compared with those without respiratory gating, the signal-to-clutter ratio and correlation coefficients of PFI with respiratory gating improved by 3.99 ± 1.71 dB ($p < 0.01$) and 0.39 ± 0.17 ($p < 0.01$), respectively; the corresponding mean square error decreased by 1893.9 ± 763.16 ($p < 0.001$); which were significantly consistent with the ground truths without respiratory motion disturbances. The continuity and visualization of *in vivo* arterioles in liver and spleen were clearly enhanced, and their perfusion details were also accurately characterized by PFIs with respiratory gating.

Quantitative results demonstrated that the out-of-plane serious distortion and other negative disturbances induced by the respiratory kinetics were effectively removed, and the accuracy and robustness of DCEUS-based PFI were significantly improved by the proposed scheme. The proposed automatic scheme benefits clinicians in providing accurate diagnoses and in developing appropriate therapeutic strategies for abdominal diseases.

Factors affecting patients' response to anti-IL5 monoclonal antibodies treatment in severe eosinophilic asthma

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Background: Although Mepolizumab, an anti-IL5 monoclonal antibody, has been approved as a maintenance treatment for severe asthmatics with elevated serum eosinophils, a number of patients don't respond optimally to this treatment in our practice despite otherwise meeting criteria for initiating treatment. The primary objective of this study is to find patient characteristics that could predict poor response to Mepolizumab in patients with eosinophilic asthma.

Method: We completed a retrospective chart review of all asthmatic patients who are currently or have been treated with Mepolizumab since January 2016. Patients who have stopped Mepolizumab due to failure to improve asthma exacerbations rates or to reduce of dose of systemic corticosteroids use were identified as non-responders. We have collected information regarding serum eosinophils levels, body mass index (BMI), pre-treatment forced expiratory volume in one second (FeV1), presence of sinus disease and presence of obstructive sleep apnea (OSA) in these patients. These variables were compared between responders and non-responders to identify factors that could influence response to Mepolizumab.

Results: 55 patients were included with 15 patients identified as non-responders. The mean serum eosinophil level was 0.80 x 10^9/L and 0.65 x 10^9/L in non-responders with a mean difference of 0.15 x 10^9/L (CI: -0.28 , 0.58). The mean BMI was 26.5 kg/m^2 in responders and 31.6 kg/m^2 in non-responders with a mean difference of -5.1 kg/m^2 (CI: -10.9 , 0.74). The mean FeV1 was 1.71 L in responders and 1.93 L in non responders with a mean difference of -0.22 L (CI: -0.56 , 0.13). 17 patients had nasal disease in the responder group (42%) while 5 patients had nasal disease (33%) in the non-responder group with an OR of 1.47 (CI: 0.67 , 3.25). 9 patients had OSA in the responder group (23%) while 7 patients had nasal disease in the non-responder group (47%) with an OR of 0.33 (CI: 0.15 , 0.74).

Conclusion: Presence of OSA is associated with poor response to Mepolizumab in asthmatics otherwise meeting criteria for initiating an anti-IL5 monoclonal antibody.

8 and 16 Weeks of Chronic Tobacco Smoke Exposure Negatively Impacts Morphological Characteristics of Motor Axons and Neuromuscular Junctions in the Diaphragm of Mice

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Chronic tobacco smoke (TS) exposure is the leading cause of preventable disease worldwide. Importantly, TS-related diseases are accompanied by similar skeletal muscle abnormalities. These abnormalities manifest differently in the diaphragm than in locomotor muscle, yet both contribute to poor patient outcomes. Recent findings have implicated TS-induced denervation as a mechanism contributing to skeletal muscle abnormalities; however, these results were only examined in locomotor muscle. As such, the objectives of this study were to resolve the impact of TS exposure on morphological features of diaphragm motor axons and neuromuscular junctions (NMJ), and to investigate whether this impact is progressive in nature. 15-wk-old male C57Bl/6 mice were randomly assigned to 8 or 16 weeks of TS exposure, with respective control groups being exposed to ambient air. 8 and 16 weeks of TS exposure attenuated body growth ($p<0.0005$). 8 weeks ($p<0.0001$), but not 16 weeks, of TS exposure reduced muscle mass normalized to respective controls in all muscles harvested. The diaphragm underwent a muscle mount protocol involving immunofluorescent staining to visualize motor axons and NMJs. Subsequent imaging by confocal microscopy unveiled an increased fraction of NMJs lacking axonal input ($p<0.05$) and/ or pre-synaptic nerve terminals (characterized as denervation) ($p<0.005$) in both TS-exposed groups. Motor axon diameter was reduced following 16 weeks ($p<0.05$), but not 8 weeks, of TS exposure. Reduced compactness of AChR structures ($p<0.005$) in the absence of AChR fragmentation was observed in both TS-exposed groups, but was exacerbated in 16-wk-exposed mice. Our results illustrate for the first time that TS exposure negatively impacts specific morphological characteristics of motor axons and NMJs in the diaphragm muscle, and that aspects of this impact are progressive in nature. As such, future therapeutic interventions should target TS-induced denervation to mitigate diaphragm abnormalities, and the associated consequences, in patients with TS-related diseases.

CD109 is a cell-extrinsic regulator of cutaneous IL-17 producing γδT cells

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Objectives: IL-23/IL-17 immune axis is a central network in the progression of psoriasis. CD109 has been shown to be downregulated in psoriatic lesions, yet its impact on the IL-23/IL-17 immune axis is unknown. Here we hypothesized that CD109 is a negative regulator of cutaneous IL-23/IL-17 in the context of psoriasis-like disease.

Methods: Comparing wild-type and CD109-deficient mice, we used in vitro and in vivo approaches to assess changes in the IL-23/IL-17 immune axis at steady-state and during imiquimod-induced dermatitis, a mouse model of psoriasiform inflammation. Unbiased transcriptional profiling of skin samples was performed using Nanostring gene expression technology and cytokine production was assessed by intracellular cytokine staining and ELISA. Bone marrow chimeras and cytokine neutralization studies were performed to identify the cellular source of CD109 and the relevant cytokines driving cutaneous inflammation.

Results: Here we show that, compared with wild-type mice, CD109 deficient mice exhibit spontaneous epidermal hyperplasia and selective activation of the IL-23/IL-17 immune axis including aberrant neutrophil recruitment and IL-23-dependent accumulation of dermal and epidermal IL-17 producing γδT cells. In addition, CD109-deficient mice are significantly more sensitive to imiquimod-induced skin inflammation. Using co-culture studies and bone marrow chimeras we demonstrate that CD109 acts in a STAT3-dependent, but cell-extrinsic manner to regulate IL-17 production by γδT cells.

Conclusions: Our studies have revealed CD109 as an endogenous regulator of skin homeostasis and the IL-23/IL-17 immune axis. Further, we demonstrate that expression of CD109 limits IL-17 production in a T cell-extrinsic manner and controls psoriasiform inflammation. Studies are now underway to dissect the specific cellular and molecular pathways in which CD109 acts to maintain tissue homeostasis and control the cutaneous inflammatory response.

Lung Cancer in the Inuit Region of Nunavik, Québec: Descriptive Epidemiology of Patients Diagnosed between 2005 and 2016

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Rationale
Canada's Inuit have the highest lung cancer incidence worldwide, but knowledge remains limited about their demographics, cancer histologies, and outcomes. All Nunavik lung cancer patients are diagnosed and treated at the McGill University Health Centre (MUHC) in Montreal. We used the MUHC lung cancer registry to describe these patients and to compare those from Hudson (population 7,366) and those from Ungava (population 6,135), the two sub-regions in Nunavik, as well as between patients from Nunavik and Montreal.

Methods
We included all adult patients newly diagnosed with primary lung cancer between January 1st, 2005-December 31st, 2016, who resided in Nunavik or Montreal, excluding those diagnosed outside the MUHC. We ascertained vital status through the provincial health insurance registry, regardless of cause or time since diagnosis. We performed descriptive statistics and univariable comparisons using Chi-square, Fisher's, or Wilcoxon tests.

Results
Amongst 91 patients from Nunavik, median age was 68 years and 40/91 (44%) were women. The Hudson sub-region accounted for 67/91 (74%) of Nunavik's lung cancer cases. Age, histologic types, and mortality amongst patients were similar between Hudson and Ungava, whereas fewer were women in the Hudson sub-region (Hudson, 25/67, 37%; Ungava, 15/24, 63%; p=.03). Of Montreal's lung cancer patients, 1477/3134 (47%) were women (p=.55 versus Nunavik) and median age was 70 years (p=.02 versus Nunavik). In terms of histology, Nunavik's patients were more likely to have small cell carcinoma (23% vs 10%; p<.001), and squamous cell carcinoma (42% vs 18%; p<.001), and less likely to have lung adenocarcinoma (18% vs 47%; p<.001). The proportion with Stage III or IV disease in Nunavik (46/72, 64%; missing=19) was similar to Montreal (1582/2404, 66%; p=.70; missing=730). Crude mortality was higher in Nunavik (77/91, 85%) versus Montreal (2274/3134, 73%; p=.01).

Conclusion
We observed differences in patients' gender distribution between Hudson and Ungava. Moreover, Nunavik patients were younger than Montreal patients, had different histologic subtypes, and possibly, higher all-cause mortality. Further research is being done to explore these differences and compare diagnostic and treatment trajectories.