AllerGen Poster Competition

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ALLERGEN POSTER COMPETITON

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Abstract #61

DEEP TCR REPERTOIRE SEQUENCING REVEALS RELATIVE CHANGE IN PEANUT SPECIFIC CLONOTYPE IN SUBJECTS UNDERGOING RUSH ORAL IMMUNOTHERAPY

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BACKGROUND
Oral immunotherapy is an emerging therapy currently under investigation for the treatment of food allergy [1]. Underlying mechanisms are thought to involve a switch in the food specific T cell response from Th2 to eitherTh1, Tr1 and/or Treg. It is unknown whether this change in response results from re-education of existing pathological food-specific T cells or from their replacement by new healthy T cells (change of guard hypothesis).

METHODS
The objective was to evaluate the clonal distribution of peanut specific T cell in subjects with peanut allergy and follow changes in clonotype with treatment using a high-throughput T cell receptor (TCR) sequencing platform. Peripheral blood mononuclear cells (PBMCs) from three subjects undergoing rush oral immunotherapy in a previous trial [2] and three control subjects on avoidance diet were cultured with peanut extract at baseline and at 9 and 18 months. Carboxyfluorescein succinimidyl ester (CFSE)-low peanut proliferating T cells were then isolated by fluorescence-activated cell sorting (FACS) and TCR analysis was performed.

RESULTS
The CFSE-low proliferating fraction was found to be comprised of between 2,000 and 12,000 different T cell clones. However, only between 15 and 25% of proliferating T cells (from 100-400 different clones) were consistently found at all three time points and probably represented true peanut-specific T cells. While the relative frequency of these peanut-specific clones was stable over time in subjects on avoidance diet (R=0.633 to 0.760), it was found to change in subjects undergoing oral immunotherapy (R= 0.123 to 0.350), following two characteristic patterns.

CONCLUSIONS
Using a deep TCR sequencing platform, we found that only a fraction of CFSE-low peanut proliferating T cells were consistent in time and likely to represent true peanut specific T cells. Oral immunotherapy was associated with changes in relative frequency of clones within this fraction which would support the change of guard hypothesis.

ACKNOWLEDGEMENTS
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Abstract #62

IMPACT OF AIR POLLUTION ON PHYSICIAN OFFICE VISITS FOR COMMON CHILDHOOD CONDITIONS IN ONTARIO, CANADA

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BACKGROUND

Children are particularly sensitive to air pollutants, due to factors such as ongoing lung development and choice of activities [1]. We evaluated the impact of fine particulate matter ($PM_{2.5}$) on physician office visits for common conditions in children in Ontario, Canada.

METHODS

$PM_{2.5}$ and temperature measurements were obtained from satellite data for all of Ontario [2]. Physician office visits were stratified into two groups based on the literature: air pollution-sensitive (acute respiratory infections, allergic rhinitis, asthma, bronchiolitis, diabetes, otitis media) and air pollution-insensitive (gastroenteritis, injuries). Claims data were obtained for every month in 2010 from health administrative databases for children 0-14 years of age. Age- and sex-standardized morbidity ratios (SMRs) were calculated by region in Ontario. Spatial Poisson regression models were used to analyze the relationship between $PM_{2.5}$ and physician office visits, with temperature as a covariate.

RESULTS

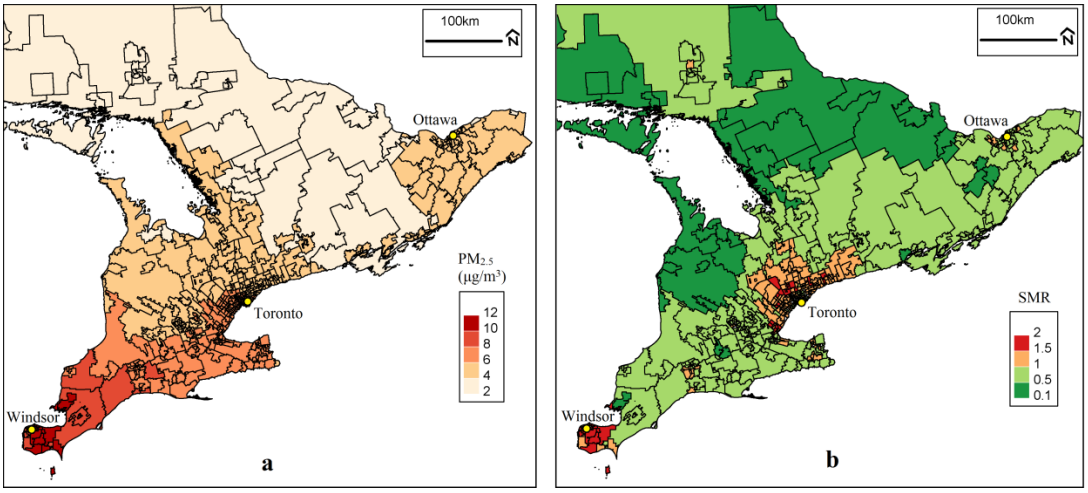
Crude rates of physician office visits are presented in Table 1. As expected, fine particulate was significantly associated with monthly rates of physician office visits for air pollution-sensitive conditions, and not for insensitive conditions. Fitted SMRs for air pollution-sensitive conditions are presented in Figure 1. SMRs for sensitive and insensitive conditions were strongly positively correlated (r = 0.53), and data were spatially autocorrelated. This suggests an underlying spatial process that influences physician office visit rates for common childhood conditions, both for air pollution-sensitive and -insensitive conditions.

Table 1. Crude rates of air pollution-sensitive and air pollution-insensitive conditions in Ontario for each month in 2010

	Crude rates of physician office visits[a]											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec
Air pollution-sensitive	8.05	8.84	8.94	7.80	7.21	6.55	5.15	4.73	6.57	7.52	9.27	10.89
Air pollution-insensitive	1.48	1.52	1.61	1.55	1.63	1.63	1.34	1.29	1.34	1.38	1.54	1.22

[a]Number of claims per 100 population aged 0-14 years.

Figure 1. Distribution of (a) fine particulate matter ($PM_{2.5}$, in $\mu g/m^3$) and (b) fitted sex-standardized morbidity ratios (SMRs) from spatial Poisson regression for physician office visits for air pollution-sensitive conditions; all by Forward Sortation Area (FSA) in Southern Ontario in July 2010



CONCLUSIONS

In this analysis, $PM_{2.5}$ was significantly associated with physician office visits for air pollution-sensitive conditions. Areas with high $PM_{2.5}$ levels and SMRs higher than 1 were identified; children with air pollution-sensitive conditions in these areas may benefit from targeted air pollution reduction interventions. Additionally, future analysis should evaluate the role of household income and access to care in influencing the spatial pattern of primary health care utilization for common childhood conditions across Ontario.

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Abstract #63

TOBACCO SMOKE INDUCES CHANGES IN IL-1 FAMILY IN BRONCHIAL EPITHELIAL CELLS OBTAINED FROM ASTHMATIC INDIVIDUALS

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BACKGROUND

Exposure to tobacco smoke (ETS) induces epigenetic modifications including DNA methylation [1]. In asthma, it has been shown that those modifications affect immune cell differentiation by down regulating expression of specific pro-inflammatory cytokines [2-4]. Interleukin 1 (IL-1) is recognized to be increased in asthma [5] and by cigarette smoke [5, 6]. Based on previous genetic association [7, 8] and DNA methylation signature of receptors in asthma and/or atopy the aim of this study is to evaluate the changes in expression and methylation pattern induced by ETS for IL-1 subunit alpha (*IL-1A*) and beta (*IL-1B*), receptors type I (*IL-1R1*), type II (*IL-1R2*) and antagonist (*IL-1RA*) and for interleukin 33 (*IL-33*) in lung tissue.

METHODS

Primary epithelium cells isolated from bronchial biopsies of mild asthmatics and non-asthmatics individuals were exposed to whole tobacco smoke according to method described [9]. Level of mRNA was measured by qRT-PCR and methylation was assessed by bis-pyrosequencing for *IL-1A*, *IL-1B*, *IL-1R1, IL-1R2, IL-1RA* and *IL-33*.

RESULTS

ETS increased mRNA level of *IL-1A and IL-1B* in both asthmatic and non-asthmatic individuals. *IL-33* showed a significant decrease in gene expression following ETS in asthmatic individuals but not in non-asthmatics. *IL-1R1* was decreased in non-asthmatic individuals but no change was observed in asthmatics. *IL-1R2* and *IL-1RA* increased in both asthmatic and non-asthmatic individuals. We observed DNA methylation differences in *IL-1R1* promoter between ETS and non-ETS cells.

CONCLUSIONS

Modifications of genes expression induced by tobacco smoke could modify IL-1 family resulting in an increase of inflammation in lung tissues of asthmatic and non-asthmatic individuals. These changes may be induced by DNA methylation. Efforts to better interpret and integrate data from genetics and epigenetics are needed to better understand the biology of asthma as well as a better comprehension of the impact of tobacco smoke in the inflammatory component of asthma.

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Abstract #64

LINEAGE SPECIFIC ROLE OF *SHIP1* IN DEVELOPMENT OF ALLERGIC AIRWAY INFLAMMATION

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BACKGROUND
The PI3K pathway is a potent mediator of several functions associated with asthma pathogenesis, including supporting leukocyte survival, activation, migration and cytokine release. Proper negative regulation of this pathway is integral in order to restrict overactive immune responses. Negative regulation of PI3K is predominantly controlled by the lipid phosphatases PTEN and SHIP-1. *Inpp5d* (*Ship1*) deficient mice develop spontaneous airway inflammation and have enhanced sensitivity to allergen induced airway inflammation. We hypothesized that deleting *Ship1* expression specifically in lineages known to be crucial for adaptive Th2 responses would uncover more subtle effects that could either positively or negatively regulate disease severity in a mouse model of allergic airway inflammation (AAI).

METHODS
Ship1 expression was deleted in B cell, T cell and dendritic cell (DC) lineages and the resulting $Ship1^{\Delta B\ cell}$, $Ship1^{\Delta T\ cell}$ and $Ship1^{\Delta DC}$ mice were exposed to house dust mite (HDM) antigen over an 18-day period. Infiltrating leukocytes in the bronchoalveolar lavage (BAL) and lung, serum antibody levels and Th1 and Th2 cytokine responses were quantified to assess disease severity.

RESULTS
Deletion of *Ship1* in either the B cell, T cell or DC lineages did not result in spontaneous airway inflammation, and loss of *Ship1* in the B cell linage did not affect HDM-induced AAI. Surprisingly, loss of *Ship1* in either of the T cell or DC lineages protected from development of AAI by skewing the HDM-induced immune response to a Th1 phenotype instead of the characteristic Th2 phenotype associated with allergic asthma.

CONCLUSIONS
While loss of *Ship1* expression throughout the hematopoietic populations leads to spontaneous lung inflammation, selective deletion of *Ship1* in T cells and DCs impairs the formation of an adaptive Th2 response and protects from the development of AAI.

ACKNOWLEDGEMENTS
This work was supported by AllerGen NCE Inc. and the Canadian Institutes of Health Research (CIHR).

Abstract #65

CO-EXPOSURE TO ALLERGEN AND DIESEL EXHAUST ENHANCE INFLAMMATORY RESPONSES IN HUMAN AIRWAY SUBMUCOSA

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BACKGROUND

Asthma is a chronic condition described by inflammation of the airways and lungs. Diesel exhaust (DE) is a major contributor to ambient particulate matter (PM) air pollution. There is rising evidence that PM acts as adjuvant on the immune responses and may lead to augmentation of allergic inflammation [1, 2]. We aim to elucidate if DE increases allergen-induced inflammation and cellular immune response in the airways of atopic human subjects.

METHODS

15 volunteer participants with allergy to house dust mite allergen (Der p 1), birch or Timothy grass were recruited. In a randomized fashion, subjects inhaled DE (300µg $PM_{2.5}/m^3$) or filtered air for 120 minutes. One hour following the exposure, the extract of an aeroallergen to which the individual is sensitive, or placebo (sterile saline), was instilled into contralateral lung segments through bronchoscopy. Endobronchial biopsies from these same segments were then acquired 48 hours after each exposure. This was repeated 4 weeks later in each subject with the alternative inhalant. Thus, biopsies under 4 different conditions were created: filtered air + saline (FAS), DE + saline (DES), filtered air + allergen (FAA) and DE + allergen (DEA). Biopsies were processed and embedded in glycol methanlacrylate acrylic resin and serial sections were cut to 2µm and used for immunostaining with monoclonal antibodies to tryptase and eosinophil cationic protein (ECP). The percent positivity and distribution of activated mast cells (tryptase+) and eosinophils (ECP+) were quantified in the bronchial submucosa by Aperio ImageScope software.

RESULTS

The percent positivity for tryptase expression: FAS=0.54±0.05, DES=0.51±0.18, FAA=0.63±0.24, DEA= 0.94±0.23. The percent positivity for ECP expression: FAS=0.35±0.17, DES=0.38±0.11, FAA=0.61±0.14, DEA=0.73±0.33. Data are presented as mean ± SEM (n=6).

CONCLUSIONS

Our preliminary data suggest that DE may enhance the inflammatory response to allergen in atopic individuals. This data is novel in the context of human lung tissue.

ACKNOWLEDGEMENTS

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Abstract #66

TRANSCRIPTIONAL NETWORKS IN WHOLE BLOOD OF ASTHMATICS

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BACKGROUND

Allergen inhalation challenge causes significant changes in the blood transcriptomes of mild atopic asthmatic individuals [1]. Systems biology approaches have been used to identify transcriptional networks that reflect underlying disease processes. Such networks provide insights into the correlation patterns among genes and may identify specific molecular processes associated with asthmatic responses. Transcriptional networks can be identified using blood expression data and are associated with allergen-induced asthmatic responses.

METHODS

14 participants (8 early responders and 6 dual responders) with mild, atopic asthma underwent a cat allergen inhalation challenge as part of the AllerGen Clinical Investigator Collaborative. The subjects' whole blood samples were collected immediately prior to allergen challenge (pre-challenge) and 2 hours after the challenge (post-challenge). Whole blood transcriptional profiling was performed using Affymetrix GeneChip® Human Gene 1.0 ST Arrays. The correlation networks (modules) were identified using weighted gene correlation network analysis (WGCNA) [2]. Pathway analysis was performed using GeneGo.

RESULTS

21,727 mRNA transcripts were profiled across 28 samples (14 pre- and 14 post-challenge). Highly expressed mRNA transcripts (10,044, mean expression > log (base2) 6) were retained for WGCNA. WGCNA identified nine modules many of which were associated with various immune cell-types. A gene module consisting of 384 genes was significantly (p=0.0008) associated with the late phase asthmatic response and also significantly (p=1e-07) correlated with the compositional abundance of T cells. Pathways analysis of these genes indicated T cell receptor signaling pathway, TCR and CD28 co-stimulation in activation of NF-kβ and ICOS pathway in T-helper cells as the top significant pathways (FDR=10%).

CONCLUSION

Transcriptional networks can be identified using whole blood expression data. Many transcriptional networks were associated with various cell-types frequencies, which may indicate the role of cell-specific gene expression in the development of asthmatic responses. Validation of these transcriptional networks will be performed using a larger asthma blood expression dataset.

ACKNOWLEDGEMENTS

This research is supported by AllerGen NCE Inc.

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Abstract #67

IL-4 AND IL-13 REGULATE TLR EXPRESSION AND EOSINOPHIL-BASOPHIL DIFFERENTIATION OF CORD BLOOD CD34$^+$ PROGENITOR CELLS

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BACKGROUND
Intrauterine environmental exposures have been shown to influence neonatal immunity and subsequent allergic disease development (1). We have previously shown that cord blood (CB) progenitor cells of high atopic risk infants have reduced toll-like receptor (TLR) expression and produce fewer lipopolysaccharide (LPS)-stimulated eosinophil-basophil (Eo/B) colonies, compared to low-atopic risk infants. In the present study, we investigated whether a surrogate *ex vivo* T_H2 milieu (i.e., either IL-4 or IL-13), could represent an underlying mechanism to explain our previous findings.

METHODS
CB CD34$^+$ cells from healthy donors were cultured with IL-4 or IL-13 (in combination with LPS) and assessed for TLR-2, TLR-4, and TLR-9 expression using flow cytometry, as well as Eo/B differentiation using methylcellulose cultures. Pharmacological inhibitors were added to the methylcellulose cultures to determine the effect of blocking IL-4 or IL-13 signalling in CB CD34$^+$ cells in relation to Eo/B colony forming unit (CFU) formation.

RESULTS
Stimulation of CD34$^+$ cells with IL-4 or IL-13 trended to decreased expression of TLR-2 ($p=0.063$), whereas IL-4, but not IL-13, reduced Eo/B CFU formation in the presence of LPS. The latter was found to be dependent on IL-4Rα and not IL-13Rα1.

CONCLUSIONS
Thus, the responsiveness of CB CD34$^+$ progenitor cells to LPS is differentially regulated by the T_H2 cytokines, IL-4 and IL-13, and may be related to TLR expression on these cells. Therefore, *in utero* interactions between placental-derived pro-allergic cytokines and neonatal progenitor cells influences CD34$^+$ phenotype and function, with implications for Eo/B-mediated inflammatory responses in early life.

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Abstract #68

BLOOD BIOMARKERS OF THE LATE PHASE ASTHMATIC RESPONSE USING RNA-SEQ

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BACKGROUND

Asthmatic individuals respond differently, but reproducibly, to allergen inhalation challenge. Some individuals develop an isolated early response (ER), while others develop a dual response (DR). Peripheral blood cell transcriptome signatures can discriminate isolated early responders from dual asthmatic responders undergoing allergen inhalation challenge.

METHODS

35 individuals (17 ERs and 18 DRs) participated in the allergen inhalation challenge. Blood samples were obtained prior to and 2 hours post allergen inhalation challenge. HiSeq-Illumina paired-ends 100bp sequencing was performed. A UCSC transcriptome using both the UCSC gene and gene-isoform transcripts was created using the RSEM (RNA-Seq by Expectation Maximization) package. RSEM uses Bowtie to align read files to the reference transcripts and estimates the expected number of counts per transcript. The biomarker pipeline consisted of 20x5-fold deep cross-validation using limma voom (linear models for microarrays and RNA-Seq using variance modeling at the observational level) for differential expression and elastic net for classification. Gene set enrichment analysis was performed using GeneGo.

RESULTS

Classification performance of the pre-challenge classifier across the 100 panels included an AUC of 0.76±0.02, a sensitivity of 0.70±0.03 and a specificity of 0.72±0.02. There were 511 gene transcripts identified across the 100 panels. Pathway analysis of the 511 gene transcripts identified lectin induced complement pathway, integrin inside-out signaling, alternative complement pathway and function of MEF2 in T lymphocytes as the top ranked pathways. Classification performance of the post-challenge classifier across the 100 panels included an AUC of 0.62±0.002, a sensitivity of 0.67±0.002 and a specificity of 0.54±0.003. There were 301 gene transcripts identified across the 100 panels. Pathway analysis of the 301 gene transcripts identified clathrin coated vesicles formation, CDC42 in cellular processes and regulation of actin cytoskeleton by Rho GTPases as the top ranked pathways.

CONCLUSION

The pre-challenge classifier out-performed the post-challenge classifier in the internal deep cross-validation as depicted by the classification performance measures. The lower performance of the post-challenge classifier in discriminating ERs from DRs may be due to a dilution of signal, given that both responder groups are undergoing an immune response to allergen inhalation. Incorporating changes in cellular composition and gene isoform expression estimates may improve classification performance and also reveal new insights into the mechanisms of the late phase asthmatic response.

Abstract #69

CHARACTERIZATION OF IGE RECEPTOR EXPRESSION IN ASTHMATIC AND NON-ASTHMATIC AIRWAY EPITHELIAL CELLS

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BACKGROUND
Bronchial asthma is an immune disease in which cytokines and IgE play important roles. Respiratory syncytial virus (RSV) is one of the causes of bronchiolitis and viral-induced asthma, especially in young children. Viral infections can shift the Th1/Th2 balance in the airway epithelium thus affecting the expression of IgE receptors, high affinity receptor FcεRI and low affinity receptor FcεRII (CD23). FcεRI is a multi-chain receptor comprising of four polypeptide chains: one alpha (α), one beta (β), two gamma (γ) units, whereas FcεRII has two isoforms: CD23a and Cd23b. We have previously shown that RSV infections increase IL-33 and ST-2 expression in airway epithelium. In this study we examined the expression for IgE receptors in airway epithelial cells after viral infection and cytokine stimulation.

METHODS
Confluent monolayers of primary human airway cells were infected with RSV or exposed to IL-33. Total RNA and protein lysates were collected for qPCR and western blotting to examine IgE receptor expression. Membrane expression of IgE receptors was quantified using flow cytometry.

RESULTS
At baseline, asthmatic cells demonstrated significant increase in FcεRIβ and CD23b mRNA expression and FcεRI α protein expression compared to non-asthmatic cells. Exposure to IL-33 and RSV regulated the mRNA expression for both FcεRI and FcεRII/CD23 compared to untreated controls. Asthmatic cells demonstrated further increase in FcεRI and FcεRII mRNA expression with challenges compared to non-asthmatic cells. IL-33 and RSV co-exposure increased the FcεRI γ surface protein expression compared to untreated controls.

CONCLUSIONS
Our group has observed an imbalance between IL-33 and ST-2 expression in airway epithelial cells from asthmatic donors. Elevated levels of IL-33 in asthmatic epithelium can be associated with higher expression of IgE receptors. Studies of IgE receptor regulation and function in airway epithelial cells will be important in understanding the underlying mechanisms of viral induced asthma exacerbations.

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Abstract #70

THE ALLERGIC RHINITIS – CLINICAL INVESTIGATOR COLLABORATIVE (AR-CIC) – OPTIMIZING THE NASAL ALLERGEN CHALLENGE (NAC) MODEL

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BACKGROUND
We sought to optimize the Nasal Allergen Challenge (NAC) model to ensure reliability and repeatability of results by modifying the qualifying criteria and allergen concentration during the challenge.

METHODS
20 Allergic Rhinitis (AR) participants underwent NAC to determine the concentration at which a Total Nasal Symptom Score (TNSS) of 10/12 OR a Peak Nasal Inspiratory Flow (PNIF) reduction of 50 % was achieved. 4-fold increases in allergen concentration were administered every 15 minutes until qualification criteria were met. The Qualifying Allergen Concentration (QAC) reached was used as a single challenge dose at the subsequent NAC visit. 10 additional ragweed allergic and 4 non-allergic participants were qualified at a TNSS of 8/12 AND a PNIF reduction of 50%. Cumulative Allergen Concentration (CAC) of all incremental doses was used during the subsequent NAC visit. Participants recorded TNSS and PNIF at baseline, 15 minutes, 30 minutes, 1 hour and hourly afterwards up to 12 hours post-challenge during the NAC visit.

RESULTS
QAC study participants qualifying only based on PNIF reduction had significantly lower TNSS scores than those qualifying on TNSS only or TNSS+PNIF ($p<0.01$). Participants in both studies' NAC visit reached peak TNSS at 15 minutes post-challenge followed by a gradual symptom decline, while the "PNIF only" group had significantly lower TNSS compared to others. All 3 groups experienced a decline in peak TNSS following NAC compared to screening, although groups qualifying on TNSS and TNSS+PNIF maintained their PNIF scores.

CONCLUSION
The NAC model is well-suited to study AR symptoms. TNSS and PNIF are complementary and must be integrated in the qualifying criteria. Further protocol modifications, such as with multiple allergen challenges during the NAC visit, may produce even more repeatable results. Through optimizing the NAC protocol, the model achieves reproducible results and becomes more reliable; suitable for testing new medications in clinical trials.

AllerGen Poster Competition

Abstract #71

THE EARLY LIFE GUT MICROBIOTA AND ATOPIC DISEASE

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BACKGROUND
Asthma is the most prevalent of all childhood diseases and accounts for the majority of hospitalizations and school absences in children [1]. Current mouse model research has identified the early life gut microbiota as a potential therapeutic target for the prevention of asthma and atopic diseases [2; 3; 4]. We hypothesize that the early life gut microbiota could play a similar preventative role against atopic disease development in humans.

METHODS
1262 children enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study with complete skin prick test and wheeze data at one year were grouped into four clinically relevant phenotypes: atopy + wheeze, atopy only, wheeze only, and control. Bacterial 16S rDNA from 3-month and 1-year stool samples of 319 children in these four phenotypes was extracted, amplified, and subjected to high throughput Illumina sequencing. Quantitative polymerase chain reaction (qPCR) and short chain fatty acid (SCFA) analysis were also conducted on 44 children in the two extreme phenotypes (atopy + wheeze vs. control).

RESULTS
16S sequence analysis of our sample cohort (319 subjects) identified bacterial populations that differed in abundance in the atopy + wheeze group at 3-months of age but not at 1-year of age. Additionally, significant changes in the abundance of certain bacterial genera were found in the atopy + wheeze group when compared to controls by qPCR at 3-months of age only. Changes in stool short chain fatty acid production between the atopy + wheeze group and the control group were also observed at 3-months of age only.

CONCLUSIONS
Shifts in the relative abundance of certain gut bacterial populations and differences in the levels of stool SCFAs before 3-months of age are associated with atopy and wheeze at one year of age.

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AllerGen Poster Competition

Abstract #72

MEDIATORS OF ALLERGIC RHINITIS: OPTIMIZATION OF RNA ISOLATION, REVERSE TRANSCRIPTION, AND QPCR PROTOCOLS

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BACKGROUND
Optimizing methods for the study of allergic rhinitis (AR), especially when using samples likely containing small amounts of material for analysis, ensures the integrity of results that may potentially enhance the understanding of AR disease mechanisms. In order to conduct future mRNA expression analysis, examining the differential expression of AR mediators such as *IL33*, *TSLPR*, *HPGDS*, and *CRTH2* at baseline and 6 hours following Nasal Allergen Challenge (NAC) in allergic individuals, this study aims to optimize the RNA isolation, reverse transcription (RT), and qPCR protocols used for the study of nasal mucosal samples.

METHODS
Several RNA isolation and RT kits were evaluated using nasal scrapings from healthy individuals, similar to those collected from allergic participants. These kits were evaluated based on the yield and purity of RNA and cDNA, assessed using spectrophotometry, qPCR amplification, and gel electrophoresis. Reference gene analysis using cDNA isolated from allergic participants was conducted using qPCR and the statistical software GenEx (MultID). Primer design and evaluation of primers for the targets of interest—*IL33*, *TSLPR*, *HPGDS*, and *CRTH2*—was also pursued.

RESULTS
RNA isolation and RT kit optimization determined that the Life Technologies-Qiagen (LT-Q) kit combination produced cDNA with maximal purity and qPCR efficiency compared with the other kit combinations evaluated. Reference gene analysis demonstrated that expression of ubiquitin C (UBC) showed limited variability among the differing conditions (time point and study) of nasal sample collection. Primer evaluation yielded inconsistent results.

CONCLUSIONS
Future processing of nasal scraping samples should use the optimal LT-Q kit combination. Following successful primer evaluation, the expression levels of the targets of interest in the allergic nasal mucosal cDNA samples at both baseline and 6h post-NAC will be conducted via the optimized qPCR reaction, using UBC as a reference gene.

Abstract #73

UNCOVERING T CELL-SPECIFIC DIFFERENTIAL EXPRESSION PATTERNS ASSOCIATED WITH POLLEN EXPOSURE IN INDIVIDUALS WITH ALLERGIC RHINITIS

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BACKGROUND
Investigating transcriptomics in whole blood is a promising avenue of research for helping to understand the allergic response [1]. However, the heterogeneity of peripheral whole blood significantly complicates the interpretation of whole blood expression data. Statistical deconvolution approaches, which can model and infer the sample composition and the cell type-specific expression, may provide a powerful means of studying complex tissues, such as whole blood, in an integrated fashion [2].

METHODS
14 individuals with allergic rhinitis were simultaneously exposed to ragweed pollen in the Environmental Exposure Unit. Peripheral blood samples were collected using PAXgene Blood RNA tubes before and after the 3 hours of pollen exposure. Gene expression profiling was performed using Affymetrix GeneChip® Human Gene 1.0 ST Arrays. Publicly available expression data (E-GEOD-48558) was used to estimate the cellular composition from whole blood expression profiles. The estimated proportions were compared against those measured by an automated hematology analyzer.

RESULTS
The estimated proportions of lymphocytes, granulocytes and monocytes were compared against the observed proportions. The prediction was good in lymphocytes (R^2=0.70, RMSE=0.036) and granulocytes (R^2=0.75, RMSE= 0.046), but relatively poor in monocytes (R^2=0.52, RMSE=0.030). No significant changes in cellular proportions between pre-challenge and post-challenge samples were identified. 261 (110 up-regulated and 151 down-regulated) differentially expressed probe sets were identified comparing pre and post-challenge samples at a false discovery rate (FDR) of 10%.

CONCLUSIONS
Statistical deconvolution is accurate in predicting the cellular proportions of lymphocytes and granulocytes but relatively poor in predicting monocytes. Allergen exposure causes significant changes in the blood transcriptomes of participants with allergic rhinitis undergoing pollen exposure. The inferred proportions will be used for cell type-specific significance analysis of microarrays (csSAM), to assess differential expression in T cells. The $CD4^+$ T cell expression profiles from E-GEOD-43497, a similar study of allergic rhinitis, will be used to validate our findings.

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AllerGen Poster Competition

Abstract #74

THE REGULATION OF INTERLEUKIN-13 RECEPTORS IN AIRWAY EPITHELIAL REPAIR

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BACKGROUND
Persistent airway epithelial damage and elevated levels of interleukin (IL)-13 are two characteristics often observed in asthma. Although IL-13 is known to be a central mediator of airway remodelling, we have shown that IL-13 is critical to normal airway epithelial repair via the release of Heparin-Binding Epidermal Growth Factor (HB-EGF) and activation of EGF-Receptor. The effects of IL-13 are mediated through two receptors: IL-13 receptor α1 (IL-13Rα1)/IL-4 receptor α subunit (IL-4Rα) and IL-13 receptor α2 (IL-13Rα2). In the current investigation we examined the roles of these receptors in normal airway epithelial repair, particularly focusing on their cellular distribution in response to injury signals.

METHODS
Confluent monolayers of Human Airway Epithelial (1HAEo-) cells were subjected to either mechanical injury or IL-13 stimulation. Cellular distributions of IL-13 receptors were examined using immunofluorescence and flow cytometry. Receptor functions were disrupted in 1HAEo- using IL-13Rα1 and IL-13Rα2 neutralizing antibodies followed by either IL-13 stimulation or wounding. Downstream effector molecules of IL-13 receptors were detected via western blot and ELISA.

RESULTS
Cell surface expression of IL-13Rα1 was unchanged in response to injury signals while membrane expression of IL-13Rα2 appears to be tightly regulated and decreased by both IL-13 stimulation and mechanical wounding at early time points. The regulation of receptor trafficking is tightly related to the functions of IL-13 receptors. When IL-13Rα1 function was disrupted, downstream molecules of IL-13Rα2 including HB-EGF and TGF-β were significantly upregulated in response to injury signals. This suggested that IL-13 receptors interacted to regulate each other.

CONCLUSIONS
The regulation of IL-13 receptor expression and trafficking is critical to IL-13 signaling and normal airway epithelial repair. Our data indicates that there may be interactions between IL-13Rα1 and IL-13Rα2 that functionally regulate their downstream signaling pathways. An imbalance of this tightly regulated relationship may contribute to the dysfunctional repair phenotype observed in asthma.

AllerGen Poster Competition

Abstract #75

MANAGING ANAPHYLAXIS IN ADULTS: A REVIEW OF ALL CASES PRESENTED IN A SINGLE YEAR AT AN EMERGENCY DEPARTMENT

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BACKGROUND
To assess anaphylaxis rate and management in adults presenting to an emergency department (ED).

METHODS
As part of the Cross-Canada Anaphylaxis Registry (C-CARE), charts of all ED visits to the Montreal General Hospital between March 2011 and February 2012 were reviewed to identify anaphylaxis cases. Cases were identified based on ICD 10 coding for either anaphylaxis or allergic reaction and only cases fitting the definition of anaphylaxis were included. Multivariate logistic regressions were used to identify factors associated with epinephrine use for moderate/severe cases.

RESULTS
Among 37,730 ED visits, 98 anaphylaxis cases [0.26 %, (95%CI 0.21%, 0.32%)] were identified. The median age was 31.5 years (IQR 26.4, 44.0) and 33.7% (95% CI 24.6%, 44.0%) were males. Food was responsible for 63.3% (52.9%, 72.6%) of reactions, drugs for 18.4% (11.5%, 27.7%) and venom for 4.1% (1.3%, 10.7%). The trigger was unidentified in 14.3% (8.3%, 23.1%).

Among all cases 95.9% (89.3%, 98.7%) were moderate (difficulty breathing/stridor/wheezing) or severe (hypoxia/cyanosis/circulatory collapse/incontinence/neurological symptoms). Prior to ED arrival, 25% (1.3%, 78.1%) of mild and 20.2% (12.9%, 30.0%) of moderate/severe reactions received epinephrine compared to 25% (1.3%, 78.1%) and 39.4% (22.9%, 42.4%) after arrival. In 51.1% (40.6%, 61.4%) of moderate/severe reactions, no epinephrine was given. In non-drug induced anaphylaxis, epinephrine auto-injectors (EAI) were prescribed in 52.5 % (41.1%, 63.7%). Older individuals presenting with moderate/severe reactions were less likely to receive epinephrine [Odds ratio= 0.96 (0.92, 0.99)].

CONCLUSIONS
Given the striking underuse of epinephrine in anaphylaxis management, especially in older individuals, educational programs are required to better implement current guidelines.