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I. PROGRAMME A – GENE-ENVIRONMENT INTERACTIONS

#	AllerGen Trainee	Institution	AllerGen Researcher/ Supervisor	Abstract Title
1A	**Akhabir,** Loubna	James Hogg Research Laboratories, **University of British Columbia**	Dr. Andrew Sandford	Identification of functional SNP in asthma genes: IL 1RL1
2A	**Ali,** Salman	Child and Family Research Institute (CFRI), **University of British Columbia**	Dr. Stuart Turvey	Functional characterization of human variants of NFKBIA: a regulator of immune responsiveness implicated in susceptibility to infectious and inflammatory disease
3A	**Kam,** Sarah	**University of British Columbia,** James Hogg Research Centre, St. Paul's Hospital	Dr. Scott Tebbutt	Functional genomics of the peripheral blood response to allergen inhalation challenge
4A	**Lambert,** Marie-Helene	**Universite du Quebec a Chicoutimi (UQAC)**	Dr. Catherine Laprise	Association between filaggrin family member genes, asthma, atopy and atopic asthma with atopic dermatitis history in the subjects from the Saguenay-Lac-Saint-Jean founder population
5A	**North,** Michelle	**University of Toronto**	Dr. Jeremy A. Scott	Examining the role of arginase in air pollution-induced exacerbation of asthma
6A	**Pitt,** Tracy	Winnipeg Children's Hospital, **University of Manitoba**	Dr. Allan Becker	Characteristics of atopic asthma and no-atopic asthma in the Study of Asthma, Genes and the Environment (SAGE) cohort at 11-13 years
7A	**Pui,** Mandy	**University of British Columbia**	Dr. Chris Carlsten	Flow cytometry to identify leukocyte sub-populations in blood and induced sputum in asthmatic and healthy volunteers exposed to diesel exhaust

1A. Identification of functional SNP in asthma genes: IL1RL1

AllerGen Programme A: Gene-Environment Interactions
Loubna Akhabir, Andrew Sandford
University of British Columbia
Supervisor: Andrew Sandford

OBJECTIVE/PURPOSE:
Our aim is to identify causal variants for the Il1RL1 gene previously associated with asthma and related phenotypes as well as perform functional assays to uncover the mechanism underlying its involvement in the disease pathogenesis. IL1RL1 has been shown to be sufficient to induce experimental allergic airway inflammation using transgenic and knockdown mouse models. Its expression has been shown to increase in murine and human asthmatic lungs; the ligand for IL1RL1 is Interleukin-33 (IL33). The signaling cascade resulting from the binding of TSLP and IL33 is crucial in eosinophilic inflammation characteristic of asthma. The *IL1RL1* gene lies in chromosome 2 in the midst of a cytokine gene cluster with *IL1R1*, *IL1RL2*, *IL18R1* and *IL18RAP*: all encoding for proteins involved in the immune response characteristic of asthma. The region is in relatively high linkage disequilibrium, thus an excellent candidate for narrowing down the asthma association signal to one or more causal SNPs.

METHODS:
Firstly, a putative causal SNP is identified based on previous association data, or linkage disequilibrium with associated SNPs, conservation scores and putative binding of regulatory proteins.
DNA samples from asthmatics and controls are then genotyped for the candidate SNP using Taqman technology in order to relate genotypes to potential alteration of gene expression.
Gene expression assays will be performed to compare levels of expression between the different genotypes as well as between the two SNP alleles. These real time-polymerase chain reaction (RT-PCR) experiments will be conducted for both IL1RL1 isoforms in order to also assess their differential expression depending on our candidate SNP genotype. If changes in expression are observed, we will perform electrophoretic mobility shift assays (EMSA) in order to test if the differential expression is due to the differential binding of a regulatory protein depending on the SNP allele. In order to further confirm that the SNP site is in an important region for gene expression regulation we will perform formaldehyde-assisted isolation of regulatory elements (FAIRE); a method which discriminates between DNA sequences depending on the presence or lack of nucleosome structures. The absence of nucleosome indicates that the region is active and accessible to regulatory elements and thus important for gene regulation.

FINDINGS:
We have selected the Il1RL1 SNP rs1420101 based on the fact that it was the most significant signal in a genome-wide study about eosinophil counts and the same SNP associated with asthma in ten populations in the same study. During the optimization phase of our gene expression assays, we confirmed differential expression of the IL1RL1 isoforms in RNA samples from blood of asthmatic children as well as controls. The next step is to relate that differential expression to the SNP genotype as well as continue with RT-PCR to compare allele-specific expression.

DELIVERABLES:
The overall objective of this research is to enhance our understanding of the pathogenesis of asthma by narrowing down genetic association signals to specific causal variants. Not only will this strengthen the evidence for IL1RL1 being an asthma gene but it will also help untangle the association signal emmanating from this region.

RELEVANCE:
The ultimate goal of these functional studies is to reach a greater understanding of the molecular pathogenesis of asthma and eventually pave the way for novel therapies targeting the source of inflammation rather than life-long therapies aimed at dampening inflammation and easing symptoms. The findings from our study will be amenable to publication in medical journals, and thus communicated to clinical scientists and other researchers to complement our research findings and eventually target our candidate gene for potential therapeutic development.

2A. Functional characterization of human variants of *NFKBIA*: a key regulator of immune responsiveness implicated in susceptibility to infectious and inflammatory disease

AllerGen Programme A: Gene-Environment Interactions
Salman Ali[1], Aaron Hirschfeld[1], Rachel Victor[1], Edgardo S. Fortuno III[1], Tobias R. Kollmann[1] and Stuart E. Turvey[1]
[1]BC Children's Hospital and Child & Family Research Institute, University of British Columbia
Supervisor: Stuart Turvey

OBJECTIVE/PURPOSE:
Genetic association studies have identified several polymorphisms in genes of the innate immunity cascade that appear to influence susceptibility to asthma and other inflammatory diseases. However, most candidate genes have not been functionally characterized for their impact on human immune responsiveness. An excellent candidate for functional investigation is *NFKBIA* which encodes IκBα—the major negative regulator of NFκB. Single nucleotide polymorphisms (SNPs) in the promoter region of *NFKBIA* have been implicated in various infectious and inflammatory diseases. Specifically, the linked promoter SNPs rs2233406, rs3138053 and rs2233409 have been implicated in sarcoidosis, trachoma, acute respiratory distress syndrome, invasive pneumococcal disease, Graves' disease and respiratory syncytial virus. We investigated the mechanistic and functional impact of the promoter variants of *NFKBIA* on human immune responsiveness.

METHODS:
Using a coding SNP that was in high linkage with *NFKBIA* SNPs rs3138053/rs2233406/rs2233409, we designed and validated an allele-specific PCR assay that could detect subtle differences in allele ratios between the major (ACC) and minor (GTT) promoter variants of SNPs rs3138053/rs2233406/rs2233409. Peripheral blood mononuclear cells of homozygous (ACC/ACC) and heterozygous (ACC/GTT) individuals were stimulated with LPS and live cultures of *Streptococcus pneumoniae* (serotype 14) for 3 and 4 hours. PBMCs of *NFKBIA* homozygotes and heterozygotes were stimulated with various Toll-like-receptor (TLR) ligands of the innate immune cascade to assay for differences in the innate immune response.

FINDINGS:
NFKBIA heterozygotes (ACC/GTT) displayed 1.21 (1.14-1.27 95% CI) - 1.26 (1.18-1.34 95% CI) fold higher expression of the major allele transcript (ACC) relative to the minor allele transcript (GTT). At 3 hours post stimulation, *NFKBIA* homozygotes (ACC/ACC) produced higher level of *NFKBIA* mRNA than heterozygotes (ACC/GTT) following stimulation with LPS (1.4 fold, p=0.0095) or *S. pneumoniae* (1.51 fold, p=0.024). Higher TNF-α secretion was seen from the peripheral blood mononuclear cells (PBMCs) of heterozygotes (ACC/GTT) as compared to homozygotes (ACC/ACC) when stimulated with Pam3CSK4 (2.29-fold increase; $p<0.01$) and 3M-002 (3.30-fold increase; $p<0.001$).

DELIVERABLES:
We have shown that the observed association of *NFKBIA* variants with infectious and inflammatory conditions has functional consequences. Individuals heterozygous for SNPs rs3138053 /rs2233406/rs1050851 display allelic imbalance, reduced levels of *NFKBIA* expression, as well as a hyper inflammatory innate immune response.

RELEVANCE:
Functional genomic studies such as this will help realize AllerGen's goal of '*discovery of the causes of, and ways to prevent, control or eliminate allergic and related immune diseases*' by:

- Generating convincing evidence that the genetic variant is functionally relevant and likely to contribute to the development of the clinical phenotype.
- Providing insight into the mechanism underlying the genetic association and, therefore, greatly enhancing our knowledge of the disease pathogenesis.
- Identifying molecular pathways that can be targeted to prevent or treat allergic disease.

3A. Functional Genomics of the Peripheral Blood Response to Allergen Inhalation Challenge

AllerGen Programme A: Gene-Environment Interactions
S.H.Y. Kam[1], J. Ruan[1], G.M. Gauvreau[2], P.M. O'Byrne[2], J.M. FitzGerald[3], S.J. Tebbutt[1]
[1]UBC James Hogg Research Centre, [2]McMaster University, [3]University of British Columbia
Supervisor: Scott J. Tebbutt

OBJECTIVE/PURPOSE:
In asthmatic individuals, airway narrowing represents the early phase of the asthmatic response to allergen inhalation challenge; early phase onset can be detected within ten minutes of allergen inhalation, reaches a maximum within thirty minutes, and typically resolves within three hours. In 50-60% of allergic asthmatic adults, the early response is followed by the late phase asthmatic response, which usually starts between three and four hours after allergen inhalation challenge, and is characterized by cellular inflammation of the airway, increased lung tissue permeability, and mucus secretion. Despite tremendous interest, the pathways leading to the late response are not completely understood. Understanding these pathways is important for evaluating allergic diseases such as asthma. In contrast to the more transient isolated early response, development of the late response is associated with the hallmark inflammatory features of chronic allergic disease.

METHODS:
Adult subjects participating in ethically approved allergen challenge studies were recruited following informed consent. Inclusion criteria included non-smokers with stable, mild to moderate atopic asthma, free of other lung diseases. Subjects were required to have a FEV_1 of greater than 70% of predicted, baseline methacholine PC_{20} of less than 16 mg/ml. Subjects meeting these inclusion criteria along with either the development of an isolated early asthmatic response (at least 20% fall in FEV_1 within two hours after allergen inhalation) or the dual asthmatic response (early response as above plus late asthmatic response – at least 15% fall in FEV_1 between three and seven hours after allergen inhalation) were studied. Peripheral blood was drawn just prior to inhalation challenge and 2-3 hours post-challenge. Gene expression analysis was performed using Affymetrix GeneChip® Human Gene 1.0 ST Arrays and the data were analyzed using Partek Genomics Suite and Ingenuity Pathway Analysis (IPA).

FINDINGS:
1783 genes were differentially expressed between pre- and post-inhalation challenge ($p \leq 0.01$). 364 genes remained significant at an FDR of 10%. Within this set, the *DNAJC1* gene ($p=7.2e-5$) has been previously identified in GWAS (genome-wide association studies) as associated with asthma. Gene ontology showed perturbed activity in mast cell secretory granules and immunoglobulin biosynthesis. The top biological functions included cell-mediated and humoral immune responses.

DELIVERABLES:
The peripheral blood transcriptome was perturbed between pre-allergen inhalation challenge and 2-3 hours post-challenge, with a focus on immunological functions. *DNAJC1* was identified to be a gene for possible further investigation. Additional recruitment of subjects is underway to identify more specific biological pathways that may be relevant to the onset of the late asthmatic response.

RELEVANCE:
This research will act as an initial step in identifying genes and pathways that may be involved in the more clinically severe late asthmatic response that follows the early response in more than half of the asthmatic population. The discovery of these biological pathways will allow for a better understanding of why some individuals develop a dual response instead of an isolated early response. It will also indicate potential therapeutic targets that can be utilized to minimize the late asthmatic response, leading to better treatments for people with asthma and other allergies.

4A. Association Between Filaggrin Family Member Genes, Asthma, Atopy and Atopic Asthma with Atopic Dermatitis History in the Subjects from the Saguenay–Lac-Saint-Jean Founder Population

AllerGen Canadian Allergy and Immune Diseases Training Initiative - Programme A: Gene-Environment Interactions
Marie-Hélène Lambert[1,2], Karine Tremblay[3], Anne-Marie Madore[1,2]
[1]Université du Québec à Chicoutimi; [2]Université Laval; [3]Université de Montréal.
Supervisor: Catherine Laprise[1]

OBJECTIVE/PURPOSE:
To perform an association study between the filaggrin (*FLG*) and the filaggrin family member 2 (*FLG2*) tagging single nucleotide polymorphisms (tagSNPs) and asthma, atopy, atopic asthma, as well as these affections in the presence of atopic dermatitis (AD).

METHODS:
Five tagSNPs covering *FLG* have been genotyped in 237 trios from the Saguenay–Lac-Saint-Jean population using a Sequenom panel. In addition, a genome-wide association study (GWAS) has also been done for the same trios in the large-scale GABRIEL project (www.gabriel-fp6.org/). The polymorphisms (SNPs) included in *FLG* and *FLG2* as well as those in the 3' and 5' UTR regions were extracted. Six SNPs were extracted for *FLG* (for a total of 11 SNPs when including the Sequenom panel) and 2 SNPs for *FLG2*. The association study for all the affections was done using a family-based association test (FBAT). The results were corrected using the Li and Ji method (1).

(1) Heredity. 2005 Sep;95(3):221-7.

FINDINGS:
Positive associations were found between a haplotype block formed by *FLG* rs2184951 and rs12730241 (H1) and asthma and related phenotypes (see results in Table 1).
Table 1: Association of *FLG* haplotype and tagSNPs with asthma and atopy (A) and with asthma and atopy that co-occur with the presence of a personal history of atopic dermatitis (B)

A

SNP	Allele	Asthma			Atopy			Atopic asthma		
		S:E(S)	Z	p	S:E(S)	Z	p	S:E(S)	Z	p
rs3126085	A	32.0:50.2	-3.48	**0.0005**	35.0:49.8	-2.94	**0.0033**	25.0:37.2	-2.74	**0.0061**
	G	148.0:129.8	3.48		141.0:126.2	2.94		109.0:96.8	2.74	

B

SNP/Haplotype	Allele	Asthma and AD			Atopy and AD			Atopic asthma and AD		
		S:E(S)	Z	p	S:E(S)	Z	p	S:E(S)	Z	p
rs3126085	A	14.0:24.0	-2.94	**0.0033**	16.0:24.5	-2.59	0.0097	10.0:18.0	-2.74	**0.0062**
	G	66.0:56.0	2.94		62.0:53.5	2.59		52.0:44.0	2.74	
H1	T G	75.0:66.0	2.61	0.009	75.0:64.0	3.33	**0.0009**	60.0:51.0	2.94	**0.0033**

Positives associations were also found between *FLG2* rs2065954 and rs3818831 and asthma (p=0.0033 and p=0.0016), atopy (p=0.00009 and p=0.00006) and atopic asthma (p=0.0004 and p=0.0002) in all cases in the presence of AD as well as AD alone (p=0.0016 and p=0.0007 respectively).

DELIVERABLES:
To conclude, *FLG* and *FLG2* are genes associated with asthma. Functional studies will be necessary to document the molecular structure (sequence) and role of these genes in asthma and the impact of the genetic variants.

RELEVANCE:
Identification of associated genes is fundamental to document the molecular nature of asthma in order to increase knowledge of the pathophysiology of this complex trait.

5A. Examining the role of arginase in air pollution-induced exacerbation of asthma

Programme A: Gene-Environment Interactions
Michelle L. North[1,2,3,4], Hajera Amatullah[3,4,5], Nivedita Khanna[2,3,4], Bruce Urch[1,4], Mary Speck[4], Hartmut Grasemann[1,6], Frances Silverman[1,2,3,4,5], and Jeremy A. Scott[1,2,3,4,5]
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Supervisors: Dr. Jeremy A. Scott and Dr. Frances Silverman

OBJECTIVE/PURPOSE:
Imbalances in the metabolism of L-arginine are known to be involved in asthma through increased competition between the arginase and nitric oxide synthase (NOS) pathways. Nitric oxide (NO) plays an important role as a bronchodilating signaling molecule in the airways. The expression of arginase 1 is increased in human asthma and murine models of allergic airways inflammation (North *et al.*, 2009). In animal models, increased arginase activity contributes to airways hyperresponsiveness (AHR) to methacholine, an important clinical feature of asthma, and pharmacologic inhibition improves airways function. Ambient particles and ozone are major constituents of urban air pollution and contribute to asthma exacerbations. However, the mechanisms underlying the exacerbation of allergic airways disease following air pollution exposure remain to be elucidated. As there is recent evidence that arginase expression is augmented in cigarette smoking asthmatics who voluntarily expose themselves to pollution, we hypothesized that arginase is a major enzyme in the response to air pollution in this susceptible population. The aim of this study was to ***examine the role of arginase in the response to air pollution in animal models of allergic airways inflammation***.

METHODS:
We used sub-acute (16-day) and chronic (12-week) murine models of ovalbumin (OVA)-induced allergic airways inflammation as models of asthma. All mice were sensitized to OVA, and then randomized to aerosol challenge with PBS (control; OVA/PBS) or OVA (allergic airways inflammation; OVA/OVA). Twenty-four hours after the final OVA or PBS challenge, mice underwent a combined exposure to concentrated ambient fine particles plus ozone (CAP+O_3), or filtered air. Following exposure, mice were treated with either the arginase inhibitor *S*-boronoethyl L-cysteine (BEC), or vehicle (PBS). After determination of airways responsiveness to methacholine using the flexiVent, tissues were harvested for Western blotting, activity testing and immunohistochemistry.

FINDINGS:
Exposure to CAP+O_3 augmented the AHR in the OVA/OVA mice with no significant effect on the OVA/PBS controls in both the subacute and chronic models. Expression of arginase 1 and total arginase activity were significantly augmented in OVA/OVA mice exposed to CAP+O_3, compared to filtered air. Immunohistochemistry revealed that arginase 1 expression was specifically up-regulated in the peribronchiolar region following CAP+O_3 exposure in OVA/OVA mice. Treatment with BEC significantly reduced the pollution-induced AHR in CAP+O_3-exposed OVA/OVA mice in both the sub-acute and chronic murine models to control levels.

DELIVERABLES:
Arginase 1 is up-regulated following environmental exposures in murine models of allergic airways inflammation and is functionally important in air pollution-induced airways hyperresponsiveness. This pathway should be explored further in humans using controlled air pollution exposures.

RELEVANCE:
As the economic costs of air pollution are staggering and predicted to increase even further, therapies to prevent air pollution-induced exacerbations of asthma are needed. This study demonstrates complete ablation of airways hyperresponsiveness in two mouse models through inhibition of arginase, suggesting that this pathway is a promising candidate for future therapies. This research also lends biological evidence to support public policy limiting air pollution exposure in this vulnerable population.

6A. Characteristics of Atopic Asthma and Non-Atopic Asthma in the Study of Asthma, Genes and the Environment (SAGE) Cohort at 11-13 years

AllerGen Programme A: Gene-Environment Interactions
J. Pitt[1], A.B. Becker[2], A. Kozyrskyj[3]
[1]Winnipeg Children's Hospital - Winnipeg/CA, [2]Health Sciences Centre, Children's Hospital of Winnipeg - Winnipeg/CA, [3]University of Alberta - Edmonton/CA
Supervisor: Dr. A.B. Becker

OBJECTIVE/PURPOSE:
Atopic and non-atopic asthma represent distinct phenotypes of childhood asthma. We hypothesize that there is an association between overweight/obesity and asthma in the absence of atopy and sought to further characterize these phenotypes at 11-13 years of age in a Canadian cohort.

METHODS:
The SAGE cohort includes 109 children with allergist-diagnosed atopic asthma (AA), 38 non-atopic asthmatics (NAA) and 185 control patients at the age of 11-13 years. Information on asthma symptoms and home environment was obtained from questionnaires. Anthropometric measurements and blood pressures were obtained. Spirometry was performed and cholesterol, LDL, HDL were measured. Statistical analysis was performed with SPSS-17.

FINDINGS:
NAA (7.8%) was less common than AA (22.4%).NAA vs. AA was 9.9%/20.8% in females, 6.2%/23.6% in males. Mean FEV1% predicted (87.8% vs. 87.3%) did not differ between the NAA's and AA's. Wheezing episodes in the last year (2.3 vs. 2.7 p=0.426) and episodes of sleep disturbances due to wheeze (0.7 vs. 0.5 p=0.280) were not significantly different. NAA's had slightly, but not significantly higher total cholesterol (4.0 mmol/L vs. 3.9mmol/L), LDL (2.2mmol/L vs. 2.0mmol/L) and lower HDL (1.4mmol/L vs. 1.49mmol/L). For children with BMI> 85%ile (n=144), NAA's had higher mean cholesterol (p=0.08). NAA's also had marginally higher systolic blood pressure (115.5 mmHg vs. 113mmHg) but not statistically significant. Mean waist circumference (75.7 cm vs. 71.2 cm) and weight (54.6 kg vs. 50.7 kg) were higher in the NAA group but also not statistically significant. Early life tobacco exposure had an important influence on our subtypes. Interestingly, mothers of non-atopic asthmatics smoked fewer cigarettes/day in the first year of life (1.11 vs. 1.71 p=0.025).

DELIVERABLES:
There is a trend towards higher cholesterol levels in overweight NAA's. There are no significant differences for asthma control between NAA's and AA's or blood lipids. AA's do have a higher burden of maternal tobacco smoke exposure in the first year of life than their NAA counterparts. This may suggest that environmental tobacco exposure is a risk factor in sensitization of AA's. We speculate that Vitamin D levels (a metabolite of cholesterol) may differ between the groups and analysis related to this is ongoing.

RELEVANCE:
It is increasingly important to better define phenotypes of asthma, especially in children. These findings will help to direct a focus for future and ongoing AllerGen research including the CHILD Study.

7A. Flow cytometry to identify leukocyte sub-populations in blood and induced sputum in asthmatic and healthy volunteers exposed to diesel exhaust

AllerGen Programme A: Gene-Environment Interactions
Mandy Pui [1], John C. Lay, DVM, PhD [2], Neil E. Alexis, PhD [2], Christopher Carlsten [1]
[1] University of British Columbia [2] University of North Carolina at Chapel Hill
Supervisor: Christopher Carlsten

OBJECTIVE/PURPOSE:
To identify five leukocyte types (in blood and induced sputum) and bronchial epithelial cells (in sputum only) using multi-colour flow cytometry in healthy and mildly asthmatic volunteers exposed to diesel exhaust.

METHODS:
Mild asthmatics and normal controls were recruited as study subjects. This crossover study was double-blinded, randomized and counter-balanced to the order of three conditions: diesel exhaust with anti-oxidant, diesel exhaust with placebo, or filtered air with placebo. The subjects were exposed to either filtered air or diesel exhaust (300ug $PM_{2.5}/m^3$) in a state-of-the-art diesel exhaust exposure facility. An anti-oxidant, N-acetylcysteine (600mg), or a placebo was taken orally for five days preceding, and on the day of the exposure. Each subject was exposed to each of the three conditions. Peripheral blood samples were taken pre-exposure, and also at 2, 6, and 30 hours after the beginning of exposure. Sputum induction was performed by inhalation of hypertonic saline according to ATS guidelines pre-exposure, and also at 6, and 30 hours after the beginning of exposure.
FACSCanto II (BD Biosciences) was used for flow cytometry. A 5-colour, 12-marker (CD3/CD9/CD14/CD16/CD19/CD20/CD45/CD56/CD83/CD206/CD326/HLA-DR) combination was used to identify dendritic cells, macrophages, monocytes, neutrophils, eosinophils, and bronchial epithelial cells. Direct immunolabelling was performed on whole peripheral blood. After incubation, red blood cells were lysed. Remaining cells were washed and resuspended in PBS with 0.5% paraformaldehyde. Sputum plugs were homogenized with 0.1% DTT, filtered, and then centrifuged to remove supernatant. Sputum cells were resuspended in PBS at 1 million per mL. Direct immunolabelling was performed. After incubation, cells were washed and resuspended in PBS with 0.5% paraformaldehyde. Spectral compensation for flow cytometry was performed using an automatic calibration technique (BD CompBeads). Cellular debris was eliminated on the SSC/FSC scattergram. A gating strategy was designed to identify the leukocyte sub-populations and bronchial epithelial cells. Surface markers were chosen based on differential cell-specific expression according to existing literature.

FINDINGS:
The CD45 marker is expressed on all leukocytes. Each cell type of interest has unique scattergram (SSC/FSC) characteristics and/or CD45 expression levels, which are similar but not identical in blood and in sputum. Each cell type has a unique expression pattern of surface markers in blood and in sputum. For example, eosinophils express CD9 whereas neutrophils do not. Findings for the cellular effects of diesel exhaust and anti-oxidants are pending.

DELIVERABLES:
Performing white blood cell differential by standard cytology is common but is poorly reproducible, and labour-intensive. Flow cytometry is superior to standard cytology in identifying rare cells, assessing expression of surface markers, and being automated for quality control and efficiency. Multi-colour flow cytometry has previously been employed to identify leukocyte sub-populations in blood with some success, but without a well-standardized strategy. Flow cytometry has been used to identify lymphocytes, and to a lesser extent, phagocytes in sputum, but has rarely been used to identify the rarer sub-populations such as dendritic cells, eosinophils, and bronchial epithelial cells. The findings of this study suggest that a standardized strategy can be created to identify bronchial epithelial cells in sputum, as well as leukocyte sub-populations in blood and in sputum.

RELEVANCE:
Efforts to understand mechanisms of health effects due to ambient air pollution, in order to develop remediation strategies to protect exposed populations (for example, anti-oxidants), are dependent on high-quality and efficient techniques for characterizing cellular effects in the intact human model. Refining the methods described above allows for such detailed assessment of blood and sputum in the context of a controlled human exposure to diesel exhaust.

II. PROGRAMME B – DIAGNOSTICS & THERAPEUTICS

#	AllerGen Trainee	Institution	AllerGen Researcher/ Supervisor	Abstract Title
1B	**Ajamian,** Farnam	**University of Alberta**	Dr. Darryl J. Adamko	Respiratory syncytial virus replication induces indoleamine 2,3-dioxygenase (IDO) activation in human dendritic cells
2B	**Allakhverdi,** Zoulfia	**CHUM Research Center,** Notre-Dame Hospital	Dr. Guy Delespesse	Regulation of Thymic Stromal Lymphopoietin (TSLP) Receptor Expression
3B	**Bennett,** Jami	**University of British Columbia,** Biomedical Research Centre	Dr. Kelly McNagny	Adhesion molecules in experimental peanut allergy
4B	**Blanchet,** Marie-Renee	**University of British Columbia,** Biomedical Research Centre	Dr. Kelly McNagny	CD103 in the development of experimental asthma
5B	**Blouin,** Evelyne	Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Quebec	Dr. Louis-Philippe Boulet	Increased methacholine sensitivity after eucapnic voluntary hyperpnea
6B	**Brown,** Meghan	**University of Toronto**, The Hospital for Sick Children	Dr. PJ Subbarao	Lung clearance index as a marker of early asthma symptoms in infants
7B	**Brown,** Meghan	**University of Toronto**, The Hospital for Sick Children	Dr. PJ Subbarao	Lung clearance index as a marker of peripheral airway disease in children under 5 years of age
8B	**Bruenahl**, Christian	**McMaster University**, Brain-Body Institute	Dr. Petra Arck	Fetal origin of allergic asthma: insights on mechanistic cues and therapeutic targets arising from a mouse model of prenatal stress challenge
9B	**Des Cormiers,** Annick	Centre de recherché de l'Institut universitaire de cardiologie et de pneumologie de Quebec	Dr. Louis-Philippe Boulet	Sleep disturbances in a Canadian population with asthma or chronic obstructive pulmonary disease (COPD)
10B	**Gold,** Matthew	**University of British Columbia,** The Biomedical Research Centre	Dr. Kelly McNagny	CD34 Function in intracellular signaling and mucosal inflammatory disease development
11B	**Hirukawa,** Alison	**University of British Columbia,** The Biomedical Research Centre	Dr. Kelly McNagny	Analysis of Tie2 function in mast cells
12B	**Ilarraza,** Ramses	**University of Alberta**	Dr. Darryl Adamko	Unique properties of RV in an *in vitro* model of asthma exacerbation

#	AllerGen Trainee	Institution	AllerGen Researcher/ Supervisor	Abstract Title
13B	**Kanagaratham,** Cynthia	**McGill University**	Dr. Danuta Radzioch	The protective effect of fenretinide against allergic asthma
14B	**Khanna,** Nivedita	**University of Toronto**	Dr. Jeremy A. Scott	Importance of routes of exposure in the development of immune response to peanut
15B	**Loeffler,** Daniela	Child and Family Research Institute, **University of British Columbia**	Dr. Tobias R. Kollmann	A bacterial immune-prophylactic approach against asthma for infants and children
16B	**Loewen,** Mark	**University of Manitoba**	Dr. Allan Becker	The role of perfluoctanoic acid (PFOA) in airway hyperresponsiveness
17B	**Loubaki,** Lionel	Centre Recherche, Institut Universitaire de Cardiologie et de Pneumologie de Quebec, **Universite Laval**	Dr. Jamila Chakir	Bronchial fibroblasts modulate CD4+Tcells phenotype towards Th17 in asthma
18B	**Maltby,** Steven	**University of British Columbia,** The Biomedical Research Centre	Dr. Kelly McNagny	CD34 is required for the infiltration of inflammatory cells into the mouse colon during DSS-induced colitis
19B	**Marino,** Rafael	**McGill University**	Dr. Danuta Radzioch	Immunoregulatory role of secretory leukocyte protease inhibitor in allergic asthma
20B	**Protudjer,** Jennifer	**University of Manitoba**	Dr. Allan Becker	A comparison of Manitoba CHILD participants and the general Manitoba population
21B	**Reece,** Pia	**McMaster University**	Dr. Judah Denburg	Cord blood hemopoietic progenitor cell toll like receptor expression and function: A mechanism underlying allergic inflammation in early life?
22B	**Skappak,** Christopher	**University of Alberta**	Dr. Darryl Adamko	Virus memory induces airway hyperreactivity through eosinophil activation
23B	**Yang,** Jasemine	**University of British Columbia,** James Hogg Research Centre	Dr. Delbert Dorscheid	IL-13Ra2 / AP-1 complex signaling mediates airway epithelial repair without effects on remodeling pathways

1B. Respiratory Syncytial Virus Replication Induces Indoleamine 2,3-dioxygenase (IDO) Activation in Human Dendritic Cells

AllerGen Programme B: Diagnostics and Therapeutics
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OBJECTIVE/PURPOSE:
Induction of IDO in dendritic cells (DCs) depletes the essential amino acid, tryptophan, and generates a family of catabolites known as kynurenines (KYN). IDO activity is reported to have immunomodulatory effects, including the selective induction of apoptosis in T-helper 1 (Th1) lymphocytes, an effect not seen with Th2 cells that are dominant in allergic asthma. Infants hospitalized for RSV-related bronchiolitis have increased risk of developing asthma (48% vs. 8% in control). Induction of IDO activity by RSV may explain the link between RSV bronchiolitis and asthma pathogenesis. IDO is induced by various cytokines and a number of non-airway viruses; however, RSV has not yet been studied. We hypothesize that RSV induce IDO activation in human dendritic cells (DCs).

METHODS:
Primary human dendritic cells (DCs) were infected with sucrose gradient purified RSV with a multiplicity of infection (MOI) rate of 1.0. Flow cytometry and confocal microscopy were used to confirm infection. We measured KYN in culture media by a spectrophotometric method using Ehrlich reagent. We blocked RSV infection with the RSV-mAb, Palivizumab, and UV-inactivation to determine a role for infection. The potent competitive inhibitor of viral RNA polymerase, Ribavirin, was used to block RSV replication and protein synthesis. To evaluate dependency of RSV-induced IDO induction on different cell signaling pathways, we used a variety of specific inhibitors including MEK inhibitor I (120 nM), MEK inhibitor II (4 μM), SB202190 (p38-MAPK, 3 μM), JNK inhibitor II (1 μM), IKK inhibitor II (Wedelolactone, 30 μM), IKK inhibitor III (BMS-345541, 3 μM) and relevant negative controls.

FINDINGS:
DCs incubated with RSV showed a 35% shift in flow cytometry compared to uninfected control DCs (n=12) thus confirming infection of DCs. KYN, as a marker of IDO induction, was increased 13.2 fold in supernatants of infected DCs compared with control DCs (43.6 vs. 3.3 μM, n=6). Inactivation of virus by Palivizumab or UV resulted in 99% decrease in levels of KYN compared to controls (n=3). Infecting DCs with higher MOI of UV-inactivated RSV (up to 20, n=3) did not induce IDO. Addition of Ribavirin to culture media reduced KYN release in a dose-dependent manner with 50% reduction at 220 μM (n=3), without having any blocking effect on positive controls (IFN-γ induced KYN release) at similar concentrations. Except for SB202190, none of the specific inhibitors of signaling pathway including NF-κB, JNK-MAPK and MEK/ERK-MAPK showed any significant inhibitory effect on IDO induction by RSV (n=3). SB202190, the specific inhibitor of P38-MAPK, blocked 51% (IC_{50}= 300nM) and 92% (3 μM) of KYN release (n=3); negative controls showed no inhibitory effect.

DELIVERABLES:
Our data showing IDO to be induced in DCs following infection with RSV is novel. Further, the observation that induction was dependent on viral replication was unexpected. Although NF-κB is reported to have a role in IDO induction, our data suggest that RSV-induced kynurenine release may occur through an NF-κB-independent pathway.

RELEVANCE:
These data support our hypothesis that RSV plays a role in the development of an immune response towards a Th2 pattern. Prevention of RSV infection could decrease the incidence of asthma. We expect to be publishing these novel findings in 2010.

2B. Regulation of Thymic Stromal Lymphopoietin (TSLP) Receptor Expression

AllerGen Programme B: Diagnostics and Therapeutics
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OBJECTIVE/PURPOSE:
TSLP plays a major role in the induction and effector phases of allergic diseases by acting on dendritic cells, mast cells (MCs) T cells and $CD34^+$ hemopoietic progenitor cells. Whereas the cellular origin and the mechanisms regulating TSLP production are well documented, little is known about the regulation of TSLP receptor expression. We analyzed the regulation of TSLP-R with several cytokines, TLR ligands and drugs commonly used to treat asthma.

METHODS:
Neonatal $CD34^+$ cells were examined by two-colour analysis for the expression of CD34 and TSLP-R after 48 hours of incubation with or without: 1) cytokines (IL-1, TNF, IL-4, IFN-γ, TGF-β) used alone or in combination; 2)inflammatory mediators (PGE2, LTC4 and PGD2); 3) bacterial products (SAC, LPS and PGN); 4) TLR ligands (TLR3, TLR5, and TLR7); 5) dexamethasone and isoproterenol.

FINDINGS:
Expression of TSLP-R on $CD34^+$ cells was markedly enhanced by IL-1/TNF; this effect was suppressed by IL-4, IFN-γ and TGF-$\beta$and augmented by PGE2. TSLP-R was induced by SAC and PGN but was not affected by LPS or other TLR ligands. Most interestingly, dexamethasone slightly induced TSLP-R and IL-7Rα expression and markedly increased the effect of IL-1/TNF. The enhancing effect of dexamethasone was also observed on $CD14^+$ and $CD56^+$ neonatal cells. Isoproterenol had no effect on the regulation of TSLP-R.

DELIVERABLES:
Taken together, our data may explain the synergistic effect of IL-1/TNF on the response of $CD34^+$ cells to TSLP. They further show that TSLP-R expression is markedly regulated by the inflammatory and cytokine environment. The biological consequences of glucocorticoid-induced upregulation of TSLP-R will be examined.

RELEVANCE:
Currently, there is no disease-modifying treatment for allergic diseases. Indeed, steroids, the main therapeutic tool for improving the quality of patients' life, do not prevent irreversible tissue-remodeling and the loss of pulmonary function. Current observations indicate that steroids upregulate the receptor of pro-inflammatory cytokine TSLP on the surface of $CD34^+$ cells. The effect of current anti-allergic treatment on the proinflammatory activity of $CD34^+$ cells should be taken into account and these cells could be considered as target for the development of novel therapeutic approaches for atopic diseases.

3B. Adhesion molecules in Experimental Peanut Allergy

AllerGen Programme B: Diagnostics and Therapeutics
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Biomedical Research Centre, University of British Columbia
Supervisor: Kelly McNagny

OBJECTIVE/PURPOSE:
Adhesion molecules are critical for appropriate localization of leukocytes and induction adaptive immune responses throughout the body. Our aim is to better understand the role of cell trafficking and adhesion molecules in an experimental model of peanut allergy.

METHODS:
Peanut allergy was induced in mice on the C57Bl/6 background (IA^b) with 4 weekly oral gavage feedings of peanut protein and cholera toxin. After a two-week rest period, sensitized animals were challenged by intraperitoneal injection with purified peanut protein and monitored for anaphylaxis. Clinical indicators of peanut allergy include decreased body temperature, scratching, swollen eyes, decreased movement and responsiveness, and moribund condition. We evaluated plasma histamine, total IgE, peanut-specific IgE, and peritoneal albumin levels as *in vivo* indicators of mast cell degranulation and vascular permeability.

FINDINGS:
In our survey of molecules involved in leukocyte trafficking, we analyzed mice deficient in CD34, CD103, PSGL-1, E-selectin, and L-selectin. We also analyzed mice lacking IL-7Rα to evaluate the requirement for T cells and B cells in peanut allergy. CD34 is well known as a surface marker for hematopoietic stem cells, but is also expressed on the mast cells, eosinophils and DCs and is required for efficient migration. We speculated that efficient migration of each cell type could require CD34 in the peanut allergy model. CD103 is the α- chain of an integrin expressed by DC and T cells which allows binding to mucosal epithelial cells, with a reported role in mucosal immunity. We anticipated a requirement for CD103 in generating a sufficient immune response to induce peanut-specific IgE, and anaphylaxis. Surprisingly, our data did not support these hypotheses and ***we found that CD34 and CD103 deficient animals are fully susceptible to induction of experimental peanut allergy***. Next, we performed similar experiments to evaluate the requirement for localization of immune cells prior to sensitization in peanut allergy. IL7-$R\alpha^{-/-}$ mice have defects in T cell homing and T and B cell development. These mice exhibited significantly decreased clinical and immunological indicators of anaphylaxis. Similarly, mice deficient in L-Selectin (an adhesion molecule required for localization of naïve lymphocytes to lymphoid tissue) were significantly protected from peanut-induced anaphylaxis. Mice deficient in PSGL-1 and E-Selectin, which are primarily involved in the rolling and tethering of activated lymphocytes to the vessel endothelium at sites of inflammation, were intermediately susceptible to peanut allergy. The phenotype of P-$Selectin^{-/-}$ mice is being assessed, but based on its role in PSGL-1 binding, we expect a similar phenotype to $PSGL\text{-}1^{-/-}$ mice. ***In summary, we conclude that adhesion molecules required for the appropriate localization of lymphocytes prior to sensitization (L-Selectin) and those required for efficient formation of adaptive immune cells (*IL7-Rα*) are required for induction of peanut induced anaphylaxis. In contrast, adhesion molecules involved in inflammatory cell mobility (CD34, CD103, PSGL-1, E-Selectin, and probably P-Selectin) are less critical. This likely reflects the fact that during acute antigen exposure, mast cell degranulation and subsequent anaphylaxis do not require leukocyte trafficking***.

DELIVERABLES:
Our data suggest that, with the exception of L-Selectin, most of the molecules known to play a role in inflammatory homing do not play a major role in acute allergen-induced anaphylaxis and would, therefore, be poor targets for therapy. Future studies will focus on how L-Selectin inactivation leads to amelioration of peanut allergies. We will test relevant methods of interfering with this site-specific function and attempt to block antigen transit/priming without breaking oral tolerance to other antigens routinely encountered in the gut.

RELEVANCE:
Food allergy and peanut allergy in particular, is a major health challenge for many young people in Canada. Understanding the role of immune cell function and localization is critical to our ability to modulate mucosal inflammation and disease. Our findings will inform future efforts to generate therapies for food allergic patients and identify or eliminate potential therapeutic targets for food allergy, ultimately enhancing the therapeutic options and quality of life for affected patients. These data will be published in peer-reviewed journals, presented in abstracts and seminars, and reported as part of the CanGoFAR project summary to deliver the findings to the community and relevant policy makers.

4B. CD103 in the development of experimental asthma.

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor: Kelly McNagny

OBJECTIVE/PURPOSE:
CD103 (Alpha-E Beta-7 integrin) is expressed on various cell types involved in the development of asthma, including dendritic cells (DCs) and CD4 and CD8 T regulatory cells. This integrin binds E-cadherin on epithelial cells and plays a role in regulating the migration and proliferation of cells. Also, $CD103^{+}$ dendritic cells have been reported to direct the development of naïve T cells into T regulatory cells. However, little is known about the exact role of CD103 in the development of asthma. The objective of this project is to investigate the role of this integrin in asthma pathogenesis, in the hope of finding new molecular targets for the treatment of asthma.

METHODS:
Asthma was induced in wild type C57Bl/6 and $Cd103^{-/-}$ mice. Briefly, mice were sensitized to Ovalbumin (OVA) through two 100µl intraperitoneal injections of 0.02% OVA coupled to aluminium hydroxide on days 1 and 8. Mice were then challenged intranasally with 50µl of 2% OVA on days 22, 23, 24, 26 and 28, and sacrificed on day 29. The assessment of airway inflammation was performed by analysis of the broncho-alveolar lavage (BAL) content as well as the hematopoietic content of collagenase-digested lungs, where the numbers of lymphocytes, macrophages, neutrophils, eosinophils, myeloid dendritic cells, lymphoid dendritic cells, plasmacytoid dendritic cells and T regulatory cells were determined. Cytokine production in recall to OVA was tested in both lung hematopoietic cells and the draining lymph nodes. Finally, airway hyperresponsiveness was tested *via* a methacholine challenge, using a flexiVent apparatus.

FINDINGS:
The analysis of airway inflammation revealed that lack of CD103 expression leads to worse asthma compared to wild type mice, as characterized by an increase in total BAL cells, in the percentage and total numbers of eosinophils in the BAL, in cytokine production in recall to OVA and by an increase in airway resistance in response to methacholine. CD103 expression did not seem to affect the accumulation of DCs or T regulatory cells in the lung tissue at baseline and in response to OVA. However, lack of CD103 leads to an expansion of the myeloid dendritic cell population in the lung at baseline, which could account for the exacerbated disease observed in these mice.

DELIVERABLES and RELEVANCE:
In light of these results, we believe that a further understanding of CD103 function in the lung could lead to an interesting new molecular pharmacological target. Also, as this molecule is expressed specifically on dendritic cells and T regulatory cells, this research could lead to new ways of intervening in the process by which these cells drive the asthmatic response rather than a normal,non-allergic response.

5B. Increased Methacholine Sensitivity after Eucapnic Voluntary Hyperpnea

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor : Louis-Philippe Boulet

BACKGROUND:
Eucapnic voluntary hyperpnea (EVH) and methacholine inhalation test (MIT) are commonly used to evaluate airway responsiveness in athletes. EVH and MIT are frequently administered consecutively. However, it has been suggested that EVH could influence airway responsiveness to methacholine.

OBJECTIVE/PURPOSE:
To evaluate the effects of EVH on the methacholine response in athletes with and without airway hyperresponsiveness (AHR) compared to control subjects.

METHODS:
Two MITs (one single and one preceded by EVH) were conducted in random order in 10 athletes with AHR (PC20 ≤ 16 mg/mL), 16 athletes without AHR (PC20 >16 mg/mL), and 11 control subjects.

FINDINGS:
In athletes with AHR, the PC20 (expressed as the mean of double concentrations (DC) ± standard error) was 0.7 ± 1.2 DC lower when MIT followed EVH (p=0.015), while there was no significant difference in athletes without AHR (-0.4 ± 0.8 DC) nor in control subjects (0.4 ± 0.7 DC). When grouping subjects based on the EVH response, those with a positive response (FEV1 fall ≥ 10%) had a mean PC20 0.8 ± 1.1 DC lower when MIT followed EVH (p=0.0008) while in those with no response to EVH (FEV1 fall < 10%), airway responsiveness was not influenced by this test (0.05 ± 0.76 DC).

DELIVERABLES:
EVH slightly increases the response to the subsequent MIT in athletes with AHR to methacholine or with a positive response to EVH, but not in those without AHR, nor in control subjects. These two challenges should, therefore, be conducted in two different sessions.

RELEVANCE:
This research is in accordance with AllerGen's mission because, by bringing to light the importance of proceeding with the bronchial challenges in two different sessions, it ensures reliable test results. These findings are going to be submitted to a scientific journal for publication.

6B. Lung clearance index as a marker of early asthma symptoms in infants

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor: Dr. Padmaja Subbarao

OBJECTIVE/PURPOSE:
Although asthma is thought to have its onset in early life, this has not been documented with objective measures in part due to the lack of sensitive objective measures, especially in young children. The multiple breath inert gas washout (MBW) test is able to detect peripheral airway obstruction through the measurement of lung clearance index (LCI) during tidal breathing, and holds great promise as an early diagnostic tool. Higher LCI values are indicative of greater ventilation inhomogeneity thought to represent peripheral airway disease. LCI is elevated in older children with asthma, yet its utility in detecting early disease in children under the age of five has not been adequately investigated.

METHODS:
We assembled a cohort of wheezy infants undergoing lung function testing and MBW testing. LCI was calculated as the average measure of at least two technically acceptable washouts, preformed before pulmonary function testing. We compared the sensitivity of LCI derived from sulfur hexafluoride MBW with spirometric flow measurements including forced vital capacity (FVC), forced expiratory volume in 0.5 seconds (FEV0.5) and forced expiratory flow between 25% and 75% expired volume (FEF25-75) in 11 children with wheeze and 9 healthy controls under 3 years of age. Healthy subjects were recruited as part of the pilot cohort of the Child Healthy Infant Longitudinal Development (CHILD) Study at the Toronto site.

FINDINGS:
LCI was higher in asthmatic infants than in healthy infants (mean difference 0.82 (95% CI 0.192 to 1.452, $p<0.01$). FVC, FEV0.5 and FEF25-75 z-scores were not significantly lower in asthmatic children (mean difference 0.07 (95% CI -1.04 to 1.19); 0.45 (95% CI -1.68 to 0.776); 0.42 (95% CI -1.712 to 0.862)). Abnormal FVC, FEV0.5 and FEF25-75 results (>+/<-1.96 z-scores) were each detected in 2 (17%) of wheezy infants, while abnormal LCI values (>7.8) were detected in 4 (33%) of children with wheeze.

DELIVERABLES:
Such preliminary data suggests that LCI may be a more sensitive and practical marker of early small airway disease than forced flow measures in infants. While a larger data set is required to confirm these results, peripheral airway abnormalities are detectable in children with wheeze under the age of 3 years.

RELEVANCE:
This research contributes to legitimization of the usefulness of early monitoring of lung function in infants and preschool children, as small airway abnormalities caused by infant wheeze are detectable even in this young population. Our conclusions suggest that the MBW may be a particularly novel diagnostic and monitoring tool requiring more investigation to determine its possible clinical relevance. Furthermore, this study, and the planned follow-up projects, will generate much needed normative LCI data for the infant population. These findings will be presented at the American Thoracic Society Annual Conference in May 2010, and will be included in a planned publication in 2010.

7B. Lung clearance index as a marker of peripheral airway disease in children under 5 years of age

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor: Dr. Padmaja Subbarao

OBJECTIVE/PURPOSE:
Evaluation of airway disease in young children has hitherto been limited by the need for cooperation when performing conventional lung function tests. The Multiple Breath Washout test (MBW) has thus gained recent attention in part for its ability to detect ventilation inhomogeneity through the measurement of lung clearance index (LCI) while the subject is breathing normally, requiring little participation and making it practical for young children. While LCI has been suggested as a marker of peripheral airway diseases such as cystic fibrosis (CF) in older children, its utility in infant and preschool populations must still be established. This study aimed to examine the sensitivity of LCI as a marker of early peripheral airway disease in children with CF and wheeze compared to healthy controls.

METHODS:
MBW testing using sulphur hexafluoride (SF6) inert gas has been completed on 14 children with asthma and 25 healthy children recruited as part of the pilot cohort of the Child Healthy Infant Longitudinal Development (CHILD) Study under the age of 3.5 years, and 17 children with CF under the age of 5 years. Subjects were required to inhale an inert gas mixture until the concentration of SF6 was in equilibrium, and then this gas is washed out during tidal breathing of room air until the end-tidal concentration of SF6 was $1/40^{th}$ of its equilibrium concentration. LCI was calculated as the average number of lung volumes required to washout the inert gas of at least two technically acceptable washouts.

FINDINGS:
LCI was higher in children with CF compared to healthy controls (mean difference 1.61 (95% CI 0.714 to 2.5); $p<0.001$), and in children with wheeze compared to healthy controls (mean difference 0.75 (95% CI 0.31-1.19), $p<0.001$). However, when the analysis of LCI is limited to those children under 3.5 years, the difference between healthy children and those with CF is no longer significant (mean difference 0.23 (95% CI -0.24 to 0.7), $p<0.33$). A significant difference in LCI remains between infants with wheeze and healthy controls.

DELIVERABLES:
This preliminary data suggests that LCI is sensitive enough to distinguish between airway disease and health even in young children. More data is needed to determine whether the usefulness of LCI in infants is dependent on the disease being considered. It may be that our CF cohort does not demonstrate symptoms of peripheral airway disease until after the age of 3 years.

RELEVANCE:
This study sheds light on both the difficulty of lung function testing in infants and the promise of the MBW to aid in this problem. Our preliminary data shows that LCI is sensitive enough to detect airway abnormalities in young children with wheeze, suggesting its value as an early diagnostic and monitoring tool. With further investigation, LCI may also help define the differences in early disease progression between asthma and more severe diseases such as CF. These findings will be presented at the American Thoracic Society Annual Conference in May 2010, and will be included in a planned publication for the new year.

8B. Fetal origin of allergic asthma: insights on mechanistic cues and therapeutic targets arising from a mouse model of prenatal stress challenge

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor: Petra Arck

OBJECTIVE/PURPOSE:
Prenatal stress challenge is a pivotal environmental factor which has been proposed to increase the vulnerability of offspring to develop chronic immune diseases in later life. The aim of our research is to identify biomarkers and mechanisms involved in stress-triggered fetal programming of allergic asthma. We and others were able to show that prenatal stress challenge results in decreased levels of progesterone during pregnancy. Thus, in the present study, we aimed to analyze the effect of prenatal exposure to stress during late gestation in mice on (i) placental integrity and function; and (ii) fetal development *in utero*; Next, (iii), we screened the offspring for immune cells and cytokine levels and focused on the frequency, methylation status and function of $CD4^{+}CD25^{+}$forkhead box P3 (foxP3) $^{+}$ T regulatory cells. Further (iv), we tested if supplementation of the dams with a progesterone derivative would ameliorate the severity of asthma in the offspring.

METHODS:
In four subsequent experiments, BALB/c-mated BALB/c mice were exposed to sound stress for 24 hours on gestation day (gd) 12.5 and 14.5. In one experiment, pregnant mice were sacrificed on gd 16.5, maternal serum was analyzed for hormone levels and placentas were morphologically and functionally analyzed. Further, fetal development was scored gender-dependently using the Theiler classification. In the second experiment, litters were born and six weeks after birth, allergic asthma was experimentally induced by sensitizing the offspring with Ovalbumin (OVA), followed by nasal OVA-allergen provocation. Sensitized offspring from non-stressed mothers and non-sensitized mice served as controls. Offspring were screened for immune cell frequency and phenotype in lungs, bronchioalveolar fluid (BAL) and lung-draining lymph nodes. We analyzed cytokine concentrations in the BAL. The foxP3 methylation status analysis of CD4+ cells proceed after CD4+ cells have been isolated out of draining lymph nodes using MACS techniques. Third, stress-challenged pregnant females were treated with a progesterone derivative (dydrogesterone), followed by Theiler analyses of the offspring on gd 16.5. Fourth, vulnerability towards asthma was evaluated in adult offspring from pregnancies where stressed dams were treated with dydrogesterone.

FINDINGS:
Stress challenge resulted in decreased serum levels of maternal progesterone and testosterone, and increased serum levels of estradiol associated with placental endocrine dysfunction, such as low expression of Proliferin in Trophoblast Giant Cells. Fetal development was impaired upon stress challenge, especially profound in females. Prenatally stressed female adult offspring revealed an increased susceptibility toward asthma, mirrored by an increased airway response, influx of inflammatory cells and increased T helper (Th) 2 cytokines in the BAL. We observed decreased frequencies of regulatory T cells ($CD3^{+}CD4^{+}CD25^{+}foxP3^{+)}$ in prenatally stressed adult offspring. Methylation analyses of the *foxP3* gene are underway. Progesterone supplementation abrogated the impaired intrauterine development as well as the susceptibility toward asthma in the female offspring.

DELIVERABLES:
Our study revealed that prenatal stress in mice severely interferes with the intrauterine development, resulting in offspring with an increased vulnerability toward asthma-like symptoms. These effects were particularly profound in female offspring. Such susceptibility is already present *in utero.* Given the decrease of Treg is seen in prenatally stressed adult offspring, epigenetic alterations, *e.g.,* of the *foxP3*, can be envisioned to account for this increased risk for asthma.

RELEVANCE:
Supplementation of progesterone during stress-challenged pregnancies abrogates the gender-dependent increase in susceptibility toward asthma. These findings provide crucial insights relevant to new therapeutic implications.

9B. Sleep disturbances in a Canadian population with asthma or chronic obstructive pulmonary disease (COPD).

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor: Louis-Philippe Boulet

OBJECTIVE/PURPOSE:
To compare the self-reported prevalence of sleep duration and quality in patients with asthma, chronic bronchitis (CB), or undefined COPD in the Canadian population.

METHODS:
This cross-sectional survey was done using the *Public Use Microdata File Canadian Community Health Survey (CCHS) Questionnaire for Cycle 1.1 (2000-2001).* Ninety-eight percent of the Canadian population was represented by a sample of 133,000 persons, aged 12 or older.

FINDINGS:
A higher frequency of difficulty falling or staying asleep most of the time was observed in people with asthma (19.1%), chronic bronchitis (29.7%), or COPD (30.9%) compared to the general population (GP: 12.8%). Fewer patients with these conditions reported finding their sleep "refreshing" most of the time (A: 50.7%; CB: 42.1%; COPD: 45.1%) compared to those without these ailments (62.3%). A difference was also observed in regard to difficulty in staying awake most of the time during the day (A: 8.3%; CB: 10.5%; COPD: 11.0%; GP: 5.7%) and in the degree to which chronic fatigue was reported (A: 1.7%; BC: 3.2%; COPD: 5.2%; GP: 0.8%). Canadians with asthma and COPD report more sleep disturbance and chronic fatigue than healthy people.

DELIVERABLES:
Eventually, this study will be published in a journal and will be presented at both national and international conferences.

RELEVANCE:
This study will help optimize treatment in respiratory diseases. A better knowledge base will result in better treatment. Asking questions about quality of sleep will provide physicians with a better understanding of their patient. This type of question will indicate to them how the disease impacts patient's lives.

10B. CD34 Function in Intracellular Signaling and Mucosal Inflammatory Disease Development

AllerGen Programme B: Diagnostics and Therapeutics
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The Biomedical Research Centre, University of British Columbia
Supervisor: Kelly M. McNagny

OBJECTIVE/PURPOSE:
CD34 is a cell surface sialomucin that has been the subject of extensive interest, largely based on its use as a marker for hematopoietic stem cells (HSCs) and vascular endothelia. Despite the almost ubiquitous use of CD34 as a HSC marker, little is known about its cellular function. Our lab was the first to show that CD34 is also highly expressed on mature murine mast cells, and we and other groups have found it to be expressed on eosinophils and dendritic cells. We found that mast cells derived from *Cd34*$^{-/-}$ mice exhibit a marked increase in cell-cell aggregation. Moreover, when *Cd34*$^{-/-}$ mice were challenged in a mouse model of asthma, immune cell accumulation in the lung was drastically reduced, while the number of immune cells in the lung at baseline were similar to that of their wild-type counterparts. We have since found that deletion of the CD34 gene in mice renders these animals resistant to a wide range of other mucosal inflammatory diseases, including hypersensitivity pneumonitis (HP), ulcerative colitis, salmonella infection and intestinal tumor development. Our objectives are to examine the specific role of CD34 in cellular function and to see whether or not CD34 is a viable therapeutic target to treat mucosal inflammatory diseases.

METHODS:
Bone marrow mast cells will be derived from wild-type and *Cd34*$^{-/-}$ mice, and basic mast cell functions will be analyzed after c-kit and/or FceRI stimulation observing any changes in migration, degranulation, polarization, cytokine production and the phosphorylation of downstream signaling proteins. For *in vivo* studies, we have generated mice that lack the mouse CD34 gene and instead express, in all of the appropriate tissues, the human CD34 gene (*hCd34*^{tg}). These mice offer a unique ability to test therapeutic agents targeting human CD34 function in a mouse model and provide us with a robust preclinical model. Disease severity will be assessed by quantifying infiltrating cell numbers and differential counts from bronchoalveolar lavage (BAL) and asthmatic mice will be evaluated for airway hyper-responsiveness using a Flexivent apparatus.

FINDINGS:
Preliminary experiments have suggested that CD34 plays an important role in c-kit signaling events and FceRI induced degranulation. Initial testing of our *hCd34*^{tg} mice has shown that expression of the human CD34 gene in CD34 deficient mice is sufficient to regain susceptibility to both allergic asthma and HP in mouse CD34-deficient animals. These findings suggest that human CD34 serves a similar function to mouse CD34 in both animal models.

DELIVERABLES and RELEVANCE:
We show that in mast cells, CD34 plays an important role in regulating cellular signaling through both the c-kit and FceRI pathways. In addition, we have demonstrated that expression of human CD34 serves a similar function to mouse CD34 in both asthma and HP, providing a proof-of-concept to assess therapeutics targeting human CD34 in *hCd34*^{tg} mice as a humanized mouse model to treat these diseases. Allergic asthma affects more than 10% of all North Americans and is a major cause of hospitalization of children. Current therapeutics are largely ineffective for chronic asthma and the most potent therapies can carry a number of side effects. CD34 could represent a new therapeutic target, and since we have shown that CD34 plays a role in the susceptibility to a wide range of mucosal inflammatory diseases, it is likely that it could serve as a viable treatment for a number of diseases.

11B. Analysis of Tie2 Function in Mast Cells

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor: Kelly M. McNagny

OBJECTIVE/PURPOSE:
Mast cells are most widely acknowledged as a central mediator of allergic reactions. Recent literature has also implicated mast cells in a variety of biological and pathological conditions, spurring an interest in the genetic regulation of mast cell function and development. In a survey of global gene expression of bone marrow derived mast cells (BMMC) in relation to a $Lin^{-}Sca1^{+}cKit^{+}$ (LSK) bone marrow population we identified higher Tie2 mRNA expression. Tie2 (gene name *Tek*) is a receptor tyrosine kinase more commonly known for its expression on endothelial cells and a receptor for angiopoietins including Ang1 and 2. Our objective is to explore the function of Tie2 in mast cell development and biology.

METHODS:
A. Employ a microarray to survey global gene expression of BMMC

B. Generate Tie2-deficient mast cells from $Tie2^{-/-}$ embryonic stem (ES) cells and assess their ability to migrate, degranulate and adhere to substrates

C. Evaluate the ability of $Tie2^{-/-}$ mast cells to reconstitute *mast cell-deficient mouse models (eg, $Kit^{W/Wv}$)*

D. Determine the effect of Tie2 stimulation or inhibition *via* various ligands, in BMMC cultures

FINDINGS:
- BMMC express Tie2 mRNA and Tie2 protein at the membrane surface
- ES cell derived $Tie2^{-/-}$ and $Tie2^{+/-}$ mast cells do not possess any observable morphological abnormalities
- Tie2 expressed on BMMCs is functionally-active Ang1 and Ang2 receptor

DELIVERABLES:
-Microarray analysis of BMMC
-Derivation of $Tie2^{-/-}$ mast cells from $Tie2^{-/-}$ ES cells
-Functional analysis and profiling of BMMC activities and intracellular signaling response following stimulation with Tie2 ligands

RELEVANCE:
Given the importance of mast cells in the pathology of human disease, analysis of the genetic factors regulating mast cell function and development may provide insight into suitable therapeutic targets.

12B. Unique properties of RV in an *in vitro* model of asthma exacerbation.

AllerGen Programme B: Diagnostics and Therapeutics
Ilarraza R, Wu Y and Adamko D.
Pulmonary Research Group, Department of Pediatrics, University of Alberta
Supervisor: Darryl Adamko

OBJECTIVE/PURPOSE:
A vast majority of asthma exacerbations are linked to viral infections, 60% of which are caused by rhinovirus (RV). We hypothesize that eosinophils are key effector cells in asthma exacerbations. As such, using an *in vitro* model of human cells, we have shown that respiratory syncytial virus (RSV) and RV induce eosinophil degranulation when co-cultured with T-cells and autologous monocyte-derived dendritic cells (moDC), concurrent with moDC-dependent CD4+ CD45RO+ T-cell activation. While our original hypothesis has been that such activation is mediated solely by antigen-presentation (AP), our recent data also suggest that RV may be the major pathogen associated with asthma attacks because of its unique ability to interact directly with T-cells.

METHODS:
Human monocytes, T-lymphocytes, autologous moDC and eosinophils (EOS) were isolated from blood from atopic donors. moDC were cultured with RSV, RV, or sham (1 hour), then washed, and incubated with autologous T-cells (7 days). To determine the role for antigen presentation, some moDC were treated with the inhibitor chloroquine before virus culture. In some, no moDc were added and T lymphocytes (CD4, CD8 or a mix) were exposed to RSV, RV or UV-inactivated RV (RV-UV). T-cell activation and apoptosis were measured by flow cytometry (CD25 expression and Annexin-V/TO-PRO-3 staining, respectively), and proliferation by BrdU incorporation (Cell Proliferation ELISA, Roche). EOS cysteinyl-leukotriene (Cys-LT) release was measured by ELISA (Cayman Chemical). Cytokine release was measured by Searchlight (Pierce).

FINDINGS:
RV presence induced a 5-fold increase in Cys-LT release from EOS co-cultured with T-cells and moDC (n=6), while RSV did not have any effect (n=2); such induction was even higher when moDC were omitted. In T-cell and moDC co-cultures, RV induced greater increases in IL-1beta, IL-10, IL-12p70, IL-13 and IFN-gamma compared with RSV (n=2). T-cells, and more evidently CD8+ T-cells, showed increased apoptosis when incubated with RV but not with RV-UV or RSV (n=2). RV but not RV-UV or RSV directly induced proliferation of T-cells in the absence of moDC (n=5-6). Virus-loaded moDC induced proliferation of T-cells, but, unlike RSV, RV was insensitive to chloroquine (n=5).

DELIVERABLES:
Our results suggest that RSV and RV induce EOS and T-cell activation differentially. RSV induces proliferation in a MHC-II restricted antigen-presentation-dependent manner, while for RV this is not the sole pathway. In addition, based on cytokine and Cys-LT release, RV appears to be more potent compared to RSV. These novel data will lead to a publication.

RELEVANCE:
Our data are relevant to help understand why RV is so potent in the induction of asthma exacerbation in allergic patients, and will lead to novel potential targets for improved preventative strategies.

13B. The protective effect of fenretinide against allergic asthma

AllerGen Programme B: Diagnostics and Therapeutics
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[1]Department of Human Genetics, McGill University, Montreal, Quebec, Canada
Supervisor: Radzioch, D.

OBJECTIVE/PURPOSE:
Fenretinide [N-4-hydroxyphenyl, 4HPR] is a synthetic retinoid derived from vitamin A with pro-apoptotic and anti-inflammatory properties. Studies have shown that fenretinide is an effective antineoplastic agent, which regulates cell growth and differentiation, inhibits the proliferation of cancer cells and increases apoptosis. We have recently demonstrated that fenretinide also has a protective effect against cystic fibrosis lung disease and juvenile osteoporosis. Fenretinide has also been shown to be effective in treating acne, rheumatoid arthritis and macular degeneration. The effect of fenretinide as a potential treatment for allergic asthma has not been evaluated in asthmatics. The goal of this proposed study is to determine if fenretinide would be able to alleviate the symptoms associated with allergic asthma by normalizing inflammatory mediators.

METHODS:
Hyperresponsive to methacholine, atopic mice of A/J strain were sensitized using ovalbumin (OVA) once a week for three consecutive weeks (100µg in Alum, i.p). The mice were age-matched and separated in number into two study groups and two control groups. The study groups were orally treated with fenretinide solubilized in 95% ethanol and resuspended in elemental Peptamen diet at a dose of 60mg/kg/day for a period of 4 weeks. The control groups were treated with the drug vehicle resuspended in Peptamen diet. During the last week of treatment, both the control and study groups were split into two additional groups and underwent 30 minute-challenges, for three consecutive days, with either a 1% OVA solution or PBS. Forty-eight hours after the last OVA challenge, resistance of the respiratory system of the mice was measured using a Buxco plethysmograph system and Harvard Apparatus ventilators. Total IgE in plasma was measured by ELISA. Lung histopathology was observed using H&E and PAS staining. The levels of inflammatory Th2 cytokines were measured using specific Th2 specific bead*lytes* by Luminex 100LS.

FINDINGS:
Our results demonstrated a protective effect of the tested drug against allergic asthma in a mouse model. Vehicle treated OVA challenged mice exhibit high values of airway resistance, plasma IgE concentration, immune cell infiltration into the airways, and Th2 cytokines concentration compared to PBS challenged animals. On the other hand, fenretinide treated OVA challenged mice had a statistically lower respiratory resistance in response to methacholine. In addition, fenretinide treatment also abrogated the recruitment of eosinophils to the area surrounding the blood vessels and airways. Similarly, a decrease in goblet cell hyperplasia was also observed through histopathology after drug treatment. Certain Th2 cytokines showed differential expression levels between the vehicle treated and drug treated OVA challenged animals. However, no difference was observed in the level of plasma IgE between the control and study groups.

DELIVERABLES:
The synthetic retinoid, fenretinide, has been demonstrated to have great potential as a therapeutic agent. Understanding the cellular and molecular mechanisms responsible for this effect, as well as any potential side effects, is of great importance in the development of this compound for clinical use in allergic asthma.

RELEVANCE:
The data presented herein will facilitate the development of fenretinide as a therapeutic compound in the treatment of allergic asthma and provide the foundation for the identification of drug targets for the development of effective future drugs. Our findings will be communicated to decision-makers and end-users through articles in scientific journals and clinical trials.

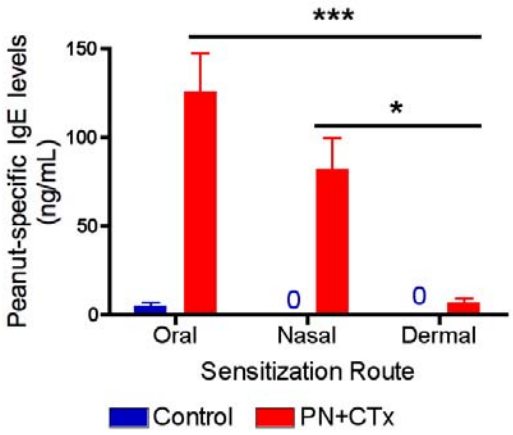
14. Importance of Routes of Exposure in the Development of Immune Response to Peanut

AllerGen Programme B: Diagnostics and Therapeutics
Nivedita Khanna[1,2] and Jeremy A. Scott[1,2,3]
[1]Divisions of Occupational and Respiratory Medicine, Department of Medicine, University of Toronto; [2]Keenan Research Centre in the Li Ka Shing Knowledge Institute and the Gage Occupational and Environmental Health Unit, St. Michael's Hospital Research Centre; [3]Occupational and Environmental Health Program, Dalla Lana School of Public Health, University of Toronto
Supervisor: Jeremy A. Scott

OBJECTIVE/PURPOSE:
Immediate hypersensitivity reactions to food are a major health concern for Canadians due to severity of reactions they elicit and their increasing prevalence. Currently 6% of children develop food allergy. Peanut (PN) hypersensitivity is one of the major causes of food-related anaphylaxis. We tested the hypothesis that the route of initial exposure to food antigen dictates the nature of the immune response and hence the development of an allergic response/anaphylaxis *vs.* tolerance upon subsequent or secondary exposure. The aim of this study was to: 1) establish three independent animal models of peanut exposure *via* the oral, dermal and inhalational routes that will allow us to 2) investigate whether sensitization or tolerance develops following gastrointestinal, dermal or inhalational exposures, following initial exposures *via* the alternate route.

METHODS:
Female Balb/c mice (8-weeks old) were sensitized to 1 mg of PN protein in combination with 5 µg cholera toxin as an adjuvant or PBS on days 0 and 14 *via* the oral, dermal or intranasal routes; and challenged with crude peanut extract (CPE) by oral gavage (2 mg/dose), dermally (10 µg/dose) or intranasally (500 µg/dose) on days 28, 30, 32, 35, 37 and 39. Mice were assessed for allergy/anaphylaxis (*i.e.,* rectal temperature at 10 minute intervals, clinical scoring for symptoms of anaphylaxis) for 40 minutes after the last allergen challenge and then euthanized for: 1) general (*e.g.,* measurement of PN-specific immunoglobulins, plasma histamine and inflammatory cell infiltration into the target organ), and 2) route-specific end-points (*i.e.,* wheal diameter after dermal and pulmonary function testing using the flexiVent after nasal challenge). Data are expressed as the mean±SEM (n=12-16/group).

FINDINGS:
Upon secondary challenge, oral sensitization elicited the highest levels of PN-specific IgE, followed by the nasal and dermal routes (**Figure**). PN sensitization and challenge by all three routes resulted in similar increases of plasma histamine upon secondary challenge. Overall, the oral challenge route elicited the greatest immune response, while the dermal challenge elicited the least. Only the oral sensitization route was able to elicit any significant clinical signs of anaphylaxis. In these mice, the scores were greatest for the oral challenge, followed the dermal and nasal routes, respectively (*i.e.,* oral>dermal>nasal).



by

DELIVERABLES:
Oral sensitization is the most likely to elicit a significant allergic response to peanut upon secondary challenge, while dermal sensitization is less likely. Nasal sensitization results in an intermediate likelihood.

RELEVANCE:
Numerous strategies have been proposed for the treatment of peanut allergies but there is still no safe, effective, specific therapy for the peanut-sensitive individual. The aim of current strategies to control food-related anaphylaxis is through desensitization of the affected individual with high doses of the allergen. Comparison of the immune and cellular mediators of tolerance and anaphylaxis *via* the different routes may help to identify the causative mediator/cell population that could lead to novel therapeutic targets for intervention.

15B. A bacterial immune-prophylactic approach against asthma for infants and children

AllerGen Programme B: Diagnostics and Therapeutics
Daniela I.M. Loeffler, Charis Segeritz, Bing Cai and Tobias R. Kollmann
Child and Family Research Institute, University of British Columbia
Supervisor: Tobias R. Kollmann

OBJECTIVE/PURPOSE:
The prevalence of asthma in Canada (and worldwide) has been increasing over the last 20 years with currently over 3 million Canadians suffering from it. Asthma appears to result from environmental influences directing a genetically predisposed host towards a pro-allergic, Th2-dominated immune response. Studies in humans and animals identify the time around birth and early infancy as a period during which the decision of pro-allergic versus non-allergic immune responses to environmental stimuli appears to be made. Presumably this is the reason why the incidence of first diagnosis is highest in infants and children, although asthma can occur at any age. The very fact that all three components (environmental, genetic and developmental) are necessary for asthma to occur also offers the opportunity to intervene early in life through *e.g*. vaccination against allergies. Vaccination as a strategy to prevent or cure asthma is a tremendous opportunity. An ideal vaccine would be one that prevents or cures asthma after only one dose and protects for life. It is well established that the whole heat-killed bacterium *Listeria monocytogenes* (*Lm*) given as an adjuvant along with model allergens effectively prevent allergic sensitization and/or allergic inflammation in adult animals following local allergen challenge. We have successfully developed a novel, live, but highly attenuated neonatal vaccine platform based on the bacterium *Listeria monocytogenes* (*Lm*). Our published data suggest that our *Lm*-based vaccine platform is capable of inducing strong anti-allergic immune responses for an entire life only after one dose given to newborn mice. Now we have investigated whether our *Lm*-based vaccine platform will provide protection from allergic reactions upon challenge with the allergen, after only one immunization given around birth. Our specific aim addressed the following objective: **Do our *Listeria monocytogenes* vaccine strains producing model allergen ovalbumin (OVA) and inducing a strong Th1 response, prevent allergic reactions upon challenge with the allergen in a neonatal mouse model?**

METHODS:
We focused on assessing protection against OVA-allergic reactions in mice that were immunized *i.p*. as newborns with heat-killed *Lm* vs. those vaccinated with our live-attenuated *Lm*-OVA or *Lm* or NaCl alone. We have coupled immune-focused analysis (total and differential counts of broncho-alveolar lavages, OVA-stimulation assays on lung cells and splenocytes, measurement of IgG1, IgE and IgG2a levels in serum) with the histopathological examination of lungs. Furthermore, we started to delineate the molecular mechanisms (Realtime PCR of immunological-relevant genes) underlying the surprisingly high efficacy of the live-attenuated *Lm*-based vaccine approach.

FINDINGS:
Our novel *Lm*-based vaccine platform was particularly safe and very-well tolerated in newborns. Using this live-attenuated platform in comparison to the already established heat-killed *Lm* approach in adult mice, we determined that mice immunized as newborns with our live *Lm* vaccine platform producing ovalbumin were indeed entirely protected from allergic OVA-sensitization after just one immunization given around birth. Furthermore, our live *Lm*-based approach was far superior to heat-killed *Lm*-based approaches, as it resulted in an almost complete inhibition of the recruitment of inflammatory cells into lungs of immunized mice after OVA-challenge. Interestingly, mice immunized with our live attenuated *Lm* strain not expressing OVA but sensitized and challenged with OVA showed inhibition of the recruitment of only eosinophils, but not inhibition of any of the other inflammatory cells into the lungs upon challenge.

DELIVERABLES:
The analysis of our single dose *Lm*-vaccination strategy in mice represents the first attempt to truly test the ability of a neonatal vaccine to prevent allergic reactions in early in life, but for the entire life. We furthermore expect to be able to optimize this approach to apply our *Lm*-based vaccines against food allergies or other clinically relevant forms of allergic disease as an immune-modulatory based therapeutic intervention in previously sensitized individuals.

RELEVANCE:
It offers immediate translation into human vaccine design and current immunization policies against asthma, as *Listeria monocytogenes* has already been approved for human applications.

16B. The role of perfluooctanoic acid (PFOA) in airway hyperrepsoniveness

AllerGen Programme B: Diagnostics and Therapeutics
Mark Loewen, Sujata Basu, Andrew Halayko, Kent HayGlass, Genevieve Bondy, Allan Becker
University of Manitoba, Health Canada Food Directorate
Supervisor: Allan Becker

OBJECTIVE/PURPOSE:

Evidence is emerging that human exposure to environmentally ubiquitous perfluorooctanoic acid (PFOA), used commonly for its household stain repellency characteristics, is associated with immunologic changes. Data from adults near an industrial PFOA disposal site demonstrated a strong negative correlation between blood PFOA concentrations and some immune responses. We propose to test if early life exposures to PFOA are playing a role in modifying airway responses.

METHODS:

Although several animal exposure models have been used to test ingestible PFOA toxicity using gavage methods at high concentrations, we chose to expose timed-pregnant Balb/C dams from GD-2 at more environmentally relevant concentrations (4mg/kg diet PFOA Sigma Aldrich) mixed into the diet (Purina 5001). Dams were allowed to eat either a control or contaminated diet ad-libitum (~ 4-6g/day) through pregnancy and lactation. Upon weaning, lung mechanics of the exposed and control dams were measured using a flexiVent and liver weights measured. Dams were not sensitized to allergen.

FINDINGS:

Baseline lung mechanics and airway responsiveness of PFOA-exposed, non-sensitized mice were not significantly different from controls, however, liver weight as a function of body weight was significantly higher in exposed dams compared to controls (9.4% vs 5.5% p=0.0003).

DELIVERABLES and RELEVANCE:

The importance of studying the effects in pregnancy and early life was demonstrated by observations of PFOA exposures in pregnant mice where offspring died, while the mother seemed unaffected. In that study, mortality of the pups was attributed to pulmonary abnormalities.

We have demonstrated that environmentally relevant exposures to PFOA in pregnant mice yield significant increases in liver weight. We will investigate lung function in the offspring of these dams. Pups from exposed and control dams are being weaned on the same diet as their mother. The exposed and control groups have been divided into 2 further groups one of which has been sensitized intraperitoneally at day 29 and 46 and intranasally at days 46, 47 and 48 with ovalbumin. Cytokines and WBC in BALF, IgE in blood and lung mechanics using flexiVent will be measured and reported.

17B. Bronchial fibroblasts modulate CD4+Tcells phenotype towards Th17 in asthma.

AllerGen Programme B: Diagnostics and Therapeutics
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Centre Recherche, Institut Universitaire de Cardiologie et de Pneumologie de Québec, Université Laval,
Supervisor: Jamila Chakir

OBJECTIVE/PURPOSE:
In asthma, CD4+T cells are selectively recruited into the bronchial mucosa. CD4+ T cells consist of different subsets that express lineage specific transcription factors and play different roles either in initiating and supporting the development of immune response, but also in orchestrating and regulating them. The aim of our study was to evaluate the effect of T cells-bronchial fibroblasts interaction on CD4+T cell phenotype.

METHODS:
Human bronchial fibroblasts were isolated from mild steroid naïve asthmatics and non-atopic healthy controls. CD4+T cells were purified from the peripheral blood of healthy and asthmatic subjects. Co-culture of confluent healthy (HF) or asthmatic bronchial fibroblasts (AF) with T cells were performed. CD4 + T cell total RNA was purified and GATA-3, Foxp3 and RORc expression was detected by quantitative PCR. Th17 (IL-17,IL-22) lineage specific cytokines profile was also evaluated.

FINDINGS:
Co-culture of T cells with bronchial fibroblasts significantly stimulated RORc in asthmatic T cells only, whereas Foxp3 and GATA-3 were not affected in both asthmatic and healthy T cells. IL-6 and IL-23 expression either by AF and HF were also significantly increased by the co-culture when TGF-β expression was not affected. In CD4+ T cells, IL-17 and IL-22, Th17 lineage specific cytokines were significantly increased by the co-culture with AF.

DELIVERABLES:
Interaction between bronchial fibroblasts and T cells seems to specifically promote a Th17 cell profile in asthma. These results suggest that cellular interactions, particularly between T cells and fibroblasts, may play a pivotal role in the regulation of the inflammatory response in asthma.

RELEVANCE:
Our study confirms the concept that structural cells play an important immunoregulatory role and may reveal novel pathways that underpin airway inflammation and remodelling in chronic asthma and identifies potential targets for therapeutic intervention where there is an unmet clinical need.

18B. CD34 is Required for the Infiltration of Inflammatory Cells into the Mouse Colon during DSS-induced Colitis.

AllerGen Programme B: Diagnostics and Therapeutics
Steven Maltby, Carolin Wohlfarth, Michael R. Hughes and Kelly M. McNagny.
The Biomedical Research Centre, University of British Columbia
Supervisor: Kelly McNagny

OBJECTIVE/PURPOSE:
Eosinophil infiltration of gut tissue plays a key role in the pathogenesis of inflammatory bowel diseases (IBD), such as ulcerative colitis. Using a model of allergic asthma, we previously demonstrated that eosinophil migration requires surface expression of the sialomucin CD34, and that *Cd34* deletion dampens asthmatic responses in mice. Since CD34 is critical for eosinophil migration, we investigated a role for CD34 in the migration of inflammatory cells into the colon using a mouse model of IBD.

METHODS:
To induce ulcerative colitis, we treated animals with 3.5% dextran sodium sulfate (DSS) and monitored the appearance of clinical symptoms including weight loss, rectal bleeding and diarrhea. Mice were sacrificed after eight days of treatment and we measured colon length, enumerated hematopoietic lineage subsets infiltrating gut tissue by flow cytometry and prepared colon sections for histology to determine the severity of gut pathology. In order to determine the significance of CD34 expression on hematopoietic cells in the development and progression of IBD, we reconstituted wild type mice with *Cd34*$^{-/-}$ bone marrow to generate chimeras.

FINDINGS:
We found that*Cd34*$^{-/-}$ mice are highly resistant to DSS-induced IBD with significantly less weight loss and colon shortening than wildtype controls. Histological analysis of *Cd34*$^{-/-}$ colons revealed less crypt loss, less tissue infiltrate, reduced tissue ulceration and overall reduced disease severity. We found that approximately 40% of the infiltrating blood cells are eosinophils and peripheral eosinophil levels are reduced following disease induction. Intriguingly, eosinophils harvested from the colon express high levels of CD34 and represent the majority of CD34^{+} cells within inflamed gut tissue. Protection from DSS-induced IBD is largely recapitulated in mice reconstituted with *Cd34*$^{-/-}$ bone marrow, demonstrating the requirement for CD34 expression on hematopoietic cells in mucosal inflammation.

DELIVERABLES and RELEVANCE:
Our findings demonstrate a key role for CD34 on hematopoietic cells in the pathology of ulcerative colitis. Gut eosinophils express high levels of CD34 and, similar to our findings in allergic asthma, we demonstrated that CD34 is required for optimal eosinophil migration *in vivo* and *Cd34* deletion results in decreased gut inflammation during IBD. Taken together, our findings highlight CD34 as a potential therapeutic target for IBD treatment and suggest that therapies targeting CD34 may be sufficient to impair eosinophil infiltration into the colon.

The research was funded by the AllerGen Network Centre of Excellence (3.14). SM and MRH hold CIHR and Hearth & Stroke Transfusion Science Fellowships from the Centre for Blood Research (UBC). Kelly McNagny is a Michael Smith Foundation Scholar (Senior) and Centre for Blood Research Member.

19B. Immunoregulatory role of secretory leukocyte protease inhibitor in allergic asthma

AllerGen Programme B: Diagnostics and Therapeutics
Rafael Marino[1], Thusanth Thuraisingam[2], Pierre Camateros[1], Yong Zhong Xu[1], Jennifer Henri[1], Jingxuan Yang[3], Guoan He[4], Aihao Ding[4] and Danuta Radzioch[1].
[1] McGill University [2] University of Montreal, [3] Swiss Federal Institute of Technology Zürich, Switzerland. [4] Weill Medical College of Cornell University, New York, USA.
Supervisor: Danuta Radzioch

OBJECTIVE/PURPOSE:
Asthma is a complex and multi-factorial inflammatory disease. It is one of the most common chronic diseases among children and adolescents. Despite many advances in treatment, asthma remains a major public health issue and patients still need more effective therapeutic options with fewer side effects. The aim of this study was to evaluate the role of Secretory Leukocyte Protease Inhibitor (SLPI) in the development of phenotypes associated with allergic asthma, and the effect of resiquimod treatment on the SLPI and the possible mechanisms of action involved in the disease.

METHODS:
The importance of SLPI was assessed by evaluating airway resistance and inflammatory parameters in SLPI transgenic and knock-out mice using an ovalbumin (OVA)-induced model of acute allergic asthma.

FINDINGS:
Allergic SLPI transgenic mice showed a significant decrease in airway resistance compared to wild-type mice (6.3±1.1 vs. 8.0±2.1 cm H_20 x s/ml), the same effect was observed with inflammatory cell infiltration, eosinophil percentage (24±1.1% vs. 29±2.3%), goblet cells (6±1.4 vs. 36±4.0%) in the lungs and IgE levels (2014.1±309.2 vs. 4173.2±685.6 ng/ml) in plasma. Allergic SLPI knock-out mice displayed significantly higher values compared to wild-type mice. They include lung resistance (8.6±2.7 vs. 6.6±0.5 cm H_20*s/ml), inflammatory cell influx, eosinophils (36.0±2.7 vs. 29.0±1.5%), goblet cells (40±4.1 vs. 30±1.4%), cytokine levels in the lungs and plasma IgE levels (3598±204.7 vs. 2763±220.3 ng/ml). Expression of SLPI decreased inflammation in the lungs, plasma IgE levels, and lung resistance, whereas the ablation of SLPI has the opposite effect. Treatment with resiquimod improved airway resistance and inflammation in the lungs in SLPI knock-out and wild type, demonstrating that its effect is independent of the expression of SLPI.

DELIVERABLES:
SLPI plays an immunoregulatory role in the respiratory tract by reducing the inflammatory process and by improving lung physiology in a model of acute allergic asthma. We intend to identify the role of SLPI in the pathophysiology of allergic asthma using, for the first time, SLPI transgenic mice and previously described SLPI knock-out mice. We also want to evaluate whether or not treatment with resiquimod directly modifies the expression of SLPI.

RELEVANCE:
Due to many functions attributed to SLPI and the lack of genetic polymorphism, this protein represents an important candidate that should be explored for therapeutic purposes in asthma. This study will lead to a better understanding of the molecular mechanism involved in allergic asthma and will improve the design of novel therapeutic approaches.

20B. A Comparison of Manitoba CHILD Participants and the General Manitoba Population

AllerGen Programme B: Diagnostics and Therapeutics; CHILD
Protudjer JLP[1,2,], Luo, JC[1,2] and Becker, AB[1,2]
[1]Manitoba Institute of Child Health, [2]University of Manitoba
Supervisor: AB Becker

OBJECTIVE/PURPOSE:
Low socioeconomic status (SES) is a risk factor for a broad array of health outcomes. Moreover, we sought to describe the socio-environmental status of participants in the Vanguard and cohort of the Canadian Healthy Infant Longitudinal Development (CHILD) Study and compare these participants' characteristics to similar characteristics of the general Manitoba population.

METHODS:
Nation-wide CHILD Study plans to recruit pregnant women at 4 Canadian sites, including Winnipeg, where 5000 participants will be recruited over the next two years. Participants (including those in the 'Vanguard', pilot group) completed questionnaires about their health, environment and SES. These data were described using descriptive and x^2 analyses. General Manitoba population data were obtained from Statistics Canada and Manitoba Health and Healthy Living.

FINDINGS:
To date, 100 women (52 Vanguard) have completed questionnaires. Participants were 30.8±4.3 years old; this is similar to the average maternal age (29.7 years) at delivery for the general Manitoba population. Post-secondary training was higher amongst CHILD Study participants than the general Manitoba population (81.1% vs. 38.8% college/undergraduate and 14.9% vs. 5.1% graduate degrees, respectively). Using Statistics Canada's income adequacy quartiles, the majority of CHILD Study participants were from upper middle class families. Notably, Vanguard participants were more likely to own their own homes than cohort participants (93.8% vs. 71.4%; $p<0.015$). Vanguard participants were also more likely to rate their social standing as higher as compared to cohort participants (96.6% vs. 38.5%; $p<0.001$). In Manitoba, 1 in 5 women smoke. In the CHILD Study, 8.8% of women currently smoke.

DELIVERABLES:
Compared to the general Manitoba population, CHILD Study participants are of higher SES and are less likely to smoke, however, more recent CHILD Study cohort participants represent a slightly broader demographic than Vanguard participants.

RELEVANCE:
Continued efforts to recruit pregnant women from a broad demographic will provide the CHILD Study with a nationally representative study population, which will serve to better understand genetic and environmental influences on early life development. This will be of importance to the AllerGen-supported CHILD Study.

21B. Cord Blood Hemopoietic Progenitor Cell Toll Like Receptor Expression and Function: A Mechanism Underlying Allergic Inflammation in Early Life?

AllerGen Programme B: Diagnostics and Therapeutics
Pia Reece[1], Lynn Crawford[1], Adrian J. Baajtes[1], Michael M. Cyr[1], Roma Sehmi[2], Judah A. Denburg[1]
McMaster University
Supervisor: Judah Denburg

OBJECTIVE/PURPOSE:
Neonatal immune responses to environmental stimuli, mediated *via* TLR, may determine the development of atopy in childhood. Since hemopoietic mechanisms are involved in development and maintenance of allergic inflammation, we investigated alterations in progenitor expression and differentiation profiles after stimulation with TLR agonists.

METHODS:
Freshly isolated, CD34-enriched human CB cells were stimulated with 10µg/mL lipopolysaccharide (LPS) or 5µM CpG ODN overnight. Flow cytometric analyses were used to evaluate surface and intracellular expression of TLR-2, TLR-4, TLR-9, as well as the hemopoietic cytokine receptors (HCR) IL-5R, IL-3R and GM-CSFR; methylcellulose cultures were performed to assess CD34+ cell differentiation capacity into Eo/B CFU.

FINDINGS:
After TLR agonist stimulation, CD34+ cell TLR-2, -4 and TLR-9 percentage expression increased significantly ($p=0.005$), whereas HCR expression decreased ($p=0.01$); however, mean fluorescence intensity of all receptors was found to be increased. Stimulation with a combination of TLR agonists and hemopoietic cytokines induced increased IL-5- and IL-3-responsive Eo/B CFU ($p=0.02$), when compared to hemopoietic cytokine stimulation alone.

DELIVERABLES:
CB CD34+ progenitor cells significantly express TLR, and TLR ligation directly affects both TLR and HCR expression. These receptor alterations allow modulation of progenitor cell differentiation capacity into eosinophils and basophils, key cells involved in allergic inflammation. These findings may highlight an alternate innate immune pathway of microbial influence on the development of allergic inflammation in early life.

RELEVANCE:
These findings may suggest that activation of TLR-mediated hemopoietic mechanisms during the neonatal period could be a forerunner for the development of infant atopy and allergic inflammation, thereby providing a novel therapeutic target for preventative measures against infant allergy.

22B. Virus Memory Induces Airway Hyperreactivity Through Eosinophil Activation

AllerGen Programme B: Diagnostics and Therapeutics
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Department of Pediatrics[1] and Medicine[2], University of Alberta, Edmonton, Alberta, Canada
Supervisor: Darryl Adamko

OBJECTIVE/PURPOSE:
Asthma is the most common chronic respiratory disease in children. Asthma exacerbation occurs when the airways acutely become obstructed, usually the result of airway inflammation. The Inflammation is caused by a unique mix of cells, and includes eosinophils. The majority of asthma exacerbations occur after a viral infection such as a common cold. Why asthmatic children develop such severe reactions to viruses is unclear. Our previous work suggests asthmatic patients develop severe airway obstruction because they have too many eosinophils in their airways before virus infection. The virus triggers these eosinophils to release harmful mediators and cause airway damage. We believe that in humans, it may be the mere presence of virus antigen that stimulates memory cells to activate the eosinophils. We hypothesize that memory T cell proliferation and eosinophil activation will occur in response to any airway virus for which immune memory exists, and that removal of the eosinophils will prevent airway hyperreactivity (AHR). In addition, we believe that this model is representative of virus-induced asthma exacerbation. As part of our project to develop non-invasive diagnostics using the metabolomic profile of urine through Nuclear Magnetic Resonance (NMR) spectroscopy, we are saving the urine samples from these animals. We hypothesize that there will be relevant differences between the urine profiles of each animal group, which will be applicable to humans.

METHODS:
Our study used two groups of guinea pigs (GPs), sensitized and non-sensitized to ovalbumin (i.p.). Both groups were infected (i.n.) with parainfluenza virus (PIV) and allowed to recover. A month after their first virus exposure, and they were challenged with ovalbumin (aerosol) to simulate an allergen exposure. 2 weeks later, OVA-exposed GPs were inoculated with either the same PIV, sham or a UV-inactivated PIV. All GPs were studied 5 days following exposure to virus for airway responsiveness, inflammation, virus titers and lymphocyte memory to the virus. Urine was collected by open bladder puncture for NMR analysis from all GPs.

FINDINGS:
GPs with primary exposure to live PIV demonstrated splenic lymphocyte proliferation *in-vitro*, thus confirming immune memory to PIV. Both sensitized and non-sensitized GPs develop AHR and inflammation following primary and secondary PIV. The sensitized animals are more eosinophilic. Only the sensitized animals develop increased AHR in response to a secondary UV-inactivated PIV. These results support our previous *in-vitro* work that suggested the virus antigen is presented to memory T cells, which then stimulate the eosinophils. This data also suggests that the development of AHR is related to the timing of virus exposure and the degree of eosinophil stimulation, not the degree of virus infection. Urine metabolomic profiles are being analyzed.

DELIVERABLES:
Guinea Pigs develop a memory response to PIV and upon re-exposure to PIV viral antigens this immune memory appears to cause increased AHR and inflammation in only the sensitized animals. These novel data will lead to a publication.

RELEVANCE:
This study will identify the cellular pathway that is involved in a virus induced asthma exacerbation. Knowing this pathway will allow researchers to develop targeted methods that may prevent virus induced asthma exacerbations. Metabolomic characterization of virus induced asthma exacerbations in a Guinea Pig model will enable us to identify relevant markers to be studied in the human version of the disease.

23B. IL-13Rα2 / AP-1 Complex Signalling Mediates Airway Epithelial Repair without Effects on Remodeling Pathways

AllerGen Programme B – Diagnostics and Therapeutics
J.S. Yang, S. Allahverdian, G.K. Singhera, R.E. MacRedmond, and D.R. Dorscheid.
James Hogg Research Centre at Providence Heart & Lung Institute – UBC
Supervisor: D.R. Dorscheid

OBJECTIVE/PURPOSE:
The airway epithelium serves as a physical defense barrier to the external environment for the underlying tissue and suffers frequent injury as a result. The initial response to injury is inflammation followed by debris clearance and repair. Although IL-13 is known to be a key cytokine in mediating inflammatory and remodeling responses *via* STAT-6 and EGR-1, our laboratory has demonstrated that IL-13 is critical to airway epithelial repair *via* the autocrine release of HB-EGF and activation of EGF-R. IL-13 signals through two receptors, IL-13Rα1/IL-4R and IL-13Rα2. IL-13Rα2 has previously been thought to act exclusively as a decoy receptor, however findings from our laboratory show that IL-13Rα2 can act as a signaling receptor and is involved in mediating airway epithelial repair. Differential signaling via IL-13Rα1 or IL-13Rα2 may determine a remodeling versus repair response to injury in airway epithelium.

METHODS:
IL-13Rα1 and IL-13Rα2 functions were disrupted in Human Airway Epithelial (1HAEo-) cells using specific IL-13Rα1 and IL-13Rα2 blocking antibodies and siRNA's. 1HAEo- cells were also transfected with AP-1 specific and scramble siRNA. Following specific antibody blocking or siRNA transfection, 1HAEo- cells were either stimulated with IL-13 (10ng/ml) or mechanically injured. Supernatants and protein lysates were then collected at different time points. Expressions of phospho-STAT-6, STAT-6, EGR-1, and AP-1 were detected via Western blotting, while HB-EGF release in supernatants was quantified using ELISA. Furthermore, AP-1activity in 1HAEo- cells after IL-13 stimulation or mechanical injury was measured using an AP-1-luciferase assay.

FINDINGS:
IL-13 stimulation resulted in upregulation of phospho-STAT6, EGR-1 and AP-1 expression. AP-1 expression correlated with activity as determined by AP-1 luciferase assay. Following mechanical injury, the expression of phospho-STAT6 and EGR-1 was inhibited when IL-13Rα1 function was disrupted, while induction of AP-1 expression is unchanged. In contrast, when IL-13Rα2 function was disrupted, HB-EGF and AP-1 expression was inhibited while STAT-6/EGR-1 signaling remains intact. Gene silencing of AP-1 had no effect on phospho-STAT6 expression in response to injury, however HB-EGF expression was significantly inhibited compared to Scr siRNA treated cells.

DELIVERABLES:
Our data indicates that IL-13 mediates repair of airway epithelial cells *via* IL-13Rα2 and AP-1, while remodeling responses downstream of STAT-6 and EGR-1 are signaled *via* IL-13 Rα1.

RELEVANCE:
Strategies directed towards augmentation of the IL-13Rα2/AP-1 pathway may lead to novel therapies which target the dysfunctional repair phenotype in asthmatic epithelium without adverse effects on airway remodeling.

III. PROGRAMME C – PUBLIC HEALTH, ETHICS, POLICY AND SOCIETY

#	AllerGen Trainee	Institution	AllerGen Researcher/ Supervisor	Abstract Title
1C	**Bahreinian,** Salma	**University of Alberta**	Dr. Anita Kozyrskyj	Does Chronic Stress Predict the Development of Asthma in Pre-adolescents?
2C	**Ben-Shoshan**, Moshe	**McGill University Health Centre**	Dr. Ann Clarke	The Prevalence of Sesame Allergy: A Cross-Canada Study
3C	**Cooney,** Mathieu	Manitoba Institute of Child Health, **University of Manitoba**	Dr. Allan Becker	Wheeze in the absence of asthma at age 8-10 is not associated with atopy in Manitoba children
4C	**Harrington,** Daniel	**McMaster University**	Dr. Susan Elliott	Who's afraid of the big bad peanut? Perceived societal risks of food allergy and anaphylaxis from the perspective of affected and unaffected Canadians
5C	**Houlbrook,** Doug	**University of Manitoba**	Dr. Allan Becker	Association of parental FEF25-75% during a methacholine challenge and Children's PC20 and diagnosis of asthma
6C	**Huang,** Henry (Hao)	**University of Manitoba**	Dr. Allan Becker	The relationship of children sensitized to peanut and parental asthma in Study Asthma Genes and the Environment (SAGE)
7C	**Peer,** Miki	**University of Toronto**	Dr. Claudio N. Soares	Does perceived stress in pregnant immigrant women predispose their infants to allergic disease development? A work-in-progress
8C	**Protudjer,** Jennifer	**University of Manitoba,** Manitoba Institute of Child Health	Dr. Allan Becker	Subsequent childhood asthma and wheeze amongst small-for-gestational-age infants in Manitoba and India: An International Partnership Initiative
9C	**Soller,** Lianne	**McGill University**	Dr. Ann Clarke	Determining the prevalence of milk, egg, and wheat allergies in the Canadian population
10C	**Xu,** Sophia	**McMaster University**	Dr. Susan Waserman	Challenges and strategies in managing food allergy: a patient and allergist perspective

1C. Does Chronic Stress Predict the Development of Asthma in Pre-adolescents?

AllerGen Programme C: Public Health, Ethics, Policy and Society
Bahreinian S[1], Ball GDC[1], MacNeil BJ[2], HayGlass KT [2], Becker AB[2], Kozyrskyj AL[1]

[1]Dept Pediatrics, University of Alberta; [2]University of Manitoba

Supervisors: Kozyrskyj AL[1,] Ball GDC[1]

OBJECTIVE/PURPOSE: Pediatric asthma has risen over recent decades in developed countries, affecting almost 10% of children. Canada ranks near the top of the list of countries with high rates of asthma and allergic diseases. Predicting which children will develop asthma remains a challenge, but both higher weight and stress may play a role. An association between obesity and asthma has been reported in previous studies among adolescents and school-age children; this connection is more apparent in girls. Children exposed to maternal stress in early life are more likely to develop asthma. If the stress exposure becomes chronic and the body is unable to respond appropriately, allostatic load/overload (AL) can develop. AL is defined as the physical price paid by the body under chronic stress. To our knowledge, the impact of AL on the development of asthma in pre-adolescents has not been investigated. The overall aim of this research is to determine the influence of AL on the development of asthma. We hypothesize that (1) exposure to AL increases the risk of developing asthma in pre-adolescence, and (2) the contribution of each AL measure is not equal in predicting the development of asthma.

METHODS: This study is a prospective evaluation of children enrolled in a novel birth cohort study, the Study of Asthma, Genes and the Environment (SAGE). Healthy children were recruited in the nested case-control study component of SAGE at the age of 8-10 years and were followed until pre-adolescence (age 12-13) to assess the development of new asthma. At study onset and follow-up, all children were examined by a pediatric allergist to assess the development of asthma; several clinical assessments were made, and a fasting blood sample was taken to assay biochemical markers. We created an index of AL using 7 markers: systolic and diastolic blood pressure, waist-to-hip ratio, total cholesterol, high density lipoprotein cholesterol (HDL), cortisol & dehydroepiandrosterone sulphate (DHEAS). An AL score was created for each child by summing the number of biomarkers in the top quartile; higher score = higher level of stress.

FINDINGS: A total of 477 children without asthma were recruited into the study at age 8-10. Overall, 10.1% of the 306 children followed until the age of 12-13 years developed asthma. At age 8-10 years, 18.7% of children had an AL score higher than 2. There was no statistically significant difference between the mean AL score according to sex, urban/rural location and family history of asthma (all $p>0.05$). However, overweight children had significantly higher mean AL scores compared to non-overweight children ($p<0.001$). None of the risk factors of sex, urban/rural residence, overweight or family history of asthma at baseline had a relationship with asthma development after the follow up period (all $p>0.05$). Subsequent analyses are planned to determine the association between AL and the development of asthma in logistic regression models, which adjust for these factors and additional covariates.

DELIVERABLES: This research provides a measure of 7 physical and biomedical components, which can be routinely measured in all children and has the potential to be used as a marker to identify children at risk for developing asthma in the future. We also provide a statistical model that can predict the probability of asthma development regarding the AL score after adjusting for other possible covariates for each individual.

RELEVANCE: Using AL as a predictor of asthma in school-aged children could provide clinicians with an opportunity to implement interventions aimed at preventing asthma. From a practical standpoint, developing a parsimonious model of AL would be the most useful and easiest for clinicians to adopt in their everyday practice.

2C. Treatment of allergic reactions to peanut in recent versus initial reaction

AllerGen Programme C: Public Health, Ethics, Policy and Society
Ben-Shoshan M[1], Nguyen Luu N[2], Alizadehfar R[1], Soller L[2] , Fragapane J[2], Joseph L[2, 3] St. Pierre Y[2], Harada L[4], Fortin C[5], Allen M[6] and Clarke AE[2,7]
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[7]Division of Allergy and Clinical Immunology, Department of Medicine, McGill University Health Center.
Supervisor: AE Clarke

OBJECTIVE/PURPOSE:
Although studies suggest underuse of epinephrine in food related allergic reactions, it is not clear whether treatment may differ over time in those who have already had an allergic reaction. We sought to characterize treatment of the most recent allergic reaction to peanut versus the initial allergic reaction.

METHODS:
Individuals with an allergist-confirmed peanut allergy were recruited from the Montreal Children's Hospital and Canadian food allergy advocacy organizations. Data were collected on initial allergic reactions to peanut and most recent reaction to peanut during the year prior to study entry.

FINDINGS:
Among 180 individuals reporting both an initial allergic reaction and a recent allergic reaction to peanut, epinephrine was administered in 8.9% (95% CI, 5.2-14.0%) and 17.2% (95% CI, 12.0-23.5%) respectively. Treatments excluding epinephrine were given in 35.6% (95% CI, 28.6-43.0%) of initial reactions and in 62.2% (95% CI, 54.7-69.3%) of most recent reactions. Among those treated only outside health care facilities (HCFs) no participant received epinephrine in initial reactions versus almost 9% (95% CI, 3.9-16.6%) in most recent reactions. However, in initial reactions, 44.8% (95% CI, 26.4-64.3%) of those treated, only in HCFs received epinephrine compared to 20% (95% CI, 2.5-55.6%) in recent reactions. Almost 1/3(95% CI, 15.6-48.7%) of participants with a severe reaction did not receive any treatment for the initial reaction compared to 6.7% (95% CI, 0.8-22.1%) of those with a recent reaction.

DELIVERABLES:
Although there is higher use of epinephrine in recent reactions compared to initial reactions, it is still administered in only 40% of severe allergic reactions. Further, our results suggest decreased epinephrine use over time in those treated initially in HCFs concurrent with increased use of other treatments such as anti-histamines.

RELEVANCE:
Given that prompt administration of epinephrine is the principal therapy for food-related anaphylaxis, it is crucial to develop and distribute guidelines and education programs that would contribute to increase epinephrine use inside and outside HCFs.

3C. Wheeze in the Absence of Asthma at Age 8-10 is not Associated with Atopy in Manitoba Children

AllerGen Programme C: Public Health, Ethics, Policy and Society
Cooney MF[1], Protudjer JLP[1,2], Kozyrskyj AL[2,3] & Becker AB[1,2]
[1]Manitoba Institute of Child Health, [2]University of Manitoba, [3]University of Alberta
Supervisor: AB Becker

OBJECTIVE/PURPOSE:
Atopy in preschool children with recurrent wheeze is the best predictor for persistent asthma. However, there are few studies in which the association between wheeze and atopy in children without asthma has been examined. A recently modified Asthma Predictive Index (API) which included allergic sensitization to aeroallergens and to foods found a higher prevalence of atopy in high-risk children compared to normal children with intermittent wheezing. Since atopy is considered a risk marker for wheeze phenotypes used in the diagnosis of asthma, we predicted that atopy would not be associated with wheeze when asthma was absent.

METHODS:
Children in the 1995 Manitoba Birth Cohort (SAGE) nested case-control study were assessed at age 8-10 years by a pediatric allergist both clinically and by questionnaire. Skin-prick tests to common allergens were performed to determine the presence of atopy. Children underwent methacholine challenge for airway hyperresponsiveness. Parent-reported history of wheeze ever was ascertained using the question "Has your child ever had wheezing or whistling in the chest at any time in the past?" The association between atopy and recurrent wheeze was determined using the odds ratio (OR) and 95% confidence interval (CI).

FINDINGS:
723 (404 [55.9%] boys) children were involved in this study (mean age 9±0.5). 246 (34.1% [149 (36.9%) boys]) had pediatric allergist-diagnosed asthma. 236/714 (33.1%) children assessed had current wheeze based on allergist notes and 420 (58.4%) had parent-reported wheeze ever; these were not mutually exclusive. There was a significant association between atopy and parent-reported wheeze ever (OR 2.16; 95% CI 1.59-2.94), physician-noted wheeze with a cold (OR 2.23; 95% CI 1.65-3.00) and without a cold (OR 1.82; 95% CI 1.33-2.50). Physician-noted wheeze without a cold was more strongly associated with atopy in girls (OR 2.41; 95% CI 1.48-3.93) compared to boys (OR 1.46; 95% CI 0.96-2.22). In the absence of asthma, the association between atopy and parent-reported wheeze ever, physician-noted wheeze with a cold and without a cold was lost. Further stratification by PC_{20} category did not yield significant associations.

DELIVERABLES:
There was an association between atopy and wheeze in the presence of an asthma diagnosis, particularly amongst girls. All associations between atopy and wheeze were lost in the absence of an asthma diagnosis.

RELEVANCE:
Atopy is an important diagnostic marker in the pediatric clinical assessment of wheeze. As predicted, wheeze not used in the diagnosis of asthma was not associated with atopy. These results support the use of a modified API that includes skin-prick tests for its positive predictive value.

4C. Who's afraid of the big bad peanut? Perceived societal risks of food allergy and anaphylaxis from the perspective of affected and unaffected Canadians

Programme C – Public Health, Ethics, Policy and Society
D.W. Harrington[1], S. J. Elliott[1], M. Ben-Shoshan[2], S. Sheth[2], S. B. Godefroy[3], J. Fragapane[2], L. Soller[2], M. H. Allen[4], M. Allen[5], C. Dufresne[6], L. Harada[4], S. Waserman[7], A. Clarke[2].
[1]McMaster University; [2]McGill University Health Centre; [3]Health Canada; [4]Anaphylaxis Canada; [5]Allergy/Asthma Information Association; [6]Association Quebecoise des Allergies Alimentaires; [7]Division of Allergy and Clinical Immunology, McMaster University.
Supervisor: Susan Elliott

OBJECTIVE/PURPOSE:
The purpose of this research is to explore the determinants of the perceived risks associated with food allergy and anaphylaxis.

METHODS:
Households (n = 3,666) were selected at random, as part of the SCAAALAR national food allergy prevalence survey, and data were collected *via* telephone. In addition to determining allergy status in the household, respondents were queried on attitudes and opinions toward environmental health risks in general, and food allergy/anaphylaxis risks in particular. Multivariate logistic regressions, weighted to the age-sex structure of the Canadian population, were used to determine the characteristics of respondents who ranked the risks of food allergy and anaphylaxis as 'High' or 'Moderate' to the Canadian public.

FINDINGS:
One-fifth of the sample reported having at least one food allergy in the household. Almost 70% of respondents ranked the risks of food allergy as high or moderate risks, compared to just over 60% for anaphylaxis. Determinants include well-established demographic predictors of health risks perceptions (*e.g.,* age, gender). Other important covariates suggest that general attitudes towards environmental health risks in general, knowledge about foood allergies, and worldviews are significant predictors of food allergy and anaphylaxis risk. In terms of risk experience, only respondents with multiple food allergies in the household significantly ranked the perceived risks as high or moderate (OR: 2.77, 95% CI: [1.56, 5.27]). Broad regional differences in risk perception were observed in this survey. Respondents from Quebec resported a greater degree of perceived societal risk for food allergy (OR: 2.07, 95%CI: [1.63, 2.63]) and anaphylaxis (OR: 1.34, 95%CI: [1.08, 1.67]).

DELIVERABLES:
This study is informed by theories of risk perception that have established the importance of understanding risk perceptions for explaining how the public responds to risk. In the context of food allergy, and anaphylactic food allergy, there is an attendant need to develop appropriate policy responses that can protect allergic individuals, while accommodating the general population. This research contributes to this need by characterizing the societal response to the prevalence of food allergies and anaphylactic food allergies.

RELEVANCE:
This study has started to uncover some important determinants of food allergy- and anaphylaxis-related risk perception. This knowledge is crucial for understanding what these risks mean to affected and unaffected populations in Canada. Analyses also revealed marked differences between Quebec and other provinces. It is becoming increasingly apparent that the policy environment in this province in particular is a key determinant of the experience and perception of allergy-related risk. Results from this research indicate that both the perceptions of affected *and* unaffected populations are modified in this context. Policymakers need to consider these impacts as advancements in regulations and policy emerge in this area.

5C. Association of Parental FEF25-75% during a Methacholine challenge and Children's PC20 and diagnosis of Asthma

AllerGen Programme C: Public Health, Ethics, Policy and Society
Houlbrook D, Becker AB, Ramsey CD
University of Manitoba
Supervisor: AB Becker

OBJECTIVE/PURPOSE:
There is evidence that paternal and maternal lung function influence children's pulmonary function. To date, the most widely used pulmonary function test (PFT) to assess airway responsiveness is the PC20 (concentration of methacholine required to decrease FEV1 by 20%). However, other measures of parental lung function may be better predictors of airway responsiveness or asthma development in their children.

METHODS:
The study population included children born in 1995 in Manitoba, who were enrolled in a nested case-control birth cohort study (Study of Asthma Genes and the Environment (SAGE). Both index children and their parents underwent PFTs and methacholine challenges to determine their PC20. Parents often had a normal PC20 but a change in flow at mid-lung volumes (forced expiratory flow at 25-75% vital capacity) was noted. Therefore, we measured parental PC30 (concentration of methacholine required to decrease FEF 25-75% by 30%) and its association with their child's PC20 and physician diagnosed asthma. A positive PC30 was measured at a concentration of ≤ 8 mg/ml. Children were assessed at age 12-13 by a pediatric allergist for a diagnosis of asthma. Odds ratios (OR) were calculated to examine the relationship between parental PC30 and a child's PC20 and asthma. This relationship was further explored by gender.

FINDINGS:
Overall 114 children and their parents were included in this analysis. Of the 114 children, 47 were females (21 asthma) and 67 were boys (23 asthma). No significant relationship was seen between children diagnosed with asthma and having a parent with a PC30 ≤ 8 (OR 1.07; 95% CI 0.49-2.37) or both parents PC30 >8.
112 children [45 females (23 with PC20 ≤ 8) and 67 males (46 with PC20 ≤ 8)] fit the criteria for having a PC20 and participating parents. In children with a PC20 ≤ 8 there was no relationship between having one parent with PC30 ≤ 8 (OR 1.18; 95% CI .54-2.63) or both parents having a PC30 >8.
When we examined gender specific relationships, there was no association between fathers PC30 and having a son diagnosed with asthma (OR 1.32; 95% CI 0.5-3.5) or daughters with a diagnosis of asthma (OR 1.07; 95% CI 0.33-3.48). Similarly, there was no significant association between the father's PC30 and their child's PC20 being ≤ 8 mg/ml.
In moms with a PC30 ≤ 8 mg/ml there was no association with sons that had a PC20 ≤ 8 mg/ml (OR 1.0; 95% CI 0.51-2.0); However moms with a PC30 ≤ 8 were significantly more likely to have a daughter with a PC20 ≤ 8 (OR 3.56; 95% CI 1.55-8.21). In addition, there was a significant relationship between mom's PC30 being ≤ 8 and a daughter having physician diagnosed asthma (OR2.34; 95% CI 1.04-5.44). The relationship was in the same direction between maternal PC30 and a son's diagnosis of asthma, however this was not significant (OR 2.0; 95% CI 0.99-4.05).

DELIVERABLES:
There was no relationship between children's asthma and PC20 and parental airway responsiveness measured as PC30. There was a statistically significant relationship between daughter's airway responsiveness and asthma and mothers' airway responsiveness as a PC30 FEF 25-75%.

RELEVANCE:
Maternal mid-flow responsiveness may be helpful in identifying risk for the development of pediatric asthma. Mid lung flow changes in mothers may represent a maternal genetic influence on the development of asthma in children.

6C. The relationship of children sensitized to peanut and parental asthma in Study Asthma Genes and the Environment (SAGE)

AllerGen Programme C: Public Health, Ethics, Policy and Society
HUANG H, Chooniedass R, Kozyrskyj AL & Becker AB
Supervisor: Becker AB

OBJECTIVE/PURPOSE:
Children with asthma most often have associated allergy, and peanut allergy with asthma is more common in children whose parents have asthma. It is not clear how common peanut allergy is among these children. The St. John's cohort, India has found 6.2% peanut sensitivity children whose mothers have asthma. Children are at greater risk for severe life threatening reactions and hospitalization.

METHODS:
The SAGE cohort is a study of children born in 1995 in Manitoba. We created a nested case-control cohort of 723 children for asthma and allergy at 8 years of age. Atopy was defined as having at least one positive skin test, to common inhalants and to peanut (wheal diameter≥3mm.) Chi-square test and Fisher's exact test was applied, the likelihood (odds ratio, OR) of parental asthma of non-peanut allergic children compared to parental asthma of peanut sensitized children was determined.

FINDINGS:
In the cohort, 220 (30.4%) parents were diagnosis with asthma. 718 children skin tested, 333 (46.4%) were atopic with 42 (5.8%) sensitized to peanut. Peanut sensitized children are more likely to have asthma (OR=2.8, 95%CI 1.5-5.2). Among 246 (34.1%) children with asthma, 6 (2.5%) children who had a parent with asthma were sensitized to peanut (OR=0.8, 95% CI 0.3-1.9) when compared with children whose parents did not have asthma.

DELIVERABLES:
Peanut allergy is more common in children who have asthma, but there is not an additional significant association with parental asthma. Diagnosing peanut allergy in an early childhood is an early marker for increased asthma risk.

RELEVANCE:
Better understanding of the relationship between childhood asthma and peanut allergy can reduce asthma morbidity and hospitality, which could also improve the life quality of asthmatic children. These data emphasize the importance of AllerGen's research and societal agenda.

7C. Does Perceived Stress in Pregnant Immigrant Women Predispose their Infants to Allergic Disease Development? – A Work-in-Progress

Programme C – Public Health, Ethics, Policy and Society
Miki Peer[1,2,3], Meir Steiner[1,2,3,4], and Claudio N. Soares[2,3,4]
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Supervisor: Claudio N. Soares

OBJECTIVE/PURPOSE:
To examine whether prenatal perceived stress and/or physiologic maternal stress responses are associated with the development of allergies in infants, in a diverse group of immigrant women.

METHODS:
Sixty immigrant women will be recruited early in pregnancy and followed up to 1 year postpartum. Three study visits (<24 weeks gestation, 32-36 gestation, and 1 year postpartum) and two brief phone calls (at 3- and 6-months postpartum) will be used to collect information about maternal health including perceived stress, depressive symptoms, social support, and biomarkers of stress reactivity (salivary cortisol). Information on infant birth outcomes and cord blood (for measurement of IgE) will be collected at the time of delivery. Infant atopy (assessed via skin-prick testing and clinical history) will be assessed at 1 year of age, along with information on the infant's health and stress response (salivary cortisol).

FINDINGS:
To date, thirty women have been recruited into the study. Preliminary data illustrate a wide range of depressive symptoms, perceived stress, and social support. Four women reported high levels of depressive symptoms (>11 on the EPDS) and five reported high levels of perceived stress (>22 on the PSS-10), but only one woman reported both. Thus, stress and depressive symptoms appear to be distinct phenomena in this population. Women who experienced a very large number of negative life events in the previous 6 months or reported low levels of perceived social support tended to endorse either high levels of stress or depression in early pregnancy. Participant recruitment and testing are on-going.

DELIVERABLES:
A large portion of our (diverse) sample of immigrant women reported high levels of perceived stress and/or depressive symptoms during early- to mid-pregnancy. Whether these adverse perinatal mental states (and their associated dysregulated stress responses) contribute to the development of allergic disease in infants is under active, prospective investigation.

RELEVANCE:
"Canada's Immigration Program" (Canadian Library of Parliament, October 2004) reports that Canada has the highest per capita immigration rate in the world. Given the finding that pregnant immigrant women display higher prevalence of depression and anxiety disorders (Stewart et al., 2008), the investigation of health *outcomes* in this population is warranted. A better understanding of the effects of perinatal factors on susceptibility to allergic disease in the infant can lead to development of interventions when plasticity in physiologic development is still relatively abundant (Shanks & Lightman, 2001).

Canada's Immigration Program (October 2004) - Library of Parliament, at http://www.parl.gc.ca/information/library/PRBpubs/bp190-e.htm URL accessed 17 September 2007.

Stewart DE, Gagnon A, Saucier J-F, Wahoush O, and Dougherty G. 2008. Postpartum Depression Symptoms in Newcomers. *Can J Psychiatry* 53(2): 121-124.

Shanks N & Lightman SL. 2001. The Maternal-Neonatal Neuro-Immune Interface: Are there long-term implications for inflammatory or stress-related disease? *J Clin Invest* 108: 1567-1573.

8C. Subsequent Childhood Asthma and Wheeze amongst Small-for-Gestational-Age Infants in Manitoba and India: An International Partnership Initiative

AllerGen Programme C: Public Health, Ethics, Policy and Society
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Supervisor: AB Becker

OBJECTIVE/PURPOSE:
The global prevalence of asthma and wheeze are increasing. Concurrently, the incidence of infants born small-for-gestational-age (SGA) is rising. Evidence describing associations between these two conditions are conflicting. We sought to better understand this phenomenon in two distinct populations: Manitoba, Canada and Bangalore, India.

METHODS:
1995 Manitoba Birth Cohort nested case-control study: Gestational period and birth weight were obtained *via* parent-report and subsequently classified as per Canadian SGA guidelines. At 8-10 years, both children's asthma status and presence of wheeze were ascertained *via* pediatric allergist assessment. Parental-reported data also included wheeze (ever, current [in past year], or during various activities) as per the International Study of Allergies and Asthma in Children (ISSAC) questionnaire, and demographic data. *Bangalore Cohort:* Gestational period at birth and birth weight were measured at birth. SGA babies were classified as per the World Health Organization's SGA guidelines. At 2-7 years, presence of wheeze was ascertained *via* physician assessment/prescription record as well as per the ISSAC questionnaire. Asthma status was not assessed. All data were analyzed using descriptive statistics and x^2 tests.

FINDINGS:
In Manitoba, 725 children (406 [56.0%] boys) were assessed. Mean gestational age at birth was 39.5±2.12 weeks (non-significant [NS] differences by gender). Mean birth weight was 3.38±0.64 kg; girls were significantly smaller than boys ($p<0.006$). 114 (16.1%) children (62 [54.4%] boys) were SGA. At ages 9.06±0.64 years, 246 (34.1%) of children (149 boys) had asthma. No associations were identified between SGA and asthma. No associations were identified between SGA and wheeze, when considering both genders combined or amongst boys only. Amongst girls, those who were SGA were significantly more likely to have wheeze-related sleep disturbances than girls who were non-SGA (OR 0.33; 95% CI 0.12-0.94; $p<0.03$).

In Bangalore, 432 children (207 [48.0%] boys) were assessed for wheeze-like symptoms. Mean gestational age at birth was 38.68±1.62 weeks and mean birth weight was 2.87±0.49 kg, with NS differences by gender for either of the variables. The mean age of the study subjects was 3.78±1.30 years. 130 (30.2%) children (62 [47.7%] boys) were SGA. 71 (16.4%) of children (28 [38.4%] boys) had a doctor diagnosis of wheeze. SGA children had twice the risk of developing wheeze at follow-up (OR 2.19; 95%CI 1.30-3.68; $p<0.003$). After stratification by gender, these associations were only significant amongst boys (OR 3.24; 95% CI 1.44-7.31; $p<0.003$).

DELIVERABLES:
Children born SGA are at higher risk of developing wheeze-like symptoms, especially among the Indian boys. There is a small, yet significant association between SGA and wheeze-related sleep disturbances in Manitoban girls.

RELEVANCE:
Understanding the associations between SGA and wheeze may lead to enhanced pediatric clinical assessments. Public policy ought to target prevention of SGA.

9C. Determining the Prevalence of Milk, Egg, and Wheat Allergies in the Canadian Population

AllerGen Programme C – Public Health, Ethics, Policy and Society
Soller L[1], Fragapane J[1], Ben-Shoshan M[2], Harrington DW[3], Alizadehfar R[2], Joseph L[1,4], St. Pierre Y[1], Godefroy S[5], Elliott SJ[3] and Clarke AE[1,6]

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Supervisor: AE Clarke

OBJECTIVE/PURPOSE:
Milk and egg are the most common allergens in childhood. Recent reports also indicate that wheat may contribute to a significant number of food-related anaphylactic events. However, there have so far been no Canadian studies to assess the prevalence of these three important allergens. Our objective was to estimate the prevalence of milk, egg, and wheat allergies in the Canadian population.

METHODS:
We performed a cross-sectional, nationwide, telephone survey by adapting a questionnaire used by Sicherer *et. al.* in the United States to assess the prevalence of other food allergies (*JACI 2003;112:1203 & JACI 2004;114:159*). Telephone numbers were randomly selected from the electronic white pages and a letter describing the study was mailed to all selected households. Respondents were eligible to participate if they were 18 years or older, were living in the household, and appeared to have no language-mental-hearing barrier to understanding the questions. To optimize response rates and minimize selection bias, up to ten attempts were made to contact households, and calling was done on different days and at different times during the day. Individuals were asked whether they had an allergy to milk, egg, and/or wheat.

FINDINGS:
Of 10,596 households surveyed, 3666 responded, representing 9667 individuals (35% response rate). Of these, 202 (2.09% [95% CI, 1.81,2.39%]) reported an allergy to milk, 77 (0.8% [0.63,0.99%]) to egg, and 74 (0.77% [0.6,0.96%]) to wheat. Based on self-report, egg allergy was more prevalent in children than in adults, and wheat allergy was more prevalent in adults than in children. Both egg and wheat allergies were more prevalent in households with a post-secondary graduate. Important regional differences between allergies to milk, wheat and egg were also evident, with Quebec showing a lower prevalence of these three allergens compared to most other regions of Canada.

DELIVERABLES:
Milk, egg and wheat allergies appear to be relatively common in the Canadian population. However, results for milk and wheat allergy are not consistent with the literature (Keets *et al.* 2009 and Dias *et al.* 2009). The unusually high prevalence of milk and wheat allergy in adults may be due to participant confusion with lactose intolerance and celiac disease, respectively. Currently, our research team is contacting participants from the telephone survey in order to validate their self-report of allergy to milk, egg and/or wheat. Additional analyses will adjust the prevalence by ethnicity and other demographic factors that may partially explain the observed regional differences.

RELEVANCE:
This is the first nationwide Canadian study to determine the prevalence of milk, egg, and wheat; three allergens which affect many Canadians and may cause life-threatening anaphylactic reactions. Because of the potential danger associated with having a food allergy, it is crucial to undertake novel research studies to better understand the natural history, diagnosis, and management of food allergy so that we may improve the quality of life of allergic Canadians.

10C. Challenges and strategies in managing food allergy: a patient and allergist perspective

AllerGen Programme C: Public Health, Ethics, Policy and Society
S.Xu, S.Waserman, M. Kastner, K. Stawiarski, L. Connors.
McMaster University Medical Center
Supervisor: S.Waserman

OBJECTIVE/PURPOSE:
Research has shown that outpatient management of food allergy is suboptimal. Few studies have compared allergists' and patients' perspectives on food allergy management. Our goal was to assess the educational experiences, learning needs, and confidence level of food allergic patients; and to examine allergists' teaching priorities, challenges and strategies for managing these patients. Our data provides feedback to health care providers that may lead to better patient-centered care in allergy clinics.

METHODS:
This is a two-part observational study: an anonymous questionnaire completed by patients or caregivers in the clinic; and a qualitative interview with allergists. Using convenience sampling, patients were recruited from allergy clinics in Southern Ontario. Patients of any age with documented food allergy, who were seen by an allergist, were considered for inclusion. Outcomes were the learning needs of patients, and the management challenges faced by allergists and strategies to address them. Recorded allergist interviews were analyzed using content analysis of grounded theory methodology. Quantitative data were analyzed using descriptive statistics and frequency analysis.

FINDINGS:
Preliminary data were collected from 49 food allergic families (mean age was 8.5 years) and 5 allergists from community and academic centers in four cities. Sixty-one percent of patients were shown how to use an auto injector with a trainer and 51% were asked to demonstrate its use. Fifty one percent of patients did not feel very confident about when to give an auto-injector, or how to administer it correctly (59%) even though on average, it was their fifth visit with the allergist. Regarding learning needs, the majority wanted more current research information on food allergy prevention and cure. Although some wanted more information on "support groups" (29%) and auto injector practice (20%), 57% did not feel anything was missing from their clinic visits. Allergists' top priorities were teaching allergen avoidance and management of acute reactions. Major challenges were ensuring correct technique and empowering people to use auto injectors. Strategies included frequent follow up, practice with auto-injectors, and providing website or CD information. Allergists indicated that anaphylaxis management needs to be incorporated into first aid courses and teacher/daycare provider training. In conclusion, both allergists and patients identified building confidence to use epinephrine auto-injectors as a priority. Recommendations for improvement included frequent practice during clinic visits with a trainer and advocating for training outside the clinic. Patients' need for further information on allergy research and better social support would require allergists to be more proactive in linking families with reputable web resources and community support groups.

DELIVERABLES:
Our project will enable us to share educational experiences and goals of at least 100 food allergic patients in clinics across Southern Ontario. This is valuable feedback to participating allergists. Furthermore, data from allergist interviews will enable allergists to compare experiences and strategies to manage challenges in educating food allergic patients.

RELEVANCE:
Our study provides direction for improvement during allergy clinic visits, and strategies to address common challenges allergists face. This in turn will help improve patient care and quality of life for families living with food allergy. We aim to share our findings with patients and families through Anaphylaxis Canada, and with pediatricians across the country at the 2010 Canadian Pediatric Society Annual Conference.

IV. NON-ADJUDICATED POSTERS

#	AllerGen Trainee	Institution	AllerGen Researcher/ Supervisor	Abstract Title
1NA (A)	**Chaudhuri**, Sri	**University of Toronto**	Dr. Miriam Diamond	Exploring the linage between phthalates and asthma by measurement and modeling
2NA (A)	**Konya,** Tedd	**University of Toronto**	Dr. James Scott	Validation of a new wipe method for the collection of indoor dust samples for the characterization of endotoxin and beta-glucan
3NA (A)	**Mykhaylova,** Natalia	**University of Toronto**	Dr. Greg Evans	Biologically-relevant air quality evaluation system
4NA (A)	**Shu,** Huan	**Simon Fraser University**	Dr. Tim Takaro	Potential sources of phthalate exposure in a Vancouver birth cohort at three months of age and socio-economic status
5NA (A)	**Speck,** Mary	**University of Toronto**	Jeffrey Brook	Improved tools for assessing long-term indoor and outdoor exposures in epidemiologic studies of allergy and asthma
6NA (A)	**Urch,** Bruce	**University of Toronto**	Jeffrey Brook	Associations between inhaled Endotoxin/β-D-Glucan levels and airway/systemic neutrophils after controlled exposures to coarse and fine particles: Effect of atopy on responses
7NA (B)	**Grewal,** Rajdip	**University of Toronto,** The Hospital for Sick Children	Dr. Padmaja Subbarao	Exhaled nitric oxide measurement in infants at birth and three months of age
8NA (B)	**Plante,** Sophie	Centre Recherche, Institut Universitaire de Cardiologie et de Pneumologie de Quebec, **Universite Laval**	Dr. Louis-Philippe Boulet	Evaluation of regulatory T cell function in allergic asthmatic and allergic non-asthmatic subjects before and after low dose allergen challenge
9NA (B)	**Singhera,** Gurpreet	**University of British Columbia,** James Hogg Research Centre	Dr. Delbert Dorscheid	Novel anti-viral effects of CLA in airway epithelium
10NA (C)	**Dostaler,** Suzanne	**Queen's University**	Dr. Diane Lougheed	Development of a work-related asthma screening questionnaire and management algorithm
11NA (C)	**Simons,** Elinor	**The Hospital for Sick Children**	Dr. Teresa To	Environmental Contributions to Asthma among Canadian Children: The Population Attributable Fraction

1NA(A) Exploring the Linage between Phthalates and Asthma by Measurement and Modelling

Allergen Programme A : Gene-Environment Interactions
Sri Chaudhuri[1], Miriam Diamond[1], Ryan Allen[2,6], Michael Brauer[3,6],Jeffrey Brook[1,4,5,6], Jason Curran[2], Miriam Diamond[1,5], Greg Evans[1], Zhimei Jiang[4],Tedd Konya[1], Natalia Mykhaylova[1], Mary Speck[1,5]James Scott[1,5,6], Huan Shu[2], Timothy Takaro[2,6], Stuart Turvey[3,6], Bruce Urch[5], Amanda Wheeler[7]
[1]University of Toronto; [2]Simon Fraser University; [3]University of British Columbia; [4] Environment Canada; [5]St. Michael's Hospital; [6]AllerGen Investigator; [7]Health Canada
Supervisor: Miriam Diamond

OBJECTIVE/PURPOSE:
Phthalates, a group of organic chemicals with a common chemical structure, are ubiquitous in household and personal care products and in the blood and urine of virtually any citizen of any country that has tested for them (Silva et al. 2004, Wormuth et al. 2006). Numerous studies have found an association between phthalate exposure and the development of allergies and asthma. In a meta-analysis of the literature, Jakkola and Knight (2008) reported that phthalates (particularly the mono-ester metabolites) with side chains from C4-10, at relatively high doses, act as an adjuvant by inducing IgE and/or IgG1 antibodies. Several occupational studies and epidemiological studies with children have found varying levels of association between exposure to particular phthalates, allergies, asthma and rhinitis. Another major concern regarding phthalates is that low level exposure in rodent studies induces “phthalate syndrome” which appears to be disturbingly similar to human testicular dysgenesis syndrome (Nat Acad Sci 2008, Swan et al. 2005).

The goal of our research within the larger CMP-1 study is to investigate the relationship between phthalate concentrations in homes and urinary concentrations in infants enrolled in the CHILD pilot study “mini-CHILD” in Vancouver. Our contributions to this study are: (1) to determine optimal sampling methods for phthalates in homes that relate to exposure where the latter is being quantified via urinary metabolites measured in infants by Takaro and co-workers, and (2) to explore the factors responsible for home-to-home and room-to-room variations in phthalate levels by developing a mechanistic model capable of estimating household phthalate concentrations.

METHODS:
We will conduct an intensive sampling campaign to measure phthalate concentrations in a small number of homes in Toronto. The sampling regime will be designed to capture room-to-room and seasonal variations in phthalate concentrations. We will evaluate the use of window wipes as a sampling method intended to avoid inadvertent sampling of phthalate-rich surfaces such as vinyl flooring and wall paper. We will meet the second objective by further developing our mechanistic model of indoor chemical dynamics that is intended to predict indoor concentrations and to allow the understanding of factors affecting concentrations (Zhang et al. 2009).

RELEVANCE:
Phthalates are in the public eye as new studies report on widespread phthalate exposure and potential links to adverse health effects. The Canadian government gave notice in June 2009 of a new regulation that restricts concentrations of 6 phthalates to less than 1000 mg/kg in soft children’s toys that are likely to be mouthed (Canada Gazette June 20, 2009).

This study will contribute knowledge that could be used for policies for further risk management measures, or not, under the Canadian Environmental Protection Act (hence its funding through the Chemical Management Plan). We anticipate that the study will also result in guidance that can be used by individuals to minimize presumed personal exposure to phthalates.

2NA(A) Validation of a New Wipe Method for the Collection of Indoor Dust Samples for the Characterization of Endotoxin and Beta-glucan

AllerGen Programme A: CMP-1 (Chemical Management Plan Study 1)
Tedd Konya[1], Juliet Ewaze[1], Bruce Urch[1], Huan Hsa[2], Jason Curran[2], Mary Speck[5], Ryan Allen[2,6], Michael Brauer[3,6], Jeffrey Brook[1,4,6], Miriam Diamond[1], Greg Evans[1], Timothy Takaro[2,6], Amanda Wheeler[7], James Scott[1,5,6]
[1]University of Toronto; [2]Simon Fraser University; [3]University of British Columbia; [4] Environment Canada; [5]St. Michael's Hospital; [6]AllerGen Investigator; [7]Health Canada
Supervisor: James Scott

OBJECTIVE/PURPOSE:
The aim of this study is to find a cost-effective method, which is comparable to the gold-standard vacuum method, for the collection of indoor dust samples for the characterization of endotoxin and beta-glucan. Under the Chemical Management Plan (CMP)-1 project, we tested the efficiency of TefTex, a pyrogen-free cloth wipe, to collect dust vs. the vacuum method and compared endotoxin and beta-glucan levels in both.

METHODS:
Trained research technicians sampled 15 Vancouver area homes as part of the CMP1-mini-CHILD Study. Wipe samples were collected from the tops of doors and door frames inside the child's bedroom and the family's most used living room. Vacuum dust samples were collected in the same rooms where wipes were collected. A standardized floor area was sampled (m^2) and if an insufficient sample was obtained, a larger area was sampled. The vacuum-collected sample from the bedroom was a composite of mattress and floor dust. Pyrochrome End-Point Assay was used for endotoxin analysis and Glucatell Diazo Endpoint Assay for β-Glucan analysis (Associates of Cape Cod). Statistical analyses included linear regression and multiple linear regression analyses.

FINDINGS:
The endotoxin results for the wipe and the vacuum-collected dust were statistically correlated (r=0.433, p=0.0168). The sampled floor area did not significantly influence the correlation when included as a covariate (p=0.4), and that association remained significant (model r=0.045, p=0.0495). Beta-glucan results for the wipe were significantly correlated with vacuum-collected dust samples (r=0.527, p=0.003). When adjusting for floor type the r increased slightly to 0.565 (p=0.006).

DELIVERABLES:
These results suggest that TefTex wipes can provide accurate point-in-time endotoxin and beta-glucan measures within the home environment. Although this study used trained research technicians, the protocol can be modified to allow study subjects to sample in their own homes. With the low cost per wipe and the lack of need for a research technician, the TefTex wipe can be a cost-effective alternative to the traditional and more labour-intensive vacuum method.

RELEVANCE:
These results can help AllerGen investigators that are studying the health of humans in relation to their indoor environment. The wipe-based method provides an easy, cost-effective means of obtaining biological data and can provide a practical alternative to the objective measurement of indoor environmental exposures. Also, the wipes can be mailed to obtain data from homes in remote regions of Canada that would otherwise be inaccessible.

3NA(A) Biologically-relevant Air Quality Evaluation System

AllerGen Programme A: Gene-Environment Interactions
Natalia Mykhaylova, Chemical Engineering and Applied Chemistry
University of Toronto
Supervisor: Greg Evans

OBJECTIVE/PURPOSE:
The level of concern about indoor air quality and exposure patterns has been increasing significantly over the past decade primarily due to increased awareness of the indoor environment acting as a sink for many hazardous compounds; the combined health effects of which are still not completely understood. The absence of standard monitoring strategy and reliable biomarkers to evaluate the degree of bioavailability further complicates the situation. Although body burden of many allergy-associated air contaminants is currently a subject of detailed investigations, the relative contributions of the inhalation, dermal and ingestion exposure pathways are yet to be elucidated. To address this issue, a monitoring approach was proposed, which would facilitate the linking of sources of major pollutants with their health effects. The suggested design would constitute a cost-efficient, easily deployable indoor air monitoring kit for evaluation of population exposure in epidemiological studies.

METHODS:
The contaminants of greatest concern have been evaluated based on their physiochemical properties, indoor and outdoor concentration, body burden, primary exposure route, toxicity, source, industry use trends as well as ease of collection, extraction and measurement. Trends between the values of physiochemical parameters and the reported indoor air and dust concentrations as well as body burden have been examined. The analysis of dominant exposure pathways was then used to propose biologically-relevant sampling and screening strategy. The potential for utilization of the proposed method as surrogate for *in vivo* bioavailability and exposure was also investigated.

FINDINGS:
A prototype system for sampling and bioavailability screening of allergy-associated air pollutants was designed and validated *in silico* using Quantitative Structure Property Relationship.

RELEVANCE:
The proposed system would be instrumental in linking the types and quantities of allergens and allergy-associated air pollutants with exposure. Used primarily as an exposure diagnostic test for known and emerging air contaminants, it can also offer insights into pathways associated with allergic/immune diseases. Finally, the proposed system would improve the quality of life of allergy sufferers by providing feedback on the air contaminants present in their homes, possible sources and mitigation strategies. It is anticipated that it would also provide incentive for cleaner industrial processes, commercial products and improved public policies.

4NA(A) Potential sources of phthalate exposure in a Vancouver birth cohort at three months of age and socio-economic status

AllerGen Programme A: Gene-Environment Interactions
Huan Shu[1]; Tim Takaro[1]; Ryan Allen[1]; Kate Bassil[1]; Mike Brauer[2]; Walter Piovesan[1]; Roxanne Rousseau[2]; Jasper Stoodley[1]; Stuart Turvey[2]
Simon Fraser University[1], University of British Columbia[2]
Supervisor: Tim Takaro

OBJECTIVE/PURPOSE:
Phthalate is a group of semi-volatile synthetic chemicals that under normal conditions often leach into the environment (Sathyanarayana et. al., 2007) Exposure to phthalates may contribute to the development of an inflammatory response and be a factor in the development of allergic disease through direct or adjuvant mechanism. Phthalates are being used in the production of a variety household and personal care products, such as children's toys; as a chemical stabilizer in cosmetics; in infant care products, and in building materials and other home products (Jaakkola & Knight, 2008, Sathyanarayana et. al., 2007)
The MiniCHILD project has piloted recruitment procedures, indoor environment inspections, and traffic related air pollution models, resulting in the testing and refinement of effective protocols and methods to be implemented in the Canadian Health Infant Longitudinal Development (CHILD) birth cohort. (AllerGen Annual Report, 2007-8)
This project investigated the associations between exposures to phthalates from the indoor environment, personal care products, and interactions with socioeconomic status.
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2. Sathyanarayana, S., Karr, C. J., Lozano, P., Brown, E., Calafat, A. M., Liu, F., et al. (2008). Baby care products: Possible sources of infant phthalate exposure. *Pediatrics, 121*(2), e260-268. doi:10.1542/peds.2006-3766.
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METHODS:
Using urine samples from 63 MiniCHILD subjects at three months of age, we examined the concentration of seven phthalate metabolites (monobutyl phthalate (mBuP); monobenzyl phthalate (mBzP); mono-ethyl *phthalate* (mEtP); mono-2-ethyl-5-oxohexyl phthalate (mEOHP); mono-2-ethylhexyl phthalate (mEHP); mono-2-ethyl-5-hydroxyhexyl phthalate (mEHHP); monoethyl phthalate (mMeP)) and their association with exposure information from questionnaires; with demographic information from Canada Census 2006. Analysis used regression models and geographic information systems.

FINDINGS:
Based on preliminary results, we found higher levels of certain phthalate metabolites associated with personal care product such as baby lotion, shampoo; work with hazardous materials; and lower socioeconomic status. Additional analysis will focus on interactions between exposure domains.

DELIVERABLES AND RELEVANCE:
Exposure to phthalates may contribute to the development of an inflammatory lung response and be a factor in the development of allergic disease through direct or adjuvant mechanisms. Children are exposed to phthalates early in life. The CHILD cohort will enable examination of this exposure in the context of allergen, endotoxin, mould and other exposures that contribute to the development of asthma.

5NA(A) Improved Tools for Assessing Long-Term Indoor and Outdoor Exposures in Epidemiologic Studies of Allergy and Asthma

AllerGen Programme A: CMP-1 (Chemical Management Plan Study 1)
Mary Speck[1,5], Ryan Allen[2,6], Michael Brauer[3,6],Jeffrey Brook[4,5,6], Jason Curran[2], Miriam Diamond[5], Greg Evans[5], Zhimei Jiang[4],Tedd Konya[5] Natalia Mykhaylova[5], James Scott[1,5,6], Huan Shu[2], Timothy Takaro[2,6], Stuart Turvey[3,6], Bruce Urch[5], Amanda Wheeler[7]
[1]St. Michael's Hospital; [2]Simon Fraser University; [3]University of British Columbia; [4] Environment Canada; [5]University of Toronto; [6]AllerGen Investigator; [7]Health Canada
Supervisor: Jeffrey Brook

Rationale: In epidemiologic studies, accurate and precise exposure assessment is a critical component in the assessment of associations between environmental exposures and adverse health outcomes. Moreover, environmental risk assessment and management depend on reliable assessments of exposure distributions across diverse geographic areas and populations. There are many challenges associated with assessment of exposures in both outdoor and indoor environments, including costs and potential burden on individuals under study. These issues were the subject of an AllerGen-sponsored workshop on Environmental Exposures held in Banff, Alberta, in February 2008. An AllerGen research program that is focusing on improving exposure assessment methodologies has emerged from this workshop. The purpose of the poster is to describe this overall program, which is funded by Health Canada.

OBJECTIVE/PURPOSE:
1) Characterize temporal and spatial variations in house dust and health-relevant constituents such as traffic-generated air pollution markers, indoor biological pollutants, and phthalate plasticizers; and 2) Develop cost-effective exposure assessment methods, including measurements and models, for use in large studies of environmental factors of relevance in the development of asthma and allergy.
Aims: Leveraging off of the national Canadian Healthy Infant Longitudinal Development (CHILD) Study, and an ongoing CHILD pilot study ("mini-CHILD") in Vancouver, the program is undertaking research to:

- Better understand the potential for residential dust samples to characterize long-term exposure in studies of chronic health effects among infants and children.
- Develop novel approaches in sampling techniques to assess variability in house dust constituents and associated long-term exposures and determine if a cost-effective method can be recommended for contemporary or future studies.
- Assess spatial (both between-home and within-home) and temporal variability in dust collected and relate this data on dust constituents exposure and received dose estimated, in part, from phthalate metabolites in urine.
- Assess temporal (seasonal) and spatial (within and between home and between city) variability in the infiltration of traffic-related compounds in to the home environment.
- Assess the contribution of traffic-generated pollution to indoor exposures by measuring the levels and variability of relevant organic traffic markers in house dust and in personal and indoor air fine particle samples and examining the relationship with other indictors of traffic exposure.
- Develop exposure models through comparison of the biological and phthalate burden of house dust in different cities, varying housing stock, climate and home ventilation characteristics.

RELEVANCE:
The new insights and exposure methods developed through this program will allow for more effective use of the exposure data being obtained in the CHILD Study**.** Furthermore, the knowledge and understanding generated will potentially lead to simple, cost-effective exposure measurement techniques that may be used in future studies or at later time points in the CHILD Study.

6NA(A) Associations between Inhaled Endotoxin/β-D-Glucan Levels and Airway/Systemic Neutrophils after Controlled Exposures to Coarse and Fine Particles: Effect of Atopy on Responses

AllerGen Programme A: CMP-1 (Chemical Management Plan Study 1)
Bruce Urch[1,2], Mary Speck[1], James Scott[1,3], J. Ewaze[1], Jeffrey R. Brook[1,3,4], Diane Gold[5], Behrooz Behbod[5], Frances Silverman[1,2,3]
[1]Gage OEHU, University of Toronto/St. Michael's Hospital; [2]Institute of Medical Science, University of Toronto; [3]Dalla Lana School of Public Health, [4]Environment Canada; [5]Harvard School of Public Health.
Supervisor: Jeffrey R. Brook

OBJECTIVE/PURPOSE:
It is well recognized that particulate matter (PM) air pollution is associated with significant respiratory health effects. PM is a complex mixture of solid particles (dust, smoke, fly ash, pollen, fungal material) and liquid droplets (aerosols, fumes) that vary in size, composition, chemical properties and origin. Current air quality standards include PM with an aerodynamic diameter < 10 μm (PM_{10}) and < 2.5 μm ($PM_{2.5}$) but not a coarse ($PM_{10-2.5}$) standard. Innate immunity and inflammatory pathways have been shown to involve molecular structures called pathogen-associated molecular patterns, such as endotoxin and β-D-glucan. We describe ambient and concentrated bioaerosol (endotoxin and glucan) concentrations in controlled exposures to coarse and fine concentrated ambient particles (CAPs) and the effect of atopy on airway and systemic inflammatory responses.

METHODS:
We recruited 19 healthy adult non-smokers from the University of Toronto campus and Greater Toronto Area. Particles were drawn from an inlet located beside a busy street in downtown Toronto. In a randomized block design, participants were exposed for 130 minutes to one fine CAPs and two coarse CAPs and filtered air. Induced sputum was carried out the morning after exposures. Blood was taken for a complete blood count before, 1-hr after, and the morning after exposures. During exposures, integrated gravimetric PM mass concentrations were measured as well as ambient and concentrated endotoxin and glucan levels. Endotoxin and glucan were analyzed using Pyrochrome and Glucatell Diazo Endpoint assays, respectively. Spearman correlation coefficients were calculated for the bioaerosol and neutrophil data to investigate the relationships between these variables. Subjects were characterized as having atopy or not. Atopy was defined as at least one positive skin prick test (wheal ≥ 3 mm).

FINDINGS/DELIVERABLES: Exposure glucan levels were weakly associated with sputum neutrophils (r=0.41, p=0.19, n=13). A stronger association was observed for coarse CAP exposures (r=0.55, p=0.16, n=8) and for subjects with atopy (r=0.90, p=0.016, n=7). A similar pattern was seen with the absolute 24-hr change in blood neutrophils. Exposure glucan levels were weakly associated with absolute neutrophils (r=0.33, p=0.086, n=28). A stronger association was observed for coarse CAPs exposure (r=0.44, p=0.064, n=18) and for subjects with atopy (r=0.68, p=0.14, n=7). Weaker but positive associations were shown between endotoxin and sputum as well as blood neutrophils. Exposure levels of glucan (7.8 ng/m^3) and endotoxin (6.2 ng/m^3) were significantly correlated (r=0.59, p=0.001). Ambient levels of glucan (1.3 ng/m^3) and endotoxin (1.0 ng/m^3) were also highly correlated (r=0.75, p<0.0001).

RELEVANCE:
Few studies have considered the biologic component of PM exposure and effects on inflammation and, furthermore, the effects of specific size fractions of PM on inflammatory measures. Particle size is important because size affects the transport and removal of particles from the air and more importantly their deposition within the respiratory tract. Exposure levels of endotoxin and glucan that were about 6-fold greater than ambient levels were associated with both sputum and blood neutrophils. One suggested mechanism of PM-induced health effects is a local respiratory inflammation followed by a systemic inflammatory state. This study highlights the importance of atopy in response to biologic components of PM and provides valuable information to research on the health effects of bioaerosols in studies such as the Canadian Healthy Infant Longitudinal Development (CHILD) Study.

7NA(B) Exhaled Nitric Oxide Measurement in Infants at Birth and Three Months of Age

AllerGen Programme B: Diagnostics and Therapeutics
R Grewal , C Keast, N Rampersad, S French, S Balkovec and P Subbarao
The Hospital for Sick Children, Toronto
Supervisor: P. Subbarao

OBJECTIVE/PURPOSE:
Exhaled nitric oxide (eNO), synthesized from L-arginine by nitric oxide synthase (NOS), is an indicator of airway inflammation and can be measured non-invasively. Inducible NOS (iNOS) is activated during states of inflammation of the airways. Many cells use iNOS to produce NO, including eosinophils, the predominant cell found in the sputum of adult allergic asthmatics. In the respiratory tract, iNOS is localized in the airway epithelial tissue, where NO is largely produced. Conditions causing inflammation in the airways have been associated with higher eNO values. Our aim was to follow the trajectory of measured eNO values in a cohort of healthy infants at birth and at three months of age.

METHODS:
In infants, online eNO is generally measured using multiple-breath sampling during quiet tidal breathing. This is accomplished by performing the test when the subject is asleep. During the test, the child inhales NO-free air, supplied by the DENOX88 NO-free air supplier. NO-free air is delivered *via* a facemask covering the subject's oral and nasal cavity. The level of NO in their exhaled air is measured for one minute using the Ecophysics CLD88sp eNO analyzer.
Infants were recruited as part the AllerGen Canadian Healthy Infant Longitudinal Development (CHILD) Study. The focus of this study is to investigate the development of allergies and asthma in children by establishing the roles of genetic and host factors and their interaction with a range of environmental aspects. The CHILD Study is a prospective longitudinal birth cohort of 5000 children, with recruitment starting at 18 weeks *in utero*. The following results were gathered from the CHILD Study pilot cohort.

FINDINGS:
In total, 41 infants were born during the CHILD Study pilot period. Of the 41 eligible infants, 32 infants had birth eNO measured (mean = 6.3 SD = 3.8). Of the 41 eligible infants, 31 had eNO measured at 3 months (mean = 12.8 SD = 6.05). In terms of longitudinal tracking data, 22 of the 41 infants had eNO levels done both at birth and at 3 months.

DELIVERABLES:
The conclusions from this study will aid in the development of protocols and standardized methodology for the AllerGen CHILD Study. In addition, normative data will be generated.

RELEVANCE:
Based on the findings from this study, establishing non-invasive methods to characterize inflammation among infants is possible. The mean eNO at birth is significantly lower than published norms and may be related to the age at time of testing. Continued follow-up is needed to understand the relevance of the eNO values and trajectory from birth.

These findings will be presented at the American Thoracic Society Conference in May 2010.

8NA(B) Evaluation of regulatory T cell function in allergic asthmatic and allergic non-asthmatic subjects before and after low dose allergen challenge

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Supervisor**:** Jamila Chakir

OBJECTIVE/PURPOSE:
Regulatory T cells (Treg) contribute to the maintenance of immunological tolerance in the airways. There is evidence for impaired function of these cells in asthma and allergy. However, there is no information about the profile and the modulatory effect of allergen exposure on these cells in asthma and rhinitis. The objective of this study was to evaluate and compare the function of Treg in allergic asthmatic and allergic non-asthmatic subjects before and after low-dose allergen challenge.

METHODS:
Three groups of subjects were recruited: 1) healthy controls without allergy or asthma; 2) allergic asthmatic; and 3) allergic non-asthmatic subjects. Allergic asthmatic and allergic non-asthmatic subjects were subjected to low-dose allergen challenge (inhalation of low doses of allergen on 3 consecutive days). Evaluation of respiratory function and blood collection were performed at baseline and following the last allergen challenge. Treg were isolated using $CD4^{+}$ $CD25^{high}$ and $CD127^{low}$ staining. Suppressor function was measured by flow cytometry. Cytokines (IL-10 and TGF-□) were measured by ELISA.

FINDINGS:
The suppressor function of Treg in healthy controls was higher than in allergic asthmatic or allergic non-asthmatic subjects (mean ± sem: 78.8% ± 5.7% of inhibition vs 44.2% ± 8.1% and 37.0% ± 6.8%, $p<0.004$ and $p<0.003$ respectively). In allergic non-asthmatic subjects, the regulatory function of Treg was higher after allergen challenge (37.0% ± 6.8% at baseline vs 59.8% ± 3.1%, $p=0.01$). There was no change in Treg function in asthmatic subjects. Serum IL-10 decreased in allergic asthmatic after allergen challenge but not in allergic non-asthmatic subjects.

DELIVERABLES:
These results show that there is a defect in the regulatory function of Treg in allergic compared to non-allergic subjects. Low-dose allergen challenge stimulates the suppressor function of Treg in non-asthmatic allergic subjects but not in asthmatic allergic subjects. (Supported by Allergen NCE)

RELEVANCE:
There is a need to understand mechanisms that govern immune regulation in allergy and asthma. Increasing activity of Treg would be beneficial in controlling asthma and allergy and would have very important clinical implications.

9NA(B) Novel anti-viral effects of CLA in airway epithelium.

AllerGen Programme B: Diagnostics and Therapeutics
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Supervisor: Delbert R Dorscheid

OBJECTIVE/PURPOSE:
Conjugated linoleic acids (CLA) are fatty acids that are natural constituents of foods derived from ruminant animals. CLA as a dietary supplement can augment both innate and adaptive immune responses by reducing pro-inflammatory cytokine responses. Respiratory syncytial virus (RSV) is a leading cause of serious respiratory tract infections and asthma exacerbations in infants and young children throughout the world. The purpose of this study was to determine whether CLA could directly reduce viral infection, viral induced inflammation and cytopathic effect in airway epithelium.

METHODS:
Human airway epithelial ($1HAE^{O-}$) cells, normal human bronchial epithelial cells (NHBECs) grown in monolayer and Air liquid interphase (ALI) cultures were pre-treated with 30 μM of CLA mixture or linoleic acid (LA) control for 48 hours, followed by Respiratory Syncytial Virus (RSV) infection at MOI_3. Viral infectivity was analyzed by FACS analysis and immunohistochemistry (IHC). Total protein lysates were collected for Western blot analysis of RSV proteins, ICAM-1 and PARP (p85) protein expression. The inflammatory cytokines IL-6 and IL-8 were measured in the cell-free conditioned media by standard ELISAs.

FINDINGS:
CLA was not found to be directly cytotoxic to AEC compared to LA treatment as assessed by LDH assay. CLA pre-treatment reduced viral protein expression compared to LA treated cells. This effect was observed at 24 and 48 hours post infection. Reduced viral load was associated with reduced epithelial injury as determined by PARP cleavage/apoptosis. CLA pre-treatment reduced RSV-mediated upregulation of ICAM-1 protein expression in AEC.

DELIVERABLES and RELEVANCE:
Our data demonstrates that pre-incubation of airway epithelial cells with CLA in a model of chronic nutritional supplementation can result in reduction in viral infection and virus-induced apoptosis. As ICAM-1 binds RSV G- and F-proteins to facilitate viral entry and is upregulated by RSV infection, reduction in viral entry in CLA treated cells may be mediated at least in part by reduced expression of ICAM-1. This indicates a novel anti-viral effect of CLA independent of cell-mediated immunity.

10NA(C) Development of a Work-related Asthma Screening Questionnaire and Management Algorithm

Programme C – Public Health, Ethics, Policy and Society
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Supervisor: M.D. Lougheed

OBJECTIVE/PURPOSE: Work-related asthma (WRA) is under-recognized and delays in recognition contribute to long-term morbidity. The objective of the project is to develop a work-related asthma (WRA) exposure questionnaire and algorithm for use by primary care providers in the assessment of individuals with asthma. It is hypothesized that these tools will increase recognition of WRA, improve adherence with WRA treatment guidelines, and expedite diagnoses and appropriate WRA compensation.

METHODS: The American College of Chest Physicians Consensus Statement on WRA (2008) was consulted and a search of databases and websites (PubMed, OVID, National Institute for Occupational Safety and Health (NIOSH), Ontario Workplace Safety and Insurance Board (WSIB), and University of British Columbia Centre for Health and Environment Research) was undertaken A): to compile existing WRA instruments and items; published studies assessing the validity and reliability of these instruments and items; and a comprehensive list of potential occupational exposures; and B): to examine the relevant time frame for the work history; and evidence-based diagnostic and management strategies for inclusion in the management algorithm; and C): to determine an appropriate validation strategy for the tools. An Advisory Committee (AC) consisting of respiratory medicine providers, occupational lung disease experts and epidemiologists was convened. AC members provided feedback on the identified instruments, items and preliminary findings. A questionnaire and a management algorithm were drafted for assessment of feasibility and content and face validity by members of the AC. The instruments will be piloted with primary care physicians, occupational asthma specialists and asthma patients to examine construct validity and the feasibility of responding to the questionnaire.

FINDINGS: A total of 10 instruments providing more than 70 items relating to occupation and exposure were assembled. These items exhibited considerable overlap; however addressed a number of constructs *e.g.*: education, type of job, duration of employment, occupational relationship of symptoms, seasonal variation of symptoms, exposures (agents, duration, frequency, level), and exposure avoidance. A list of 21 categories (with examples) of known offending agents was compiled. Two draft questionnaires were designed: i) a short version (1 page, 6 items) designed to supplement the 6 screening WRA questions in the Ontario Lung Association's (OLA) Primary Care Asthma Program (PCAP) care map and ii) a stand-alone 'full' version containing all 12 items (2 pages) for use in non-PCAP primary care locations. Questionnaire and algorithm validation is in progress.

DELIVERABLES: Deliverables will include a WRA screening questionnaire and algorithm with content and construct validity which is feasible to implement in primary care within paper or electronic medical records, including PCAP sites. The overall impact of these tools on early detection of WRA will subsequently be evaluated in a prospective study of detection of WRA in the primary care setting.

RELEVANCE:
Development and validation of a WRA screening questionnaire and algorithm are expected to lead to increased and timely recognition of WRA, prompt adherence with evidence-based guidelines for the management of WRA, and facilitate timely, accurate adjudication of compensation claims. Implementation of these tools may reduce the burden of WRA and improve the quality of life of individuals with WRA. In addition, these may be useful research tools for future studies of WRA. Project findings will be disseminated to stakeholders by presentation at national and international meetings and by peer-review publication.

11NA(C) Environmental Contributions to Asthma among Canadian Children: The Population Attributable Fraction

Canadian Allergy & Immune Diseases Advanced Training Initiative Award, Programme C – Public Health, Ethics, Policy and Society
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The Hospital for Sick Children, Toronto
Supervisors: Dr. Teresa To and Dr. Sharon Dell

OBJECTIVE/PURPOSE:
We calculated the population attributable fraction (PAF) of Canadian childhood asthma due to modifiable environmental exposures, with the goal of prioritizing recommendations for avoidance of potential contributing factors.

METHODS:
We conducted a systematic review of the literature to find Canadian childhood asthma incidence and prevalence, Canadian prevalence of exposure to airborne pollutants and indoor allergens, and international estimates of the relative risk of physician-diagnosed asthma [used to determine the attributable risk (AR)] for PAF calculation by the formula:

$$PAF = \frac{AR * \text{Exposure prevalence}}{\text{Asthma incidence}} * 100\%$$

FINDINGS:
The Canadian childhood asthma incidence was between 2.8 and 6.9%. Canadian exposure prevalences were: PM_{10} 16%, outdoor $PM_{2.5}$ 7.1%, indoor $PM_{2.5}$ 1.7%, outdoor NO_2 25%, indoor NO_2 3.3%, O_3 22%, SO_2 0.1%, CO 0.1%, environmental tobacco smoke (ETS) 9.0%, cat 22%, dog 12%, mouse 17%, cockroach 1.7%, dust mite 30%, moisture 14%, and mould 33%. Median odds ratios of physician-diagnosed asthma used to determine the AR were above 1.00 for PM_{10}, $PM_{2.5}$, NO_2, CO, ETS, mouse, cockroach, moisture, and mould. PAF estimates for incident asthma among preschool children were: PM_{10} 11%, outdoor $PM_{2.5}$ 1.2%, indoor $PM_{2.5}$ 0.30%, outdoor NO_2 1.4%, indoor NO_2 0.19%, and ETS 4.0%. PAF estimates for prevalent asthma calculated for other exposures were above 0 for mouse (3.8%), cockroach (0.22%), moisture (4.5%), and mould (10%).

DELIVERABLES:
This systematic review suggests contributions to childhood asthma development from exposure to particulates, NO_2, ETS, mouse and cockroach allergens, mould, and moisture. The associations with cat, dog and dust mite allergen exposure appear to be more complex.

RELEVANCE:
The evaluation of environmental contributions to childhood asthma in Canada will further AllerGen's mission to improve the quality of life for allergic disease sufferers by suggesting targets for modification of risk factor exposures through changing individual and societal behaviours. With a more accurate picture of environmental contributions to childhood asthma, we will prioritize public policy recommendations for changes in air quality standards. These findings will be communicated to decision-makers by publication and generation of policy recommendations in partnership with our colleagues in Health Canada and Environment Canada.