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¹ Please note that the poster abstract by J. He, *Multidimensional Epigenomic Effects of Short-Term Diesel Exhaust on Asthmatics*, is not included in this booklet. However, it will be presented as a poster during the conference.

² Please note that the poster abstract by J. Thiele, *Identification of suitable qPCR reference genes during IL-5 induced cord blood eosinophilopoiesis*, is not included in this booklet. However, it will be presented as a poster during the conference.

³ Please note that the poster abstract by A. Des Cormiers, *Development of Shared-Decision Aids in Asthma Management*, is not included in this booklet. However, it will be presented as a poster during the conference.

I. PROGRAMME A: GENE-ENVIRONMENT INTERACTIONS

#	AllerGen Trainee(s)	Institution	AllerGen Researcher(s)/ Supervisor(s)	Abstract Title
1A	Alton, Megan E.	Department of Pediatrics, University of Alberta	Dr. Anita L. Kozyrskyj	The effect of maternal history of drug use and distress during pregnancy on childhood allergic disease
2A	Amatullah, Hajera North, Michelle L. Urch, Bruce	Department of Medicine & Gage Occupational and Environmental Health Unit, University of Toronto	Dr. Jeremy A. Scott	Comparative cardiopulmonary effects of size-fractionated airborne particulate matter
3A	Azad, Meghan	Department of Pediatrics, University of Alberta	Dr. Anita L. Kozyrskyj	Impact of SES on cytokine responses in asthmatic versus healthy control children
4A	Bernstein, Kylie I. Protudjer, Jennifer L.P.	Manitoba Institute of Child Health & Department of Pediatrics and Child Health, University of Manitoba	Dr. Allan B. Becker Dr. Anita L. Kozyrskyj	Non-atopic asthma and atopic asthma are associated with reduced pulmonary function by age 7 years
5A	Chaudhuri, Sri R.	Environmental Chemistry Research Group, Department of Geography, University of Toronto	Dr. Miriam L. Diamond	Indoor measurements and multimedia modeling of phthalates: Toronto Intensive (TI) homes
6A	Curran, Jason	Simon Fraser University & University of British Columbia	Dr. Ryan Allen	An assessment of hopanes in settled house dust as indicators of exposure to traffic-related air pollution in Windsor, Ontario
7A	Dawoud, Dina	UBC James Hogg Research Centre, University of British Columbia	Dr. Denise Daley	Assessing the reproducibility of clusters across independent asthma cohorts
8A	Giles, Luisa V.	School of Human Kinetics & School of Environmental Health, University of British Columbia	Dr. Christopher Carsten	The effects of pre-exposure to diesel exhaust on a 20km cycling time trial performance and cardiovascular and pulmonary parameters in endurance-trained males
9A	Hirota, Jeremy Warner, Stephanie Hackett, Tillie-Louise	UBC James Hogg Research Centre, University of British Columbia	Dr. Darryl Knight	Identification and functional characterization of an airway epithelium inflammasome
10A	Kam, Sarah H.Y.	UBC James Hogg Research Centre, University of British Columbia	Dr. Scott J. Tebbutt	Genomic response profiles in peripheral blood of asthmatics undergoing allergen inhalation challenge differentiate isolated early from dual responders
11A	Kanagaratham, Cynthia Marino, Rafael Camateros, Pierre	Departments of Human Genetics and Experimental Medicine, McGill University	Dr. Danuta Radzioch	Seven novel candidate genes responsible for airway hyperresponsiveness
12A	MacIntyre, Elaina	School of Environmental Health, University of British Columbia	Dr. Michael Brauer Dr. Christopher Carsten	Traffic-related air pollution and candidate genes of oxidative metabolism: preliminary results from TAG (Traffic pollution, Asthma, Genetics project)

#	AllerGen Trainee(s)	Institution	AllerGen Researcher(s)/ Supervisor(s)	Abstract Title
13A	McGarry, C. Chau, J. Karimi, Khalil	Brain Body Institute & Department of Medicine, McMaster University	Dr. Paul Forsythe	Prenatal environmental influences in a murine model of cockroach allergy
14A	North, Michelle L. Khanna, Nivedita	Department of Medicine & Gage Occupational and Environmental Health Unit, University of Toronto	Dr. Jeremy A. Scott Dr. Frances Silverman	Hypertrophic airway smooth muscle mass correlates with increased airways responsiveness in a chronic murine model of allergic asthma
15A	Park, Julie Tripp, Ben Zamar, David Akhabir, Loubna	UBC James Hogg Research Centre, University of British Columbia	Dr. Denise Daley	Performance of whole genome amplified DNA samples using Illumina 610-Quad genotyping array
16A	Pui, Mandy	University of British Columbia	Dr. Christopher Carsten	Diesel exhaust-associated changes in airway reactivity and innate immunity in asthmatic and healthy volunteers
17A	Salas, Scarlet M. Unruh, Claire R. Protudjer, Jennifer L.P.	Manitoba Institute of Child Health & Department of Pediatrics and Child Health University of Manitoba	Dr. Allan B. Becker	The relationship of fast food, breastfeeding and asthma in adolescents
18A	Sbihi, Hind	School of Environmental Health, University of British Columbia	Dr. Michael Brauer	Estimating the variability of endotoxin, (1,3)- β -D-glucan, and hopanes in repeated samples of settled house dust
19A	Shu, Huan Rousseau, Roxanne	Simon Fraser University & University of British Columbia	Dr. Tim Takaro	Potential sources of phthalate exposure in the Vancouver CHILD Study at three months of age
20A	Singh, Amritpal Kam, Sarah H.Y. Ruan, Jian	UBC James Hogg Research Centre, University of British Columbia	Dr. Scott J. Tebbutt	Genomic response profiles in peripheral blood of allergic rhinitis subjects exposed to ragweed pollen in the Environmental Exposure Unit
21A	Smith, Steve G. Obminski, George	Division of Respiriology, Department of Medicine, McMaster University	Dr. Gail M. Gauvreau	Evaluation of peroxisome proliferator-activated receptors (PPARs) effects on eosinophil migration
22A	Stefanowicz, Dorota Hackett, Tillie-Louise	UBC James Hogg Research Centre, University of British Columbia	Dr. Darryl Knight	Alterations in histone acetylation in asthmatic airway epithelial cells
23A	Thomas, Andrew Urch, Bruce Speck, Mary	Gage Occupational and Environmental Health Unit, University of Toronto and St. Michael's Hospital, Toronto	Dr. Frances Silverman	Eosinophilic inflammation due to volatile organic compounds in controlled human exposure studies
24A	Tremblay-Vaillancourt, Vanessa	Département des sciences fondamentales, Université du Québec à Chicoutimi	Dr. Catherine Laprise	Association study between <i>IL1R2</i> pathway genes and asthma related phenotypes in two independent familial studies
25A	Wang, Rongrong	School of Environmental Health, University of British Columbia	Dr. Michael Brauer	Assessment of the temporal stability of land use regression models for traffic-related air pollution

1A The Effect of Maternal History of Drug Use and Distress in Pregnancy on Childhood Allergic Disease

AllerGen Programme A: Gene-Environment Interactions

Megan E Alton, Suzanne C Tough, Piushkumar J Mandhane, & Anita L Kozyrskyj,
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Supervisor: Anita L Kozyrskyj

OBJECTIVE/PURPOSE:

Substance use during pregnancy has been shown to be detrimental for child developmental outcomes. However, little research has been done on the effect of substance use during pregnancy on childhood allergic disease. We investigated the impact of maternal substance use and distress during pregnancy on the development of allergic disease in children.

METHODS:

Data were accessed from the Community Perinatal Care trial of 791 children and their mothers in Calgary. This included information on maternal distress, alcohol, smoking, and illicit drug use, and child health outcomes collected *via* questionnaire during pregnancy and the post partum period. Logistic regression analysis was used to investigate the association between substance use (illicit drug use, alcohol, and smoking) and distress during pregnancy with asthma and allergy development at age 3 in female and male children. Effects were adjusted for out-of-home child care, breastfeeding, prenatal vitamin use, preterm birth, and maternal education level.

FINDINGS:

When adjusted for alcohol use and smoking during pregnancy, maternal education level, and other confounds, history of maternal drug use (Odds Ratio [OR]:5.19, 95% Confidence Interval [CI]: 1.36-19.78), severe maternal distress during pregnancy (OR:5.85, 95% CI: 1.27-27.02), and prenatal vitamin use (OR:0.01, 95% CI:0.02-0.49) were significantly associated with asthma in girls. In boys, an association was found between severe distress (OR:3.61, 95% CI: 1.05-12.36) and allergies that was independent of history of drug use, alcohol use and smoking in pregnancy, maternal education level and other confounds.

DELIVERABLES:

As substance use continues to increase in women of child-bearing age, the behavioral and biological ramifications of its use and interaction with distress during pregnancy need to be considered as important environmental exposures in the development of childhood allergic disease.

RELEVANCE:

We propose that improved prenatal care and extensive psychological screening should be provided to future mothers. In-depth therapies for drug addiction and stress may help to alleviate symptoms in pregnant women and reduce the risk of asthma and allergies in children.

2A Comparative Cardiopulmonary Effects of Size-Fractionated Airborne Particulate Matter

AllerGen Programme A: Gene-Environment Interactions

Hajera Amatullah^{1,2,3}, Michelle North^{2,3,4}, Neeraj Rastogi^{5,6}, Bruce Urch^{1,2,3,4,6}, Frances Silverman^{1,2,3,4,6},
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⁵Department of Chemical Engineering and Applied Chemistry, Faculty of Applied Science and Engineering, University of Toronto, Toronto, ON. ⁶Southern Ontario Centre for Atmospheric Aerosol Research, Toronto, ON.

Supervisor: Jeremy A. Scott

OBJECTIVE/PURPOSE:

Strong epidemiological evidence exists linking particulate matter (PM) levels with hospital admissions of patients with cardiorespiratory symptoms. For regulatory purposes, PM is generally categorized into three size fractions: Coarse (aerodynamic diameter (AD): 2.5 - 10 µm), Fine (AD: 0.1 - 2.5 µm), and Ultrafine (AD: ≤ 0.1 µm). PM size is important in determining the extent of infiltration into the respiratory system and systemic circulation. Our objective was to evaluate the role of particle size in PM-associated toxicity by investigating the differential effects of the three PM size fractions on pulmonary and cardiovascular function in naïve mice.

METHODS:

Naïve female BALB/c mice were exposed to coarse, fine or ultrafine PM for 4 hours using our nose-only exposure system in conjunction with the Harvard Ambient Particle Concentrator. These exposures were conducted as part of the 'Health effects of Aerosols in Toronto (HEAT)' campaign. Control mice were exposed to HEPA filtered lab air (FA) in the same nose-only exposure system. Following the exposures, mice underwent assessment of pulmonary function using the flexiVent system. ECGs were also recorded using the flexiVent system.

FINDINGS:

PV curves exhibited a slight downward shift for coarse and fine PM exposures compared with FA controls, which was reflected by their reduced quasistatic compliance. Total baseline resistance was augmented by exposure to coarse and fine PM, but not ultrafine PM. Maximum responsiveness to methacholine was similarly augmented with coarse and fine PM. Total BALF cell count was significantly increased following coarse PM exposure. Ultrafine PM alone had significant effect on heart rate and heart rate variability compared with FA controls.

DELIVERABLES:

A proper understanding of the differential effects of size fractions of PM is critical to determining the health risk of air pollution. In mice with no predisposition to disease, coarse and fine PM primarily augmented airways responsiveness and lung function whereas ultrafine PM exhibited cardiovascular effects (*i.e.*, reduced heart rate variability).

RELEVANCE:

Our findings highlight that PM can induce significant respiratory and cardiovascular changes in naïve mice and these effects were size-specific. A strong understanding of the mechanisms responsible for these pathways in naïve mice can then be translated into studies of susceptible populations, such as asthmatics. Coarse and fine PM are currently regulated in Canada. Our findings support a similar consideration of the risks of exposure to ultrafine PM.

3A Impact of SES on Cytokine Responses in Asthmatic Versus Healthy Control Children

AllerGen Programme A: Gene-Environment Interactions

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³Department of Psychology, University of British Columbia, Vancouver, BC

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Supervisor: Anita L Kozyrskyj

OBJECTIVE/PURPOSE:

Chronic exposure to a low-income environment from birth is associated with the development of persistent asthma, and a protective effect has been observed among those children whose families move out of poverty. Since the biological mechanisms underlying these associations are poorly understood, we sought to examine trajectories of socioeconomic status (SES) throughout childhood and their relationship to the production of asthma-related cytokines.

METHODS:

Peripheral blood samples were collected from 286 children in the Study of Asthma, Genes and Environment (SAGE) birth cohort. Mononuclear cells were stimulated with bacterial endotoxin (lipopolysaccharide) *in vitro* prior to measuring the production of the following asthma-related cytokines by multiplex ELISA: IL-10, IL-6, IL-1 β , TNF- α , CCL2 and CCL22. Parents reported on family SES, indicating the number of bedrooms in the family home for each year of the child's life. Using longitudinal latent-class modeling techniques, five distinct trajectories of childhood SES were identified. Associations among SES trajectories and cytokine responses were examined using linear regression, adjusting for gender, atopy, asthma, family history of atopy, and region of residence (urban, rural or First Nations reserve).

FINDINGS:

SES trajectory groups were independently associated with production of IL-6. Chronic low SES was associated with the highest levels of IL-6 production, while "increasing SES" (a trajectory showing low early-life SES that increased throughout childhood) was associated with the lowest levels of IL-6 production. These associations were restricted to boys with a family history of atopy, and were strongest in those with atopic asthma (versus non-atopic asthma or healthy controls). Male gender was independently associated with increased TNF- α and CCL2, atopic asthma was independently associated with increased IL-10, and First Nations residence was independently associated with decreased IL-10 and CCL2.

DELIVERABLES:

We plan to disseminate these findings by journal publication; a manuscript is currently in preparation. We further intend to expand our investigation to include additional innate and adaptive immune stimuli, including asthma-associated viral antigens.

RELEVANCE:

The effects of chronic poverty on child health have long been recognized, and recent studies have shown that chronic low SES is specifically associated with the development of asthma (while moving out of poverty during childhood has a protective effect). Our findings indicate that cytokine responses may at least partially explain this relationship, since chronic low SES during childhood was associated with increased production of IL-6 (a pro-inflammatory cytokine linked to asthma exacerbations), while decreased production was observed in children who experienced the "increasing" SES trajectory. Thus, our study provides a biological explanation for the established association between SES and childhood asthma, and emphasizes the advantage of using trajectory (versus static) measures of SES. Finally, our findings broadly suggest that interventions aimed at decreasing SES disparities during childhood have the potential to limit or reverse the detrimental effects of exposure to poverty during early life, with important implications for public health policy.

4A Non-Atopic Asthma and Atopic Asthma are Associated with Reduced Pulmonary Function by Age 7 Years

AllerGen Programme A: Gene-Environment Interactions

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Supervisors: Allan B. Becker and Anita L. Kozyrskyj

OBJECTIVE/PURPOSE:

Studies have demonstrated decreased pulmonary function in children with asthma by the pre-adolescent years. Asthma is strongly related to atopy, and atopy may play some role in modifying pulmonary function. We hypothesized that atopy amplifies differences in pulmonary function between children with and without asthma.

METHODS:

The Canadian Asthma Primary Prevention Study (CAPPS) is a cohort of children at high risk for asthma based on immediate family history who were born in Winnipeg or Vancouver. Participants were identified during mothers' third trimesters of pregnancy. 545 families were randomized to control (266; 48.8%) or to multi-faceted intervention (279; 51.2%). Intervention occurred during pregnancy and the first year of life and consisted of avoidance of exposure to house dust mites, pets and environmental tobacco smoke, promotion of breastfeeding and delayed introduction of solid foods. Follow-up assessment at 7 years old included physician assessment for diagnosis of asthma, skin testing, measurement of baseline and post β -agonist pulmonary function and airway responsiveness.

FINDINGS:

At 7 years, 367 children (53.1% boys; 174 control; 193 intervention) had complete assessments. Asthma was more prevalent in the control vs. intervention (23.0 vs. 14.9%, OR 0.44; 95% CI 0.25-0.79). There was no significant difference in atopy prevalence between the control (41%) vs. intervention (45%) groups, although atopy prevalence was higher in children with vs. without asthma (71.0% vs 36.4% $p < 0.001$). Baseline FEV1 was significantly lower for children with vs. without asthma (90.7 ± 12.3 S.D. % predicted vs 95.1 ± 10.6 % predicted; $p = 0.003$) as was FEV1/FVC (0.84 ± 0.08 vs. 0.88 ± 0.06 ; $p < 0.001$) and airway hyperresponsiveness (AHR), measured as PC20 methacholine (2.3 ± 2.3 mg/mL vs 4.3 ± 2.9 mg/mL, $p < 0.001$). Differences in pulmonary function were also apparent for different asthma phenotypes. Children with non-atopic asthma tended to have lower baseline FEV1 (88.9 ± 12.0 vs. 92.6 ± 12.6 % predicted, $p = 0.15$) and FEV1/FVC (0.83 ± 0.08 vs. 0.85 ± 0.08 , $p = 0.23$) compared to those children with atopic asthma. Post β agonist children with non-atopic asthma had lower FEV1/FVC compared to children with atopic asthma (0.82 ± 0.08 vs. 0.85 ± 0.08 , $p = 0.059$). Children with atopic asthma had greater AHR than those with non-atopic asthma (PC20 1.6 ± 1.5 mg/mL vs 2.9 ± 2.6 mg/mL; $p = 0.06$).

DELIVERABLES:

Baseline pulmonary function is lower in children with asthma compared to children without asthma as early as 7 years of age. The reduced post β agonist FEV1/FVC ratio in children with asthma suggests that remodeling of airways has occurred by age 7. For children with non-atopic asthma, we speculate that the reduced FEV1/FVC pre and post β agonist may reflect airway changes present from birth.

RELEVANCE:

Better understanding of the heterogeneity of asthma phenotypes, such as atopic vs non-atopic asthma in childhood is important in order to consider most appropriate approaches to therapy. Healthcare providers should be encouraged to consider asthma phenotypes when considering management strategies.

* *Co-first authors*

5A Indoor Measurements and Multimedia Modeling of Phthalates: Toronto Intensive (TI) Homes

AllerGen Programme A: Gene-Environment Interactions

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Supervisor: Miriam L. Diamond

OBJECTIVE/PURPOSE:

Phthalates (PAE), a diverse group of organic esters are an ubiquitous class of plasticizers. They are found in a variety of consumer products such as personal care items, residential materials, and medical applications. To date, several studies have indicated an association between PAE exposure and allergy and asthma development in children. The purpose of the current study is: (1) to determine optimal indoor sampling methods of PAE and other semi-volatile organic compounds (SVOCs) that relate to exposure and (2) to develop a mechanistic multimedia model capable of estimating indoor chemical concentrations and fate, and explain factors responsible for spatial variations.

METHODS:

An intensive sampling campaign was executed in 5 Toronto homes over 4 days in September 2010. The measurements, slated to be repeated again in early 2011, were organized into: (1) building characterization parameters including i) air-exchange-rate (AER), ii) temperature, iii) particulate matter (PM) concentration, and iv) dwelling dimensions, (2) household concentrations of six PAE and other SVOCs in dust, surface films, and air (collected using both standard and novel techniques), and (3) participant specific exposure measures including urine samples to monitor PAE metabolites. The homes also underwent the CHILD Home Environment Assessment, and additional dust samples were collected following CHILD protocol to permit direct comparison of dust sampling methods. Development of the multimedia model is focusing to incorporate features uniquely relevant to exposure and dust.

FINDINGS:

The September 2010 TI measurement campaign collected 154 dust, window film, urine, and air samples. 96 hours of continuous temperature, PM, and AER measurements were also conducted. The average temperature and PM 2.5 μm concentrations in the homes ranged from 20°C to 26°C, and 3 $\mu\text{g}/\text{m}^3$ to 29 $\mu\text{g}/\text{m}^3$, respectively. Average temperature differences between rooms ranged from 0.5°C to 2°C. Between homes, average PAE concentrations in window films varied by an order of magnitude with levels as high as several ng/cm^2 of surface. In 80% of the homes heavier molecular weight di(2-ethylhexyl) phthalate (DEHP) had the highest concentration in window films, whereas lighter molecular weight diethyl phthalate (DEP) was found to be the lowest. This trend was also observed in PAE film levels between rooms. Eight urinary PAE metabolites, tested in daily urine samples, demonstrated an order of magnitude difference between participants. Further indoor measurements, PAE concentrations in the sampled media, and initial model results will also be discussed.

DELIVERABLES:

The detailed TI study will contribute to evaluating the sampling techniques described in the Methods, aimed at assessing personal exposure. Furthermore, the use of passive samplers to measure indoor air PAE concentrations is a new application with the potential for widespread use. The multimedia indoor model is being designed to effectively predict and explain phthalate concentrations and offer a cost-effective assessment tool.

RELEVANCE:

The 2-fold objective of the current research will improve the ability to identify indoor sources, and explain fate, transport and exposure of PAE and other SVOCs. This will allow for the suggestion of interventions or preventative measures to minimize exposure to these chemicals. The insight gained through this work will also permit effective use of exposure data obtained from CHILD and potentially lead to straightforward and cost-effective exposure measurement techniques that may be used in future time points in CHILD.

6A An Assessment of Hopanes in Settled House Dust as Indicators of Exposure to Traffic-Related Air Pollution in Windsor, Ontario

AllerGen Programme A: Gene-Environment Interactions

Jason Curran¹, Jeff Brook², Tim Takaro¹, Amanda Wheeler³,
Zhimei Jiang², Alice Grgicak-Mannion⁴, Mary Speck⁵, Ryan Allen¹

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OBJECTIVE/PURPOSE:

Traffic-related air pollution (TRAP) has been linked with adverse health effects in studies that use residential proximity to major roads or land use regression (LUR) models as surrogates of personal exposure. Indicators of TRAP levels inside residences may be useful in epidemiologic studies because individuals spend the majority of time in that microenvironment. We investigated hopanes, markers of primary particle emissions from both gasoline and diesel engines, in house dust as an alternative approach for assessing exposure to TRAP in Windsor, Ontario.

METHODS:

Data were collected from the homes of 28 CHILD Study participants (age range 10 – 13 yrs). Settled house dust was collected from the floor in the main activity room of each participant's home, and the dust was analyzed for a suite of hopanes by gas chromatography-mass spectrometry. We calculated correlations between total dust hopane concentrations and estimates of annual average NO₂ concentrations derived from an existing LUR model in Windsor. Infiltration efficiency, calculated from continuous outdoor and indoor PM_{2.5} measurements at each home, and the presence of an attached garage were considered as a potential modifiers of the hopane-NO₂ relationships.

FINDINGS:

Hopanes were consistently present in detectable quantities in house dust. Annual average outdoor NO₂ estimated from the LUR was moderately correlated with hopanes in house dust ($r = 0.46$; $p < 0.05$). The correlations did not vary by infiltration efficiency or the presence of an attached garage. Hopanes measured in settled house dust show promise as an indicator of long-term exposure to traffic-related air pollution.

DELIVERABLES:

The knowledge and understanding generated through this work will allow for more effective use of the exposure data being obtained in the national Canadian Healthy Infant Longitudinal Development (CHILD) Study. Furthermore, the methods and techniques developed will potentially lead to simple, cost-effective exposure measurement techniques that may be used in future time points in the CHILD Study.

7A Assessing the Reproducibility of Clusters Across Independent Asthma Cohorts

AllerGen Programme A: Gene-Environment Interactions

Dina Dawoud¹, Allan Becker², Anita Kozyrskyj³, Andrew Sandford¹, Catherine Laprise⁴, Denise Daley¹
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Supervisor: Denise Daley

OBJECTIVE/PURPOSE:

Asthma is a complex disease, displaying phenotypic and genetic heterogeneity. Previous publications have expressed the need to provide detailed characterizations of asthma phenotypes in an effort to reduce some of the phenotypic heterogeneity in genetic studies. Detailed characterization is usually done by classifying subjects into different categories based on a set of asthma phenotypes. However this classification varies across studies. The purpose of this analysis is to determine if using five common variables, general and consistent clusters are produced across independent asthma cohorts.

METHODS:

Four independent cohorts were considered in the analysis namely, the Canadian Asthma Primary Prevention Study (CAPPS), the Study of Asthma Genes and the Environment (SAGE), the Saguenay-Lac-Saint-Jean familial sample (SLSJ) and the Busselton Health Study. The cohorts were classified as childhood (CAPPS and SAGE) vs. adult (SLSJ and Busselton). Clustering was done on five common standardized variables: sex, atopy, airway hyperresponsiveness (AHR), wheeze in the last 12 months and body mass index (BMI). Ward's hierarchical clustering and k-means clustering were run. To test for differences between clusters, the analysis of variance (ANOVA) was used for the continuous variables and chi-square tests for the categorical variables.

FINDINGS:

Five clusters were formed for each cohort, and it was observed that within the asthmatic clusters (*i.e.*, the cluster that captured the largest portion of asthmatics), the majority are atopic and most, if not all, suffered with wheeze in the past 12 months. In the childhood cohorts it was found that BMI was the only variable that was not significant across clusters. In the Busselton case-control sample, all variables, including BMI, were significantly different. Reproducibility of clusters across the childhood cohorts was found, where clusters were matched based on the proportions observed for each variable within a cluster. Some similarities among clusters were also observed across the childhood and adult cohorts.

DELIVERABLES:

The analysis found reproducibility of clusters across the independent cohorts.

RELEVANCE:

Defining distinct and reproducible subsets based on asthma phenotypes that are generalizable and reproducible across studies, allows us to identify homogeneous subsets of asthma patients. These distinct subsets can be used in genetic studies to increase the genetic homogeneity among individuals in the subset, which will increase the statistical power in these studies.

8A The Effects of Pre-Exposure to Diesel Exhaust on a 20km Cycling Time Trial Performance and Cardiovascular and Pulmonary Parameters in Endurance-Trained Males

AllerGen Programme A: Gene-Environment Interactions

Luisa V. Giles, Michael S. Koehle and Christopher Carlsten
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School of Environmental Health, Faculty of Medicine
University of British Columbia, Vancouver, BC
Supervisor: Christopher Carlsten

PURPOSE:

To determine the effect of pre-exposure to dilute diesel exhaust (DE) on 20km time trial (TT) performance, cardio-respiratory responses during exercise and pulmonary function.

METHODS:

Eight endurance-trained males aged 29 ± 6 yrs (mean \pm SD) years of normal height (1.79 ± 0.10 m) and weight (72.6 ± 5.4 kg) participated. They attended the lab on 3 occasions. Day 1 consisted of a maximum exercise test, a 20km time trial (TT) familiarisation. Days 2 and 3 consisted of a 60-minute exposure followed by a 20km TT. Exposures were to either filtered air (FA) or diluted diesel exhaust (DE) at a concentration of $PM_{2.5}$ of $300\mu g/m^3$. The order was randomised and participants were blinded to the condition. Pulmonary function was assessed before and after exposure, and after exercise. Minute ventilation (V_E), tidal volume (V_T), breathing frequency (F_B), heart rate and oxyhaemoglobin saturation (measured by pulse oximetry: SpO_2) were collected during the TTs. Differences in duration of TT, mean power during TT's, and the effect of condition order were each analysed using paired T-tests. Repeated-measures ANOVAs were used to assess the effect of DE exposure on physiological measures: ventilatory data, SpO_2 and heart rate during time trial performance were analysed using condition (FA vs. DE) and time (quarter (Q) 1, 2, 3 and 4 of the time trial). Pulmonary function (FEV_1 , FVC and FEV_1/FVC) was analysed using a 2 (condition: FA vs. DE) \times 2 (delta pulmonary function: Δ baseline and post exposure, Δ post exposure and post exercise) repeated-measures ANOVA. Post hoc analyses were performed using paired t-tests.

FINDINGS:

There were no main effects of exposure condition (FA and DE) on either time trial duration (33.0 ± 1.4 min vs. 32.5 ± 0.9 min) or mean power output (256.0 ± 28.2 w vs. 265.1 ± 19.8 w). There were also no significant differences in V_E , V_T , F_B and SpO_2 during a 20km TT that followed a 60-minute exposure to filtered air or diesel exhaust. However, heart rate was significantly higher ($p = 0.023$; 157.3 ± 7.3 vs. 163.9 ± 7.5 , for FA and DE respectively) during time trials that followed exposure to diesel exhaust. There were main effects of exposure condition on the change in FEV_1 ($p = .013$) and FEV_1/FVC ($p = 0.008$) which tended to be less in the diesel condition. When assessing the effects of exercise and exposure, the improvement in FEV_1 and FEV_1/FVC was lower in diesel compared to filtered air.

DELIVERABLES:

Our ongoing work regarding DE-related asthma pathophysiology depends on a clear understanding of the effect of exercise on our protocols. This preliminary work is the first step in a comprehensive assessment of the interaction between exercise and the DE-mediated cardiorespiratory pathophysiology.

RELEVANCE:

The interaction of air pollution and exercise on the cardiovascular and respiratory system is poorly understood. Exercise may have positive effects on the immune system, which may lead to the prevention and management of chronic illnesses. However, as exercise in air pollution causes a several-fold increase in particle deposition in the respiratory tract, exercise in a polluted environment may exacerbate the effects on the respiratory system. Therefore, before advising those with allergies/immune system complications about exercise, it is important to gain a better understanding of air pollution and exercise on the cardio-respiratory system.

9A Identification and Functional Characterization of an Airway Epithelium Inflammasome

AllerGen Programme A: Gene-Environment Interactions

Jeremy Alexander Hirota, Stephanie Warner, Tillie-Louise Hackett
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University of British Columbia, Vancouver, BC
Supervisor: Darryl Knight

OBJECTIVES:

Intracellular danger sensing protein complexes called inflammasomes have been identified and functionally characterized in inflammatory cells. The nod-like receptor protein 3 (NLRP3) inflammasome consists of caspase-1, apoptosis associated speck like protein containing a caspase recruitment domain (ASC), and NLRP3, and is activated by danger signals including oxidative stress. NLRP3 inflammasome activation leads to caspase-1 mediated production of mature cleaved IL-1 β .

We have chosen a novel direction that will begin to explore the role of inflammasomes in airway epithelial cells. We have examined the consequences of ambient urban particulate matter (PM10) exposure on airway epithelium and the mechanisms of NLRP3 inflammasome activation. Our hypothesis is that the airway epithelium contains a functioning NLRP3 inflammasome that contributes to IL-1 β production in response to PM10 that can exacerbate existing airway inflammation in asthmatics.

METHODS:

We used human primary airway epithelial cells, a murine model, and clinical biopsy samples to identify and functionally characterize the NLRP3 inflammasome in airway epithelium. Immunoblots and confocal microscopy were performed to confirm the presence of NLRP3 and caspase-1 protein. Functional characterization was performed by exposing primary airway epithelium cultures to PM10 and assessing IL-1 β production in culture supernatant. Interventions were performed with glyburide and Z-YVAD-FMK to inhibit NLRP3 and caspase-1, respectively. *In vivo* exposure of WT and NLRP3 *-/-* mice to PM10 was performed with outcome measurements of IL-1 β and cellular inflammation in lung lavage fluid. Lastly, clinical samples of non-asthmatic and asthmatic human airways were stained for NLRP3 and caspase-1.

FINDINGS:

Immunoblot and confocal microscopy confirmed NLRP3 and caspase-1 proteins are expressed in primary airway epithelial cells *in vitro*. PM10 exposure to cultures resulted in NLRP3 inflammasome mediated production of IL-1 β . NLRP3 *-/-* mice were protected from PM10 induced elevations in lung IL-1 β and cellular inflammation. Immunohistochemical staining of human bronchus demonstrates NLRP3 and caspase-1 expression in the airway epithelium.

DELIVERABLES:

NLRP3 and caspase-1 proteins are expressed in airway epithelium and form a functional inflammasome that is activated by PM10 exposure. The resulting production of IL-1 β from the airway epithelium may contribute to exacerbations of existing airway disease or may facilitate sensitization and development of new disease.

RELEVANCE:

Our studies provide insight into a previously unexplored multiprotein complex in the airway epithelium, the NLRP3 inflammasome. These results may present a new approach to stem the progression of airway inflammation in the airways of asthmatics in an easily accessible therapeutic target, the airway epithelium. These results may also be relevant to other allergic or autoimmune disease processes that display aberrant pro-inflammatory cytokine profiles.

10A Genomic Response Profiles in Peripheral Blood of Asthmatics Undergoing Allergen Inhalation Challenge Differentiate Isolated Early from Dual Responders

AllerGen Programme A: Gene-Environment Interactions

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Supervisor: Scott J. Tebbutt

OBJECTIVE/PURPOSE:

In asthmatic individuals, airway narrowing represents the early phase of the asthmatic response to allergen inhalation challenge, which 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, usually starting between three and four hours after allergen inhalation challenge, and characterized by cellular inflammation of the airway, increased lung tissue permeability, and mucus secretion. The pathways leading to the late response are not completely understood.

METHODS:

Adult asthmatic subjects (18-55 years of age, with stable, mild allergic asthma, n=8) underwent allergen inhalation challenges using cat allergen. All subjects had an early asthmatic response of $\geq 20\%$ fall in FEV₁, and four of these subjects also had a late phase response of $\geq 15\%$ fall in FEV₁ or a reduced post-challenge methacholine PC₂₀ (dual responders). Peripheral blood was drawn using PAXgene Blood RNA tubes just prior to inhalation challenge and two 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:

When we compared pre- and post-allergen challenge data, the numbers of differentially expressed probe-sets ($p \leq 0.005$) were 688 for isolated early responders (ER) and 442 for dual responders (DR), with 11 overlapping. 370 probe-sets were significant at a false discovery rate (FDR) of 5% in an ANOVA model that included the interaction of pre-/post-challenge and response type. Using IPA, the top biological functions identified for ER (post- versus pre-challenge) included protein trafficking and cellular function and maintenance. For DR, the top functions were cell death, hematological system development and function, and lymphoid tissue structure and development. The top canonical pathways identified for DR also included granzyme B signaling, T helper cell differentiation, and various apoptotic signaling pathways.

DELIVERABLES:

Peripheral blood genomic profiles associated with isolated early response and dual response are significantly different following allergen inhalation challenge. The biological processes for DR indicate a more severe outcome involving cell death and changes to the immune system.

RELEVANCE:

This study presents a first 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.

11A Seven Novel Candidate Genes Responsible for Airway Hyperresponsiveness

AllerGen Programme A: Gene-Environment Interactions

C. Kanagaratham¹, R. Marino², P. Camateros², D. Radzioch¹

¹Departments of Human Genetics and ²Experimental Medicine, McGill University, Montréal, QC
Supervisor: Danuta Radzioch

OBJECTIVE/PURPOSE:

Pulmonary airways naturally respond to bronchoconstricting stimuli by narrowing; however, in asthmatics this response is more forceful and can occur at lower concentrations of the stimuli. This feature, known as airway hyperresponsiveness (AHR), is an intermediate phenotype of asthma. The heritability of AHR has been shown through its familial aggregation and through the use of inbred strains of mice. However, since AHR is a polygenic trait, identifying the specific genes responsible for it proves to be a difficult task. Here we show that using a unique genetic panel ideal for the dissection of polygenic traits we are able to identify several candidate genes responsible for AHR.

METHODS:

We have at our disposal a panel of 35 AcB/BcA recombinant congenic strains of mice (F1) created by a cross between airway hyperresponsive and hyporesponsive strains, A/J and C57BL/6J respectively. Each recombinant strain is fully inbred and contains 12.5% of the genome from one parental strain on the background of the other parental strain.

1. Informative strains which exhibit the phenotype (Penh) of the minor genetic donor were identified from the AcB/BcA panel and used to create F2 strains by backcrossing to the major genetic donor and to an unrelated strain with the same phenotype. F2 mice were phenotyped and genotyped for custom SNPs at 4Mbp intervals.

2. QTL analysis was done to identify candidate genes. The list of candidate genes was narrowed by selecting for genes containing non-synonymous mutations that are expressed in the lungs and found in regions non-identical by descent.

FINDINGS:

1. Three informative strains for AHR were identified from the AcB/BcA panel: AcB64, BcA85, and BcA86. The BcA86 cross was selected for F2 mapping and F2 crosses were created by backcrossing to parental strain, C57BL/6J, and unrelated strain, C3H/HeJ.

2. Three QTLs containing a total of 232 genes were identified in the BcA86 F2 crosses. Using our gene selection criteria, the 232 genes were narrowed to seven candidate genes. The authenticity of the function of these genes in AHR is currently in the process of being validated.

DELIVERABLES:

Our approach provides a manageable list of candidate genes for AHR for which homologous genes can be identified in humans. This approach can be applied to identify candidate genes for other intermediate phenotypes of asthma, such as total and specific IgE and eosinophil counts.

RELEVANCE:

Genetic studies such as ours will help further AllerGen's mission to improve the quality of life of allergic/immune disease sufferers by allowing for targeted treatments and medications. Furthermore, identifying genes involved in allergy can be used to screen for individuals who are susceptible to developing disease. This allows for these individuals at risk to take the necessary measures to prevent disease.

12A Traffic-Related Air Pollution and Candidate Genes of Oxidative Metabolism: Preliminary Results from TAG (Traffic pollution, Asthma, Genetics)

AllerGen Programme A: Gene-Environment Interactions

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Supervisors: Michael Brauer and Christopher Carlsten

OBJECTIVE/PURPOSE:

Traffic-related air pollution has been linked to incident asthma in birth cohorts across Canada and Europe. Unfortunately, there has been little success assessing gene-environment interactions due to small sample sizes. We have combined data from two Canadian (CAPPS, SAGE) and four European birth cohorts (BAMSE, PIAMA, GINI, LISA) to examine the association between traffic-related air pollution and incident asthma, and potential modification by oxidative stress genes.

METHODS:

Logistic regression was used to estimate odds ratios for the association between nitrogen dioxide (a marker for traffic-related air pollution) and parent report of physician diagnosed asthma at 7 or 8 years, stratified by candidate gene and adjusted for gender, city and cohort.

FINDINGS:

Complete data on traffic-related air pollution, candidate genes and asthma / wheeze symptoms were available for 4,902 children. 380 (7.6%) children had asthma at the 7/8 year follow-up and 366 (7.3%) children had both asthma at the 7/8 year follow-up and parent reported wheeze symptoms in the previous 12 months. For glutathione S-transferase P1 (rs1799811), the point estimate for the association between air pollution and asthma was elevated for children with the minor allele in all analyses (pooled and individual cohort) when compared to results for children with the major allele – although these results were not statistically significant [OR(95%CI) 2.01(1.03-3.94) for minor; 1.11(0.77-1.59) for major]. Findings were similar but less consistent for a second glutathione S-transferase P1 SNP (rs947894) [1.50(1.04-2.18) for minor; 1.22(0.93-1.61) for major] and for tumor necrosis factor (rs1800629) [1.22(0.72-2.09) for minor; 1.24(0.90-1.73) for major].

DELIVERABLES:

Associations between air pollution and asthma stratified by genotype will be presented. Sensitivity analyses will examine different definitions of asthma and wheeze.

RELEVANCE:

This study could identify a population that, due to their genetic profile, is at increased risk of developing asthma when living in areas of high traffic exposure. Genetic screening could be used to identify these children in early life to target education and awareness programs. These findings will be disseminated through our website, national and international conferences, peer review publications, and invited presentations.

CAPPS (Canada) – Canadian Asthma Primary Prevention Study

SAGE (Canada) – Study of Asthma, Genes and Environment

BAMSE (Sweden) – Children, Allergy, Milieu, Stockholm, Epidemiological Survey

PIAMA (The Netherlands) – Prevention and Incidence of Asthma and Mite Allergy

GINI (Germany) – German Infant Nutritional Intervention Program

LISA (Germany) – Lifestyle Related factors, Immune System and the Development of Allergies

13A Prenatal Environmental Influences in a Murine Model of Cockroach Allergy

AllerGen Programme A: Gene-Environment Interactions

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Pulmonary Research Group, Faculty of Medicine & Dentistry,
University of Alberta, Edmonton, AB
Supervisor: Paul Forsythe

OBJECTIVE/PURPOSE:

Allergic asthma is a chronic condition characterized by airway inflammation, hyper-responsiveness and remodelling. While genetics contribute to atopy, epidemiological studies suggest that maternal atopy is a much stronger indicator of allergic diseases in the offspring, than paternal atopy. As such, genetic predisposition alone is insufficient in understanding the underlying cause of asthma. Environmental interactions also play a large role in mediating asthma progression, potentially through epigenetic modifications, which may very well be the missing component in understanding disease development and progression. It is known that maternal stress is associated with alterations in the immune function of offspring and has been demonstrated to enhance severity of the allergic airway response in an ovalbumin sensitized murine asthma model. Conversely, maternal probiotic treatment has been proposed as a potentially novel therapeutic strategy for asthma. We have established a mouse model of cockroach allergy as a tool to help understand mechanisms underlying the influence of intrauterine and postnatal environmental factors on the susceptibility to and severity of allergic airway inflammation.

METHODS:

Pregnant mice were exposed to aversive sound stress for 24 h on days 12 and 14 of pregnancy or were treated with the a lactobacillus species *via* gavage (1x10⁹cfu) on alternate days throughout pregnancy and the weaning period. Offspring were sensitized with cockroach antigen intranasally from postnatal day 2 to day 8 and then challenged with cockroach intranasally on days 42 to 45. Airway hyper responsiveness and inflammation were assessed 24 hours following the last cockroach challenge. Spleen, mesenteric and bronchial lymph nodes were collected and T cell and dendritic cell populations assessed by FACS.

FINDINGS:

Offspring from stressed mothers were demonstrated to have a more severe allergic airway response than those from control mothers with significantly increased eosinophil influx into the airways following antigen challenge and enhanced responsiveness to methacholine. Conversely, offspring from probiotic treated mothers exhibited a decrease in the characteristic symptoms of an asthmatic response following antigen challenge. Furthermore offspring of probiotic treated mothers showed increased levels of regulatory T cells in lymph nodes following antigen challenge.

DELIVERABLES:

Evidence of efficacy in murine model using clinically relevant antigens and sensitization route

RELEVANCE:

We have demonstrated that prenatal environmental factors can have both detrimental and beneficial influences on the severity of the allergic airway response. We have also established an animal model that uses clinically relevant antigens and sensitization routes that can serve as a tool to determine the mechanisms underlying these effects and to test potential therapeutic strategies.

14A Hypertrophic Airway Smooth Muscle Mass Correlates with Increased Airways Responsiveness in a Chronic Murine Model of Allergic Asthma

AllerGen Programme A: Gene-Environment Interactions

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[†] Deceased

* Authors contributed equally to this work.

Supervisors: Jeremy A. Scott and Frances Silverman

PURPOSE:

The increase in airway smooth muscle (ASM) mass observed in asthma results from muscle hypertrophy and hyperplasia, yet there is little evidence correlating these changes with functional effects.

METHODS:

We performed a ventilator-based assessment of respiratory mechanics and responsiveness to methacholine in murine models of acute (3 wk) and chronic (12 wk) ovalbumin-induced airway inflammation. Using the Flexivent system and constant-phase modeling to differentiate between airway and peripheral tissue responses, we correlated functional changes (central airways Newtonian resistance [R_N], peripheral tissue resistance [G] and tissue elastance [H]), with the relative contribution of bronchial smooth muscle cell proliferation, hypertrophy and apoptosis to increased ASM mass.

FINDINGS:

Indices of increased airways hyper-reactivity were observed in both the acute and chronic models. Morphometric analyses of treated (ovalbumin-sensitized and –challenged; OVA/OVA) and control (ovalbumin- sensitized and saline-challenged; OVA/PBS) lungs, showed an increase in ASM area of chronic, but not acute, OVA/OVA mice, which correlated directly with the airways Newtonian resistance in response to methacholine. In contrast, in the acute model, none of the parameters of responsiveness to methacholine correlated with the airway smooth muscle content. Rather, the acute model exhibited significantly greater active proliferation of bronchial smooth muscle, which correlated with increased parameters of peripheral lung tissue dampening and elastance. Concomitant diminished apoptosis was also observed. This response resolved completely in the chronic 12 week model where the increased muscle mass that was laid down demonstrated hypertrophic growth.

DELIVERABLES:

We demonstrate a distinct temporal response in murine airways to antigenic challenge with ASM proliferation and diminished apoptosis occurring in the acute model, leaving a hypertrophied ASM mass that correlates with increased airways Newtonian resistance in chronic OVA/OVA treated mice.

RELEVANCE:

Identification of a functionally relevant hypertrophic smooth muscle mass highlights the possibility of regulation of airway muscle hypertrophy as a novel therapeutic target in asthma.

ACKNOWLEDGEMENTS: This research was supported by the Canadian Institutes of Health Research (operating grants to JB and MW), Keenan Centre Summer Student Research Award, St Michael's Hospital (AW), AllerGen NCE and National Sanatorium Association (JAS), and CIHR/Ontario Thoracic Society Doctoral Awards (MN).

15A Performance of Whole Genome Amplified DNA Samples Using Illumina 610-Quad Genotyping Array

AllerGen Programme A: Gene-Environment Interactions

Julie Park¹, Andy Leung¹, Ben Tripp¹, David Zamar¹, Loubna Akhabir¹, Scott Brown¹, Andrew Sandford¹, Allan Becker², Anita Kozyrskyj³, Peter Paré¹, Denise Daley¹
Supervisor: Denise Daley

¹ UBC James Hogg Research Centre – Heart & Lung Institute, University of British Columbia, Vancouver, BC; ²Department of Pediatrics and Child Health, Faculty of Medicine, University of Manitoba, Winnipeg, MB; ³Department of Pediatrics, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB

OBJECTIVE/PURPOSE:

Whole genome amplification (wga) enables researchers to maximize the sample size in the genetic studies; however, it is not recommended for the Infinium bead arrays used for Genome-Wide Association Studies (GWAS). We assessed the performance and reproducibility of wgaDNA genotypes using the 610 quad Illumina Infinium chip.

METHODS:

We analyzed three types of replicates: i) 1 cord blood quadruplet (2 genomic DNAs (gDNAs) & 2 wgaDNAs from the same individual), ii) 33 triplets (1 gDNA & 2 wgaDNAs) of different sample types (*i.e.*, 12 blood, 9 mouth epithelial and 12 sterile peripheral blood samples) and iii) 5 buccal swab wgaDNA duplets. In order to ensure accuracy of the genotype calls, gDNA and wgaDNA samples were genotyped on separate plates. As a measure of reproducibility, the sample concordance rate (the proportion of concordant SNPs across all comparisons) and SNP concordance rate (the proportion of concordant pairs that were genotyped at a particular SNP), were calculated using PLINK and R scripts.

FINDINGS:

The genotyping was successful for 98.4% of gDNA samples and 89.5% of wgaDNA samples, with 592,343 SNPs (95.4%) for both gDNA and wgaDNA samples. After the standard quality control (QC) was performed on gDNA and wgaDNA separately, 562,952 gDNA and 536,647 wgaDNA SNPs survived QC. Among the samples with greater than 97% call rate, we observed a sample concordance rate of 99.995% for 4 gDNA pairs, 98.990% for 59 gDNA-wgaDNA pairs and 99.998% for 35 wgaDNA pairs. When stratified by the sample types, the reproducibility of wgaDNA compared to gDNA was 99.982%, 99.993% and 99.995% for blood, mouth epithelial and sterile peripheral blood, respectively. In terms of SNP concordance, the majority of SNPs had 100% concordance; 99.7% for gDNA-wgaDNA and 99.95% for wgaDNA pair comparisons. Among SNPs with ≥ 1 discordance, 94% of SNPs in the gDNA-wgaDNA, and 77% in the wgaDNA pair comparisons had greater than 95% SNP concordance.

DELIVERABLES:

High sample concordances (>99.9%) as well as a high number of SNPs with no discordance (>99.7%) indicated it is feasible to use wgaDNA of different sample types for GWAS with Infinium arrays. The future analysis on SNPs would reveal whether or not GC contents and the distance from telomere affect the accuracy of wgaDNA genotype calls. To our knowledge, this is the first study that investigated the performance of wgaDNA using Infinium arrays.

RELEVANCE:

GWAS have been a popular study design to investigate the common disease common variant hypothesis. Our results enable the researchers to maximize the usage of data in GWAS settings, which, in turn, will aid in identifying genetic variants and improving methods of treatment of asthma and related phenotypes.

16A Diesel Exhaust-Associated Changes in Airway Reactivity and Innate Immunity in Asthmatic and Healthy Volunteers

AllerGen Programme A: Gene-Environment Interactions

Mandy Pui¹, John C. Lay², Neil E. Alexis²
Christopher Carlsten¹

¹University of British Columbia, Vancouver BC, ²University of North Carolina at Chapel Hill
Supervisor: Christopher Carlsten

OBJECTIVE/PURPOSE:

To identify an effective method to assess changes in innate immunity due to diesel exhaust and antioxidant supplementation and to correlate findings with changes in airway reactivity in intact humans, both healthy and asthmatic.

METHODS:

Three physician-diagnosed asthmatics and eight non-diagnosed participants completed a double-blinded, randomized, crossover, counter-balanced study of 3 exposure conditions, each separated by a 2-week washout period: (1) diesel exhaust (300ug PM_{2.5}/m³ for 2 hours) with anti-oxidant (N-acetylcysteine 600mg 3x/day for 5 days preceding, and on the day of the exposure) ["DEN"], (2) diesel exhaust with placebo ["DEP"], or (3) filtered air with placebo ["FAP"]. Airway reactivity to methacholine (PC₂₀) was assessed pre-exposure and at 30 hours post-exposure. Induced sputum was collected pre-exposure, and at 6 hours post-exposure. Sputum neutrophils, macrophages, and monocytes were analyzed by two different flow cytometry methods for changes in phagocytic function: (1) fluorescein-labelled zymosan (Invitrogen), and (2) pHrodo E.coli BioParticles (Invitrogen) then direct immunolabelling with APC-cy7-CD45 antibody (BD BioSciences).

FINDINGS:

All three physician-diagnosed asthmatics, and three of the eight non-diagnosed participants reacted significantly (PC₂₀ ≤8 mg/mL) to methacholine at baseline. In every case, the lowering of PC₂₀ seen following DEP was partially or completely mitigated with N-acetylcysteine supplementation (DEN). Using the zymosan method to assess phagocytic function, the average phagocytic index in sputum macrophages increased by 4.6 units following DEP (relative to FAP baseline) and this was partially attenuated by DEN (average 3.2 units above FAP baseline). Results for the pHrodo method are pending.

DELIVERABLES:

Diesel exhaust-induced changes in airway reactivity and innate immunity have been independently shown by previous literature. However, the relationship between changes in airway reactivity and innate immunity associated with diesel exhaust has not been established. Preliminary data from this ongoing study suggests that DE-associated increases in airway reactivity are mitigated by anti-oxidant supplementation (N-acetylcysteine). This may be mediated by upregulated innate immune responsiveness as reflected by DE-induced phagocytosis in airway macrophages, which is also attenuated by N-acetylcysteine.

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 efficiency techniques for characterizing cellular effects in the intact human model. Refining the methods described above allows for such detailed assessment of sputum in the context of a controlled human exposure to diesel exhaust.

17A The Relationship of Fast Food, Breastfeeding and Asthma in Adolescents

AllerGen Programme A: Gene-Environment Interactions

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Supervisor: Allan B. Becker

OBJECTIVE:

To establish a relationship between the consumption of fast food, the duration of breastfeeding and asthma in adolescent children.

METHODS:

Data from the SAGE cohort showed that regular consumption of fast food negates the protective effect of breastfeed of asthma in children 8 years of age⁴. Information about breastfeeding was gathered from Wave 1 and information about fast food consumption from Wave 3. We reassessed the impact of breastfeeding and fast food consumption in adolescents at age 13-14 from the SAGE cohort. This included a total of 471 cases, 146 children (30.9%) with pediatric allergist-diagnosed asthma and 319 (67.7%) controls without asthma. Breastfeeding and fast food consumption were obtained from detailed questionnaire data filled out by parents of participants at age 8-10 and children at age 13-14. The association between breast-feeding, fast food consumption and asthma was determined using logistic regression.

FINDINGS:

At ages 13-14 years old, there was no significant difference in frequent fast food consumption between children with or without asthma (136/146 (93.1%) vs. 307/319 (96.2%)). In adjusted models, we found no associations between the interactive effects of breastfeeding and fast food consumption on asthma status. For example, there was neither significant relationship between less frequent consumption of fast food and breastfeeding for less than 12 weeks in these adolescents (Crude OR 0.90; 95% CI, 0.57-1.42), nor for more frequent consumption of fast food and exclusive breastfeeding 12 weeks or more (Crude OR 1.64; 95% CI, 0.67-4.03) and asthma. In relation to fast food consumption and asthma, 12 out of 319 children, who did not have asthma, and 10 out 146 children, who did have asthma, frequently ate fast food. Frequent fast food consumption was not significantly more common among children with or without asthma (10/146 (1.46%) vs. 12/319 (3.04%), $p=0.15$). Stratification by gender did not yield any statistically significant differences. Boys who were breastfed for 12 weeks or more and consumed fast food tended to be more likely to have asthma than girls (Crude OR 2.08; 95% CI, 0.67-6.48) at age 13-14. There were insufficient children who rejected frequent fast food consumption at age 13-14 and who were breastfed for less than 12 weeks. There were insufficient children who did not have frequent fast food consumption at age 13-14 and who were breastfed for less than 12 weeks for analysis.

DELIVERABLES:

In this preliminary analysis, for young adolescents we were unable to define any significant relationship between fast food consumption and breastfeeding and asthma. Further analysis will help us better understand the risk of asthma based on prolonged breastfeeding duration and consumption of fast food for children into the adolescent years.

RELEVANCE:

It is important to understand the effect of environmental influences on human health, especially for growing adolescents, such as fast food consumption.

⁴ X-M. Mai, A.B. Becker, J.J. Liem and A.L. Kozyrskyj, *Clinical & Experimental Allergy*, 2009 (39) 556-561.

18A Estimating the Variability of Endotoxin, (1,3)- β -D-Glucan, and Hopanes in Repeated Samples of Settled House Dust

AllerGen Programme A: Gene-Environment Interactions

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Supervisor: Michael Brauer

OBJECTIVE:

Young children spend the majority of their time indoors at home where it is expected that most of their exposures to biological hazards occur. House dust is a commonly used medium to assess inhalant exposure hazards. It has the advantage of representing an integrated measurement for multiple pollutants of both indoor (*e.g.*, endotoxin and ((1,3)- β -D-glucan) and outdoor (*e.g.*, hopanes as a marker of diesel exhaust) origins that may be related to asthma incidence.

Using data gathered in repeated home visits conducted within the mini-CHILD studies for children 3 months and 12-18 months old, we examined the temporal variation in house dust measurements of (1,3)- β -D-glucan, and endotoxin as well as hopanes. Our specific objectives were to measure the temporal variability of indoor pollutants and to model indoor air exposures.

METHODS:

Samples were initially collected from 56 homes when the child was about 3 months old. Dust was collected either in the bedroom or in both the bedroom and the most commonly used room.

The second home visits were conducted when children were 12 – 18 months old and included 39 homes of which 30 had dust samples collected during the first sampling period. House dust was collected with the same sampling procedures as in the initial home visit. Subsequently, we compared the concentrations of endotoxin and (1,3)- β -D-glucan over 2 home visits, and assessed the correlation between the amount of dust and each of the endotoxin and (1,3)- β -D-glucan as well as that between endotoxin and (1,3)- β -D-glucan.

FINDINGS:

House dust was not collected consistently in the two visits: only 53% of the 56 homes during the first home visit had dust collected from both bedroom and most used room, while 37 out of 39 houses sampled during the second visit had dust from both bedroom and most commonly used room.

	(1,3)- β -D-glucan (in ug/g dust)			Endotoxin (in EU/g dust)		
	GM (GSD no units)	Min	Max	GM (GSD no units)	Min	Max
Visit 1	1624 (3.6)	64	11200	183497 (2.9)	15600	3940000
Visit 2	392.9 (2.3)	34	2308	169037 (1.7)	38274	571372

In total 30 homes had repeated visits with house dust collected in the same areas. Preliminary analysis suggests that there is weak to moderate correlation in endotoxin ($r=0.2$) and (1,3)- β -D-glucan ($r=0.4$) levels per home between the two sampling periods for samples collected in the most commonly used room. However, the correlation between repeated measures in the bedroom is very weak for both endotoxin ($r=-0.05$) and (1,3)- β -D-glucan ($r=-0.15$).

DELIVERABLE:

Similar analyses will be conducted for hopanes and allergens (*Der p1 Der f1 Blag2 Feld1 Can f1*) once sample analyses have been completed. The investigation of the temporal variability can be used to understand the determinants of differences in concentrations of inhalable indoor air hazards such as home characteristics.

RELEVANCE:

The understanding generated through this work will allow for more effective use of the exposure data being obtained in the national Canadian Healthy Infant Longitudinal Development (CHILD) Study. The methods developed will potentially lead to cost-effective strategies for exposure assessment in birth cohort studies of childhood asthma

19A Potential Sources of Phthalate Exposure in the Vancouver CHILD Study at Three Months of Age

AllerGen Programme A: Gene-Environment Interactions

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¹Simon Fraser University, Burnaby, BC, ²University of British Columbia, Vancouver, BC

Supervisor: Tim Takaro

OBJECTIVE/PURPOSE:

This study is part of a pilot to the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort studying determinants of asthma and allergy in early life. Exposure to the ubiquitous plasticizers known as phthalates may contribute to the development of an inflammatory response and be a factor in the development of allergic disease through direct or adjuvant mechanisms. This project investigated the associations between exposures to phthalates from the indoor environment, personal care products, and interactions with socioeconomic status.

METHODS:

We have analyzed 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)) in urine samples from 63 subjects at age three months. We examined associations between levels of urinary phthalate metabolites and measurements of phthalates in house dust and questionnaire/home inspection indicators of phthalate sources in the indoor environment, including furnishings and personal care products. Regression and geographic analyses were applied.

FINDINGS:

Based on the preliminary results, we found higher levels of phthalate metabolites associated with flooring, personal care product such as baby lotion and shampoo; work with hazardous materials; and low socioeconomic status. The parent compound for seven phthalate metabolites were also recently measured in house dust from subjects' homes and will be included in subsequent models.

Pending Results:* We've just received the repeated visit urine results for the same cohort at 9 months of age. We will apply the same analysis methods to the repeated visit results.

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 Study cohort will enable examination of this exposure in the context of allergen, endotoxin, mould and other exposures that contribute to the development of asthma.

20A Genomic Response Profiles in Peripheral Blood of Allergic Rhinitis Subjects Exposed to Ragweed Pollen in the Environmental Exposure Unit

AllerGen Programme A: Gene-Environment Interactions

A. Singh¹, SHY. Kam¹, J. Ruan¹, AK. Ellis^{2,3}, JD. Ratz², DJ. Adamko⁴, JH. Day^{2†}, SJ. Tebbutt¹
¹ UBC James Hogg Research Centre – Heart & Lung Institute, University of British Columbia, Vancouver, BC, ²Kingston General Hospital and Queen's University, Kingston, ON, ³Department of Microbiology & Immunology, Queen's University, Kingston, ON ⁴Department of Pediatrics, Faculty of Medicine & Dentistry, Edmonton, AB
[†] Deceased
Supervisor: Scott J Tebbutt

OBJECTIVE/PURPOSE:

Preliminary analysis has shown that individuals with allergic rhinitis (AR) who are exposed to pollen exhibit either an isolated early phase response (EPR) or a dual response [DR; EPR and a last phase response (LPR)]. Such differential responses are determined by molecular mechanisms that are not fully understood. The purpose of this study is to determine genomic response profiles in peripheral blood of allergic rhinitis individuals exposed to ragweed pollen.

METHODS:

Forty-four subjects were simultaneously exposed to ragweed pollen (3500 ± 500 grains/m³) in the Environmental Exposure Unit (EEU). Peripheral blood samples were collected using PAXgene Blood RNA tubes before and after the 3h pollen exposure. Based on symptom score cards completed both during and 6-12h following the exposure, samples from 9 isolated early responders and 5 dual responders were selected for microarray analysis. 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:

458 probe sets had a significant ($p \leq 0.01$) change in gene expression in blood samples between the pre and post ragweed pollen exposure. In addition, 186 probe sets were significant at a $p \leq 0.01$ in an ANOVA model that included the interaction of pre-/post-challenge and response type. Using IPA, the top biological functions identified for post versus pre exposure included Cell Death, Respiratory Disease, and Inflammatory Response. The top biological functions for the gene list generated using the interaction ANOVA model included Cell-mediated and Humoral Immune Responses.

DELIVERABLES:

Genomic analysis in the peripheral blood has shown significant changes in gene expression after exposure to ragweed pollen relative to pre-exposure. Further analysis will show how these genes contribute to the development of the EPR and LPR.

RELEVANCE:

Genomic analysis may identify genes and pathways associated with the molecular mechanisms involved in the early and late phase responses. Such knowledge may lead to more effective research into better treatments for individuals with allergic rhinitis and other allergic conditions.

21A Evaluation of Peroxisome Proliferator-Activated Receptors (PPARs) Effects on Eosinophil Migration

AllerGen Programme A: Gene-Environment Interactions

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McMaster University, Hamilton, ON
Supervisor: Gail M. Gauvreau

OBJECTIVE/PURPOSE:

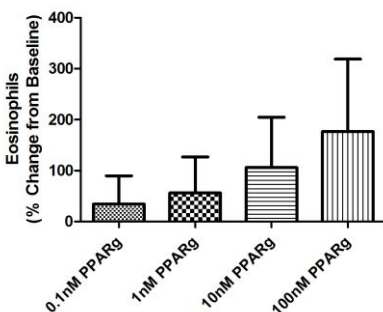
Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear transcription factors that regulate inflammatory responses. There are three subtypes, PPAR α , PPAR δ and PPAR γ . PPARs form heterodimers with retinoic X receptors and subsequently bind to portions of DNA known as peroxisome proliferator response elements (PPREs). PPREs, which are located in the promoter region of target genes, can effect gene expression. PPARs can also repress target genes independently of DNA-binding. Several researchers have suggested that the PPARs are novel anti-inflammatory targets because they inhibit migration, proliferation and reduce cytokine production in several inflammatory disease models. In murine models, PPAR α and PPAR γ agonists inhibit eosinophil influx to the lung after airway antigen challenge. PPAR α and PPAR γ agonists also inhibit eosinophil chemotaxis *in vitro*. However, the effect of PPAR γ agonists on eosinophil chemotactic activity *in vitro* has not been well described. The objective of this study was to examine the effects of PPAR γ agonists on *in vitro* migration of eosinophils collected from 8 donors with atopy.

METHODS:

Peripheral blood was diluted in McCoy's 5A and eosinophils were purified using an accuprep density gradient followed by a MACS column CD16⁺ neutrophil depletion. Purified eosinophils were resuspended in RPMI complete (10% Fetal Bovine Serum, 1M HEPES in RPMI 1640). The cells were then treated for 20min with media (RPMI complete with 0.1% DMSO), or with PPAR γ (rosiglitazone) agonist. Media was placed into the lower wells of the 48 well microchemotaxis assembly. A nitrocellulose filter with a pore size of 8 μ m was used to separate the upper and lower wells. Cell suspension at a concentration of 3x10⁶ cells/mL was placed into the upper wells. The chamber was incubated in high humidity for 90min at 37°C. The filter was fixed and stained with hematoxylin, and chromotrope 2R. The filter was then mounted on a glass slide and ten random fields from the underside of the filter were counted at 400x magnification. Data was expressed as percent change from baseline \pm standard deviation and statistical differences were determined by one-way analysis of variance.

FINDINGS:

A dose dependent, but non-statistical ($p=0.2296$), increase in eosinophil migration was observed after treatment with rosiglitazone.



DELIVERABLES:

We observed evidence for PPAR γ agonist-induced eosinophil chemokinesis.

RELEVANCE:

Our pilot experiments suggest rosiglitazone can increase migration of peripheral blood eosinophils.

22A Alterations in Histone Acetylation in Asthmatic Airway Epithelial Cells

AllerGen Programme A: Gene-Environment Interactions

Dorota Stefanowicz¹, Tillie-Louise Hackett¹, Peter Paré¹, Darryl Knight¹

¹UBC James Hogg Research Centre – Heart & Lung Institute, University of British Columbia,
Vancouver, BC

Supervisor: Darryl Knight

OBJECTIVE/PURPOSE:

The airway epithelium is the interface between the environment and the submucosa of the lung and thus is the first line of defense against inhaled exogenous agents. We have shown phenotypic difference between asthmatic and non-asthmatic epithelial cells which persist over passage suggesting that these cells have inherited and/or acquired epigenetic abnormalities. One method of epigenetic control of gene expression is chemical modification of the histone proteins that package DNA into chromatin. In particular, modification by histone acetyltransferases (HATs) and deacetylases (HDACs) result in de/acetylation of lysine residues on histone tails thus influencing the access of transcription factors and enzymes to the DNA sequence. The objective of this research is to evaluate epigenetic changes in histone acetylation and their contribution to the remodeled phenotype of the epithelium in asthma and thus the pathogenesis of the disease.

METHODS:

Bronchial epithelial cells from non-asthmatic and asthmatic subjects were obtained from fresh donor lung tissue by pronase digestion and maintained in monolayer culture. Nuclear extracts from cells were obtained using the Qproteome Nuclear Protein Kit. Extracts were analyzed for HAT and HDAC activity using commercially available assays. Tracheal tissue was analyzed for lysine acetylation on histone 3 and 4 by immunohistochemistry.

FINDINGS:

We observed no difference in HDAC activity between healthy controls and asthmatic epithelial cells. We also observed elevated HAT activity in the asthmatic samples on histone 3 (mean% HAT activity 70.4 ± 11.4), compared to healthy controls (9.5 ± 4.5 , $p < 0.01$). We found the same trend in HAT activity on histone 4 in asthmatic epithelial cells (mean % HAT activity 86.5 ± 29.1) compared to healthy controls (21.5 ± 8.2 , $p = 0.02$). Analysis of the acetylation status of H3K14, H3K18, H3K27, and H4K8 showed no significant difference in asthmatic cells although a trend is visible.

DELIVERABLES:

Elevated HAT activity in asthmatic epithelial cells indicates that the epigenome is altered in asthma.

RELEVANCE:

Our data suggest that the epigenome of the asthmatic epithelium is altered and future work is required to determine the effect of these modifications on dysregulated epithelial function in asthma.

23A Eosinophilic Inflammation Due to Volatile Organic Compounds in Controlled Human Exposure Studies

AllerGen Programme A: Gene-Environment Interactions

Andrew Thomas¹, Bruce Urch¹, Mary Speck¹, Frances Silverman¹

¹Gage Occupational & Environmental Health Unit, St. Michael's Hospital & University of Toronto, Toronto, ON

Supervisor: Frances Silverman

OBJECTIVE/PURPOSE:

To measure the ambient concentrations of volatile organic compounds (VOCs) during controlled human exposure studies and to examine the cardio-respiratory and inflammatory responses from exposure to volatile organic compounds

METHODS:

Exposures were conducted at the Gage Occupational and Environmental Health Unit (GOEHU) human exposure facility in Toronto, Ontario. Over the course of 20 exposures, nine volunteer subjects were exposed for 130 minutes to medical air, filtered air, and concentrated ambient particles (CAP). During each exposure, VOCs were collected on a multi-bed sorbent tube. The concentrations of 23 selected VOCs were subsequently quantified by gas chromatography- mass spectrometry. Blood samples were collected for a complete blood count (CBC) before and after each two hour exposure. In addition, cardio-respiratory health effects were measured at the same time points.

FINDINGS:

The majority of the VOCs analyzed were significantly correlated with traffic-related pollution sources. Several VOCs including carbon tetrachloride and hexane were associated with an increase in the absolute eosinophil count after exposure. In addition, 1, 3, butadiene and 1, 4 dichlorobenzene were associated with an increase in the absolute neutrophil count after exposure. When CAP mass concentrations were included in regression analyses, a $1\mu\text{g}/\text{m}^3$ increase in carbon tetrachloride was associated with a 15.1 mmHg and 16.8 mmHg increase in (post-pre) systolic and diastolic blood pressure respectively ($p=0.03, 0.05$). On the other hand, VOC exposure was not shown to have a significant effect on several spirometry outcomes including forced expiratory volume in one second (FEV_1).

DELIVERABLES:

Results from this study have already been presented at the Dalla Lana School of Public Health Student Research and Practice Day November 5, 2010 and can be used to further inform future human exposure studies.

RELEVANCE:

Blood eosinophil counts can reflect asthmatic activity and are useful for early detections of exacerbations. Neutrophilic inflammation has been shown to underlie symptoms and physiological changes that are characteristic of asthma. Low level exposures to volatile organic compounds could result in exacerbation of asthma symptoms. These findings have significant public health implications, as many of the VOCs measured in this study have been completely banned from production and usage, yet are still present at ambient concentrations which may cause adverse health effects.

24A Association Study Between *IL1R2* Pathway Genes and Asthma Related Phenotypes in Two Independent Familial Studies

AllerGen Programme A: Gene-Environment Interactions

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F. Demenais^{5,6,7}, C. Laprise^{1,2}

¹Université du Québec à Chicoutimi, QC ²CSSS Chicoutimi, QC ³Ontario Institute for Cancer Research, Toronto, ON ⁴University of British Columbia, Vancouver, BC ⁵Institut national de la santé et de la recherche médicale, France ⁶Fondation Jean-Dausset-CEPH, France ⁷Université de Paris, France
Supervisor: Catherine Laprise

OBJECTIVE/PURPOSE:

We previously demonstrated that *IL1R2* (Interleukin-1 receptor type II) is differentially expressed in bronchial biopsies of allergic asthmatics compared to controls and we also associated *IL1R2* single nucleotide polymorphisms (SNPs) with atopy in four Canadian/Australian independent studies (three familial and one case-control samples; n>5500). The present study is an association analysis of genes involved in the *IL1R2* pathway and asthma and related phenotypes in three Canadian studies and in a French European one (Epidemiological study on the Genetics and Environment of Asthma (EGEA)).

METHODS:

We performed association studies in a French Canadian sample (SLSJ) (253 families) and we performed the replication within the Canadian Asthma Primary Prevention Study (CAPPS) and the Study of Asthma Genes and the Environment (SAGE) Canadian samples as well as in EGEA (348 families) to identify SNPs associated with asthma, allergy and allergic asthma. Genes were selected by a review of relevant studies of the regulation of *IL1R2*. SNPs belonging to 13 genes involved in the *IL1R2* pathway were extracted from the genome wide association study performed on the different samples with PLINK software and the transmission disequilibrium was analyzed using the Family-Based Association Tests software (FBAT) in an additive genetic model with the empiric variance estimator “-e”. SNPs were subjected to a Bonferroni multiple tests correction considering the number of independent SNPs and the number of phenotypes.

FINDINGS:

After correction, we found four genes associated with asthma related phenotypes in the SLSJ and EGEA samples. In the SLSJ study, Beta-site APP-cleaving enzyme-1 gene (*BACE1*) was associated with asthma and allergic asthma (P-values ranging from 0.004 to 0.0003) and Spleen focus forming virus proviral integration oncogene gene (*SPI1*) with allergy and asthma (P≤0.005 for rs3740698). In the EGEA study, Interleukin 1 receptor type 1 (*IL1R1*) was associated with asthma and allergy (P= 0.0012 and 0.0009 respectively for rs3732131). Endoplasmic reticulum aminopeptidase gene (*ARTS1*) was associated with allergic asthma (P≤0.001 for rs13154629, rs12173167 and rs10050860).

DELIVERABLES:

The several associated SNPs in *IL1R2* pathway genes combined with the replication studies suggest that this pathway has an important role in asthma and allow us to have an overall view of the molecular biology of this pathophysiology.

RELEVANCE:

To genetically analyze biological pathways of target genes may increase our chance to find relevant predictive or therapeutic biomarkers. Indeed, results of this study reveal associations for different genes of the same biological pathways in different population, underlining the importance of this pathway in asthma. Analyzing biological pathways and making connections between the different components leads to a better integration of genetic knowledge of the pathophysiology and gets us closer to a more specific therapy.

25A Assessment of the Temporal Stability of Land Use Regression Models for Traffic-Related Air Pollution

AllerGen Programme A: Gene-Environment Interactions

Rongrong Wang¹; Sarah Henderson²; Ryan Allen³; Michael Brauer¹

¹School of Environmental Health, University of British Columbia, Vancouver, BC; ²British Columbia Centre for Disease Control, Vancouver, BC ³Faculty of Health Sciences, Simon Fraser University, Burnaby, BC
Supervisor: Michael Brauer

OBJECTIVES/PURPOSE:

Land-use regression (LUR) models have been used as a cost-effective approach for assessing intra-urban air pollution contrasts. A large number of LUR models have been used to estimate exposure to traffic-related air pollution in epidemiologic studies of childhood asthma and allergy, based on the assumption that the spatial patterns of pollution are stable over time so that a LUR model developed from a particular time point could be applied to other time points. However, this assumption of temporal model stability has not been adequately examined. This issue has specific relevance to birth cohort studies such as CHILd where models are developed in specific years and then applied to cohorts over periods of 5 – 10 years. We therefore tested the measured and modelled spatial patterns in outdoor NO₂ across Metro Vancouver over 7 years.

METHODS:

A LUR model for annual average nitrogen dioxide (NO₂) in Metro Vancouver was developed in 2003, based on measurements at 116 locations. In 2009/10, we took measurements again at the same locations and developed a new model using updated data for the same predictor variables. The temporal stability of LUR models over a 7-year period was evaluated by comparing model predictions and measured spatial contrasts between the two time periods.

FINDINGS:

LUR models from 2003 and 2009/10 explained 54% and 68% of the observed spatial variation, respectively. The 2009/10 LUR model explained 51% of the variability in the 2003 measurements, while the 2003 model explained 52% of variability in 2009/10 measurements. The 2003 model predicted similar levels of variability when applied across the 7-year period as compared to the same year of the measurements and to the 2009/10 model applied to 2003, indicating strong temporal stability.

DELIVERABLES:

- The pollution surface for NO₂ has been updated from 2003 to 2009/10, and comparisons were made for both TRAP measurements and LUR models. This will facilitate application of air pollution exposure estimates to epidemiological studies, as well as evaluation of air quality management programs.
- The results contribute to verification of the assumption that a LUR model developed from a particular time point could be applied to other time points, thus the validity of applying LUR models to cohort studies where recruitment and follow-up occurs over 5 – 10 year periods was strengthened.

RELEVANCE:

The updated 2009/10 LUR model will be used to estimate TRAP exposures for Vancouver participants in the CHILd Study cohort. Our demonstration of model temporal stability indicates that exposure estimates developed for birth year, are still valid for subsequent years of follow-up for subjects who remain at the same home address. Similarly, LUR models developed before subject recruitment can provide an estimate of birth year exposure. For subjects who move, updating exposure estimates with residential history is necessary.

II. PROGRAMME B: DIAGNOSTICS AND THERAPEUTICS

#	AllerGen Trainee	Institution	AllerGen Researcher(s)/ Supervisor(s)	Abstract Title
1B	Akhtar, Umme S.	Department of Chemical Engineering and Applied Chemistry, University of Toronto	Dr. Jeremy A. Scott	Comparative toxicity of size-fractionated ambient particulate matter in relation to composition and redox activity in human airway epithelial cells
2B	Brown, Meghan	Department of Physiology, University of Toronto , and The Hospital for Sick Children, Toronto	Dr. Padmaja Subbarao	Effects of forced expiratory maneuvers and bronchodilator on Lung Clearance Index in infants
3B	Carson, Kaitlyn Tunis, Matthew	Department of Microbiology and Immunology, Dalhousie University	Dr. Jean S. Marshall	The effect of Toll-like receptor 2 activation during the induction of oral tolerance in mice
4B	Edwards, Sarah Fraser, Kathleen Thiele, Jenny	Department of Microbiology & Immunology, Queen's University	Dr. Anne K. Ellis	Evaluation of IL-10 and TGF- β levels in primary cultures of dendritic cells as potential predictors of atopy
5B	Gold, Matthew	Biomedical Research Centre, University of British Columbia ,	Dr. Kelly M. McNagny	CD34 in intracellular signaling and mucosal inflammatory disease
6B	Hackett, Tillie-Louise Singhera, Gurpreet K.	UBC James Hogg Research Centre, University of British Columbia	Dr. Tony Bai	Intrinsic phenotypic differences of the asthmatic epithelium and its inflammatory responses to RSV and particulate matter
7B	Hirsch, Gaelle Lavoie-Lamoureux, Anouk	Faculté de Médecine vétérinaire, Université de Montréal	Dr. Jean-Pierre Lavoie	Comparison of blood neutrophils and mononuclear cells steroid responsiveness
8B	Hui, Claudia Asher, Ilan Héroux, Delia Allakhverdi, Zoulfia	Division of Clinical Immunology & Allergy, McMaster University , and CRCHUM, Hôpital Notre-Dame de Montréal	Dr. Judah A. Denburg	Effect of thymic stromal lymphopoietin (TSLP) on cord blood (CB) progenitor cell differentiation and hemopoietic cytokine receptors (HCR) expression
9B	Hui, Claudia Thong, Bruce	Division of Clinical Immunology & Allergy, McMaster University , and Laboratory on Allergy, CRCHUM	Dr. Judah A. Denburg	Induction of Thymic Stromal Lymphopoietin (TSLP) in Airway Epithelium by Recombinant Allergens
10B	Maltby, Steven Gold, Matthew Blanchet, Marie-Renée	The Biomedical Research Centre, University of British Columbia	Dr. Kelly M. McNagny	CD34 localization in eosinophils at steady state and during disease
11B	Marr, Nico	Department of Pediatrics, Child and Family Research Institute, University of British Columbia	Dr. Stuart E. Turvey	Live RSV fails to activate human TLR3, TLR4 and TLR7 in transfected HEK 293 cell lines
12B	Oldford, Sharon A.	Department of Microbiology and Immunology, Dalhousie University	Dr. Jean S. Marshall	Activated mast cells inhibit tumor growth

#	AllerGen Trainee	Institution	AllerGen Researcher(s)/ Supervisor(s)	Abstract Title
13B	Oliveria, John Paul Strinich, Tara Howie, Karen	Division of Respiriology, Department of Medicine, McMaster University	Dr. Gail M. Gauvreau	The hypothalamic-pituitary-adrenal axis in subjects with mild allergic asthma
14B	Pascoe, Christopher	UBC James Hogg Research Centre, University of British Columbia	Dr. Peter D. Paré Dr. Chun Y. Seow	Mechanical properties of asthmatics' airway smooth muscle
15B	Raja, Musherif Watson, Rick	Division of Respiriology, Department of Medicine, McMaster University	Dr. Gail M. Gauvreau	Repeated allergen challenges do not induce tolerance in asthmatic dual responders
16B	Reece, Pia Thanendran, Amudhinie	Division of Clinical Immunology & Allergy, and Department of Clinical Epidemiology and Biostatistics, McMaster University	Dr. Judah A. Denburg	Effect of maternal allergic sensitization and smoking during pregnancy on neonatal eosinophil-basophil lineage commitment
17B	Segeritz, Charis-P Loeffler, Daniela M. Cai, Bing Gold, Matthew	Child and Family Research Institute, and University of British Columbia	Dr. Tobias R. Kollmann	The immune-prophylactic vaccine <i>Lm</i> $\Delta(trpSactA)/pSPO-PS_{nly}OVA$ protects neonates from asthma
18B	Tunis, Matthew	Department of Microbiology and Immunology, Dalhousie University	Dr. Jean S. Marshall	Mast cells are not required for the induction of oral tolerance in mice
19B	Watson, Brittany Howie, Karen Obminski, George Campbell, Heather	Division of Respiriology, Department of Medicine, McMaster University	Dr. Gail M. Gauvreau	LTB ₄ release from neutrophils in allergic and non-allergic subjects
20B	Yang, Jasemine S. Wadsworth, S.J. Singhera, Gurpreet K.	UBC James Hogg Research Centre, University of British Columbia	Dr. Delbert R. Dorscheid	IL-13R α 1 and IL-13R α 2 interaction regulates airway epithelial repair pathways

1B Comparative Toxicity of Size-Fractionated Ambient Particulate Matter in Relation to Composition and Redox Activity in Human Airway Epithelial Cells

AllerGen Programme B: Diagnostics and Therapeutics

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^bDepartment of Chemistry, University of Toronto, Toronto, ON ^cGage Occupational & Environmental Health Unit, St. Michael's Hospital, Toronto, ON ^dDepartment of Medicine, University of Toronto, ON

Supervisor: Jeremy A. Scott

OBJECTIVE/PURPOSE:

Epidemiological studies have revealed associations between ambient particulate matter (PM) and allergic respiratory diseases. Recent studies have suggested that PM-associated effects are related to size and composition of inhaled particles. However, knowledge gaps remain regarding their importance. The main objective of this study was to identify the physicochemical properties responsible for the toxicological/biological effects of PM.

METHODS:

Concentrated ambient PM was collected for three size fractions: quasi-ultrafine (UF, <0.2 µm), fine (0.15-2.5 µm), and coarse (2.5-10 µm) near a busy street in Toronto during an intensive field campaign 'Health Effects of Aerosols in Toronto (HEAT)'. PM was then extracted and analyzed for various inorganic and organic chemical species. The dithiothreitol (DTT) assay was used to measure the redox activity of the particles. Human alveolar epithelial cells (A549) were exposed for 4 h. Three biological endpoints were measured: induction of antioxidant enzymes (heme oxygenase, HMOX-1), pro-inflammatory response (interleukin IL-8), and cell viability. Real time PCR was used to determine the mRNA expression of HMOX-1 and IL-8, relative to 18S. Cell viability was analyzed by using the MTT assay. Correlation analyses were assessed by the Spearman rank correlation test (significantly different when $p < 0.05$).

FINDINGS:

UF particles were more potent at inducing HMOX-1 expression than coarse and fine particles (fold increase with respect to control: UF 3.36, fine 2.17, and coarse 2.98 at PM concentration of 100 µg/ml, $p < 0.05$). Similarly, the PM-induced release of IL-8 was greatest for UF particles (3.40 vs. fine 3.10, coarse 2.72 ($p < 0.05$)). However, cell viability was least affected by UF particles (88% vs. 84% and 71% for fine and coarse ($p < 0.001$), respectively). HMOX-1 upregulation correlated positively with Fe concentrations for all ($p < 0.05$), Zn for coarse ($p = 0.003$), and Cu ($p = 0.02$), elemental ($p = 0.004$) and organic carbons (OC) ($p = 0.02$) for fine PM. IL-8 induction correlated positively with Fe for all ($p < 0.05$), Cu for fine ($p < 0.001$) and UF ($p = 0.04$), Zn for coarse ($p = 0.03$) and UF ($p = 0.02$). Furthermore, OC content for both fine ($p = 0.004$) and UF ($p = 0.05$) were correlated with IL-8 induction. No significant correlation was found between redox activity and biological responses, cell viability and chemical species.

DELIVERABLES:

In this *in vitro* study, we demonstrated that activation of antioxidant enzymes and pro-inflammatory response are affected significantly by ultrafine particles compared with that of other size fractions. HMOX-1 and IL-8 upregulation appeared to be composition dependent, specifically related to transitional metals (Fe, Cu, and Zn) and organic carbon.

RELEVANCE:

This study contributes to the current understanding of the toxic effects of ultrafine particles and emphasizes the importance of relating PM composition and size to toxicity. This understanding will help Environment Canada and Health Canada evaluate the need to create an exposure standard for ultrafine particles to supplement the current Canada-wide standard for fine PM. The improved air quality standard will reduce the risk of exacerbation of respiratory symptoms, specifically in individuals with pre-existing conditions such as asthma.

2B Effects of Forced Expiratory Maneuvers and Bronchodilator on Lung Clearance Index in Infants

AllerGen Programme B: Diagnostics and Therapeutics

Meghan Brown¹, Renee Jensen², Susan Balkovec², Felix Ratjen^{2,3,4}, Padmaja Subbarao^{2,3,4}

¹School of Graduate Studies, Department of Physiology, University of Toronto, Toronto, ON ²Division of Respiratory Medicine, Department of Paediatrics, The Hospital for Sick Children, Toronto, ON, ³University of Toronto, Toronto, ON, ⁴Physiology & Experimental Medicine, Research Institute, The Hospital for Sick Children, Toronto, ON

Supervisor: Padmaja Subbarao

OBJECTIVE/PURPOSE:

Although the Lung Clearance Index (LCI) as measured by the Multiple Breath Washout (MBW) technique is proposed to be a sensitive test of small airway function in older children, little is known about its use in infants. LCI is representative of the efficiency with which inspired gas mixes with resident gas within the lung periphery. The raised volume rapid thoracoabdominal compression technique (RVRTC), a technique used to measure forced flow measurements in infants, could be hypothesized to produce changes in LCI. Positive pressure inflations and compressions may have effects on small airway caliber and ventilation homogeneity within the lungs of infants undergoing RVRTC. Furthermore, these changes must be understood prior to understanding the effects of bronchodilator (BD) on LCI in infants undergoing a protocol involving RVRTC measurements. The objective of this project was to investigate the effect of RVRTC and BD treatment on LCI measurements in healthy infants and infants with respiratory disease including confirmed cystic fibrosis (CF), suspected CF (intermediate sweat test score and one CF allele) and wheeze.

METHODS:

We obtained LCI measurements using mass spectrometry 4% SF₆ inert gas MBW at baseline, post-RVRTC and then post-BD in 16 healthy infants (mean age; 0.52 years) and in 14 infants with respiratory disease (mean age; 1.29 years). Any LCI difference between testing conditions that was outside the 95% confidence interval of the mean LCI difference between the same two testing conditions in aged matched controls was considered to be a significant difference in LCI post-RVRTC and post-BD.

FINDINGS:

Baseline mean LCI was significantly higher in infants with respiratory disease compared to healthy controls (mean difference 1.25 (95% CI 0.342 to 2.168); $p < 0.009$). There was no significant difference in the LCI of healthy infants between testing conditions (pre or post RVRTC or BD) ($p < 0.44$). Mean LCI of infants with respiratory disease also did not significantly change between testing conditions ($p < 0.74$), although the individual response was heterogeneous. Of the 13 infants with respiratory disease, 6 (46%) showed a significant improvement (decrease) in LCI post-RVRTC. Similarly, 4 infants (31%) showed a significant worsening (increase) in LCI post-RVRTC. After BD treatment, 5 infants (38%) showed no significant change in LCI, 3 (23%) demonstrated an increase in LCI, and 3 (23%) showed a decrease in LCI.

DELIVERABLES:

LCI is able to distinguish between groups of healthy infants and infants with CF, suspected CF or wheeze. Raised volume methodology and BD treatment seems to have a heterogeneous effect on LCI in patients with lung disease but no effect in healthy controls.

RELEVANCE:

Understanding the effects of RVRTC maneuvers and BD on LCI is important to the development of the MBW technique as a tool in infant asthma diagnosis and monitoring.

3B The Effect of Toll-Like Receptor 2 Activation During the Induction of Oral Tolerance in Mice

AllerGen Programme B: Diagnostics and Therapeutics

Kaitlyn R. Carson, Matthew Tunis and Jean S. Marshall
Dalhousie University, Halifax, NS
Supervisor: Jean S. Marshall

OBJECTIVE/PURPOSE:

To determine if TLR-2 activators interfere with the induction of oral tolerance to egg protein in mice.

METHODS:

Oral tolerance was investigated in Balb/C mice by feeding the egg protein ovalbumin (OVA). Two groups of mice were fed OVA *ad libitum* in drinking water for one week while a control group was fed normal water. During the OVA feeding, one group of mice were gavage fed with 1 mg of OVA mixed with 10 µg of the TLR-2 activator Pam₃CSK₄ while the second OVA-fed group were gavage fed with OVA alone. Following the initial week of feeding, all three groups were immunized intraperitoneally (i.p.) with 10 µg OVA-alum to elicit an antibody response. Two weeks later, all mice received an i.p. boost dose of 1 µg OVA. Blood samples were obtained one week after the boost. Anti-OVA IgG1, IgG2a, IgE and IgA antibody levels were measured by enzyme-linked immunosorbent assay (ELISA). A similar protocol was adapted for peanut in order to investigate the outcome of Pam₃CSK₄ activation on the induction of oral tolerance to peanut. *In vitro* experiments have also been initiated to investigate how the activation of innate immune cells with TLR-2 activators alters the antigen response of mouse splenocytes.

FINDINGS:

Oral tolerance was successfully induced following OVA feeding for all antibodies analyzed: IgG1 ($p < 0.05$), IgG2a ($p < 0.01$), IgA ($p < 0.01$) and IgE ($p < 0.001$). Oral administration of Pam₃CSK₄ during oral tolerance induction prevented tolerance in IgG1, IgG2a and IgA antibody subclasses but did not significantly alter the IgE response.

DELIVERABLES:

This research builds a foundation of results that can initiate further research and investigation of contaminants in food products or bacterial infection and their potential outcomes on oral tolerance induction.

RELEVANCE:

Research following these preliminary findings could inform testing guidelines for contaminants in foods and the outcomes on oral tolerance induction. Through both food allergy researchers in AllerGen and links with receptor groups, we will distribute relevant information to fellow researchers. The results of this study may also be of interest to the food industry and regulatory agencies.

4B Evaluation of IL-10 and TGF- β levels in Primary Cultures of Dendritic Cells as Potential Predictors of Atopy

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Anne K. Ellis

OBJECTIVE/PURPOSE:

Maternal atopic status is known to influence later development of allergic disorders in children. It is predicted that dendritic cells (DC) isolated from umbilical cord blood will express different levels of cytokines based on maternal atopy. The cytokines IL-10 and TGF- β are both known to play key roles in the regulation of the immune response and we thus aim to evaluate their use as potential biomarkers and possible predictors for the later development of atopy.

METHODS:

Informed consent was obtained from mother's receiving planned caesarean section. An optional questionnaire, regarding the parental atopic status, was also performed at this time. Umbilical cord blood was collected using a heparin-containing syringe and isolation of mononuclear cells (MNCs) was then completed with the addition of Dextran followed by layering onto an Accuprep® gradient and collection of the resulting buffy coat. MNCs were then frozen in freezing media for temporary storage until stimulations could be performed. After flash thawing, the MNCs underwent negative DC selection using EasySep Human Pan-DC Pre-Enrichment Kit and EasySep magnetic particle selection techniques (Stem Cell Technologies). In our pilot evaluation, 8 cord blood samples were stimulated with Lipopolysaccharide (LPS) or Peptidoglycan (PG) for a period of 6 or 24 hours. Cell supernatants were collected and examined for IL-10 and TGF- β levels *via* ELISA. In a follow-up study a revised protocol was applied. DCs were plated 5 hours prior to stimulation with Control Standard Endotoxin (CSE), PG or plain media. Supernatants were collected 24 hours post-stimulation and ELISA was used to determine the amounts of IL-10 and TGF- β produced.

FINDINGS:

In the pilot study, DCs obtained from the cord blood of atopic mothers produced significantly decreased levels of TGF- β compared to those of non-atopic mothers following stimulation with peptidoglycan and a 6-hour incubation period. The pilot study did not reveal any significant differences in DC IL-10 production upon stimulation. A revised protocol has been implemented for all future DC stimulations.

DELIVERABLES:

Initial findings suggest that the potential of cord blood DCs to regulate immune response is decreased in infants at high-risk for the development of atopy compared to those of low-risk with non-atopic mothers. Additional evaluation as to the reliability of IL-10 and TGF- β as cord blood biomarkers for the prediction of atopy is currently underway.

RELEVANCE:

The discovery of reliable biomarkers in umbilical cord blood as indicators to the later development of atopy could allow for early intervention through appropriate lifestyle changes or therapeutic strategies, with the goal of slowing or preventing development of the atopic march.

5B CD34 in Intracellular Signaling and Mucosal Inflammatory Disease

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Kelly M. McNagny

OBJECTIVE/PURPOSE:

Remarkably little is known about the cellular function of the CD34 antigen, a cell surface sialomucin used extensively as a marker of hematopoietic stem cells (HSCs). We have shown that CD34 is also expressed by mature hematopoietic cells, including mast cells, eosinophils and a subset of dendritic cells (DC), and that CD34 facilitates the trafficking of these cells *in vivo*. Intriguingly, loss of CD34 expression renders mice resistant to asthma, hypersensitivity pneumonitis (HP), ulcerative colitis, salmonella infection and intestinal tumor development. In the present study, we examine the molecular functions of CD34 in mast cell signaling and test the applicability of CD34 as a therapeutic target in asthma.

METHODS:

Using bone marrow mast cells (BMMCs) derived from wild-type and *Cd34*^{-/-} mice, we assessed the role of CD34 in mast cell signaling and effector functions in response to FcεRI cross-linking or stimulation with stem cell factor (SCF), a c-Kit ligand. We used degranulation, cytokine release and *in vitro* migration assays as measures of mast cell functions. To test the effectiveness of CD34 targeting therapeutics for the treatment of asthma, we developed a preclinical animal model. Transgenic mice were generated that lack the mouse *Cd34* gene and instead express, in all the appropriate tissues, the human CD34 gene (*hCD34*^{tg}). We used a standard OVA-induced asthma model to characterize disease severity in the *hCD34*^{tg} strain.

FINDINGS:

We demonstrate that BMMCs derived from *Cd34*^{-/-} mice are hyper-responsive to FcεRI stimulation with enhanced degranulation and cytokine release. Detailed biochemical analysis revealed increased phosphorylation in several key signaling intermediaries downstream of the FcεRI cross-linking, including Syk and the β and γ subunits of the FcεRI receptor complex. In addition, we found impaired migration of *Cd34*^{-/-} BMMCs in response to a stem cell factor (SCF) stimulus. For our *in vivo* experiments, we found that expression of the human CD34 transgene in mCD34-deficient mice is sufficient to restore susceptibility to asthma.

DELIVERABLES:

Our *in vitro* analysis of CD34 function in mast cell activity revealed a novel functional role of CD34 in local inflammatory responses. Our *in vivo* experiments demonstrated that expression of human CD34 serves a similar function to mouse CD34, providing a proof-of-concept model to assess therapeutics targeting human CD34 in *hCD34*^{tg} mice as a humanized mouse model.

RELEVANCE:

Through increased understanding of novel regulatory pathways in mast cell function, we hope to facilitate the development of novel mast cell targeting therapeutics. Our data also suggest CD34 is an appropriate target to treat allergic asthma, and further studies will examine the efficacy of such therapeutics in our preclinical mouse model.

ACKNOWLEDGEMENTS: This work was funded by the CIHR, the AllerGen Network of Centres of Excellence, and the Michael Smith Foundation for Health Research.

6B Intrinsic Phenotypic Differences of the Asthmatic Epithelium and its Inflammatory Responses to RSV and Particulate Matter

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Tony Bai

OBJECTIVE/PURPOSE:

A substantial proportion of the costs associated with asthma are from hospital admissions, due to exacerbations of the disease. Within the airway, the epithelium forms the mucosal immune barrier, which is the first structural cell defense against common environmental insults such as respiratory syncytial virus (RSV) and ambient air pollution which have been shown to exacerbate patient symptoms. We proposed to characterize the phenotype of differentiated airway epithelial cultures derived from asthmatic and non-asthmatic individuals and determine their intrinsic inflammatory responses to RSV infection and ambient particulate matter exposure.

METHODS:

Air-liquid interface (ALI) cultures were generated from asthmatic (n=6) and non-asthmatic (n=6) derived airway epithelial cells. Airway tissue and patient matched ALI cultures were analyzed by immunohistochemistry for Cytokeratin-5, E-cadherin, Ki67, Muc5AC, and for apoptosis using Apotag™. ALI cultures were either infected with RSV (4×10^6 PFU/ml) or particulate matter (EHC-93, 100µg/ml) for 24, 48, and 96 hours and supernatants were analyzed for a panel of pro-inflammatory cytokines using Luminex and ELISA.

FINDINGS:

All epithelial cells were screened for contamination with viral RNA and were found to be negative for prior infections. The airway epithelium of asthmatics *in vivo* and in ALI culture demonstrated a less differentiated epithelium characterized by significantly elevated number of cells expressing the basal cell markers CK-5 and Ki67 but express less adherens junction protein E-cadherin ($p < 0.01$), though trans-epithelial resistance was no different compared to non-asthmatic donor tissues. In response to RSV infection and PM10 exposure, asthmatic ALI cultures released even higher levels of IL-6, IL-8 and GM-CSF compared to non-asthmatic cultures ($P < 0.05$). This enhanced cytokine expression was not due to increased numbers of RSV infected cells or enhanced p-p38 or NF-κB expression.

DELIVERABLES:

This parallel *ex vivo* and *in vitro* study demonstrates that the asthmatic epithelium displays an intrinsic alteration in phenotype and responds with an enhanced inflammatory profile to viral infection and ambient particulate matter.

RELEVANCE:

Our data suggest that the asthmatic epithelium is unable to form an appropriate mucosal immune barrier using the diagnostics markers in this study. The biomarkers identified in this study will help clinical researchers understand mechanisms of epithelial damage with the hope of developing therapeutic strategies to improve the quality of life for asthma sufferers.

7B Comparison of Blood Neutrophils and Mononuclear Cells Steroid Responsiveness

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Jean-Pierre Lavoie

OBJECTIVE/PURPOSE:

Neutrophils commonly infiltrate the airways of patients with COPD and severe asthma, two conditions often poorly responsive to corticosteroids. It is considered that neutrophils are corticosteroid resistant when compared to other blood cell populations. Because objective support for this view is lacking, we compared the genomic and transactivation effects of 3 commonly prescribed corticosteroids in blood neutrophils and mononuclear cells populations.

METHODS:

Neutrophils and mononuclear cells were isolated from the blood of healthy horses using a gradient technique and immunomagnetic separation, respectively. Cells were incubated *ex vivo* 5h with or without lipopolysaccharide (LPS) (100ng/mL) alone or combined with hydrocortisone (10^{-6} M), prednisolone (10^{-6} M) or dexamethasone (10^{-6} M). IL-8, and TNF α (transrepression), and glutamine synthetase (transactivation) mRNA expression was quantified by qRT-PCR.

FINDINGS:

Stimulation with all three corticosteroids similarly increased the mRNA expression of glutamine synthetase in neutrophils and mononuclear cells. They also downregulated the LPS-induced IL-8, and TNF α mRNA expression in both cell populations.

DELIVERABLES:

These preliminary results suggest that corticosteroids exert genomic effects of similar magnitude on equine peripheral blood neutrophils and mononuclear cells. We speculate that the poor response to corticosteroids observed in patients with airway neutrophilia is not explained by an intrinsic resistance of neutrophils to these drugs.

RELEVANCE:

Corticosteroid resistance is an important health concern as it complicates the clinical management of patients that may have compromised airway function. A better understanding of the effects of corticosteroids on immune cells is required in order to develop novel therapeutic strategies.

8B Effect of Thymic Stromal Lymphopoietin (TSLP) on Cord Blood (CB) Progenitor Cell Differentiation and Hemopoietic Cytokine Receptors (HCR) Expression

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Judah A. Denburg

OBJECTIVE/PURPOSE:

TSLP has been described as a “master T_H2 cytokine” with pleiotropic effects including the activation and differentiation of CB CD34⁺ hemopoietic progenitor cells. Previous studies have demonstrated that CB CD34⁺ cells express receptors for TSLP and release proinflammatory T_H2 cytokines and chemokines in a TSLP-dependent manner. It has recently been suggested that TSLP may modulate the function of CD34⁺ cells via the alterations of HCR, which have been shown to be altered in CB CD34⁺ cells of atopic at-risk infants. The aim of the current study was to evaluate the effects of TSLP on the expression of interleukin-3 receptor (IL-3R α), IL-5R α , and granulocyte-macrophage colony-stimulating factor (GMCSFR α) on CD34⁺ cells, as well as to examine the differentiation of CB CD34⁺ cells.

METHODS:

Fresh and frozen CB cells, highly purified for CD34⁺ through a magnetic-activated cell sorting technique, were stimulated for 24 hours with varying doses of TSLP (0.1, 0.5, and 1ng/ml), with and without IL-33 (1 and 10ng/ml), IL-1 (10ng/mL), and TNF (25ng/mL) then stained for surface expression of IL-3R α , IL-5R α and GMCSFR α . Mean numbers of eosinophil/basophil colony-forming cells (Eo/B CFU) was enumerated from methylcellulose cultures of TSLP-stimulated CD34⁺ cells.

FINDINGS:

After overnight stimulation with TSLP, mean expression by CB CD34⁺ cells of IL-5R α , but not IL-3R α or GM-CSFR α , was significantly enhanced by TSLP stimulation at 0.1 ng/mL TSLP (n=8, p=0.04). In addition, TSLP alone and in combination with IL-33 and IL-1 induced Eo/B CFU in methylcellulose cultures.

DELIVERABLES:

TSLP stimulation increases the expression of IL -5R α on CB CD34⁺ cells, and stimulation with TSLP promotes Eo/B differentiation.

RELEVANCE:

These findings may reflect an in utero pathway influencing CB progenitor cell eosinophilic lineage commitment, eventuating in allergic inflammation and disease in early life. This may allow for the assessment of CB CD34⁺ cells from infants to monitor allergic disease development and to identify new therapeutic targets and approaches for allergy and immune diseases, which is in alignment with AllerGen’s Programme B - Diagnostics and Therapeutics, and adds to the Biomarkers thrust within Programme B and across AllerGen’s other Programmes. Finally, this project fits with AllerGen’s strategic goal of enabling knowledge and technology exchange and exploitation (KTEE) as it allows for the collaboration of AllerGen trainees and investigators from different labs. This environment is an ideal catalyst for creativity and innovation.

9B Induction of Thymic Stromal Lymphopoietin (TSLP) in Airway Epithelium by Recombinant Allergens

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Judah A. Denburg

OBJECTIVE/PURPOSE:

TSLP has been described as a “master T_H2 cytokine” with pleiotropic effects including the modulation of dendritic cell (DC) function to promote T_H2 responses. Previous studies have demonstrated that TSLP can be elicited from human airway epithelial cells through a variety of stimuli including cytokines (e.g., IL-4/TNF α) and pathogen-associated molecular patterns (PAMPS) such as viral RNA (PolyI:C as a surrogate). It has recently been suggested that some allergens may modulate the function of airway epithelium. The aim of the current study was to evaluate the ability of two common aeroallergens, Fel d 1 and Phl p 5a, to induce TSLP production in primary bronchial epithelial cells (PBECs) from asthmatics and non-asthmatics grown at air-liquid interface.

METHODS:

We examined the induction of TSLP in PBECs from healthy controls (n=5) and allergic asthmatics (n=5). A novel bio-assay to measure TSLP induction was developed, involving the induction of thymus and activation-regulated chemokine (TARC) production from monocyte-derived dendritic cells (MoDC) in the presence, or absence, of blocking antibodies to TSLP.

FINDINGS:

Purified, low endotoxin recombinant allergens induced TSLP from normal PBECs (3.5-fold; p<0.001) and asthmatic PBECs (3.1 fold; p<0.05). Epithelial cell culture supernatant from asthmatic and non-asthmatic individuals induced TSLP-dependent TARC production in MoDC, which was significantly inhibited by the addition of anti-TSLP antibodies (p=0.02).

DELIVERABLES:

Highly purified recombinant allergens are capable of inducing biologically active TSLP from primary airway epithelium. These findings highlight and further support the role of allergens and airway epithelial cells in triggering the allergic inflammatory cascade by releasing TSLP.

RELEVANCE:

TSLP is an obvious candidate for therapeutic intervention in allergic asthma, and therefore merits investigation. However, even more important is the determination of putative allergen-derived factors which have the ability to induce TSLP through epithelial activation - as it remain possible targets of therapeutic significance (in alignment with AllerGen's Programme B - Diagnostics and Therapeutics).

10B CD34 Localization in Eosinophils at Steady State and During Disease

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Kelly M. McNagny

OBJECTIVE/PURPOSE:

The cell surface sialomucin CD34 has an emerging role in hematopoietic cell migration, notably on mast cells and eosinophils. Our research has demonstrated that CD34 surface expression promotes cell migration and CD34 ablation results in impaired homing and increased cell adhesion. As eosinophils are key inflammatory cells involved in the pathogenesis of allergic asthma and inflammatory bowel diseases (IBD), we assessed the effects of CD34 ablation in these diseases. In mouse models of both allergic asthma and ulcerative colitis, CD34 ablation results in impaired eosinophil migration, resulting in attenuated disease pathology. In our current studies, we aim to characterize CD34 expression patterns in eosinophils at steady state and during disease pathology and assess the role of CD34 in eosinophil migration.

METHODS:

Asthma was induced using a standard OVA induction model, while ulcerative colitis was induced using 3.5% dextran sulfate sodium (DSS). In both disease models, mice were characterized to assess disease severity. Under disease conditions, CD34 expression was assessed using flow cytometry in digested lung and colon samples. At steady state, CD34 expression on the cell surface and in intracellular compartments was assessed on spleen and bone marrow cells isolated from mice with transgene-induced eosinophilia (IL5^{Tg} and IL5^{Tg}Cd34^{-/-} strains).

FINDINGS:

Cd34^{-/-} mice exhibit decreased eosinophil infiltration and are resistant to both OVA-induced allergic asthma and DSS-induced ulcerative colitis. In allergic asthma, lung and alveolar eosinophils express moderate levels of surface CD34 and eosinophils purified from Cd34^{-/-} lung tissues have decreased migration efficiency *in vitro*, compared to wildtype controls. In ulcerative colitis, approximately 40% of colon-infiltrating blood cells are eosinophils, which express high levels of surface CD34. At steady state, eosinophils purified from IL5^{Tg} animals express low levels of surface CD34 within the blood, bone marrow and spleen, but high levels of surface CD34 expression in colon tissues. These findings suggest that surface CD34 is critical for optimal eosinophil migration during disease conditions and contributes to tissue pathology. Intriguingly, permeabilization of spleen and marrow eosinophils reveals high levels of intracellular CD34 expression. We propose that intracellular CD34 is rapidly exposed on the cell surface following activation, and may be a novel mechanism regulating eosinophil migration.

DELIVERABLES:

Our findings suggest key roles for CD34 in eosinophil migration during disease conditions, and suggest CD34 as a novel therapeutic target in disease. Further, our recognition of intracellular CD34 expression at steady state suggests novel mechanisms involved in eosinophil migration.

RELEVANCE:

Further characterization of factors regulating CD34 surface expression will lead to an improved basic understanding of eosinophil biology and reveal new targets to modulate eosinophil responses in disease conditions.

ACKNOWLEDGEMENTS: The research was funded by AllerGen NCE (Grant 3.14). SM received funding from the CIHR/HSF Transfusion Science Fellowships from the Centre for Blood Research (CBR) at UBC and MG received funding from the CIHR/MSFHR Transplantation Training Program. KMM is a MSFHR Scholar (Senior) and CBR Member.

11B Live RSV Fails to Activate Human TLR3, TLR4 and TLR7 in Transfected HEK 293 Cell Lines

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Stuart E. Turvey

OBJECTIVE/PURPOSE:

We focus on the role of innate immune responses to respiratory syncytial virus (RSV), the most important etiological agent of paediatric viral respiratory infection¹. There is a lack of understanding how the innate immune system—the principal immune defence mechanisms by which infants recover from viral infection²—can ‘sense’ RSV. Thus, our objective is to systematically test which innate immune receptors become activated upon infection with RSV.

METHODS:

We used transfected HEK 293 cell lines expressing selected Toll-like receptors (TLRs) and a NF-κB-inducible reporter (secreted embryonic alkaline phosphatase), and measured reporter activation upon infection with live RSV and purified PRR ligands (controls). In addition, parental cells that can express the reporter gene but lack the PRR gene in question were used to control for endogenous responses.

FINDINGS:

We demonstrate that live RSV infects HEK 293 cells but fails to activate hTLR3, hTLR4 and hTLR7. Moreover, live RSV is unable to interfere with TLR3, -4 and -7 activation in HEK293 cells mediated by their specific, purified agonists poly(I:C), *E. coli* LPS, and R848, respectively. This suggests that live RSV does not engage with these pattern recognition receptors upon infection of HEK 293 cells.

DELIVERABLES:

The present study demonstrates that live RSV can escape recognition by TLRs. In agreement with our findings, poor TLR stimulation has been suspected to be responsible for lack of antibody affinity maturation upon RSV infection and enhanced RSV disease³.

RELEVANCE:

RSV is the most important etiological agent of paediatric viral respiratory infection¹. In addition to being a significant cause for childhood morbidity and mortality^{4,5}, RSV bronchiolitis and pneumonia early in life is associated with increased risk for asthma later in life⁶. Later in childhood, RSV infection is associated with the acute exacerbation of asthma⁶. Currently available options for prophylaxis or treatment of RSV infections have considerable limitations (high costs and limited efficacy, respectively), and there is no RSV vaccine⁷. We anticipate that a clearer understanding how RSV is ‘sensed’ in a naïve host (e.g., infant) will (i) help identify novel therapeutic targets for RSV disease and asthma; and (ii) guide vaccinologists in choosing an appropriate adjuvant for the development of safe and protective RSV vaccines.

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12B Activated Mast Cells Inhibit Tumor Growth

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Jean S. Marshall

OBJECTIVE/PURPOSE:

Allergic disease affects up to 20% of the North American population and the majority of individuals seriously affected seek symptomatic relief through the use of medications such as anti-histamines and leukotriene receptor antagonists. Epidemiological evidence suggests that individuals with allergic disease have decreased incidence of certain cancer subtypes, including breast carcinoma. A hallmark of allergic disease is IgE-mediated mast cell activation. Mast cells are abundant at the periphery of breast carcinomas and a positive correlation has been noted between increased mast cell numbers and long term survival in invasive disease. We hypothesized that mast cell activation at tumor sites could inhibit tumor growth.

METHODS:

Effects of mast cell activation *via* innate pattern recognition receptors on tumor growth were investigated using an established *in vivo* B16.F10 murine melanoma tumor model system and complimentary Matrigel-tumor model systems of B16.F10 and Lewis lung carcinoma, LLC1. *In vitro* protein array and enzyme-linked immunosorbent assays were used to identify candidate mast cell secreted mediators. Mast cell dependence was assessed *via in vivo* reconstitution of *Kit*^{W-sh/W-sh} mice with bone marrow derived mast cells (BMMC) generated from wild type and gene deficient mice. Effects of mast cell activation on immune cell recruitment and tumor angiogenesis were determined by flow cytometry and histology, respectively.

FINDING:

We have demonstrated that innate activation of mast cells *via* TLR2 ligands inhibits the growth of melanoma and lung carcinoma *in vivo*. *In vivo* reconstitution studies of mast cell deficient *Kit*^{W-sh/W-sh} mice demonstrated melanoma growth inhibition occurred in an interleukin-6 dependent manner and was associated with decreased angiogenesis and increased T cell and natural killer cell recruitment. *In vitro* assays have demonstrated IgE-activated mast cells secrete multiple mediators, including large amounts of IL-6. Ongoing studies are investigating the effects of IgE-mediated allergic mast cell activation *in vivo* on breast carcinoma growth using a xenograft model of human breast carcinoma and an immunocompetent syngeneic murine breast carcinoma model. The role of specific mast cell mediators will be assessed using pharmacological inhibitors.

DELIVERABLES:

This study will utilize experimental systems to investigate a causal association between allergic disease and decreased cancer development and presents a strategic initiative linking allergy research with the cancer field. This research will provide information as to the potential benefit or adverse effects in breast carcinoma of drug classes commonly used for allergy treatment.

RELEVANCE:

The aim of this study is to identify potential mechanisms of reduced cancer risk in allergic individuals and thereby may lead to novel opportunities for the development of anti-breast cancer treatments that exploit allergic mast cell activation and/or specific allergic mediator/receptor pathways. The outcome of this study may contribute to the development of guidelines for the use of common allergy medications in those at high risk for breast cancer or undergoing breast cancer treatment. Through the use of the AllerGen Network and scientific publications, this research can be effectively disseminated and will be of great interest to pharmaceutical companies as well as to clinicians and patient advocacy groups.

13B The Hypothalamic-Pituitary-Adrenal Axis in Subjects with Mild Allergic Asthma

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisor: Gail M. Gauvreau

OBJECTIVE/PURPOSE:

Cortisol is a hormone which regulates the stress system; during stressful events, the HPA axis is activated to secrete an abundance of cortisol. Regulation of inflammation is also associated with cortisol; if there is a defect in the HPA axis response, there is increased likelihood of inflammatory diseases. Individuals with mild allergic asthma have the potential to experience severe symptoms following exposure to a sensitizing antigen, but it is unknown whether this is associated with dysregulation of HPA axis.

Study Objectives: Individuals with mild allergic asthma only require intermittent bronchodilators for treatment of asthma, and under basal conditions they can only be distinguished from non-asthmatics using bronchoprovocation. We hypothesized that dysregulated HPA axis is associated with the development of allergen-induced late asthmatic responses in subjects with mild allergic asthma.

METHODS:

Salivary samples were collected from mild allergic asthmatics (n=10) and normal, age and gender-matched non-allergic controls (n=10) immediately after awakening. Saliva was stored frozen, and assayed for cortisol using a commercial ELISA kit. Subjects with mild allergic asthma underwent allergen challenges, and all developed early and late phase asthmatic responses. The cortisol levels were compared using Mann-Whitney and Wilcoxon statistical tests.

RESULTS:

Mild allergic asthmatics had a significantly different cortisol level of 7.5 nmol/L (standard deviation 6.2 nmol/L) compared to normal controls with baseline cortisol level of 14.6 nmol/L (standard deviation 5.4 nmol/L ($p < 0.05$)). These data demonstrate that cortisol levels are lower in mild allergic asthmatics compared to normals.

CONCLUSIONS:

It was determined that baseline cortisol levels are indeed attenuated in mild allergic asthmatics as compared to normal individuals. The HPA axis is known to play a role in regulating the expression of cytokine genes in asthmatic patients, including those mediating inflammation in the airways of asthmatic subjects (IL-1, IL-6 and TNF α). Furthermore, 11 β -hydroxysteroid dehydrogenase is an enzyme that is directly responsible for decreases in cortisol levels. Thus, if allergic asthmatics truly have diminished HPA axis activity and decreased cortisol levels, 11 β -hydroxysteroid dehydrogenase, and other like-genes, will be up-regulated. This could explain, in part, how lower cortisol levels may impart risk of developing allergen-induced late phase responses in subjects with mild allergic asthma.

14B Mechanical Properties of Asthmatic Airway Smooth Muscle

AllerGen Programme B: Diagnostics and Therapeutics

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Supervisors: Peter D. Paré and Chun Y. Seow

OBJECTIVE/PURPOSE:

Airway hyperresponsiveness is a hallmark of asthma and is characterized by exaggerated airway narrowing caused by airway smooth muscle (ASM) shortening. It is largely unknown if the changes in ASM function in asthma are related to alterations in ASM mass, its force generation, ability to and velocity to shorten or length adaptation

METHODS:

ASM from human trachealis (asthmatic 8 and non-asthmatic 5) was measured when relaxed *In situ* in the tracheal ring to determine its reference length (L_{ref}). ASM strips were then mounted in a myograph capable of measuring force (F_{max}) induced by electric field stimulation (EFS) and length simultaneously. L_{ref} was used to normalize all force and length measurements, which included EFS-induced stress, the length-force relationship, the load-velocity relationship and maximal ASM shortening. Measurements of force were converted to stress by dividing by the cross-sectional area of each ASM strip. The same donors were used to determine the areas of smooth muscle, adventitia, lamina propria and total wall of intraparenchymal airways using morphometric analysis.

FINDINGS:

There were no significant differences in the EFS-induced stress, the length-force relationship, the load-velocity relationship or maximal ASM shortening between asthmatic and non-asthmatic subjects. The asthmatic ASM strips were stiffer at longer lengths and oscillatory strain reduced the isometric force in response to subsequent EFS by 19% and 36% in asthmatic and non-asthmatic subjects respectively ($p < 0.0001$). Normalized for basement membrane perimeter, the intraparenchymal airways of asthmatic donors had significantly increased areas of smooth muscle, adventitia, lamina propria, and total wall area ($p < 0.05$), compared to non-asthmatic donors.

DELIVERABLES:

The mechanical properties of human ASM from asthmatic and non-asthmatic subjects are comparable except for increased passive stiffness and attenuated decline in force generation induced by an oscillatory perturbation.

RELEVANCE:

Our confirmation that airway smooth muscle is increased in asthmatic lungs (hyperplasia or hypertrophy) aids in the pathological characterization of asthma. The novel discoveries that there is a difference in passive stiffness between asthmatic subjects and non-asthmatic subjects and that oscillatory perturbations, mimicking deep inspirations *in vivo*, result in less force reduction in asthmatic airway smooth muscle suggest a new target for treatment of airway narrowing.

15B Repeated Allergen Challenges Do Not Induce Tolerance in Asthmatic Dual Responders

AllerGen Programme B: Diagnostics and Therapeutics

Musherif Raja, Alyssa Schermel, Rick Watson, Kieran Killian, Gail M. Gauvreau
McMaster University, Hamilton, ON
Supervisor: Gail M. Gauvreau

OBJECTIVE/PURPOSE:

The overall objective of this study was to determine whether repeated exposure of antigen alters the magnitude of asthmatic responses over time by inducing immune tolerance. This is particularly important when the allergen challenge model is used in the determination of drug efficacy. By comparing changes in LAR and post-allergen methacholine PC₂₀, we 1) determined whether repeated allergen challenge alters allergen-induced responses by over short-term (<1 month) and long-term (>3 years) re-exposure; and 2) determined whether the allergen-induced response is dependent on the type of allergen.

METHODS:

Post-hoc analysis was performed on 339 allergen challenges carried out in 61 asthmatic subjects (dual responders) over the course of 70 months. The information extracted for comparison from each subject included the number of allergen inhalation challenges, the early and late maximum percent fall in FEV₁ (EAR and LAR), the type of allergen extract inhaled, and the methacholine PC₂₀ before and 24 hours after allergen challenge.

FINDINGS:

The magnitude of LAR decreased from test 1 to test 2, but this was not statistically significant. When allergen challenge #1 was compared to #2, there was no difference in the LAR:EAR ratios or methacholine PC₂₀ measured at 24 hours post allergen challenge (p>0.05). Furthermore, the asthmatic responses did not change with >2 allergen challenges.

DELIVERABLES:

The results from this study demonstrate that neither short term nor long term repeated allergen challenges induce immune tolerance, and this is independent of allergen extract. Any attenuation of late asthmatic responses is likely the result of attenuated EAR and lower doses of allergen.

RELEVANCE:

The allergen inhalation challenge is a robust model to test efficacy of drugs for treatment of asthma.

16B Effect of Maternal Allergic Sensitization and Smoking During Pregnancy on Neonatal Eosinophil-Basophil Lineage Commitment

AllerGen Programme B: Diagnostics and Therapeutics

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Departments of Allergy & Clinical Immunology and Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, ON, and School of Paediatrics and Child Health, University of Western Australia

Supervisor: Judah A. Denburg

OBJECTIVE/PURPOSE:

We have previously shown that intrauterine environmental exposures (e.g., microbial) can influence eosinophil-basophil (Eo/B) lineage commitment in infants at risk for atopy. However, it is unknown how maternal smoking affects Eo/B lineage commitment in neonates at risk for allergy. Since cigarette smoke may modulate eosinophilopoiesis, we investigated the effects of maternal smoking during pregnancy on Eo/B lineage priming.

METHODS:

Thirty-nine (19 low- and 20 high-atopic risk) *in utero* cigarette smoke exposed infant CB samples, were assessed for phenotypic alterations flow cytometry. Functional alterations were assessed by methylcellulose cultures for hematopoietic cytokine-stimulated eosinophil-basophil (Eo/B) colony forming units (CFU), with or without lipopolysacchride (LPS).

FINDINGS:

Smoke exposed high-atopic risk infants had significantly higher CB CD34⁺ cell GM-CSFR α and IL-5R α expression (P = .021). Hematopoietic cytokine costimulation with LPS induced Eo/B CFUs from both low- and high-risk infant CB CD34⁺ cells; however, this response was significantly (P = .047) increased in the high-risk CB progenitors.

DELIVERABLES:

Cigarette smoke during pregnancy alters high- atopic risk neonatal CB CD34⁺ cell phenotype and functional responsiveness. Maternal sensitization may synergize with smoking to upregulate CB progenitor cell programming and Eo/B production, resulting in allergic inflammation and disease in early life. I will be presenting this work at the American Academy of Allergy, Asthma and Immunology (AAAAI) in San Francisco, CA (March 2011).

RELEVANCE:

This work provides evidence of early maternal (genetic and environmental) influences on cord blood hemopoietic progenitor cell eosinophil- basophil lineage commitment. This may not only provide key etiological and potential therapeutic targets in the management of allergic disease in early childhood, but also demonstrates the effects of *in utero* smoke exposure on the fetus which should promote avoidance of maternal smoking during pregnancy.

17B The Immune-Prophylactic Vaccine *Lm* $\Delta(trpSactA)/pSPO-PS_{hly}OVA$ Protects Neonates from Asthma

AllerGen Programme B: Diagnostics and Therapeutics

Charis-P. Segeritz, Daniela M. Loeffler, Bing Cai, Sofia Peterson, Matthew Gold, Kathleen Wee, Kinga K. Smolen, Marie-Renée Blanchet, Kelly McNagny, Tobias R. Kollmann
Child and Family Research Institute and University of British Columbia, Vancouver, BC
Supervisor: Tobias R. Kollmann

OBJECTIVE/PURPOSE:

To determine if our prophylactic *Listeria monocytogenes* (*Lm*) based, neonatally-administered vaccine strain expressing the model allergen ovalbumin (OVA) induces a strong Th1 response, preventing asthma upon future allergen challenge.

METHODS:

Neonatal and adult mice were immunized intraperitoneally (*ip*) with the attenuated *Lm* vaccine strain *Lm* $\Delta(trpS actA)/pSPO-PS_{hly}OVA$ (*Lm*-OVA) synthesizing OVA proteins, heat-killed attenuated *Lm*-OVA, the attenuated *Lm* strain *Lm* $\Delta(trpS actA)/pSPO$ and NaCl (negative control). Six weeks after immunization with the *Lm* strains, mice were sensitized *ip* with OVA absorbed onto alum hydroxide gel. The NaCl-immunized control group received alum hydroxide gel only. Anesthetised mice were then challenged intranasally with OVA in PBS. Subsequent analysis included examination of the bronchoalveolar lavage fluids (BALF) to determine the total number of live cells and quantify the different cell types. Supernatants of BALF were harvested to determine cytokines *via* ELISA or Luminex. In addition, histological analysis of lung tissue itself was conducted to evaluate airway inflammation and pathological changes within the tissue. Goblet cells in lung tissue were stained with Alcian blue-periodic acid Schiff to test for goblet cell hyperplasia. Spleen and lung tissue was restimulated with OVA and heat-killed *Lm* in order to quantify IL-4, IL-5, IL-13, IL-17, IL-10, TGF-beta and IFN- γ cytokine production by Luminex. The concentration of IgG1, IgG2a and IgE in serum was monitored *via* ELISA analysis. Mice were also examined for airway resistance after challenge, as measured by the FlexiVent approach.

FINDINGS:

Neonatal mice immunized with the live-attenuated vaccine strain *Lm*OVA exhibited a significant lower total cell count and lower eosinophil levels in BALF compared to the asthma group. Similarly, histology analysis of the *Lm*OVA group showed significant less cell infiltration of the airway epithelium and goblet cell metaplasia. FlexiVent measurements echoed those findings and showed lower airway resistance for mice immunized neonatally with *Lm*OVA. Interestingly, all of the above mentioned trends also rang true for the *Lm*-immunized group, but did not hold up for mice immunized with heat-killed *Lm*OVA. After investigation of the various cytokine levels in BALF (*in-vivo*), spleen and lung (*ex-vivo*), as well as examination of serum antibodies, the observed protection in both, the *Lm*OVA and *Lm* group did not appear to be mediated by shifts in Th1/Th2. Mice that followed this experimental schedule as adults showed similar trends, however, protection was seen primarily in response to immunization with *Lm*-OVA, not to *Lm*.

DELIVERABLES:

- Protection from asthma is dependent on live, replicating *Lm* (no protection with heat-killed *Lm*).
- Protection from asthma can be achieved through an antigen non-specific mechanism.
- Different cytokine profiles found in *Lm*OVA- and *Lm*-immunized mice suggest that protection is brought about by two distinct pathways and may be organ-dependent.
- Immunization early in life represents a crucial immunological window in time.

RELEVANCE:

Our successful prophylactic *Lm*-based one-shot vaccination is now also being tested for its potential use as a therapeutic vaccine against allergies and cancer. In our efforts to thereby reduce morbidity and mortality caused by allergies, our lab has cloned other allergens into our attenuated *Lm* vaccine strain, especially food allergens such as peanut proteins, which are currently tested in animal models.

18B Mast Cells are Not Required for the Induction of Oral Tolerance in Mice

AllerGen Programme B: Diagnostics and Therapeutics

Matthew Tunis and Jean S. Marshall
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Supervisor: Jean S. Marshall

OBJECTIVE/PURPOSE:

Mast cells are well known mediators of allergic disease, but they have also been identified as important in maintaining peripheral tolerance in transplant models. It remains unclear what role mast cells might have in the induction of oral tolerance to foods. The objective of this research was to investigate whether mast cells are critical for oral tolerance induction.

METHODS:

C57Bl/6 mice were compared to Kit^{W-sh/W-sh} (mast cell-deficient) mice. One group from each strain was fed egg protein (OVA) in water *ad libitum* for one week while control mice were fed normal water. Two days after feeding, all mice were immunized intraperitoneally (i.p.) with 50µg of OVA-alum and boosted with 10µg OVA two weeks later. One week after the boost, blood samples were obtained from all mice. Anti-OVA IgE, IgG1, and IgG2a were measured by enzyme-linked immunosorbant assay (ELISA). In order to assess the physiological relevance of oral tolerance in mast cell-containing mice, animals were fed and immunized as described then challenged i.p. with 10mg of OVA. Anaphylaxis was measured by core temperature drop 50 minutes after OVA challenge. FcγRIII^{-/-} mice were used to assess the contribution mast cell activation by IgG in C57Bl/6 mice.

FINDINGS:

It was observed that mast cells are not necessary for oral tolerance induction. Feeding OVA to the Kit^{W-sh/W-sh} mast cell-deficient mice significantly reduced levels of anti-OVA IgE ($p < 0.001$), IgG1 ($p < 0.01$), and IgG2a ($p < 0.01$). Surprisingly, anti-OVA IgE levels were in fact significantly lower in the Kit^{W-sh/W-sh} mice than in C57Bl/6 mice ($p < 0.05$). Upon i.p. challenge with OVA, OVA-fed FcγRIII^{-/-} mice experienced significantly less temperature loss from anaphylaxis than sensitized FcγRIII^{-/-} mice ($p < 0.001$). This suggests that the oral tolerance model employed in these studies has relevant outcomes in terms of food challenge response.

DELIVERABLES:

Increased understanding of the role of mast cells in regulating allergic sensitization and tolerance can inform clinical and epidemiological studies investigating the outcomes of mast cell stabilization or activation. This work may also inform policy decisions regarding the potential benefits or risks associated with treatments that alter mast cell function.

RELEVANCE:

Current therapies for allergic disease include the use of drugs that alter mast cell numbers and function. This research helps define the role of mast cells in oral tolerance induction and suggests that altering mast cells has potential to modify oral tolerance induction with impact on development of new food allergies among Canadians. Initial dissemination of the study information is aimed at other allergy researchers and clinical groups who can evaluate the results of these experiments in the context of allergy patients.

19B LTB₄ Release from Neutrophils in Allergic and Non-Allergic Subjects

AllerGen Programme B: Diagnostics and Therapeutics

Brittany Watson, Karen Howie, Rick Watson, George Obminski, Heather Campbell, Gail M. Gauvreau
McMaster University, Hamilton, ON
Supervisor: Gail M. Gauvreau

OBJECTIVE/PURPOSE:

Neutrophils play an important role in the pathophysiology of asthma through release mediators capable of producing chronic inflammation and tissue damage. Leukotriene B₄ (LTB₄) is a pro-inflammatory lipid mediator released from neutrophils following activation by various stimuli. The sensitivity of neutrophils to physiological stimuli and subsequent release of LTB₄ in allergic versus non-allergic subjects has not been well described. The objective of these experiments is to examine the effects of physiological stimuli (GM-CSF, TNF- α , and fMLP) and a non-physiological stimulus (calcium ionophore, A23187) on LTB₄ release from neutrophils stimulated in vitro, and to compare release of LTB₄ by neutrophils of non-allergic subjects versus allergic subjects.

METHODS:

Blood was collected from 8 male and female subjects (4 non-allergic normal, 4 allergic donors) aged 25 to 40 years old. 470 μ L of whole blood and adenosine deaminase (0.01 U/ml) was added to 5 tubes. Tube 1 was stimulated with PBS for 35 minutes (baseline). Tube 2, was stimulated with 1 nM GM-CSF + 1.5 nM TNF α + 10 μ M cytochalasin B for 30 minutes, then 300 nM fMLP was added for an additional 5 minutes (physiological stimuli). Tube 3 was stimulated for 5 minutes with 10 μ M of A23187 (non-physiological stimulus). Tubes 4 and 5 were additional controls for tubes 2 and 3, respectively. After the desired incubation period, 500 μ L of cold HBSS was added and tubes were microcentrifuged at 4° C for 7 minutes. Plasma was removed and stored at -70° C. LTB₄ was measured using a commercial ELISA (R&D Systems) and the absorbance was read at 450 nm with an ELISA plate reader. The data were log transformed and expressed as LTB₄ (picograms/10⁶ neutrophils).

FINDINGS:

Following stimulation of neutrophils with physiological stimuli, blood from allergic subjects had 2-fold higher LTB₄ levels compared to blood from the non-allergic normal subjects. LTB₄ release from neutrophils stimulated with A23187 was also 2-fold higher in allergic subjects compared to non-allergic normal subjects.

DELIVERABLES:

We have evidence showing that neutrophil LTB₄ release is greater in allergic versus non-allergic normal subjects, regardless of the stimulus. This suggests that neutrophils in allergic individuals have been primed to respond. A larger sample size is necessary to confirm these findings.

RELEVANCE:

These findings improve our understanding of immune cell responses in allergic and healthy individuals and the behaviour of neutrophils in terms of their activation and mediator release during allergic responses.

20B IL-13R α 1 and IL-13R α 2 Interaction Regulates Airway Epithelial Repair Pathways

AllerGen Programme B: Diagnostics and Therapeutics

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¹UBC James Hogg Research Centre – Heart & Lung Institute, University of British Columbia,
Vancouver, BC

Supervisor: Delbert R. Dorscheid

OBJECTIVE/PURPOSE:

Elevated levels of interleukin (IL)-13 and persistent airway epithelial damage are two characteristics observed in asthma. Although IL-13 is known to be a key cytokine in mediating inflammatory and remodelling responses, however our lab has reported that IL-13 is critical to normal airway epithelial repair *via* the release of HB-EGF and activation of EGF-R. The effects of IL-13 are mediated through two receptors, the complex of IL-13 receptor α 1 (IL-13R α 1)/ IL-4 receptor (IL-4R α) and IL-13 receptor α 2 (IL-13R α 2). In the current investigation we studied the roles of these receptors in normal airway epithelial repair, particularly focusing on their expression, distribution and interaction in response to injury signals.

METHODS:

Confluent monolayers of Human Airway Epithelial (1HAEO-) cells were subjected to either mechanical injury or IL-13 stimulation followed by supernatant and protein lysate collection. Caveolae/cholesterol-rich lipid rafts were isolated using sucrose gradient fractionation 24 hours post-treatment. IL-13R α 1 and IL-13R α 2 expression levels were examined *via* Western blot, while IL-13R α 2 downstream molecules including HB-EGF and TGF- β were detected by ELISA. Cellular distributions of IL-13R α 1 and IL-13R α 2 were examined using immunofluorescence. In parallel receptor functions were disrupted in monolayers using specific IL-13R α 1 and IL-13R α 2 neutralizing antibodies followed by either IL-13 stimulation or wounding. Downstream effector molecules of IL-13 receptors were detected *via* Western blot or ELISA.

FINDINGS:

Both IL-13 stimulation and mechanical injury resulted in upregulation of IL-13R α 2 expression, while induction of IL-13R α 1 expression was unchanged. Isolated fractions demonstrated that IL-13R α 1 localized in caveolae/cholesterol-rich lipid rafts at baseline and increased in expression within the lipid raft fractions in response to injury signals. IL-13R α 2 expression appeared to be tightly regulated and was only significantly expressed in lipid rafts upon stimulation. Immunofluorescence staining of IL-13R α 1 and IL-13R α 2 showed that the two receptors were distributed in different cellular compartments. IL-13R α 2 molecules were distinctly localized in cytoplasmic granules, while IL-13R α 1 molecules were diffusely distributed throughout the cytoplasm and cell membrane. The regulation of receptor expression and distribution is tightly related to the functions of IL-13 receptors. When IL-13R α 1 function was disrupted, downstream molecules of IL-13R α 2 such as HB-EGF and TGF- β were significantly upregulated in response to IL-13 stimulation and mechanical injury. This suggested that IL-13 receptors interacted to regulate each other.

DELIVERABLES:

Our data indicate that there are interactions between IL-13R α 1 and IL-13R α 2 that functionally regulate their downstream signaling pathways.

RELEVANCE:

An imbalance of this tightly regulated relationship may contribute to the dysfunctional repair phenotype observed in the epithelia of asthmatic patients.

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

#	AllerGen Trainee	Institution	AllerGen Researcher(s)/ Supervisor(s)	Abstract Title
1C	Ladouceur, Erika Anderson, Sharon	University of Victoria and University of Alberta	Dr. Miriam Stewart	"There's more people like this, so you shouldn't feel bad about yourself": Internet peer support for youth with asthma and severe allergies
2C	Ladouceur, Erika Anderson, Sharon McNiel, Debra	University of Victoria and University of Alberta	Dr. Miriam Stewart	Online support for children with asthma and allergies: "I'm not the only one left out, so I feel much better in the group."
3C	Bahreinian, Salma	Department of Pediatrics and Department of Public Health Sciences, University of Alberta	Dr. Anita L. Kozyrskyj Dr. Geoff D.C. Ball	Comorbidity with depression and overweight in children with asthma
4C	Bedouch, Pierrick	Collaboration for Outcomes Research and Evaluation (CORE), Faculty of Pharmaceutical Sciences, University of British Columbia	Dr. Larry D. Lynd	Calculating health care costs associated with asthma in British Columbia, 2002-2007
5C	Chow, Bonnie Y.L. Harrington, Daniel W. Soller, Lianne	School of Geography and Earth Sciences, McMaster University, and Divisions of Clinical Immunology/Allergy and Clinical Epidemiology in the Department of Medicine, McGill University Health Centre	Dr. Susan J. Elliott	A danger foreseen is half-avoided: Understanding the effects of food allergen labels in Canada
6C	Harrington, Daniel W.	School of Geography and Earth Sciences, McMaster University	Dr. Susan J. Elliott	Who says what? The importance of issue framing and claims-making in the construction of food allergies
7C	Houlbrook, Doug	Manitoba Institute of Child Health & Department of Pediatrics and Child Health, University of Manitoba	Dr. Allan B. Becker	Association of parental FEF25-75% during a methacholine challenge and children's diagnosis of asthma and airway hyper responsiveness
8C	Knoll, Megan Soller, Lianne	Divisions of Clinical Immunology/Allergy and Clinical Epidemiology in the Department of Medicine, McGill University Health Centre	Dr. Ann Clarke	The use of unconditional incentives in vulnerable populations for a telephone survey
9C	Luo, Jing C. Chooniedass, Rishma	Manitoba Institute of Child Health & Department of Pediatrics and Child Health, University of Manitoba	Dr. Allan B. Becker	A comparison of health outcomes and proximity to hydro substations in participants of the SAGE study
10C	Mykhaylova, Natalia	Southern Ontario Centre for Atmospheric Aerosol Research (SOCAAR) and Department of Chemical Engineering and Applied Chemistry, University of Toronto	Dr. Greg Evans	Design and testing of a portable, high time resolution system for monitoring air pollutant mixtures associated with allergy onset and development

#	AllerGen Trainee	Institution	AllerGen Researcher(s)/ Supervisor(s)	Abstract Title
11C	Peer, Miki	Institute of Medical Science, University of Toronto; St. Joseph's Healthcare Hamilton; Departments of Psychiatry & Behavioural Neurosciences and Obstetrics & Gynecology, and Department of Medicine, McMaster University	Dr. Claudio N. Soares	Depression and stress are associated with blunted cortisol activity in pregnant immigrant women
12C	Protudjer, Jennifer L.P.	Manitoba Institute of Child Health & Department of Pediatrics and Child Health, University of Manitoba	Dr. Allan B. Becker	Asthma is a low priority amidst layers of complexity faced by adolescents
13C	Simons, Elinor	Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, and Department of Health Policy, Management and Evaluation, University of Toronto	Dr. Teresa To Dr. Sharon D. Dell	How does the timing of home environmental tobacco smoke exposure during childhood affect the age of asthma development?
14C	Soller, Lianne Knoll, Megan Harrington, Daniel W.	Divisions of Clinical Immunology/Allergy and Clinical Epidemiology in the Department of Medicine, McGill University Health Centre	Dr. Ann Clarke	Prevalence of food allergy in Canada: Preliminary results from the SPACE study
15C	Tremblay, Caroline	Hôpital du Sacré-Coeur de Montréal	Dr. Catherine Lemière	Characteristics of subjects with Work-Related Asthma in Québec and Ontario

1C “There’s more people like this, so you shouldn’t feel bad about yourself”: Internet Peer Support for youth with Asthma and Severe Allergies

AllerGen Programme C: Public Health, Ethics, Policy and Society

Erika Ladouceur, University of Victoria, Victoria, BC; Sharon Anderson, University of Alberta, Edmonton, AB

Research Team: Miriam Stewart, University of Alberta, Edmonton, AB; Jeff Masuda, University of British Columbia, Vancouver, BC; Nicole Letourneau, University of New Brunswick, Fredericton, NB; Shawna McGhan, Alberta Asthma Centre, Edmonton, AB; Susan Watt, University of Toronto, Toronto, ON; Lisa Cicutto, University of Toronto, Toronto, ON
Supervisor: Miriam Stewart

OBJECTIVE/PURPOSE:

Youth with asthma and allergies experience peer pressure that results in risks such as non-adherence to health regimens. Their unique support needs have been neglected. Adolescents place high value on the support of peers who share similar chronic conditions. Youth have flocked to online social networking sites for peer support, but there are few evaluations of the value of Internet mediated support for youth. This study was undertaken to determine appropriate components and contents of an online peer support intervention for young adolescents and to evaluate intervention processes, perceived benefits and satisfaction with the intervention.

METHODS:

Three months of support were provided to youth through online synchronous chat. Support group sessions were facilitated by trained peer mentors and health professionals. In depth qualitative individual interviews and quantitative measures were used to understand the effective ingredients and impact on youth.

FINDINGS:

For a core group of youth, the online support group quickly became a safe place to share experiences about asthma and allergies. All youth reported being satisfied with the mentorship they received during the intervention. Additionally, mentors discussed feeling affirmed by the positive impacts they perceived they were having on youth. Online peer support can reach all youth with a computer and internet connection. Youth said they enjoyed the program overall but that a series of technological glitches within Ability Online was a nearly fatal barrier to the success of the intervention. Online social networking can be an important positive influence in supporting youth with asthma and severe allergies coping with challenges they face living with asthma and allergies.

DELIVERABLES:

This study is the first of its kind in its participatory approach to developing and evaluating an online peer mentorship program for youth with asthma and allergies. Peers delivered emotional and informational support that enabled participants to interpret information in ways that were relevant and responsive to their specific needs in their everyday lives.

RELEVANCE:

This intervention proved adept in engaging a notoriously difficult age group to reach. Rural youth perceived this provided them with support readily available to urban youth. The group continues to meet periodically online and has developed some outstanding future leaders who continue to mobilize knowledge. Youth believed this would be a valuable addition to conventional informational websites.

2C Online Support for Children with Asthma and Allergies: “I’m not the only one left out, so I feel much better in the group.”

AllerGen Programme C: Public Health, Ethics, Policy and Society

Erika Ladouceur, University of Victoria, Victoria, BC; Sharon Anderson and Debra McNiel, University of Alberta, Edmonton, AB

AllerGen Investigators: Miriam Stewart, University of Alberta, Edmonton, AB; Lisa Cicutto, University of Toronto, Toronto, ON; Jeff Masuda, University of British Columbia, Vancouver, BC; Nicole Letourneau, University of New Brunswick, Fredericton, NB; Shawna McGhan, Alberta Asthma Centre, Edmonton, AB; Susan Watt, University of Toronto, Toronto, ON; Carina Majaesic, University of Alberta, Edmonton, AB
Supervisor: Miriam Stewart

OBJECTIVE/PURPOSE:

Asthma is the most common chronic illness of childhood and the leading cause of hospitalization in young children. Increasingly psychosocial factors are implicated in poor asthma control, hospitalizations for severe allergies, and reduced quality of life. Recent studies reveal social isolation, gaps in social support, and limited support in the community for children with asthma and allergies and their parents. Children often hide their condition from peers, refuse to take medication in public, and are reluctant to seek assistance in trigger avoidance or managing symptoms because they lack social support. Although psychosocial interventions for children with asthma and parents exist, our review reveals that reported interventions emphasize education and information, not support; do not include support from peers; and are typically hospital/clinic-based and face-to-face rather than community-based involving innovative technology. The objectives of this study were to determine appropriate components and contents of an online peer support intervention for children (aged 7 to 12) with asthma and/or severe allergies and to evaluate intervention processes, perceived benefits and satisfaction with the intervention.

METHODS:

A synchronous weekly online support group was offered for eight weeks for 27 children (aged 7 to 12) with asthma and allergies. The intervention was hosted on an existing secure online meeting site, GoToMeeting® and children’s peer networking website. To suit the children’s and parents’ schedules; participants were offered a choice of five different times. Five peer mentors and a health professional facilitated the online sessions. Weekly sessions lasted 45 to 120 minutes’ including the time spent on Club Penguin. Qualitative interviews and standardized quantitative measures were used to understand the processes and benefits of the support program.

FINDINGS:

Participation was high in all groups (Mean= 88%, range 81-95%). Through telling about their experiences, listening to others’ experiences, and role playing, the children were introduced to practical skills like problem solving, open communication, positive ways of educating others, seeking support, and advocating for themselves. Through it all, they had fun in the support sessions and when they played together in the virtual world, Club Penguin. Quantitatively, children perceived they were receiving more support from family and friends, their support seeking coping increased, and loneliness and social dissatisfaction decreased.

DELIVERABLES:

The high participation rates indicate that children were engaged in the intervention. Findings from other chronic diseases show that online interventions may be a viable means of supporting adults and families, but ours suggests that this is a feasible and useful way of supporting younger children.

RELEVANCE:

Peer support to empower young children to successfully manage their asthma and allergies and improve their quality of life can be delivered accessibly over the Internet. Rural and urban children, with an internet connection, can join the group from the comfort of their own home.

3C Comorbidity with Depression and Overweight in Children with Asthma

AllerGen Programme C: Public Health, Ethics, Policy and Society

S Bahreinian¹, GDC Ball¹, I Colman², AB Becker³, AL Kozyrskyj¹

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Supervisors: AL Kozyrskyj, GDC Ball

OBJECTIVE/PURPOSE:

In Canada 16.6% of children are affected by asthma which may increase the risk of comorbid depressive disorders in the adolescent years. Overweight is more prevalent in children suffering from asthma or depression, yet few studies have explored the possible relationships between these three chronic conditions in children. We examined whether depression was more prevalent in children with atopic and non-atopic asthma, especially among those who were overweight. A secondary objective was to report on the interrelationship between allergic symptoms, asthma and depression status in children.

METHODS:

A cross sectional analysis was performed on data collected as a part of the nested case-control study of the Study of Asthma, Genes and Environment in Manitoba. Children enrolled in the case-control study at age 7-10 were reassessed by a pediatric allergist at 11-14 years to confirm asthma diagnosis according to the patient's history and physical exam. Atopy was defined based on skin prick testing. Depressive symptoms were assessed using children's depression inventory- short form (CDI-S). Data were analyzed using logistic regression modeling to determine likelihood of depression in asthmatic children, stratified by sex and adjusting for ethnicity, waist circumference (WC) and atopy.

FINDINGS:

431 children at 11-14 years (136 asthmatics and 295 non asthmatics) were enrolled in this study. After adjusting for the covariates, girls who had non-atopic asthma were 2.8 times more likely to have comorbid depressive symptoms (OR: 2.84, 95% CI 1.00- 8.10). For each 10 cm increase in WC of girls there was 39% to 56% increase in chance of depression in our models. In boys, neither asthma nor WC showed an association with depression.

DELIVERABLES:

This study was a first look at the association between asthma phenotypes and depression in children accounting for body adiposity. Girls who have non-atopic asthma showed higher chance of having comorbid depressive symptoms.

RELEVANCE:

Depression is an important comorbidity of asthma as a chronic condition. It is essential to diagnose and treat depressive symptoms in children with asthma, as one of the consequences of untreated depression is decreased quality of life, impaired functional status and poor asthma outcome. We recommend all health practitioners who see asthmatic girls watch for depressive symptoms and treat comorbid depression seriously.

4C Calculating Health Care Costs Associated with Asthma in British Columbia, 2002-2007

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Larry D Lynd

OBJECTIVE/PURPOSE:

To calculate the health care costs associated with asthma in British Columbia (BC).

METHODS:

A population-based cohort study was conducted using the administrative health data for the population of British Columbia (BC), Canada. The discharge abstract database (for hospitalization), medical services plan (for physician visits), and PharmaNet (for prescriptions) were used to identify a cohort of asthma patients followed from fiscal years 1997 to 2007. Patients aged 5–55 years were included in the cohort using a previously published case definition of asthma based on a combination of asthma-related hospitalization, physician visits, and drug usage (Prosser et al. 2008). The first five years of the data were used as a 'look-back' period allowing for cases of asthma to be detected; therefore costs for years 2002 to 2007 were calculated. Fee-for-service values for physician visits, and government reimbursement fees for prescribed medications were applied. The case mix method was used to calculate hospitalization costs. Since emergency department (ED) visits are not captured in the provincial data, rates were estimated from the ratio of hospitalization to ED visits in Canadian studies. All costs were reported in inflation-adjusted 2008 Canadian dollars. We used a narrow (specific) definition of asthma-related resource use as baseline and a broad (sensitive) definition in the sensitivity analysis.

FINDINGS:

There were 304,099 unique patients who fulfilled the case definition of asthma (mean age 29.2 years at the time case definition was satisfied, 56.1% female). Based on the population of BC at the midpoint of the follow-up, the prevalence of asthma was estimated to be 8.7% (from 7.9% [2002] to 9.4% [2007]) in the 5-55 y/o age group. Overall, the identified cohort of patients was responsible for \$221.9 M in direct health care costs during the 6-year period, corresponding to \$36.9 M annually. Hospitalizations/ED visits, physician visits, and medication costs accounted for 14.5%, 17.0% and 68.5% of the total cost, respectively. When the broad definition of asthma-related resource used was employed, the total cost of asthma increased by 45% to \$323.2 M. In this scenario the hospitalization/ED visits, physician visits, and medication accounted for 27.8%, 22.7%, and 49.5%, respectively. The three medication categories that accounted for the highest proportion of medication costs were inhaled corticosteroid (ICS, 40.1% of medication costs), combined ICS and beta-agonists (26.9%), and short-acting beta2 agonists (14.4%).

DELIVERABLES:

Our results, consistent with our previous report for an earlier time period (Sadatsafavi et al. 2010) indicate the substantial burden of asthma in British Columbia, and the significant share of medications in the direct cost of asthma in the province. Further analyses should focus on identifying subgroups of patients with higher costs in order to improve asthma-treatment and to guide health policies.

RELEVANCE:

Our results have direct public policy impacts. These results show an increasing prevalence of asthma in BC during the last decade with an associated increasing economic burden. How will the findings be communicated: publications/presentations. Also these findings will be integrated into future cost effectiveness models.

5C A Danger Foreseen is Half-Avoided: Understanding the Effects of Food Allergen Labels in Canada

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Susan J. Elliott

OBJECTIVE/PURPOSE:

The purpose of the research is to identify the proportion of Canadians affected by food allergies; and to investigate the determinants of consuming behaviors and perceptions of food allergen labels in affected families in Canada.

METHODS:

Randomly selected households were interviewed *via* telephone in 2008, as part of the SCAAALAR project (Surveying Canadians to Assess the prevalence of common food Allergies and Attitudes towards food Labeling and Risk). Data regarding allergy status, consumption behavior, perceptions of food allergen labels were collected. Cross-tab Chi-square (χ^2) and ANOVA tests were used to test for differences by demographic variables.

FINDINGS:

Of the surveyed households (n = 3439), 20% self-reported a food allergy in the household, 18.8% of these reported an allergic child. 31% were non-allergic, but reported experience preparing for an allergy controlled environment, thus are indirectly affected. A considerable proportion of allergic families reported not heeding current precautionary statements. The most observable trend was found by allergic status. Allergic families were less likely to find precautionary statements helpful and were also more skeptical about the use of these statements by manufacturers. These attitudes are reflected in their purchasing behavior, and as a result, they are less likely than indirectly affected households to be heeding all types of precautionary statements. Overall, respondent age, region and allergic status were found to affect purchasing behavior at varying degrees. Gender, respondent age, region, allergic status, and income were found to affect attitudes towards allergen labels.

DELIVERABLES:

This research provides a national picture of the affected population, reflects the opinions and preferences affected families have for food allergen labels, and supports Health Canada's policy change. It is also part of a larger project that adopts a mixed methods approach, an emerging paradigm in health research, which utilizes both quantitative and qualitative data collection tools. Results from this research have informed the development of a follow up qualitative data collection tool, and data collection is currently ongoing.

RELEVANCE:

This study is the first in the literature to explore national attitudes toward allergen label use in Canada, which is critical to guide policy change that is currently underway. It will provide a description of the lived experiences of affected Canadians, and their perceptions of food allergen labels. Ultimately, an effective labeling system will enable affected consumers to make safe food choices, with minimal limitations.

6C Who Says What? The Importance of Issue Framing and Claims-Making in the Construction of Food Allergies

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Susan J. Elliott

OBJECTIVE/PURPOSE:

The purpose of this research is to explore the construction of food allergy risk through Canadian print media.

METHODS:

A content analysis of 18 daily newspapers was conducted for a nine year period between January 1, 2000 and December 31, 2008 inclusive. Online textual databases (e.g., LexisNexis Academic) were used to compile a database based on key search terms (e.g., 'food allergy'), yielding 598 relevant articles. These articles were analyzed through the lens of issue framing and claims-making.

FINDINGS:

The majority of articles were framed to define food allergies as a problem or risk (37%), followed by those suggesting policy/regulatory remedies (32.6%), diagnosing the causes of food allergy (20.7%), and those making moral judgements (e.g., stories advocating or dismissing existing or emerging policies) (9.7%). Results show that advocates and affected individuals dominate discussions around policy action, while researchers and health professionals are most often diagnosing the causes of food allergy. Results also suggest that there is a competition of voices defining food allergies between advocates, affected individuals, researchers, and health professionals. This is a potential source of confusion which may be shaping public understanding of the related risks. There is also an indication that the framing of food allergies is evolving over time, and that the discussion is becoming increasingly one-sided with affected individuals leading the charge.

DELIVERABLES:

This study is informed by previous work that has established the heightened perceived risk of food allergies in the Canadian population (Harrington et al, In press). This study uses media content analysis as a way of exploring how those risks are constructed in the print media, a key source of risk information for the general public. Attribution of a message to a source, as well as the way in which that message is framed, can provide critical insight into how health risk is constructed. Indeed, this process matters for public perception, as representation of a risk issue influences whether the general population understands, attends to, or acts upon risk information.

RELEVANCE:

The literature in and around food allergies has started to recognize that various social actors and interests are involved in the social construction of food allergy. This is evidenced by differences in lay and expert understandings of related risks. This is certainly reflected in media representations of food allergies, and the results of this research extend this contention. While many voices compete to define the risks of food allergies, discussions of causes and solutions are relatively one-sided, with potential consequences for public understanding and policy. As food allergies continue to be constructed and re-constructed through claims-making and framing in the media, it is important to know who is saying what, why and to what end if we are to respond in an evidence-based manner.

7C Association of Parental FEF25-75% During a Methacholine Challenge and Children's Diagnosis of Asthma and Airway Hyper Responsiveness

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Allan B. Becker

OBJECTIVE/PURPOSE:

Parental lung function may influence children's pulmonary function. We previously explored the relationship between parental FEF 25-75% PC30 (concentration of methacholine required to decrease FEF 25-75% by 30%) and children's asthma and/or airway hyper responsiveness (AHR) at age 12-13. We found positive association between mothers mid flow and daughter's asthma adjusted for maternal diagnosis of asthma, hay fever, environmental tobacco smoke (ETS), and socioeconomic status (SES). We examined the association of parental PC30 with child asthma and AHR at age 8-10.

METHODS:

Children born in 1995 in Manitoba were enrolled in a nested case-control birth cohort study (Study of Asthma Genes and the Environment). Children were assessed at age 8-9 by a pediatric allergist for asthma and methacholine challenge was performed at this time to determine AHR. Children were assessed again at age 12-13, and their parents also underwent methacholine challenges to determine AHR. Parental FEF 25-75% PC30 (≤ 8 mg/ml MCH) was also measured. Odds ratios (OR) were calculated for the relationship between parental PC30 FEF 25-75% and child's asthma at both ages and logistic models were adjusted for maternal hay fever, asthma, ETS and SES. Models were also stratified by gender. A multinomial regression was performed for the association of parental PC30 and child's asthma at age 8-9 and 12-13 [categorized as healthy in both waves, asthma in both, asthma remission and incidental (no asthma age 8-9 but asthma age 12-13)]. Parental PC30 and child AHR was also examined in a similar fashion.

FINDINGS:

There was no association of paternal PC30 and child asthma at age 8-9 (OR1.4, 95%CI 1.44-4.47) but there was for maternal PC30 and child's asthma (adjusted for maternal asthma, hay fever, ETS and SES; OR1.36, 95%CI 1.03-1.8). When stratified by gender this was not significant for females (OR 1.29, 95%CI 0.8-2.09), and borderline for males (OR1.45, 95%CI 1-2.1). At age 12-13 there was no association with paternal PC 30 and child asthma but maternal PC30 was associated with asthma in females (OR1.93, 95%CI 1.15-3.24). Child's asthma at both ages was associated with maternal PC30 (OR 2.13, 95%CI 1.3-3.5). Maternal PC30 was not associated with remittent or incident asthma. Maternal PC30 was associated with child's AHR being moderate/severe) at age 8-9 (OR 2.08, 95%CI 1.30-3.32) and at age 12-13 (OR 2.04, 95%CI 1.28-3.26).

DELIVERABLES:

There is no significant association with paternal PC30 and asthma or AHR in children. There was an association between maternal PC30 and asthma at age 12-13 for girls and for moderate/severe AHR in girls at both ages. There was a positive association between maternal PC30 and boys with asthma at both 8-9 and 12-13 years but no association for AHR at either age.

RELEVANCE:

Maternal airway responsiveness, at least in terms of PC30 FEF 25-75%, may be helpful in identifying risk for the development of asthma especially in girls. It may also identify boys that will have persistent asthma. Mid lung flow changes in mothers may represent a maternal genetic influence on the development of asthma and pulmonary function in children.

8C The Use of Unconditional Incentives in Vulnerable Populations for a Telephone Survey

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Ann Clarke

OBJECTIVE/PURPOSE:

Poor response rates can lead to non-response bias, thereby compromising the accuracy of prevalence estimates. Our research team conducted a telephone survey of randomly selected households to estimate the prevalence of food allergy in the 10 Canadian provinces between May 2008 and March 2009 (the SCAAALAR study: Surveying Canadians to Assess the Prevalence of Common Food Allergies and Attitudes towards Food Labeling and Risk). A household response rate of only 35% was attained and new Canadians, Aboriginals, and those of lower socioeconomic status or residing in the Territories were under-represented. We are now attempting to replicate the survey focussing on these vulnerable populations (the SPAACE study: Surveying the Prevalence of Food Allergy in All Canadian Environments) and are evaluating strategies to increase the response rate. Although others have demonstrated the success of incentives, few studies have examined the use of unconditional incentives (incentives provided to every potential subject, not contingent on participation) in vulnerable populations for a telephone survey. Hence, we conducted a pilot study comparing the response rate between a population receiving an unconditional incentive and a population not receiving an incentive.

METHODS:

To target the vulnerable populations desired in SPAACE, census tracts (CTs) from all census metropolitan areas for the 2006 census were obtained. CTs with either a high proportion of low socioeconomic status Canadians or new Canadians were identified and converted into postal codes from which telephone numbers were randomly selected. Although Aboriginals and the Territories were not targeted in this pilot incentive study, these populations will be included in SPAACE. 364 households were then randomly assigned to either the incentive or non-incentive group. Both groups were mailed an information letter; the former also received the incentive (either a \$5 Maple Leaf Foods coupon or a \$5 Tim Hortons gift card) with the letter. Confidence intervals comparing the differential response and cooperation rates were calculated using a 2-sample normal approximation to the binomial distribution. The cooperation rate is a less conservative version of the response rate as the denominator excludes any non-contacts.

FINDINGS:

The response rates for the incentive and non-incentive groups were 38.8% and 31.4% respectively, yielding a between group difference of 7.4% (95% CI -2.3%, 17.0%). The cooperation rates for the incentive and non-incentive group were 47.3% and 40.0% for a difference of 7.3% (95% CI -0.9%, 15.7%).

DELIVERABLES:

Although wide CIs preclude definitive conclusions, the results suggest that unconditional incentives might increase the response rate.

RELEVANCE:

Although unconditional incentives will substantially increase the cost of SPAACE, our results suggest that the incremental increase in response rate likely merits the additional cost. With this additional investment, it is hoped that an improved response rate will result in more accuracy in the Canada's food allergy prevalence estimates.

9C A Comparison of Health Outcomes and Proximity to Hydro Substations in Participants of the SAGE study

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Allan B. Becker

OBJECTIVE/PURPOSE:

All cities use substations to distribute power. Many studies have shown that exposure to high voltage can be harmful to children's health. This study identifies participants living in a substation area and compares health outcomes based on physician diagnosed asthma, BMI and airway hyperresponsiveness.

METHODS:

The Study of Asthma Genes and the Environment (SAGE) focused on children from a 1995 birth cohort in the province of Manitoba. Participants completed questionnaires about geographic information. Through a Manitoba Hydro database, we identified 10 substations inside the city of Winnipeg. These 10 substations are assigned to 10 specific postal codes. Participants were sorted into two groups based on their postal codes: those with postal codes that matched the 10 substation postal codes and those with postal codes not attributed to substations. Researchers collected information about participants' height, weight and results of methacholine challenges. A pediatric allergist examined each participant and diagnosed their asthma status. BMI and PC₂₀ were calculated. Data were analyzed using chi-square and odds ratio.

FINDINGS:

There were 487 participants who returned for the third wave of the SAGE study. We focused on the 232 participants who reside in Winnipeg. 29% (68/232) of participants lived in a substation postal code versus 71% (164/232) with a postal code not attributed to a substation. 94 participants were diagnosed with asthma and 27.7% of these asthmatics were living in substation postal code (OR: 1.14, p-value = 0.65). Of the total population studied, the prevalence of asthma in the substation group was 38% versus 41% in the non-substation group. 29% of participants with a PC₂₀ ≤ 8 live in substation postal code (OR: 1.00, p-value = 0.99). 40% of the overweight population among the participants lived in substation postal code (OR: 0.58, p-value = 0.17).

DELIVERABLES:

Substations appear to have no significant affect on diagnosis of asthma, airway hyperresponsiveness or BMI status.

RELEVANCE:

In this modern sized cohort, there is no correlation between living in a substation area and physician diagnosed asthma. There are major societal concerns and some debate on the impact of child health related to proximity to hydroelectric substations. Medical geographers will need to consider this in addition of other socio-economic factor with studying asthma in population.

10C Design and Testing of a Portable, High Time Resolution System for Monitoring Air Pollutant Mixtures Associated with Allergy Onset and Development

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Greg Evans

OBJECTIVE/PURPOSE:

Current evidence suggests that air pollutant exposure is a risk factor for inducing and increasing the severity of upper airway allergic disease and asthma. The air pollutants most commonly linked to these disease outcomes include ozone, NO₂ and fine particulate matter (PM). The presence of mixtures of these pollutants, even at low concentrations, has been associated with direct impacts on respiratory disease biomarkers as well as indirect impacts on allergenicity of many aeroallergens. In addition, volatile organic compound (VOC) mixtures have been suggested as surrogates for allergen exposure. The current methods for monitoring of air pollutant mixtures in multiple microenvironments are still burdened by low time resolution, high cost and problems with sample integrity. For long-term indoor air quality research campaigns, gas sensor arrays are an optimum choice because they can offer versatile, low cost method for real-time monitoring and resolution of indoor pollutant mixture components. To address this opportunity, a sensor array-based system is being developed and tested for real-time monitoring of VOC mixtures, NO₂, ozone, and fine PM.

METHODS:

A prototype has been created consisting of an array of commercially available sensor technologies: three VOC sensors, three fine PM sensors, two NO₂ sensors and an ozone sensor. A variety of detection technologies are used to increase the resolution of pollutant mixtures, including chemi-resistive, electrochemical, optical and ionization sensor technologies. Temperature and humidity sensors are included for data normalization. The system is modular, allowing it to be customized for specific applications. The prototype response is being characterized in several indoor microenvironments.

FINDINGS:

In preliminary tests, the system response was found to be sensitive enough for detection of changes in ambient levels of pollutants and comparison of pollutant compositions in different microenvironments, such as residential, office and public transportation. Improved circuit design allows for baseline subtraction and measurement sensitivity regulation. The high time resolution of measurements allowed tracking of pollutant levels at different times of the day. Internal data acquisition and storage allowed for unsupervised long-term monitoring. The dependence of sensor signal on temperature and humidity was analyzed.

DELIVERABLES:

This prototype is the first step towards a portable, flexible, and low cost solution for monitoring multiple air pollutants. The system may facilitate selection of the best biomarkers for human pollutant exposure and identification of threshold pollutant levels for response endpoints.

RELEVANCE:

The proposed system would be valuable in advancing research in asthma and allergy. Air pollutant mixtures can be used as markers to identify populations at risk of development of allergies and asthma. This diagnostic profiling would potentially promote more targeted and effective air pollutant policy interventions, as well as discovery of novel chemopreventative therapies and allergy treatment strategies.

11C Depression and Stress Are Associated with Blunted Cortisol Activity in Pregnant Immigrant Women

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Claudio N. Soares

OBJECTIVE/PURPOSE:

To prospectively examine correlates and clinical implications of poor mental health in pregnant immigrant women and their offspring.

METHODS:

Seventy immigrant women in early- to mid-pregnancy are being recruited and followed up to 1 year postpartum. Information regarding maternal health, mental health (perceived stress, depressive symptoms, negative life events, and social support) and biomarkers of stress reactivity (salivary cortisol) is gathered during pregnancy study visits. Postpartum follow-up visits collect information on neonatal health outcomes, infant health during the first year of life, infant allergic status (*via* skin-prick testing), and infant stress reactivity (salivary cortisol)

FINDINGS:

Of the 65 immigrant women assessed at 19.2 ± 4.7 weeks of gestation, around 30% reported a high level of depressive symptoms (EPDS ≥ 11), perceived stress (PSS ≥ 19), and/or experiencing >2 negative life events in the previous 6 months. Perceived stress and depressive symptoms were highly correlated ($r = 0.60$) and were both negatively correlated with perceived level of social support ($r = -0.33$, $r = -0.34$ respectively). Negative life events in the previous 6 months were moderately correlated with perceived stress ($r = 0.29$), but not with depressive symptoms. Saliva samples ($n=45$) were analyzed for cortisol by ELISA to examine waking cortisol levels, cortisol awakening response (CAR) area under the curve (AUCg) and CAR mean increase, and diurnal AUCg. Timing of saliva sampling (*i.e.*, weeks of gestation), number of hours slept, and wake time were significantly associated with one or more of the cortisol variables of interest and were controlled for in subsequent correlational analyses. CAR AUCg was negatively associated with depressive symptoms ($r = -0.38$) and diurnal AUCg was associated with perceived social support ($r = 0.40$). T-tests demonstrated that women experiencing >2 negative life events in the previous 6 months had a significantly lower mean cortisol increase post-waking (1.28nmol/L vs. 3.81nmol/L).

DELIVERABLES:

A considerable number of women in our sample reported poor mental health in early- to mid-pregnancy which was associated with maternal biomarkers of stress reactivity, namely decreased or blunted cortisol responses. Further follow up of this cohort will investigate whether these factors may contribute to the development of allergic disease in the offspring.

RELEVANCE:

Accumulating evidence suggests that perinatal stress and/or depression may be associated with the development of allergies in children. A better understanding of the mechanisms underlying this association can lead to development of interventions aimed at early life, a time when plasticity in physiologic development is especially abundant and robust.

12C Asthma is a Low Priority Amidst the Layers of Complexity Faced by Adolescents

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Allan B. Becker

OBJECTIVE/PURPOSE:

Adolescence is a time of emerging independence. This period of life is even more difficult for youth with asthma. Physiological changes resultant from puberty may change asthma presentation and perception; these changes, coupled with characteristics of adolescence influence youths' perceptions of, and ways of living with asthma. Moreover, youths' perceptions and management behaviours may not align with parental beliefs about their children's asthma. We sought to better understand youths' perceptions of, and management behaviours towards asthma, as well as those of their parents.

METHODS:

Participants were recruited using convenience sampling from the case:control study of the 1995 Manitoba Birth Cohort. All were 15 years old and all lived in Winnipeg. Focus groups were conducted with boys and girls separately, as well as concurrently with parents, although in separate rooms. All focus groups followed a semi-structured interview guide and were digitally recorded and transcribed. Data were analyzed using thematic coding, which was facilitated by NVivo.

FINDINGS:

To date, we have completed two focus groups of boys with asthma, two focus groups of girls with asthma, and four focus groups with parents. Preliminary data suggest that adolescents have multiple demands, including school, peers, family and extra-curricular activities, on their time. Adolescents are also aware of peer pressure to become involved in inappropriate behaviours (drugs, alcohol, smoking) and they acknowledge the burden of trying to balance all of their activities and create their own identity. Adolescents spoke of asthma as a series of isolated events, rather than a chronic condition. Although most carried their medications on a regular basis, they were not concerned if they occasionally forgot them. They viewed asthma as a minor issue compared to other demands in their lives.

DELIVERABLES:

Layers of complexity faced by today's youth overshadow the burden and management of asthma. Youth have numerous demands on their time and rarely recognize the importance of sustained asthma management.

RELEVANCE:

Understanding that adolescents do not prioritize their asthma management is an important first step in asthma education. Healthcare professionals need to acknowledge these layers of complexity when discussing asthma action plans and management with youth. In order to improve quality of life for adolescents, it is important to stress the focus of asthma management as an on-going process rather than a series of isolated events. Studies are required to define the most effective approach(es) with these youth and to communicate these findings to healthcare providers and policy makers.

13C How Does the Timing of Home Environmental Tobacco Smoke Exposure During Childhood Affect the Age of Asthma Development?

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Advisors: Teresa To and Sharon D. Dell

OBJECTIVE/PURPOSE:

To evaluate the relationship between age of home exposure to environmental tobacco smoke (ETS) and age of asthma development.

METHODS:

In phase 1 of the Toronto Child Health Evaluation Questionnaire, parents of 5619 grades 1 and 2 students attending 283 randomly-sampled public and Catholic schools reported age of physician-diagnosed asthma development and exposure to maternal ETS during pregnancy and the first year of life. In phase 2, a nested case-control study in which half of the children had asthma or wheezing, parents of 1497 students reported any ETS exposure in all homes inhabited by the child until age 7 years. Using the Cox's proportional hazards model, we evaluated the relationship between timing of ETS exposure and age of asthma development.

FINDINGS:

In phase 1, maternal smoking was reported in 5.0% during pregnancy and 7.8% during the child's first year; these proportions were similar for the subset participating in phase 2 (5.6% and 8.4%, respectively). Yearly home ETS exposure (12-14%) decreased over the first 7 years of life. Hazard ratios adjusted for sex, parental atopy and income adequacy suggested that children exposed to maternal ETS during pregnancy (1.32, 95% CI: 1.05-1.68) and their first year (1.17, 95% CI: 0.93-1.47) developed asthma sooner. Similarly, children with any home ETS exposure in each of the first 7 years trended towards earlier asthma development. However, the total number of years exposed to maternal ETS and the number of cigarettes smoked in the house per day did not appear to be associated with the age of asthma development.

DELIVERABLES:

Home ETS exposure was associated with earlier development of physician-diagnosed asthma. Longitudinal analysis will be used to further evaluate the timing of home ETS exposure and its contribution to the age of asthma development.

RELEVANCE:

The demonstration of an association between home ETS exposure and earlier development of physician-diagnosed asthma will contribute to AllerGen's mission to improve the quality of life for allergic disease sufferers by providing an additional rationale for recommending childhood avoidance of ETS exposure and illustrating that the importance of this avoidance likely extends beyond the first few years of life. These findings will be communicated to health care professionals and decision-makers by publication and generation of policy recommendations in partnership with our colleagues in Health Canada and Environment Canada.

14C Prevalence of Food Allergy in Canada: Preliminary Results from the SPACE Study

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Ann Clarke

OBJECTIVE/PURPOSE:

We completed a nationwide study on prevalence of food allergy in Canada in 2009 (SCAAALAR). However, vulnerable populations of Canadians, such as New Canadians, Aboriginals, and those of lower socioeconomic status or living in the Territories were under-represented. Hence, we are now extending our survey to involve these populations to provide a more representative estimate of the prevalence of food allergy in Canada.

METHODS:

We performed a nationwide cross-sectional random telephone survey of Canadian households beginning in September 2010. Using 2006 Census data, we targeted postal codes known to have a high proportion of our groups of interest. We asked respondents whether any household member had an allergy to peanut, tree-nut, fish, shellfish, sesame, milk, egg, wheat, or soy.

FINDINGS:

So far, 830 households (representing 2071 individuals) completed the survey. Of these individuals, 123 reported an allergy for a prevalence of 5.9% (95%CI,4.9%,7.0%). The prevalence is higher in children [9.3% (6.5%,12.1%)] than in adults [5.1% (4.0%,6.2%)]. The prevalence for specific foods are: peanut, 1.7% (1.2%,2.3%), tree-nut, 1.3% (0.8%,1.7%), fish, 0.7% (0.4%,1.1%), shellfish, 1.8% (1.2%,2.4%), sesame, 0.4% (0.1%,0.7%), milk, 0.7% (0.3%,1.0%), egg, 0.7% (0.4%,1.1%), wheat, 0.3% (0.1%,0.6%), and soy, 0.1% (0%,0.2%).

DELIVERABLES:

5.9% of our study participants report having at least one food allergy, with children having a higher prevalence than adults.

RELEVANCE:

Extending the collection of data regarding prevalence to the vulnerable populations is essential to developing the full picture of the health, social, and economic burden of illness that food allergy represents in Canada. We hope that these results will influence policy to reduce the morbidity, mortality, and socio-economic burden of food allergy in Canada.

15C Characteristics of Subjects with Work-Related Asthma in Québec and Ontario

AllerGen Programme C: Public Health, Ethics, Policy and Society

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Supervisor: Catherine Lemièr

OBJECTIVE/PURPOSE:

To compare the characteristics of the workers assessed for work-related asthma (WRA) in two referral centres from Québec and Ontario and to compare the delay between the onset of work-related-asthma symptoms and the diagnosis of occupational asthma (OA) and work-exacerbated asthma (WEA) between Québec and Ontario.

METHODS:

Retrospective cohort study of all the workers with OA and WEA investigated between 2000 and 2007 in two tertiary centers of Québec and Ontario. The data were extracted from the clinical charts using a common data extraction sheet in both provinces. Demographic data, data on work exposure (occupation, type of occupational agent), date of symptoms occurrence, date of asthma diagnosis, date of work-related-asthma diagnosis, skin prick tests results, FEV₁, PC₂₀, and results of the specific inhalation challenge tests or the peak flow (PEF) and PC₂₀ monitoring were collected.

FINDINGS:

Four hundred and thirty six workers from Montréal and 128 from Toronto were investigated during the same time frame. In Québec, sources of referrals were the worker compensation board (50.7%), primary care (39.2%) and respiratory physician (8.7%). In Ontario referrals were issued by respiratory physician (34.4%), primary care (27.3%), the Workplace Safety and Insurance Board (18.0%) and allergist (11.7%). There was a greater proportion of workers diagnosed with OA in Ontario (55.5%) compared with Québec (44.3%) among the workers investigated for work-related asthma, $p = 0.02$. The diagnosis of OA or WEA was made mainly by specific inhalation challenges in Québec (97.7%) in contrast with Ontario (3.13%) where the diagnosis was made by serial PEF or PC₂₀ monitoring. The delay between the occurrence of work related respiratory symptoms and the diagnosis of occupational asthma was similar in both centers (Québec: 4.6 ± 5.5 years, Ontario: 4.4 ± 4.8 years, $p = 0.7$). Workers with OA appeared to show a milder asthma severity in Québec (FEV₁: 90.0% pred, PC₂₀ at work: 3.0 ± 6.3 mg/ml) compared with Ontario (FEV₁: 81.3 ± 15.2 % pred, PC₂₀ at work: 0.8 ± 4.8 mg/ml). High molecular agents were identified as causal agents in 44% of the cases of OA from Québec and 12.9% of the cases of OA in Ontario ($p < 0.001$). In both provinces, isocyanates and flour were the agents the most frequently identified as causal agents in OA.

DELIVERABLES:

There were substantial differences between workers diagnosed with OA and WEA in Québec and Ontario between 2000 and 2007. However, the delay between the onset of the respiratory symptoms and the final diagnosis is similar between the two provinces.

RELEVANCE:

Implementation of surveillance programs in workplaces of interest may allow an early detection of work related respiratory symptoms and hence may reduce the delay in diagnosing work related asthma. An early diagnosis is likely to improve the prognosis of work related asthmatics. The results of this study will be communicated to the Québec Public Health Department.

IV. NON-ADJUDICATED POSTERS

#	Researchers and/or Trainees	Institution	AllerGen Researcher(s)/ Supervisor(s)	Abstract Title
1NA(A)	Kozyrskyj, Anita L. Scott, J.A. Azad, M.B. Guttman, D.S. Mandhane, Piushkumar J. Becker, Allan B. Hayglass, Kent T. and The SyMBIOTA Team	University of Alberta University of Toronto University of Manitoba		Synergy in Microbiota Research (SyMBIOTA)
2NA(A)	Konya, Tedd Koster, B. Becker, Allan B. Kozyrskyj, Anita L. Mandhane, Piushkumar J.	University of Manitoba University of Alberta University of Toronto		Impact of environmental exposure on the bacterial community in the human infant gut: A pilot project
3NA(B)	Grewal, Radjip Jensen, Renee Brown, Meghan Bendiak, Glenda Amin, Reshma Balkovec, Susan Ratjen, Felix Subbarao, Padmaja	Division of Respiratory Medicine, Department of Paediatrics; Physiology & Experimental Medicine, Research Institute, The Hospital for Sick Children, Toronto Department of Physiology, University of Toronto	Dr. Padmaja Subbarao	Lung Clearance Index in well-controlled Cystic Fibrosis (CF) and asthmatic children compared to healthy controls
4NA	Grabenhenreich, L. Keil, T. McBride, D. Rosenfeld, L. Willich, S.N. Wahn, U. Beyer, K.	Institute of Social Medicine, Epidemiology and Health Economics; * Department for Pediatric Pneumology and Immunology; Charité University Medical Centre, Berlin, Germany		Characteristics of allergic and non-allergic families in the EuroPrevall birth cohort on food allergy
5NA(C)	Douglas, M. McGhan, Shawna Ciccuto, Lisa Befus, Dean	Alberta Asthma Centre, University of Alberta , and Department of Medicine, University of Toronto		Nationally informed refinement and implementation of best practice childhood asthma education and training for healthcare professionals

1NA(A) Synergy in Microbiota Research (SyMBIOTA)

AllerGen Theme: Cross-Programmatic Research Teams and Platforms

Kozyrskyj AL¹, Scott JA², Azad MB, Guttman DS, Mandhane PJ, Becker AB, HayGlass KT and the SyMBIOTA Team

¹Department of Pediatrics, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB

²Dalla Lana School of Public Health, University of Toronto, Toronto, ON

Supervisor: Anita L. Kozyrskyj

OBJECTIVE/PURPOSE:

Intestinal microbiota plays a crucial role in the maturation of the immune system. Antibiotic use in infancy has been linked to the development of atopic disease (allergy and asthma) in childhood. The disruption of immune system development by antibiotic-induced changes to infant microbiota is the proposed mechanism underlying this association but evidence is limited. SyMBIOTA program objectives are to determine:

- 1) if antibiotic use during infancy alters intestinal microbiota,
- 2) whether the antibiotic effect is synergistic with mode of delivery and infant diet, and
- 3) whether induced changes in microbiota are associated with future atopy in children.

METHODS:

The SyMBIOTA team is a network of interdisciplinary researchers across Canada with expertise in biomedical and clinical sciences, and population health research. Our research program will use data from 2,500 infants in the ongoing Canadian Healthy Infant Longitudinal Development (CHILD) cohort study, which recruits women during pregnancy and follows newborns until age 5 to assess development of atopic disease. Mode of delivery and infant postnatal exposures are extracted from hospital records. Maternal exposures during pregnancy and type of infant feeding are obtained from parent report. Antibiotic use will be detailed from prescription database records. These data will be linked to microbiota profiles (generated *via* high-throughput signature gene sequencing of fecal samples at age 3 months and 1 year) and major health outcomes including asthma, atopic disease and immune profiles. SyMBIOTA is the first longitudinal birth cohort study to assess the determinants and outcomes of changes to microbiota in infants using high-throughput signature gene sequencing.

FINDINGS:

Our preliminary pilot study results indicate that microbiota composition and diversity are affected by birth mode, antibiotic use, and infant diet. With our new CIHR Microbiome Initiative funding, the SyMBIOTA team aims to confirm these findings and test their association with the development of atopic disease using the larger CHILD platform.

DELIVERABLES:

We plan to disseminate our findings by journal publication and presentation at scientific meetings. To enhance potential for impact, we further intend to publish our findings in the Society of Obstetricians and Gynaecologists of Canada guidelines on caesarean section delivery, and in the Canadian Paediatric Society guidelines on acute otitis media, as evidence for judicious use of antibiotics.

RELEVANCE:

There is a global trend towards increasing prevalence of childhood asthma and allergy, especially in developed countries. Simultaneously in these populations, the rate of caesarean section delivery continues to rise, exclusive breastfeeding duration falls short of recommended standards, and more than 60% of infants will be prescribed an antibiotic by one year of age. Accumulating evidence suggests that these factors impact significantly on infant microbiota and subsequent development of atopic disease; however, the causal mechanisms remain poorly understood. SyMBIOTA aims to provide evidence for a true causal relationship, with the potential to impact clinical practice guidelines for antibiotic use, breast feeding and caesarean section.

2NA(A) Impact of Environmental Exposures on the Bacterial Community in the Human Infant Gut: A Pilot Project

AllerGen Programme A : Gene-Environment Interactions

Konya, T; Koster, B; Becker, AB; Mandhane, PJ; Befus, DA; Kozyrskyj, AL; and Scott, JA
Supervisor: James Scott

OBJECTIVE/PURPOSE:

The aim of this project was to investigate the microbial community composition of 3-month stool samples from a portion of the infants participating in the Winnipeg vanguard of the Canadian Healthy Infant Longitudinal Development (CHILD) study as a function of exposure factors including: delivery mode, feeding method, antibiotic exposure and probiotic use. Communities were analysed by denaturing gradient gel electrophoresis (DGGE).

METHODS:

Total community DNA was isolated from 13 samples and a signature region of the 16S rRNA gene was amplified by PCR using universal bacterial primers, and the resulting community PCR amplification products were separated by DGGE. Diversity and uniqueness indices were calculated as community descriptors. As well, samples were clustered on the basis of overall similarity of their community composition "fingerprints" using unweighted-pair group method with the arithmetic average (UPGMA) and support for the resulting dendrogram was determined by 1,000 bootstrap replications. Similarity measures inferred by cluster analysis were used to investigate the impact of exposures.

FINDINGS:

Eighty unique band positions were scored across all samples, with individual samples having between 4 and 14 bands each. UPGMA analyses revealed three principal clades accounting for 8 of the samples. The remaining samples did not display any discernable patterns of similarity. In this preliminary investigation, the infant who received an oral antibiotic, amoxicillin, at 3 months of age showed lower microbiota diversity than a control matched on gender, mode of delivery and type of feeding but without antibiotic exposure. The microbiota of the antibiotic-treated infant was similar to that of 2 other infants whose mothers experienced premature rupture of membranes prior to delivery and likely received antibiotics at that time. Generally, we observed lower diversity among infants delivered by Caesarean section and/or those who were solely fed by formula. The highest community diversity and greatest uniqueness were observed in an infant delivered vaginally, exclusively breastfed at 3 months and who received a probiotic supplement (BioGaia).

DELIVERABLES:

The results of this study are instructive in the formulation of a framework for the evaluation of risk factors in the SyMBIOTA microbiome research project. Additionally, these results demonstrate that DGGE can be used as a rapid method to screen for diversity and similarity of microbial communities in fecal samples.

RELEVANCE:

The microbiota of the gut is responsible for the largest, naturally occurring immunomodulatory environmental exposure we encounter in our lives. A better understanding of the normal successional colonization of the gut by microbes in early life, and the impact of disruption of this sequence on atopic disease can reveal important, potentially modifiable risk factors for atopic disease.

3NA(B) LUNG CLEARANCE INDEX IN WELL-CONTROLLED CYSTIC FIBROSIS AND ASTHMATIC CHILDREN COMPARED TO HEALTHY CONTROLS

AllerGen Programme B: Diagnostics and Therapeutics

Rajdip Grewal¹, Renee Jensen¹, Meghan Brown², Glenda Bendiak^{1,3}, Reshma Amin^{1,3}, Susan Balkovec¹, Felix Ratjen^{1,3,4}, Padmaja Subbarao^{1,3,4}

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³University of Toronto, Toronto, ON; ⁴Physiology & Experimental Medicine, Research Institute, The Hospital for Sick Children, Toronto, ON

Supervisor: Padmaja Subbarao

OBJECTIVE/PURPOSE:

Lung Clearance Index (LCI) has previously been shown to be a sensitive tool for detecting cystic fibrosis (CF) lung disease in children with normal spirometry. However, few studies have investigated whether LCI may be useful in identifying lung disease in well controlled asthmatic populations. Our objective was to investigate whether LCI was able to detect abnormalities not only in our CF population with mild lung disease but also a cohort of well controlled asthmatics. Both disease cohorts were compared to a group of age matched healthy controls.

METHODS:

We measured baseline LCI values derived from 4% SF6 mass spectrometry Multiple Breath Washout in a group of 38 healthy children aged 6 to 18 (mean age 12.6 years, SD: 3.75), 19 children with CF (mean age 10.5 years, SD: 3.1) and 9 children with well controlled asthma (mean age 7.0 years, SD: 3.8). All subjects also performed reproducible spirometry.

FINDINGS:

The mean LCI from our group of healthy children (n=38) was 6.26 (standard deviation (SD):0.46, 95% CI: 6.12 to 6.41), which is comparable to other published normative data. Mean LCI was significantly higher compared to our healthy controls in the CF cohort (mean difference 2.65 (95% CI 1.85 to 3.46), p<0.0001) but not in the asthmatic cohort (mean difference 0.10 (95% CI -0.26 to 0.46); p<0.58) and. Mean FEV1 z-scores from the asthmatic subjects were significantly different from healthy controls (mean difference 0.86 (95% CI -1.56 to -0.17); p<0.016), while there was no significant difference between FEV1 z-score of CF subjects compared to healthy controls (mean difference 0.25 (95% CI -0.29 to 0.80); p<0.25). While abnormally high LCI values were found for 11 (58%) of the CF cohort with normal FEV1, all asthmatic subjects who completed spirometry had LCI and FEV1 values within limits of normality.

DELIVERABLES:

The LCI was able to detect abnormalities in a high proportion of children with mild CF disease. Based on our results of the 9 asthmatics tested, LCI was not elevated compared to controls. Further evaluation is needed to determine if LCI is a useful tool for detecting ventilation abnormalities in asthmatic children with normal spirometry.

RELEVANCE:

This study includes the first North-American healthy cohort to undergo MBW testing, and is the first to show no significant elevation of LCI in well-controlled asthmatics compared to healthy controls. It may be that the MBW technique more accurately reflects the efficacy of asthma controller medications than spirometry.

4NA Characteristics of Allergic and Non-Allergic Families in the EuroPrevall Birth Cohort on Food Allergy

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K. Beyer*

Institute of Social Medicine, Epidemiology and Health Economics;

*Department for Pediatric Pneumology and Immunology;
Charité University Medical Centre, Berlin, Germany

OBJECTIVE/PURPOSE:

There is uncertainty about how genetic predisposition, parental behavioural factors and home environment influence onset and course of food allergies in children. Therefore, we aimed to compare allergic and non-allergic families who participate in the EuroPrevall birth cohort in terms of type of parental allergy, sociodemographic characteristics, living environment and lifestyle during pregnancy.

METHODS:

Newborn babies were recruited in 9 European countries, establishing the EU-funded EuroPrevall birth cohort. Information on allergies in parents and siblings, education, ethnicity, living environment including animals, diet and other lifestyle factors during pregnancy among others was recorded at baseline (birth). Non-responders were asked about mother's allergies and education only.

FINDINGS:

The study centre Berlin recruited 1568 families between 2005 and 2007 (54% of those approached). Non-responders reported lower educational degrees and fewer allergies in the family (33% vs. 41%). Of responding mothers/fathers 11%/10% reported asthma in their medical history, 31%/30% allergic rhinitis and 15%/8% eczema respectively. Allergic compared to non-allergic mothers avoided more often fish (93%/87%) and tree nuts (73%/63%) during pregnancy.

Allergic parents preferred synthetic (foam) mattresses for their children over coco or other natural materials. Smoking and other lifestyle and environmental factors were similarly distributed amongst allergic and non-allergic families.

DELIVERABLES:

The German sub-cohort is slightly risk-enriched in terms of allergic predisposition and has a higher educational level than the general population. Allergic and non-allergic participating families differed only to a minor extend regarding diet in pregnancy and children's mattresses.

RELEVANCE:

These factors and parental education and allergies in should be accounted for when analysing risk factors for onset and course of food allergies.

5NA(C) Nationally Informed Refinement and Implementation of Best Practice Childhood Asthma Education and Training for Healthcare Professionals

AllerGen Programme C: Public Health, Ethics, Policy and Society

¹M. Douglas, ¹S. McGhan, ²L. Ciccutto, ¹D. Befus

¹Alberta Asthma Centre, University of Alberta, Edmonton, AB; ²University of Toronto, Toronto, ON
Supervisor: D. Befus

OBJECTIVE/PURPOSE:

This project was conceived to address persistent educational gaps for children with asthma. The project aimed to ensure that the RAP online instructor's training is relevant, links with key partner programs and is accessible from all Canadian communities.

METHODS:

The phases of the project included: 1) Solicit input from national stakeholders and modify on-line course, 2) Establish national online faculty and plan recruitment and delivery, 3) Deliver the course, 4) Evaluate process and impact. Diverse and nationally representative experts (n=20) made recommendations to make the course more comprehensive, relevant, accessible, sustainable and in demand and was translated into French. Experienced facilitators (n=23) were recruited. A substantial waiting list of course participants developed *via* stakeholder networks.

FINDINGS:

The modified course included twelve interactive discussion forums with experts, exercises and an exam (n=18 graduates). Participant communities had limited access to health care for asthma (11/18) and were outside large cities (10/18). The quality of materials (4/5), learning community (4/5), organization (4/5) and guest facilitators (4.2/5) was rated high. "Using fun and engaging teaching strategies" ranked most beneficial and practical module (14/18). Graduates' confidence in their ability to serve the needs of children with asthma increased (100%) and 75% reported plans to implement RAP.

DELIVERABLES:

The Roaring Adventures of Puff ("RAP") is a comprehensive, pro-active, effective program which enables children to manage asthma and equips health professionals to skilfully engage children. This program contains two curricula: 1) For 6-12 year olds: school-based, small group setting, activity-and-game-filled sessions (www.educationforasthma.com). The online guide and tool kit was reviewed, modified and translated into French. 2) For Health Professionals: A facilitated, interactive on-line course (www.raonline.ca). The course was modified, implemented, and translated into French. 'Tips from the experts' and a module, 'diverse populations in health' were added and an online expert faculty data base was developed.

RELEVANCE:

The Online RAP instructor training program improved health professionals' skills and confidence in assisting children with asthma to develop management skills and improved quality of life. Access and relevance improved from recommendations of the national advisory committee, translation into French and discussion board contributions from expert faculty across Canada. The school-based approach was viewed as a feasible model to achieve a wide reach, reduce long term costs and impact associated with poorly controlled asthma. Communication plans are underway to promote the course and motivate decision makers to enable health educators to provide support for children with asthma in schools.

ACKNOWLEDGEMENTS: Funding from the Public Health Agency of Canada under the National Lung Health Framework Program. Online course development and pilot testing funded by AllerGen NCE, Inc. We gratefully acknowledge the many partners who helped make this project a success.