TRAINEE POSTER ABSTRACTS

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INNOVATION from cell to society^3

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AllerGen
Allergy, Genes and Environment Network
Le réseau des allergies, des gènes et de l'environnement

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The Feasibility and Safety of Collecting Exhaled Breath Condensate in Normal and Wheezy Infants

Programme A – Gene-Environment Interactions
Pediatric Asthma

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Supervisor: Dr Padmaja Subbarao

Objective/Purpose: Exhaled breath condensate (EBC) has been proposed as a non-invasive marker of lung inflammation in children. The authors have previously shown that adequate amounts of EBC could be collected within the normal range of respiratory rates and tidal volumes for infants in an vitro system. The aim of our study was to 1) determine if EBC collection was safe and feasible in infants and 2) to determine if there was a difference in the inflammatory profile of EBC between healthy controls and wheezy infants.

Methods: Wheezy infants and normal controls between 6 weeks and 3 years of age were recruited from the Infant Pulmonary Function and Echocardiogram Laboratories, respectively, at the Hospital for Sick Children, Canada. For the wheezy infants, an Ecoscreen Breath Condensate (Jaeger) apparatus was used. For the normal controls, a modified Hans-Rudolph valve (HRV) with the expiratory valve removed was connected to a modified R-Tube. Exhaled Breath Condensate was collected over 15 minutes. EBC samples were analyzed using Gas Chromatography Mass Spectroscopy (GCMS).

Findings: Patients were between the ages of 5 and 26 months. EBC samples for 18 wheezy children and 16 healthy controls were obtained. The mean (µL) ± SD collection volume was 285.25 ± 205.4 (range 50, 805) for the wheezy infants and 388.75 ± 103.481 (range 220,635) for controls. Thromboxane, leukotriene and prostaglandin concentrations were below the standard range of 0.05ng (range, 0-0.47) for the majority of samples.

Relevance: The Ecoscreen Breath Condensate apparatus and the modified R-Tube are safe and feasible methods to collect EBC in infants. However, more sensitive assays are needed for the detection of inflammatory compounds before EBC can be used as a tool to distinguish wheezy infants and healthy controls. The results of this study will provide a framework on which to build the

Exhaled Nitric Oxide and Pulmonary Function Tests in a Cohort of Infants with Prior Severe Wheezing

Programme A – Gene Environment Interactions
Pediatric Asthma

Reshma Amin, MD., University of Toronto, The Hospital for Sick Children, Susan Balkovec, RRT., University of Toronto, The Hospital for Sick Children, Colleen Keast, RRT., University of Toronto, The Hospital for Sick Children, Felix Ratjen, MD, PhD., University of Toronto, The Hospital for Sick Children, Padmaja Subbarao, MD, Msc, University of Toronto, The Hospital for Sick Children
Supervisor: Dr Padmaja Subbarao

Objective/Purpose: Infants with severe wheezing illnesses are at high risk for the development of asthma. Exhaled nitric oxide (eNO), a marker of airways inflammation, has been shown to be related to asthma severity. Our aim was to prospectively document the degree of lung dysfunction in a cohort of infants with a prior severe wheezing illness and determine if there is a correlation between lung volumes, forced expiratory volumes and exhaled Nitric Oxide.

Methods: Infants were eligible for the study if they had been hospitalized for a wheezing illness or had previously received oral steroids. Plethysmographic lung volumes and forced expiratory volumes were measured using the raised volume rapid thoracoabdominal compression techniques. Offline eNO levels were measured with the Ecophysics machine.

Findings: Data was available for 14 infants (aged 22-114 weeks, 11 male). Six infants returned for two visits. The mean z-score for FEV0.5 was 0.43 (95% CI -0.1, 0.75) for the baseline visit and 0.21 (95% CI -0.14,0.56) for the six infants at their second visit. Lung volumes (FRC mean z-scores) were elevated at baseline [1.89 (95% CI 1.6, 2.2)] and follow-up [1.1 (95% CI 0.8, 1.4)]. The mean (standard deviation) for nitric oxide was 15.0 (6.8) at baseline and 19.1 (9.8) at follow up respectively. The FRC z score and TGV significantly correlated with eNO for both baseline (p=0.0002, p=0.0097) and follow up visits (p=0.0392,0.0094). However, eNO did not correlate with forced expiratory flows.

Infants with a previous history of a severe wheezing illness who are asymptomatic at the time of assessment have a significant correlation between lung volume and eNO. Further recruitment and follow-up is required to determine whether infants with abnormal lung function and elevated eNO levels will develop asthma.

Deliverables: It is anticipated that the results of this study will aid in the development of the AllerGen birth cohort (CHILD) study.

Relevance: Our ultimate goal is to identify tools to predict a diagnosis of asthma from a cohort of infants with a history of wheezing illness and therefore, guide future therapies. We also hope that our study will contribute to clinical practice guidelines in the future.

Co-Existing Skin and Respiratory Symptoms among Four Occupational Groups

Programme C – Public Health, Ethics, Policy and Society
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Supervisor: Dr. D. L. Holness

Objective/Purpose: Co-existing occupational skin and lung disease has rarely been studied despite several case reports in the literature. We report on the prevalence and risk factors for co-existing skin and respiratory symptoms in four occupational populations.

Methods: Previously collected questionnaire data from studies of occupational skin or respiratory disease was utilized. Subjects were employed in embalming, cabinet making, soda ash processing or softwood planning. Data from equivalent questionnaire items was pooled and univariate analyses were completed.

Findings: Ninety-six percent of all subjects (n=245) were male. On average subjects were 36.4 years old (S.D. 12.7) and had been at their current place of employment for 9.3yrs (S.D. 9.1). Seventy percent of subjects (n=172) reported ever smoking. Mean lung function was approximately normal, 94.1%pred. FEV1 (S.D. 12.7) and 96.1%pred. FVC (S.D. 11.6). Skin rash was more common among embalmers (p=0.0001) compared with other occupational groups. Overall, 46% of subjects reported at least one respiratory symptom; this did not differ significantly between occupational groups. Subjects were grouped in four symptom group: no symptoms, skin symptoms only, respiratory symptoms only and both skin and respiratory symptoms. Thirty-three subjects (13%) reported both skin and respiratory symptoms. Symptom groups did not differ significantly in terms of age, lung function, smoking status, atopy or years working. However, subjects reporting both skin and respiratory symptoms were more likely to work in embalming (p=0.002) and be female (p=0.002).

Deliverables: More research into the factors associated with co-existing skin and respiratory symptom is needed. Future work will have two foci: (1) characterization of workers who present at an occupational clinic with co-existing respiratory and lung allergic disease and, (2) workplace study of the exposure-response relationship with regard to skin and respiratory allergy.

Relevance: This research furthers AllerGen's mission to improve quality of life for workers. In the past, respiratory and skin allergies have been addressed separately and the effects of exposure have been considered in organ system silos. This work provides evidence of co-existing respiratory and skin symptoms in multiple working populations. In the future we want to explore the association between co-existing respiratory and skin allergy and occupational characteristics; this work could lead to changes in the workplace exposure standards and ultimately prevent future cases of work related allergy.

Differential LPS signaling in peripheral CD4+ cells from atopic and non atopic children: the hygiene hypothesis revisited.

Programme B – Diagnostics and Therapeutics
TLR4+ T cells in children (3.11)

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Objective/Purpose: The hygiene hypothesis states that LPS exposure early in life is associated with lower incidence of atopy. Such protective effect of LPS would involve Toll-like receptor 4 (TLR-4)-expressing CD4 T lymphocytes. Age, atopic status and *in vitro* IL-4 stimulation were previously shown to alter TLR-4 expression in T lymphocytes. We sought to determine whether atopic children have impaired LPS signaling compared with normal subjects.

Methods: Lipopolysaccharides (LPS) or IL-4-stimulated peripheral blood mononuclear cells (PBMC) collected from atopic and non atopic children (7-15 years-old) were analyzed by flow cytometry for surface (CD4, TLR4, CD14, Vα24, CD1d tetramer) and intracellular (phospho p44/42 MAP kinase) protein expression. Data were confirmed by reverse transcriptase real-time quantitative polymerase chain reaction (RT-qPCR).

Finding: Upon LPS exposure, TLR-4 expression was reduced both at the protein and mRNA levels. This effect of LPS was time-dependent, but not dose-dependent. LPS stimulated CD4 cells showed significantly increased p44/42 phosphorylation. Reduced signaling was observed in CD4+ high (T 'helper') compared with CD4+ intermediate lymphocytes, which include Va24+CD1d tetramer+ invariant natural killer T (iNKT) cells. CD4+ cells derived from atopic children exhibited significantly reduced signaling as compared to age-matched controls. Reduced LPS signaling could be duplicated in non-atopic controls by pre-incubation of the cells with IL-4 for 1 hr; this did not effect TLR-4 complex gene expression. Prolonged incubation with IL-4 (24 hr) strikingly reduced CD14 expression.

Deliverables: Our data suggest impaired LPS signal transduction in circulating CD4+ T cells from atopic compared to those from non-atopic children, or in IL-4-independent T lymphocytes. This study supports the role of LPS and TLR-4-expressing T lymphocytes in the hygiene hypothesis: atopy would be mirrored by impaired responsiveness to microbial ligands.

Relevance: The role of TLR4+ CD4+ intermediate T lymphocytes in innate immunity, as well as in allergic diseases, remains unclear. Investigations on TLR-ligand sensitive and cytokine producing T lymphocytes could contribute to develop therapeutic strategies in order to limit the atopic march and development of an allergic phenotype during childhood.

The use of confirmatory testing in the diagnosis of peanut allergy in children

Programme C – Public Health, Ethics, Policy and Society

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Background: Peanut allergy is a major health problem associated with a substantial risk of severe inadvertent reactions and a compromised quality of life. However, the diagnosis of peanut allergy can be complex. It usually requires an appropriate clinical history corroborated by confirmatory testing, especially in children never exposed to peanut or with an unconvincing history of peanut allergy (only one mild symptom/more than 2 hours after exposure/not due to skin contact or ingestion). Thus, in a child never exposed to peanut or with an unconvincing history of an IgE- mediated reaction to peanut, the diagnosis could be established appropriately by one of the following: 1) a positive skin prick test (SPT) to peanut and a peanut-specific IgE ≥ 15 kU/L, 2) a positive SPT to peanut and a positive food challenge with peanut, or a 3) a peanut-specific IgE ≥ 15 kU/L, or 4) a positive food challenge.

Objective/Purpose: To describe the use of confirmatory tests by physicians across Canada to diagnose peanut allergy in children who were never exposed to peanut or with an unconvincing history of an allergic reaction to peanut and to determine factors which might be associated with the appropriate use of tests.

Methods: Children 4-18 years old who reported a physician-confirmed diagnosis of peanut allergy were recruited from the Montreal's Children Hospital (MCH) and food allergy advocacy organizations. Data were collected on participants' clinical history and confirmatory tests. The use of confirmatory diagnostic testing was evaluated in children who were never exposed to peanut or with an unconvincing history. Multivariate regression analysis was used to identify potential factors which might be associated with the appropriate use of tests.

Findings: Three hundred and twenty-eight children were recruited. One hundred and ninety-four were never exposed to peanut and 134 had an unconvincing history. In 32.9%, the diagnosis was established by a positive SPT and a peanut-specific IgE ≥ 15 kU/L, in 5.8%, by a positive SPT and a positive food challenge, and in 2.4%, by a peanut-specific IgE level ≥ 15 kU/L. In 15.2%, a positive SPT and a peanut-specific IgE < 15 kU/L or a peanut-specific IgE < 15 kU/L alone was considered sufficient by the treating physician to diagnose peanut allergy. In 35.7%, a positive SPT alone was considered sufficient. In 7.9%, no confirmatory test was used. Those recruited from the MCH (odds ratio, OR 4.99; 95% CI, 2.88-8.65), having a history of asthma (OR 2.15; 95% CI,1.28-3.62) or other food allergies (OR 2.04; 95% CI,1.15-3.62) and an increased time interval between initial reaction (when applicable) and study entry (OR 1.19; 95% CI,1.06-1.34) were more likely to have their diagnosis confirmed by the appropriate use of confirmatory tests. However, appropriate use of diagnostic tests was less likely in those with an unconvincing history (OR 0.26; 95% CI, 0.12-0.57).

Deliverables: Although several confirmatory tests are available to establish the diagnosis of peanut allergy, they are not used appropriately in a significant proportion of subjects.

Relevance: Guidelines for the diagnosis of peanut allergy should be developed and disseminated among physicians and allergy advocacy associations. They would decrease the number of children mislabelled as allergic to peanut and unnecessarily carrying adrenaline auto-injectors and adhering to restrictive diets; they would also ensure that those truly allergic are identified and educated.

Perception of Asthma as a Factor in Career Choice among Adolescent Asthmatics

Programme C, Theme VI, Work-Related Allergy and Asthma

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Objective/Purpose: Asthma is a common chronic condition that can be aggravated by workplace exposures. Adolescents with asthma should be knowledgeable about how their future occupation might affect their asthma and potentially their quality of life. Workplace exposure to sensitizers and irritants are known to induce work-related asthma (WRA) and aggravate pre-existing asthma. For adolescent asthmatics, awareness of potential workplace exposures, the importance of protective equipment, and proactive medical management is critical to minimizing WRA. We sought to assess the perception and importance of workplace exposures and high-risk occupations, protective equipment, and asthma as a factor in career choice among adolescent asthmatics.

Methods: In total, 101 participants with physician-diagnosed asthma between the ages of 16 to 22 were recruited from the community using paper-based flyers posted on university and college campuses, as well as community center and public notice boards. Participants completed a pilot-tested questionnaire consisting of 31 items, which elicited demographic data, assessed their asthma medications, asthma symptom history, perception of asthma as a career choice factor, and their opinion of protective equipment in the workplace.

Findings: The majority of participants were female (54%), had an average age of 19±2 years, and an 11±6 year history of asthma. Asthma was not an important factor in career plans for 65% of participants and 44% were unaware of occupations that could exacerbate WRA. Family physicians were involved in asthma care for 80% of participants. Only 14% of participants involved allergists and pulmonologists in their asthma care. Adolescents were more likely to discuss asthma and their career plans with their parents (44%) or friends (30%) than their family physician (14%; $p<0.001$).
While 54% of participants agreed that protective equipment was important in the workplace, those currently in university, and those experiencing exacerbations triggered by fumes and exercise were statistically more likely to agree that protective equipment was important in the workplace ($p<0.05$).

Deliverables: Awareness of occupational and work-exacerbated asthma risks and the importance of asthma in career plans are suboptimal among adolescent asthmatics. Family physicians are most responsible for asthma care, but adolescents are less likely to discuss asthma and their career choices with their family physician than family and friends. A minority of participants sought specialist care for their asthma, and a narrow majority agreed that it was important to wear protective equipment in the workplace.

Relevance: In order to improve the quality of life for adolescent asthmatics, counselling must aim towards identifying occupational asthma risk factors and higher risk occupations. By understanding the perception of asthma and career choice among adolescent asthmatics, the goal of intervention will move away from occupational avoidance and career limitation. Optimal intervention will involve prevention efforts to increase awareness of inherent occupational risks, allowing for informed career choice decisions, strategies for protective measures to minimize workplace exposures, and counselling towards effective medical management and symptom control.

To achieve accurate physician assessment of the occupational aspirations of adolescent asthmatics, physicians involved in their care will be presented with our research findings and analysis through research conferences, peer-reviewed publications, and hospital research seminars.

Important Clues on the Mechanism of Action of Anti-Asthma Synthetic Toll-like Receptor (TLR) 7 Ligand generated from Gene Expression Analysis in Two Strains of Mice

Programme B – Diagnostics and Therapeutics
Therapeutics and Drug Discovery (Theme IV)
Camateros, P., Moisan, J., Henri, J., Radzioch, D. from McGill University
Supervisor: Radzioch, D.

Objective/Purpose: Results generated using murine models of allergic asthma, have demonstrated that treatment with the TLR7 ligand Resiquimod (S28463, R-848) prevents increases in methacholine induced lung resistance and elastance following ovalbumin sensitization and aerosol challenge. Resiquimod treatment also prevented the increase in systemic IgE levels, eosinophilia in both lung sections and in bronchoaloveolar lavage fluid, and airway remodelling. In order to further explore the mechanism of action of Resiquimod in a model of allergic asthma we performed a GeneChip analysis of A/J and C57BL/6 lungs. The goal of the proposed studies is to provide insights into the molecular mechanisms involved in the regulation of gene expression by this promising treatment; these may help identify new therapeutic targets downstream of the receptor-ligand interaction.

Methods: A/J and C57BL/6 mice were sensitized to ovalbumin (OVA) by three weekly injections (100µg in Alum, i.p.) and, one week later, challenged by aerosol (1% OVA in PBS or PBS control) on three consecutive days. One group of mice from each strain received 100µg of Resiquimod by i.p. injection 24 hours before each OVA challenge. Three hours following the final challenge, lung RNA was collected and processed for analysis with Affymetrix Mouse Genome 430 v2 expression arrays, or lung cells were isolated for flow cytometry. Expression data was then analyzed by the RMA method and the BioConductor R packages. KEGG pathway analysis was performed with EASE and significance was assessed by bootstrap analysis. NK cells were identified as DX5 and CD11b double positive leukocytes.

Findings: Our analysis of the generated data resulted in several interesting findings. OVA sensitization and challenge leads to the differential expression of hundreds of genes, in both strains of mice when compared to mock challenged animals. Treating OVA sensitized and challenged mice with Resiquimod lead to significant transcriptional changes involving several hundred genes which were not affected by OVA challenge alone. As expected, most of the genes whose expression was affected by OVA challenge in OVA sensitized mice showed an increase in expression. Much of the gene expression which has been significantly up-regulated following OVA challenge was significantly lowered in animals treated with Resiquimod. However, Resiquimod led to significantly elevated expression of several gene clusters when compared to OVA challenged but untreated animals, indicating that these genes are specifically activated by Resiquimod treatment. Analysis of the pathway distribution of the genes whose expression was altered by Resiquimod treatment allowed the identification of several overrepresented KEGG pathways including “Natural Killer Cell Mediated Cytotoxicity”, “Cell Adhesion Molecules”, “Cytokine-Cytokine Receptor Interaction”, and “Leukocyte Transendothelial Migration”. These pathways suggested the recruitment of NK cells into the lungs of treated animals which was confirmed by flow cytometry analysis.

Deliverables: The synthetic TLR7 ligand Resiquimod has been demonstrated to have great potential as a therapeutic agent for the treatment of allergic asthma. Understanding the cellular and molecular mechanisms responsible for this effect, as well as any potential side effects, is of great importance in the development of this compound for clinical use.

Relevance: The data presented herein will facilitate the development of TLR7 ligands as a new class of therapeutic compounds in the treatment of allergic asthma and provide the foundation for the identification of drug targets for the development of more effective future drugs.

DataBiNS: a Web-based tool for visualization of non-synonymous SNPs in biological pathways

Programme A - Genes and Early-Life Determinants

Fong Chun Chan, Edward Kawas, Mark D. Wilkinson and Scott J. Tebbutt

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Supervisors: Drs. Scott J. Tebbutt and Mark D. Wilkinson

Objective/Purpose: To develop a bioinformatics tool (DataBiNS) that can automatically gather multiple types of scientific data and knowledge from disparate web-based sources, relating to non-synonymous coding single nucleotide polymorphisms (nsSNPs). The collected data is visualized through a web application to allow more efficient study of the information.

Methods: The necessary components of the workflow tool were constructed through the BioMoby framework. The components were then meshed together through an open-source workflow management tool called Taverna. In order to make use of the data retrieved through the workflow, a web application built on the Java Platform; Enterprise Edition (J2EE) was created that executes the Taverna software in the background, which in turn executes the BioMoby framework workflow. The results are displayed through fundamental web technologies/languages such as Cascading Style Sheets (CSS), and Asynchronous JavaScript and XML (AJAX).

Findings: The use of an automated workflow tool, through the Taverna software, rather than manual surfing of the internet, significantly reduces the effort required to retrieve information. Furthermore, the friendly user interface of the web application allows for easier study of the data to find interesting correlations between specific outputs.

Deliverables:

1. The combination of executing a Taverna workflow programmatically and then displaying the results as a web-application is something that, to our knowledge, is innovative. Even though the framework we have developed to display the results are specific for the DataBiNS workflow, it can be generalized to accept any Taverna-based workflow, displaying the results as a webpage. This framework would no longer require users to rely on the default dataviewer provided by Taverna which makes studying the results more difficult. Additionally, the modularized nature of the workflow allows us to propose and develop new BioMoby services to add to the workflow in order to expand the information retrieved. The amount of information we can add to the workflow is unlimited, in theory, but in reality hardware limitation hinders the information we can retrieve.
2. DataBiNS can be accessed at: http://bioinfo.icapture.ubc.ca:8090/DataBiNS/ (best used with Firefox or Safari web browsers). Links to DataBiNS will also be provided at www.genapha.ca.

Relevance: One of the major problems with bioinformatics is the disparate sources of data available. While each individual source provides important information on the subject in question, the data is difficult to connect to other sources. The project's aim is not to serve as an individual source of information, but rather to conglomerate various sources into one location. In other words, the researcher is able to retrieve to a single location important information that would normally require having to jump from one source to another source.

AllerGen Animations

Programme A – Gene-Environment Interactions
Genes and Early-Life Determinants
and KTEE funding

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Objective/Purpose: Our objective is to develop innovative three-dimensional graphical animation tools to help engage and educate society about the relevance and importance of genetics and the many roles genes may play in complex, chronic diseases such as asthma. The animations will visualize the action of beta-2 agonists (reliever medication) on airway smooth muscle cells via the beta-2 adrenergic receptor, simultaneously exploring the possible role of genetic variation (single nucleotide polymorphisms – SNPs) in the gene that codes for this receptor. An additional animation will more clearly show how an individual's genotype for specific asthma/allergy-relevant SNPs would be ascertained in future point-of-care diagnostic devices, such as the AllerChip.

Methods: The initial animations were created at Arkitek Studios in Seattle, using 3D Studio Max software, and were rendered to a variety of output formats, including QuickTime Movies, Windows Media Player files and Flash files. Low resolution formats have been uploaded to the Genapha website (www.genapha.ca) and YouTube (www.youtube.com).

Findings: Initial focus-group testing has been very positive and we have received many excellent suggestions as to how content from the animations can be used for more specific applications. A finding of the initial reviews was the observance by some researchers and educators (*e.g.*, asthma educators, and staff from other related institutions [Heart and Stroke Foundation, Genome BC]) that people will generally struggle to keep up with the changing field of genetics and with the technology involved with discovering/explaining genetic processes and how they relate to pharmacogenetics and complex disease. Medical school training programmes were recognized by the initial focus groups as an area where the animations could be of potential benefit. Download statistics from YouTube include over 2,000 views of the genetic testing animation (with 5 subsequent links to other websites), and nearly 400 views of the asthma pharmacogenetics animation (with 3 website links).

Deliverables: We have developed innovative three-dimensional graphical animations that describe concepts such as 'Asthma Pharmacogenetics' and 'Genetic Testing'.

http://genapha.icapture.ubc.ca/animations/animation1.jsp

http://genapha.icapture.ubc.ca/animations/animation2.jsp

Relevance: We are currently developing and focus-group testing several application-specific tools that use content from these animations. Such tools will help enable more effective engagement and education of end-user groups, including medical undergraduate students, asthma educators and patients, and school-children.

The Association between Maternal Distress and Serum Cortisol Levels in Children: Differential Outcomes by Asthma Diagnosis

AllerGen Program: SAASSI
Dreger, L., MacNeil, B., HayGlass, K.T., Becker, A.B., & Kozyrskyj, A.L., University of Manitoba
Supervisor: Dr. A.L. Kozyrskyj

Objective/Purpose: Asthma and exposure to maternal distress have both been shown to correlate with changes in the stress response systems of children whereby, in response to an acute stressor, maternal distress is associated with increased cortisol levels and asthma is associated with an attenuated cortisol response. In this project, the combined effects of asthma and maternal distress were examined in relationship to serum cortisol levels in children and it appears to be the first reported study to include all three variables in a single investigation. We hypothesized that children with asthma who were exposed to recurrent maternal distress beginning in the postnatal period would have a blunted cortisol response to an acute stressor and that non-asthmatic children who were exposed to the same level of maternal distress would experience an increased cortisol response.

Methods: Using a representative sample (n = 503) of the 1995 Manitoba birth cohort, serum cortisol levels (ng/ml) were obtained at age 7 -10 years following the acute stressor of an invasive medical examination. Maternal distress was indicated by a physician diagnosis of a depressive or anxiety disorder or by a prescription history of related medications as reported in the mothers' healthcare records. Children's asthma status was determined via examination by a pediatric allergist. A multiple linear regression analysis was conducted to determine the predictive effects of asthma status and exposure to maternal distress on serum cortisol levels in response to the acute stressor.

Findings: Results of the asthma testing revealed that 188 children had asthma and 315 did not. Of the children never exposed to maternal distress (39.2%), mean cortisol levels were 46.8 ng/ml for those with no asthma and 45.8 ng/ml for those with asthma. Children exposed to maternal distress only after the first year of life (43.9%) had mean cortisol levels of 51.8 ng/ml (no asthma) and 49.2 ng/ml (asthma). Exposure to postnatal maternal distress only (3.4%) resulted in mean levels of 76.1 and 68.8 ng/ml, while exposure to persistent maternal distress resulted in values of 59.4 and 41.0 ng/ml for children with no asthma and with asthma, respectively. The multiple linear regression analysis indicated a significant interaction between asthma status and exposure to maternal distress such that children who were exposed to recurrent maternal distress (beginning postnatally) responded to an acute stressor with elevated cortisol levels if they had no asthma, whereas children with asthma responded with blunted cortisol levels. Exposure to recurrent maternal distress predicted a 25.9% increase in cortisol for children without asthma and a 5.2% decrease in cortisol levels for children with asthma.

Deliverables: The finding that exposure to postnatal maternal distress differentially affects stress response functioning in children with and without asthma who are exposed to recurrent maternal distress, points to a possible underlying relationship between the neuroendocrine system and the immune system. Whether maternal distress mediates this relationship cannot be determined by the present study as the measure of the children's stress response functioning was taken following the development of asthma symptoms. A follow-up investigation with this birth cohort may help to elucidate the possible mediating effect of maternal distress on asthma symptoms if it is discovered that the children who go on to develop asthma at a later age had lower cortisol levels at the time of the current analysis.

Relevance: Our results reinforce the need for the screening of depression and anxiety disorders in new mothers through programs such as Manitoba's Families First postnatal screening program. These types of programs are designed to assist at risk families with caring for their newborn, which may help to reduce or prevent asthmatic symptoms and the associated, health-threatening dysregulation of stress response systems in children.

Allergic Asthma: Air Pollution and Allergen Interactions

Programme A – Gene-Environment Interactions
Environments/Populations and Society

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Gage Occupational and Environmental Health Unit[1], St. Michael's Hospital[2], University of Toronto[3], The Toronto Hospital[4], McMaster University[5], The James Hogg iCAPTURE Centre[6], The Hospital for Sick Children[7], Environment Canada[8], Health Canada[9], and Harvard School of Public Health[10]

Objective/Purpose: To determine: 1) airway reactivity to inhaled allergen and 2) airway and systemic inflammatory cellular and molecular responses after exposure to urban concentrated ambient fine particles (CAP) + ozone (O_3).

Methods: The study is conducted at the CAP exposure facility at the Gage Occupational & Environmental Health Unit, Toronto. Allergic asthmatic non-smokers 18-49 yrs old are exposed to "real life" ambient pollutants (150 µg/m^3 CAP + 200 ppb O_3) and filtered air (FA), in a randomized crossover design. Exposures are 1-hr in duration, followed by an allergen inhalation challenge (AIC) to determine the allergen provocation concentration causing a 20% decrease in FEV_1 (PC_{20}).
The exposure protocol includes 3 consecutive days of testing, with a 4-week washout period between exposures. Day-1 tests include a methacholine challenge (MC) and sputum induction. Day-2 is the exposure day and tests include: pre-exposure blood sampling (CBC, cytokines/mediators, markers of bone marrow stimulation, IgE, surfactant proteins A & D), spirometry & lung volumes; post-exposure spirometry, lung volumes & symptoms, followed by an AIC. Day-3 is the 24-hr post-exposure testing, including a MC, sputum induction, blood sampling, and symptom assessment.

Findings: To date, 50 individuals have been pre-screened, 11 screened on site, while only 5 met all the entry criteria. Among the 5 enrolled, 4 completed the study. The post exposure MC showed one doubling dose decrease in PC20 (increase in bronchial hyper-reactivity) in one patient after exposure to CAP+O_3 and in 3 subjects after FA. For the allergen challenge, two subjects had a one doubling dose increase in allergen response after exposure to CAP+O_3 when compared to FA, while the others had similar responses for both. Bone marrow stimulation was evaluated with methylcellulose assays for eosinophil/basophils colony-forming unit (Eo/B CFU). There was a trend suggesting an increase in Eo/B CFU in the presence of IL-5 and GM-CSF stimulation after exposure to CAP+O_3 when compared to results after exposure to FA ($p = 0.06$ and $p = 0.17$, respectively). A small increase in the eosinophil count was seen in 3 subjects 24 hours after both exposures to FA and CAP+O_3. Sputum data was available for only 2 subjects. One subject had an increased eosinophil response after FA while the other had an increase after CAP+O_3. Subject 1 was virtually deficient in functional SP-D & A obtained from induced sputum before & after both exposures, but 24-hrs post CAP+O_3 exhibited high levels of cleaved or non-functional SP- and was not detectable using ELISA; some small fragments of SP-A may also have been present. Subject 2 exhibited intact SP-D & A pre & post for both exposures; and ELISA for SP-D increased after both exposures.

Conclusions, relevance and future directions: The results provided to date are only descriptive. So far we have not seen a clear trend pointing to an increased bronchial hyper-reactivity on methacholine or allergen specific challenge after exposures to CAP+O_3, when compared to exposures with FA. A small increase in the 24-hr eosinophil count seen after both exposures is likely related to the allergen exposure in the previous day, and there is no suggestion that it is different among exposures to CAP+O_3 compared to FA. There is a trend showing an increased Eo/B CFU after exposure to CAP+O_3 but the number of subjects is too small to reach any statistical significance. Based on the results to-date and to facilitate recruitment, we have modified the protocol. The changes include: elimination of the lengthy allergen challenge (a day-long follow-up); exposures lengthened 2 hours; exposures will include CAP+O_3 and O_3 alone, in a crossover design, with no FA. From a public health perspective, it is important to understand the interaction between air pollutants and allergy. This knowledge is critical for risk management and the development and implementation of Air Quality Standards.

Oral Treatment with *Lactobacillus reuteri* attenuates the allergic airway response in mice through induction of regulatory T cells

Programme B - Diagnostics and Therapeutics
Mechanisms and Biomarkers

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Objective/Purpose: The therapeutic potential of probiotics organisms in atopic diseases is an area of increasing interest. Previously, we have demonstrated that oral treatment with live *L. reuteri* can attenuate airway inflammation and hyperresponsiveness in response to antigen challenge in a murine model of asthma. The objective of the current study was to delineate the immunomodulatory pathways underlying these effects.

Methods: BALB/c mice were treated daily with *L. reuteri* ($1x10^9$ cfu) for 9 days by gavage. FACS analysis was used to determine $foxp3^+CD4^+CD25^+$ T cell populations in spleens following treatment with *L. reuteri* or vehicle control. Purified $CD4^+CD25^+$ T cells from spleens of treated mice were transferred into ovalbumin-sensitized mice that were subsequently exposed to intranasal antigen challenge. The airway responsiveness to methacholine, influx of inflammatory cells to the lungs, and cytokine levels in bronchoalveolar lavage fluid of recipient mice were assessed.

Findings: Mice receiving 9 days oral treatment with *L. reuteri* demonstrated a significant increase in the percentage of $foxp3^+CD4^+CD25^+$ T cells in the spleen. Antigen induced airway hyper-responsiveness, eosinophil influx to the airways and inflammatory cytokine levels in bronchoalveolar lavage fluid were suppressed by adoptive transfer of $CD4^+CD25^+$ T cells from spleens of animals fed *L. reuteri* but not from vehicle controls.

Deliverables: This study identifies key pathways responsible for *L.reuteri* induced attenuation of the allergic airway response in mice.

Relevance: The insight into the mechanism of action of probiotics provided by this study will aid in the assessment and selection of candidate probiotics strains that have the potential to provide a novel immunomodulatory strategy for the treatment of allergic disorders such as asthma.

The burden of work-related asthma in Canada: a comparison of two surveillance programs

Programme C – Public Health, Ethics, Policy and Society
Environment, Health and Allergic Disease

NA Garzia, M Koehoorn, PA Demers, SM Kennedy
Supervisor: SM Kennedy

Objective/Purpose: The first objective of this study was to assess asthma in relation to work and compare results using data from two national surveillance programs with different asthma and work information. The second objective was to estimate the burden of work-related asthma (WRA) in Canada.

Methods: Data was obtained from two national surveillance programs; Canadian Community Health Survey 2002/03, Cycle 2.1 and the National Population Health Survey, Longitudinal Household Component (1994/95-2002/03), referred to as Survey 1 and 2 respectively. Survey 1 is cross-sectional and Survey 2 is longitudinal (5 survey cycles); the sample populations included respondents between 15 and 65 years of age and working full-time in 2002/03 (Survey 1: n= 56,217; Survey 2: n=5,707). For both surveys, current asthmatics were identified as those who self-reported "yes" to "do you have asthma?". Survey 2 respondents were also asked "when were you diagnosed with this asthma?" to identify adult-onset or childhood-onset asthma. For exposure assessment, occupational codes were linked to an asthma-specific job exposure matrix (JEM) to determine high risk (HR) and low risk (LR) job groups for occupational asthma using current job among Survey 1 respondents. Using longitudinal data, job risk groups were assigned to both job held at time of asthma-onset for most adult-onset asthmatics and current job held for childhood-onset asthmatics among Survey 2 respondents. Both surveys provided estimates that are intended to reflect the prevalence across Canada's population, enabling us to estimate the burden of WRA by estimating the number of prevalent asthma cases attributable to working in HR jobs. Estimates were derived separately for men and women, and by age of asthma-onset.

Findings: In Survey 2, men and women had a higher asthma prevalence in HR jobs compared to LR jobs, whereas the opposite finding was seen among men and women in Survey 1. The results from Survey 1 are likely biased by the healthy worker effect, because the data is cross-sectional and only information on current asthma status and current job was available. Further assessment of asthma in relation to HR jobs among Survey 2 indicated a large burden of childhood-onset asthma among women and a large burden of adult-onset asthma among men. The estimated number of prevalent childhood-onset asthma cases attributable to HR jobs among women, suggest that approximately 17,267 of 5.4 million working women may be suffering from work-exacerbated asthma. The estimated number of prevalent adult-onset asthma cases attributable to HR jobs among men, suggest that approximately 18,816 of 7.4 million working men may be suffering from occupational asthma.

Deliverables: This study shows that Canadian surveillance programs must at least collect information on age of asthma-onset and job held at time of asthma-onset, regardless of survey design, for estimating the burden of WRA and minimizing potential bias of the healthy worker effect. Improved surveillance is necessary for Canada, as this study indicates that many thousands of asthmatics in Canada suffer from asthma that is either "caused" or "exacerbated" by their working exposures.

Relevance: This study identifies limitations in Canada's surveillance programs. With improved surveillance data we can identify at-risk groups and implement workplace intervention to alleviate the social and personal burden of WRA in Canada. Findings have been and will continue to be communicated to key stakeholders through meetings and lay summaries on occupational health websites (e.g. UBC - WorkSafeBC Partnership, Centre for Health & Environment Research).

Interactions between *Aspergillus fumigatus* Conidiospores and Airway Cells

Programme A – Gene-Environment Interactions

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Supervisor: Dr. Scott J. Tebbutt

Objective/Purpose: *Aspergillus fumigatus* is a ubiquitous mould associated with a spectrum of diseases ranging from aspergillomas and invasive aspergillosis, to rhinosinusitis, asthma, and allergic bronchopulmonary aspergillosis. The rise in prevalence of asthma and allergic diseases, and the growing immunosuppressed or immunocompromised population at risk of fungal infections, has brought this important pathogen and allergen increased attention, but much of its basic biology remains unknown. Our research studies the interaction between host and pathogen/allergen at the cellular level and focuses on internalization of conidiospores by airway epithelial cells, with the aim of determining how this finely tuned interaction is altered in asthma.

Methods: We have developed a cell-culture model of infection using the bronchial epithelial cell line 1HAE, and genetically engineered *A. fumigatus* conidiospores expressing high levels of green fluorescent protein (GFP). Conidiospores are visualized by fluorescence microscopy following infection of 1HAE cell monolayers. Treatment with an antibody raised against *A. fumigatus* allows discrimination between extracellular and internalized conidia, and thus quantification of spore uptake. A nystatin protection assay, in which the anti-fungal agent nystatin is used to kill extracellular spores but not internalized ones, also allows quantification of spore uptake. We have used fluorescence-activated cell sorting (FACS) to select and isolate 1HAE cells infected with conidiospores.

Findings:

1. Fluorescence microscopy and the nystatin protection assay indicate a time dependent uptake of *A. fumigatus* spores, with internalization reaching up to 50% of bound spores within six hours of co-incubation.
2. FACS analysis indicates that up to 50% of cells bind or internalize conidiospores within six hours of co-incubation. Sorting of cells by the presence of a GFP signal is 90% accurate, based on re-sorting and microscopic visualization.

Deliverables: We have developed a cell culture model of the interaction between *A. fumigatus* spores and human airway cells. Furthermore, we have demonstrated the ability to use FACS to isolate cells directly interacting with conidiospores for further study.

Relevance: The quantification of conidiospore internalization and the ability to isolate cells in direct interaction with conidia allows further study of the host-pathogen/allergen interaction, including innate immunity responses of the host to the conidia as well as possible adaptations of the conidiospores to the intracellular host environment. We are initiating a study of the gene expression profiles of both the host cells and fungal conidiospores in FACS sorted samples. We will investigate differential genetic responses of asthmatic and non-asthmatic airway epithelial cells to *Aspergillus fumigatus.*

Baseline subject characteristics for a double-blind, placebo-controlled, randomized, crossover trial of mint tea high in rosmarinic acid in adults with nasal polyposis.

Programme B – Diagnostics and Therapeutics

Loie Goronfolah, Penelope Ferrie, Susan Waserman, Judah Denburg, Laima Kott, Paul Keith

Objective/Purpose: Nasal polyposis (NP) is a common chronic inflammatory disease of the nasal mucosa that can have a major impact on patients' lives. Rosmarinic acid is a polyphenolia phytochemical found in a variety of plants including the herbs oregano and rosemary, as well as the mints. It has been found *in vitro* to have significant antimicrobial and antiviral properties, strong antioxidant and antitumor actions, and some antiallergenic properties.

Methods: 15 subjects with bilateral nasal polyps have been enrolled to date. Subjects were randomized in a-double-blind, placebo-controlled crossover trial of mint tea high in rosmarinic acid BID vs mint tea low in rosmarinic acid BID. Each treatment period is of 4 weeks duration separated by a 4 week washout period. The first treatment period is preceded by a 2 week baseline phase.

Findings: 5 out of the 15 subjects had previous nasal polyp surgery with recurrence of their nasal polyps (33.3%). None of our patients reported sensitivity to ASA. Seven subjects(46.6%) had positive allergy skin tests. 14 out of 15 (93.3 %) reported symptoms of nasal blockage or stuffiness. 11 out of 15 (73.3%) reported that sleep is affected or disturbed. 80% of our subjects reported some impact of the disease on their sense of smell. Left Polyp size pre treatment ranged from 1 to 3 with mean of 1.73. Right polyp size pre treatment ranged from 1 to 3 with mean of 1.86(0= no polyps present, 1= polyps restricted to middle meatus, 2= polyps not restricted to the middle meatus, but not below the lower edge of the inferior turbinate & 3= polyps reaching below the lower edge of the inferior turbinate). The blood eosinophil level ranged from 0.1to 1.4 with a mean of 0.35, SD 0.31. Blood eosinophil levels were not correlated with either polyp size nor to the positivity of skin tests. Nasal peak inspiratory flow measures ranged from 30 to 240 with mean 148.6.

Conclusion: Nasal polyps are a chronic disease with high risk of recurrence after surgery. Nasal polyps have a high impact on patients' quality of life including sleep and sense of smell. This chronic condition may be useful to identify new, potentially beneficial, anti-inflammatory agents.

In vivo and *Ex vivo* Characterization of the Bronchial Epithelium from Asthmatic and Normal Individuals.

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Objective/Purpose: The bronchial epithelial cell is the first cell of contact and a physical barrier to the external environment. Detailed cellular examination of bronchial biopsies and lavage fluid has provided convincing evidence of epithelial damage and aberrant repair in asthma. Excessive epithelial damage and fragility could arise from an enhanced susceptibility to injury, inappropriate repair or a combination of both. The aim of this study was to compare the ability of bronchial epithelial cells from asthmatic and non-asthmatic subjects to differentiate in air-liquid interface (ALI) culture and characterize their response to wounding and RSV challenge.

Methods: Differentiated (ALI) cultures were generated from primary human bronchial epithelial cells obtained from non-transplantable lungs of normal (n=5) and asthmatic donors (n=3). Bronchial sections and ALI cultures generated from the same airway were analyzed by immunohistochemistry for Cytokeratin (CK) 5, CK-18, ZO-1, E-cadherin and p63. Specific staining was evaluated using ImagePro Plus software. For wounding studies ALI cultures were wounded in a cross-hatch manner and repair was followed using DCI images for 96 hours post wounding. For RSV challenge ALIs were infected with RSV (MOI3) for 24, 48, and 96 hours and supernatants were analyzed by ELISA.

Results: 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 p63 and decreased number of cells expressing the ciliated cell marker CK-18 compared to controls ($p<0.001$). Asthmatic airways and ALIs also expressed less tight-junctional protein ZO-1 and adherin junctional protein E-cadherin ($p<0.001$). When wounded Asthmatic ALI cultures were unable to repair compared to ALI cultures obtained from normal individuals. Basal expression of inflammatory cytokines IL-6 and IL-8 were significantly higher from asthmatic ALIs ($P<0.05$). However, in response to RSV infection, asthmatic ALI cultures released lower levels of these IL-6 and IL-8 compared to controls ($P<0.05$).

Conclusion: This parallel *in vivo* and *in vitro* study of pediatric asthmatic airways demonstrates that the airway epithelium is remodelled early on in the disease and displays inappropriate repair following challenge with wounding or viral infection. Our data suggests that the asthmatic epithelium is unable to form an appropriate mucosal immune barrier. Future work is required to determine the factor/s involved in this inappropriate airway remodelling.

Novel associations of genetic polymorphisms in the interleukin-1 receptor / Toll-like receptor signaling pathways with atopy and atopic asthma

Programme A – Gene-Environment Interactions
Genes and Early-Life Determinants

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Objective/Purpose: A popular explanation for the increase of asthma and other allergic diseases is the "hygiene hypothesis". The interleukin-1 receptor (IL-1RI)/Toll-like receptor (TLR) signaling pathways constitute vital components of the innate immune system which is essential for host survival because of its ability to recognize invading pathogens and mount defensive responses. The objective of the study was to identify associations of polymorphisms in IL-1RI/TLR pathway genes with atopy and asthma.

Methods: We used four large cohorts which contained a total of 5615 individuals to investigate associations of 227 single nucleotide polymorphisms (SNPs) in 19 genes in the IL-1RI/TLR signaling pathways with four phenotypes: atopy, asthma, atopic asthma and airway hyper-responsiveness (AHR). The study group consisted of three Canadian family-based cohorts and one Australian population-based case-control cohort. A general allelic likelihood ratio test was performed for each of the four cohorts as well as a joint analysis of family-based and case -control samples. P values were corrected for the effective number of independent SNPs at each locus as well as the effective number of phenotypes.

Findings: In the joint analysis SNPs in *IL1R2*, *TLR1* and *TLR5* were associated with atopy after correction for multiple comparisons. In separate analyses, the following gene-phenotype associations were observed in one cohort only: lipopolysaccharide binding protein (LBP) with atopy; interleukin-1 receptor-associated kinase 2 (IRAK2), TLR3 and TLR1 with atopic asthma; interleukin 18 receptor accessory protein (IL18RAP), TLR5, myeloid differentiation primary response gene 88 (MYD88) and LBP with AHR after correction for multiple comparisons.

Deliverables: This research has identified genes potentially involved in susceptibility to allergic disease. These genes will be used to determine gene-gene and gene-environment interactions and will be targets for other AllerGen researchers to investigate further.
Relevance: Identification of novel susceptibility genes will provide new therapeutic targets and may prove to be predictive for the development of allergic diseases.
The findings will be published in peer-reviewed journals.

Infant lung function in a cohort of infants after severe wheezing illness

Programme A – Gene-Environment Interactions
Population and Environments

C. Keast, S. Balkovec, F. Ratjen and P. Subbarao
Supervisor (P Subbarao)

Objective/Purpose: Infants with severe wheezing illnesses are thought to be at higher risk for the development of asthma. However a proportion of these infants have self-resolving wheezing illnesses that do no respond to typical anti-asthma treatment. We propose to study infants with a history of severe wheezing illness to prospectively document their degree of lung dysfunction and response to bronchodilators and to follow their course with repeat lung function.

Methods: Infants who had been hospitalized or received oral steroids for wheezing illnesses were recruited into the study. We recorded plethysmographic lung volumes and forced expiratory volumes using raised volume rapid thoracoabdominal compression techniques.

Findings: Thus far 14 infants (aged 22-114 weeks, 11 male), have been enrolled in this study. Six infants have returned for two visits. The mean z-score for FEV0.5 was 0.43 (95% CI -0.1, 0.75) for the baseline visit and 0.21 (95% CI -0.14,0.56) for the six infants at their second visit. Three infants had FEV0.5 z-scores < - 1.5. The baseline and follow-up mean FEF2575 z-scores [baseline -1 (95% CI -2,0.01), follow-up -0.56 (95% CI -1.3, 0.2)] and mean FEF85 z-scores [baseline -1.5 (95% CI -2.1,-0.8), follow-up -1.3(95% CI -2.1,-0.4)] values showed a greater degree of dysfunction. Lung volumes (FRC z-scores) were elevated at baseline [1.89 (95% CI 1.6, 2.2)] and follow-up [1.1 (95% CI 0.8, 1.4)]. The group as a whole did not have a significant response to bronchodilators at either the baseline or follow-up visit.

Infants post severe wheezing illness have normal FEV0.5 but decrements in their small airways measures. They also have elevated lung volume measurements. These data support the theory that the focus of injury in wheezing illness is in the small airways. Further recruitment and follow-up is required to determine whether infants with severe wheezing illness develop asthma.

Deliverables: It is anticipated that final conclusions based on this study will aid in the development of the Allergen CHILD Study.

Relevance: Based on findings from this study, earlier diagnostic ability to predict persistent asthma will aid in appropriate treatment and maintenance therapy of wheezing infants.
Once the final study is completed, the findings will be presented at several national meetings and published in journals aimed at the target end-user. We will also present our results to consensus group leaders in order that it may inform future guidelines for therapy.

Airway Hyperresponsiveness in a Mouse Model of Allergic Asthma Following Exposure to Concentrated Ambient Particles and Ozone: Role of Stem Cell Factor

Programme A – Gene-Environment Interactions
Environments, Populations & Society

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Objectives/Purpose: Exposure to air pollution results in exacerbation of pulmonary symptoms in asthmatics, which may be due in part to mobilization and/or activation of immune cells. However, the signaling pathway(s) responsible for the pollution-induced migration of these cells to the airways of asthmatics remains to be elucidated. The receptor tyrosine kinase c-Kit and its cognate ligand, stem cell factor (SCF), are expressed by epithelial cells in the airways of asthmatics and in specific populations of immune cells. The present study was undertaken: 1) to determine the functional consequences of exposure to pollution on airway responsiveness in a murine model of allergic asthma. 2) To determine whether c-Kit expression and/or signaling is altered by exposure to pollutants.

Methods: Female Balb/c mice (6-8 weeks old) were sensitized to ovalbumin (OVA) on days 0 and 7, and challenged with aerosolized OVA or PBS on days 14-16 (25 min/day). On day 17 freely moving control (OVA sensitized, PBS-challenged; OVA/PBS) and allergic (OVA-sensitized, OVA-challenged; OVA/OVA) mice were exposed to real-world concentrated ambient particles (CAP) using the Harvard Ambient Particle Concentrator, ozone (O_3), or the combination (CAP+O_3) for 4 hours. The final exposure conditions were: CAP: 0.2-1.5 mg/mm^3; ozone: ~2 ppm. The flexiVent system was employed to assess methacholine (MCh) responsiveness and respiratory mechanics immediately after exposure. Lung tissues were obtained and homogenized for Western blotting for SCF, c-Kit and actin (as a loading control), immunoprecipitation with anti-c-Kit and subsequent blotting for phosphorylated c-Kit.

Findings: In this sub-acute model of murine allergic asthma, there were no significant alterations of pulmonary function (as indicated by pressure-volume loops, total lung capacity) or MCh-responsiveness (central airway resistance) between the unexposed OVA/OVA and OVA/PBS animals. However, OVA/OVA mice exhibited increased responsiveness to MCh following exposure to CAP+O_3, relative to unexposed OVA/OVA ($P<0.0001$) and CAP+O_3 exposed control (OVA/PBS, $P<0.05$) mice. c-Kit protein was increased moderately in both control and CAP+O_3-exposed OVA/OVA mice, compared to their respective controls. However, phospho-c-Kit expression was increased 2-4-fold in the CAP+O_3-exposed OVA/OVA mice. Furthermore, SCF co-immunoprecipitated with anti-c-Kit antibody;supporting the activation of c-Kit by its cognate ligand.

Deliverables: The establishment of this murine pollution exposure system allows investigation of the mechanisms of pathological exacerbations caused by environmental pollutants. In this study we examined the relationship between c-Kit expression and augmentation of airway responsiveness following exposure to air pollution. This is the first data to demonstrate SCF/c-Kit signaling after pollutant exposures.

Relevance: The role of SCF and c-Kit in tissue homing of hematopoietic progenitor cells continues to be an area of vigorous research. Ongoing investigations in our laboratory seek to elucidate the interaction(s) between hematopoietic progenitor cells and specific pollutant exposures. A thorough understanding of the impact of pollution exposure on airway SCF/c-Kit expression in allergic asthma could have therapeutic implications in preventing exacerbations of severe chronic disease caused by air pollution.

Effect of Allergen Challenge and Withdrawal on Chronic Smooth Muscle Remodeling in an Equine Model of Asthma

Programme B – Diagnostics and Therapeutics
Mechanisms and biomarkers

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Supervisor: Jean-Pierre Lavoie.

Objective/Purpose: Increased airway smooth muscle (ASM) mass may contribute to airway obstruction in asthma. It is unknown if this remodeling is reversible under natural conditions once it is established. In this study, peripheral ASM was measured in heaves-affected horses, a natural model of chronic asthma, after a 30-day challenge and after a 3-month period of antigen withdrawal.

Methods: Eleven adult horses (6 heaves-affected and 5 controls) were kept in an antigen poor environment for a minimum of 3 months. They were then exposed to environmental allergens for 30 days. Diseased horses had been intermittently symptomatic for at least 3 years prior to the study. Lung biopsies were obtained under thoracoscopic guidance after antigen withdrawal (T0) and after antigen challenge (T30). Smooth muscle was stained by immunohistochemistry for smooth muscle specific α- actin and its area in the airway wall was measured and corrected for airway size using standard morphometric techniques.

Findings: All horses had normal lung function at T0 while at T30, only heaves-affected horses developed airway obstruction. Compared to controls, diseased horses had more than twice the amount of smooth muscle at T0 and T30 ($p \leq 0.01$). There was no further significant increase in the 30-day challenge period.

Deliverables: Results of this study indicate that ASM remodeling developing over years may not be reversible by only removing the allergen exposure. Alternatively, they could indicate that remodeling may require more than 3 months to reverse. Furthermore, no notable increase in ASM was seen after a month long continuous challenge in animals with pre-existing airway remodeling, suggesting that it may reach a plateau. These results also provide the basis for a long term longitudinal study of smooth muscle remodeling reversibility.

Relevance: These results suggest that ASM remodeling reversibility does not occur over a short period of time in chronic asthmatic animals, therefore highlighting the importance of early diagnosis as well as prevention of ASM hypertrophy early in the course of the disease. Furthermore, these results suggests that pharmacological intervention maybe required for ASM mass to return to normal once it is chronically established.

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CD34 Involvement in Mast Cell and Eosinophil Infiltration in Both Allergic Asthma and Melanoma Development in Mice

Programme B - Diagnostic and Therapeutics
Role of mast cells and eosinophils in allergic inflammation and fibrosis of the lung

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Objective/Purpose: In the mouse model, asthma is characterized by the infiltration of hematopoietic cells into the lung tissue and bronchial-alveolar space, associated with remodeling and airway hyperresponsiveness. Melanoma formation also involves infiltration by immune mediator cells, although interactions between these immune cells and cancer cells are more poorly understood. In both conditions, mast cells and eosinophils are prominent infiltrating cell types, and are hypothesized to directly cause tissue inflammation and damage in asthma, while leading to angiogenesis or tumor rejection in melanoma. Our group has previously shown a role for the sialomucin CD34 on mast cell migration in vivo and more recently on eosinophil migration in asthma. Our current work suggests CD34-mediated enhancement of migration is not limited to the lung, but is also required during melanoma development for cell recruitment to the tumor. These findings lead us to speculate that CD34 may play a broad role in mast cell and eosinophil migration across a diverse range of conditions.

Methods: CD34-/- and wild-type C57Bl/6 mice were injected subcutaneously with B16 melanoma cells, to induce solid tumor development. Palpable tumor masses were measured to assess tumor growth in vivo, on a nineteen day timeline. At experimental endpoints, animals were sacrificed to determine final tumor masses and tumor were processed for histology. Histological tissue preparations were stained with hematoxylin/eosin and toluidine blue for evaluation of tumor morphology and mast cells counts. Ly5.1 wild-type animals were also irradiated and reconstituted with CD34-/- or control C57Bl/6 bone marrow and allowed to recover over ten weeks. Reconstitution levels were confirmed and tumor timecourses performed in these animals to determine the role of CD34 specifically on hematopoietic cells in tumor growth.

Findings: We found that CD34-/- mice had altered tumor growth kinetics, with significantly smaller tumors at an early timepoint. At a later timepoint however, tumor growth in CD34-/- animals had exceeded growth in wild-type animals. Histology results indicated similar general morphology in all tumors, with lower mast cell infiltration observed in tumors from CD34-/- animals. Tumors grown in CD34-/--reconstituted animals exhibited the same increase in size at later timepoints, but no difference at early timepoints, compared to wild-type-reconstituted animals. A decrease in mast cell infiltration was also seen in these animals, confirmed a hematopoietic role for CD34 in this phenotype. These findings demonstrate a complex phenotype, with CD34 being pro-tumorigenic early in development, but anti-tumorigenic at later timepoints. These differences likely reflect kinetic differences in the varying CD34-expressing cell types infiltrating the tumor.

Deliverables: Our findings have demonstrated a role for CD34 in mast cell and eosinophil migration in asthma, and now in melanoma. These findings suggest a broad role for CD34 in migration of these cell types, which have been implicated in many diseases and ailments. Furthered understanding of the role of CD34 may lead to novel therapeutics to modulate cell migration, as a means of decreasing inflammation and tissue damage in a range of conditions.

Relevance: Our results suggest CD34 plays an important role in mast cell and eosinophil migration, presumably by reducing adhesion and enhancing invasiveness of these cells. This decreased homing efficiency results in a significant decrease in asthma pathology and altered tumor growth kinetics. As mast cell and eosinophils are believed to play a role in most, if not all, allergic diseases, further studies using CD34-/- will provide new understanding of cell migration in these conditions.

Genetic dissection of airway hyperresponsiveness and its impact on allergic asthma

Program B - Diagnostic and Therapeutics

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Objectives / Purpose: Asthma is a chronic, multi-factorial and inflammatory disease of the respiratory tract, due by a complex trait with a strong genetic component. Previous studies have demonstrated major gene effects underlying the traits of susceptibility or resistance to this disease, as well as of several intermediate phenotypes related to certain components of host immune/inflammatory pathways. Given that airway hyperresponsiveness (AHR) is a complex trait, which is associated with asthma susceptibility, we intend to identify the genetic loci underlying the differences in airway responsiveness to methacholine and susceptibility (A/J mouse strain) or resistance (C57BL/6 mouse strain) to ovalbumin-induced asthma, to analyze the inheritance of disease-related phenotypes that could be considered biomarkers of asthma in the informative recombinant strains and to ascertain their genetic association and functional co-segregation with the phenotypes of either asthma susceptibility or resistance.

Methods: Population: screen of 36 AcB/BcA Recombinant Congenic Strains (RCS) of mice previously developed, from which informative strains will be selected for the production of F2 generations (6 crosses with a total of 1400 mice). Phenotype: Airways Responsiveness to methacholine measured by a non-invasive method (Whole Body Plethysmograph) and correlated with an invasive technique of direct assessment of resistance. Genotype: Microsatellites and 200 custom designed Single Nucleotides Polymorphisms (SNPs) (Sequenom iPlex). Analysis of the phenotype-genotype correlations: Quantitative Trait Loci (QTL) and statistical analysis of markers' correlation with the AHR of AcB/BcA backcrosses.

Findings: The generation of the RCS was performed and previously published. 36 AcB/BcA strains were phenotyped and the analysis of the baseline airway responsiveness phenotype distribution of the RCS of mice has allowed us to confirm the existence of several chromosomal regions which have been previously identified as being significantly, or suggestively, associated with methacholine responsiveness.

These include two regions on chromosome 2, near 5 - 30 and 74 - 105cM respectively, a region on chromosome 6 near 20cM and a more distal region near 50cM, a region on chromosome 10 near 59cM, a region on chromosome 15 near 49cM, and a region on proximal chromosome 17 near 11 – 22cM. Furthermore, we have identified several novel regions that are significantly associated with airway responsiveness in our RCS panel but have not previously been reported. These include regions on distal chromosome 1, 5, 7, and 8, a proximal region on chromosome 10, a centrally and a distally located region on chromosome 12 and a region centrally located on chromosome 17.

We selected the three most informative RCS of mice and each of them was backcrossed to two strains (one parental exhibiting opposite phenotype and one genetically unrelated mouse strain with the same phenotype). We have completed the phenotyping of 1300 F_2 backcrosses (out of the 1400 F2 planned) in order to map the identified regions with greater resolution. The genotyping of these mice with our panel of polymorphic SNPs will allow us to refine our mapping.

Deliverables: We intend to identify of genes involved in complex traits that significantly contribute to asthma susceptibility which will lead to a better understanding of the molecular mechanism of the pathology of the disease.

Relevance: Understanding the inheritance of disease-related phenotypes might allow us to identify asthma biomarkers that represent a new, expedite and reliable tool for the diagnosis of asthma and improve the design of novel therapeutic approaches.

Secretion of anti-inflammatory prohormone SMR1 from rat salivary glands is regulated by the autonomic nervous system

Programme B - Diagnostic and Therapeutics
Mechanisms and Biomarkers

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Objectives/Purpose: Stressful life events are known to modulate inflammatory responses in many diseases including asthma. One mechanism by which stress regulates inflammation is the activation of the sympathetic nervous system, and consequent modulation endocrine glands. In rats, we determined that the sympathetic nervous system regulates pulmonary inflammation by modulating the function of the submandibular gland. The prohormone SMR1 (submandibular rat-1) can be cleaved to form two peptides with anti-inflammatory activities that are able to decrease allergic pulmonary inflammation. A mimic of one of these peptides is being developed as a therapeutic and is effective in rats, mice, dogs, sheep, cats, and isolated human neutrophils in models of pulmonary inflammation, food allergy, septic shock, pancreatitis, and spinal cord injury. The release of related anti-inflammatory mediators by salivary glands may be important in human disease, and the regulation of this pathway warrants investigation.

We have evaluated the effect of sympathetic and parasympathetic mimetics and cervical superior ganglionectomy on the expression, processing, and secretion of SMR1 in rats.

Methods: Male Sprague-Dawley rats were injected interperitoneally with vehicle (saline) or with sympathetic mimetic isoproterenol or parasympathetic mimetic pilocarpine. Surgical removal of the superior cervical ganglion was performed on some rats. Saliva, blood, and tissues were collected from rats and analyzed for expression of SMR1 mRNA and protein.

Findings: SMR1 is expressed in rat submandibular, sublingual, and parotid glands in multiple protein species that result in part from N-glycosylation. The SMR1 protein is also present in other tissues including testis and lung and in blood, and shows tissue-specific processing. Beta-adrenergic (sympathomimetic) stimulation causes the rapid and complete secretion of SMR1 protein from all major salivary glands into the saliva. Cholinergic (parasympathomimetic) stimulation causes the secretion of significantly less SMR1 into saliva. Removal of the superior cervical ganglion that innervates the salivary glands causes changes in SMR1 mRNA and proteins levels in the salivary glands.

Deliverables: Methods have been developed to evaluate the expression and post-translational modification of SMR1. We have determined that SMR1 secretion but not processing is regulated by the autonomic nervous system. We have determined that SMR1 is present in blood and many tissues where it may have biologically relevant anti-inflammatory functions and that the protein is secreted in response to sympathetic stimulation. This information will facilitate studies on analogous pathways in humans.

Relevance: The release of SMR1 and its fragments into saliva and plasma in response to stress may be important in regulating the response to allergic inflammation. Pharmaceutical development of SMR1 peptide mimetics will be aided by information on the endogenous regulation of SMR1and related anti-inflammatory peptides in neuroendocrine pathways. Future work will aim to evaluate the role of this stress-regulated salivary gland peptide release in immune responses to endotoxic shock and asthma.

School board policies regarding allergy management in Quebec

Programme C – Public Health, Ethics, Policy and Society
The Development, Implementation, and Evaluation of Strategies to Promote the Well-Being of Children and Youth with Allergies and/or Asthma

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Objective / Purpose: Food allergy has a major impact on a child's life at home and at school. In 2006, Ontario legislated the management of anaphylaxis in public schools by implementing Sabrina's Law. As the best approach to ensure the proper management of allergies/ anaphylaxis in schools is unknown, it is likely that practices vary widely across Canada. Our objective is to describe the approaches of Quebec school boards towards managing allergies and to determine the level of consistency between board policies and Sabrina's Law.

Methods: All Quebec school board policies that address allergies/anaphylaxis (anaphylaxis/allergy policy, food policy, health/first aid policy, medication administration policy) were reviewed independently by 2 assessors using a checklist based on Sabrina's Law. Areas of disagreement between the assessors were discussed and agreement was obtained through consensus. School board policies were retrieved mainly from board websites and supplemented with information from the Ministry of Education and the Federation of Quebec School Boards websites. Descriptive statistics were used to report data.

Findings: There are 72 public school boards in Quebec: 60 French-speaking, 9 English-speaking, and 3 have special status for First Nations people. All have a web site. Fifty-two boards have no allergy/anaphylaxis policy. Only 3 boards (2 French-speaking and 1 English-speaking) have a specific policy regarding allergy/anaphylaxis management. These 3 boards are located in different regions: 2 included large urban areas although none covers the Island of Montreal. The policies were created between 1998 and 2005. All the elements outlined in Sabrina's Law were included with the exception of 1 board not addressing the possibility of emergency administration of epinephrine by school personnel in the absence of pre-authorization by parents. However, details of implementation strategies were not always specified. Although the 3 boards require regular training on anaphylaxis for employees in direct contact with students, none explicitly describe modality of training. In addition, 17 boards (14 French-speaking and 3 English–speaking) have policies addressing some aspects of allergy avoidance/ anaphylaxis management (5 with food policy, 11 with first aid / health or medication administration policy, 1 with both). Food policies were similar: they forbid the use of peanut oil in cooking at school and recommend clear identification of foods containing peanut, nuts, milk, egg, soy, fish, shellfish, sesame and wheat.

Deliverables: Only 4% of Quebec boards have specific policies regarding allergy/anaphylaxis management. Although most boards do not have a specific allergy/anaphylaxis policy, 24% at least address allergy in other policies. These policies were complete and included almost all elements of Sabrina's Law although this legislation is not applicable in Quebec. Since few boards had policies, it was not possible to examine factors potentially associated with the existence of a policy.

Relevance: It is not known why only a small number of Quebec boards have specific policies regarding allergy/anaphylaxis. Schools in Quebec are staffed, at least part-time, by a nurse. It is hypothesized that these nurses are responsible for developing an approach to life-threatening allergies and ensuring all school personnel are properly educated. We are currently interviewing individual schools to better understand the Quebec approach. School environments will then be compared across Canada to determine if the statutory approach in Ontario is more effective than the regulatory approach in other provinces for protecting children with severe allergies.

Arginase and airway hyperresponsiveness are induced by air pollution in a murine asthma model

AllerGen Theme II: Environments, Populations & Society
Environmental effects on allergic airway disease

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Objective/Purpose: Exposure to urban air pollutants (particulate matter and ozone) can increase asthma symptoms. Induction of arginase has been proposed to contribute to the development of asthma but the role of this pathway in pollution-induced asthma exacerbations has not been explored. We examined the hypothesis that increased arginase expression and activity contributes to the development of airway hyperresponsiveness (AHR) induced by exposure to urban air pollution in a sub-acute murine model of asthma.

Methods: Female Balb/c mice were sensitized to ovalbumin (OVA) on days 0 and 7, and challenged with nebulized OVA or PBS on days 14, 15 and 16 (25 min/day), followed by exposure to concentrated ambient particles (CAP), ozone (O_3), or the combination (CAP+O_3) for 4 hours, respectively. An ozone generator was used for O_3 exposures, and the Harvard Ambient Particle Concentrator was employed to concentrate real-world ambient particles with an aerodynamic diameter between 0.1 and 2.5 μm for CAP exposures. The flexiVent system was employed to assess responsiveness to methacholine immediately after the exposure. Bronchoalveolar lavage was performed for differential cell counts and lung tissues were obtained for isolation of RNA and proteins. Expression and activity of arginase isoforms were determined in lung homogenates using Western blotting and colorimetric endpoint assays, respectively.

Deliverables: OVA/OVA mice exhibited AHR following exposure to CAP+O_3, compared with unexposed OVA/OVA mice ($P<0.0001$) and CAP+O_3 exposed OVA/PBS controls ($P<0.05$). Western blotting of lung homogenates demonstrated increased expression of arginase 1 and arginase 2 under control conditions in OVA/OVA mice, compared with OVA/PBS. Following exposure to CAP+O_3, OVA/OVA mice exhibited a significant 2-fold increase in arginase 1 protein expression, compared with the unexposed OVA/OVA group ($P<0.05$) and the exposed OVA/PBS group ($P<0.005$). Augmented increases in arginase 2 and total arginase activity were also observed in response to CAP+O_3 exposures.

Relevance: Exposure to concentrated ambient particulates and ozone up-regulates arginase in this model, which may contribute to the development of AHR. This study describes a link between L-arginine metabolism and the induction of AHR following exposure to air pollution in mice, providing a rationale for future investigations of this pathway in human air pollution related asthma exacerbations. This is a new possible mechanism that could contribute to increased susceptibility of this population to air pollution. A thorough understanding of the mechanism(s) underlying exacerbations due to air pollution could lead to new pharmacological approaches to alleviate or prevent such exacerbations.

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Potential modulatory role of eosinophil-DERIVED GLUTAMATE ON T CELL Survival and PROLIFERATION

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Background: Glutamate is an excitatory neurotransmitter in the central nervous system. Prolonged exposure to glutamate leads to neuronal apoptosis through a process called excitotoxicity. Similarly, glutamate-induced excitotoxicity is a major immunoregulatory mechanism on glutamate-receptor-expressing activated lymphocytes found in lymphoid tissue. The local concentration of glutamate may be influenced by the upregulation of glutamate transporters during inflammation. We hypothesize that eosinophils express glutamate transporters that lead to the release of glutamate that modulates T cell function.

Objective/Purpose: The main objectives of this study are to determine the expression of glutamate receptors and transporters on human eosinophils, and the effect of glutamate released from eosinophils on T cell survival and proliferation.

Methods: RT-PCR and flow cytometry were used to determine the expression of ionotropic (NMDA, AMPA and Kainate) and metabotropic (mGluR1-mGluR8) glutamate receptors. Flow cytometry (Fluo-3AM and Fura Red) was used to estimate changes in intracellular calcium. Intracellular cAMP and extracellular glutamate were measured colorimetrically. Apoptosis in T cells was determined using annexin-V staining. T cell proliferation was evaluated using CFSE and flow cytometry.

Results: Human eosinophils expressed functional mGluR2 and mGluR7. Freshly isolated human eosinophils did not express any of the 6 currently known classes of glutamate transporters. However, adhesion to fibronectin-coated plates rapidly induced the expression of the XcT cystine/glutamate antiporter system and led to the release of glutamate from eosinophils within 2hr. Co-culture of glutamate-producing eosinophils with activated T cells induced both apoptosis in T cells and inhibition of their proliferation.

Conclusions: Eosinophils express functional glutamate receptors. Glutamate released by tissue-dwelling eosinophils may modify T cell function.

Funding: AllerGen.

Role of GATA-3 transcription factor in airway epithelial cells: modulation of Toll-like receptor-4 (TLR-4)

AllerGen Program B / Theme no. 3

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Objective/Purpose: As the first cells to encounter inhaled foreign particles, airway epithelial cells (AEC) are increasingly reported as contributors to the Th2-type immune response seen in asthma. AEC express Th2-type transcription factors which affect their inflammatory phenotype, as well as their further responses to stimuli like microbial patterns. We hypothesized that atopic / allergic / asthmatic AEC exhibit an increased expression of the Th2 transcription factor called GATA-3, and that such expression would reduce the expression of bacterial lipopolysaccharide (LPS)-binding Toll-like receptor 4 (TLR-4).

Methods: Nasal mucosa biopsies were collected from atopic and non-atopic patients, outside the allergy season (n = 6 per group). Primary bronchial epithelial cells were obtained from asthmatic and control patients. Primary Normal Human Bronchial Epithelial (NHBE) cells, as well as the BEAS-2B cell line were used to further investigate the expression of GATA-3 and TLR-4 expression under various conditions. BEAS-2B cells were transiently transfected with GATA-3 overexpressing and siRNA vectors. Immunocytochemistry (ICC) in nasal tissues and real-time quantitative PCR (RT-qPCR) were used to investigate TLR-4 and GATA-3 expression.

Findings: TLR-4 is expressed by epithelial cells of the upper airways. Atopy is associated with reduced TLR-4 and increased GATA-3 gene expression in nasal biopsies. ICC confirmed submucosal leukocyte infiltration in nasal biopsies from atopic patients. Asthmatic bronchial epithelial cells exhibited increased endogenous GATA-3 and reduced TLR-4 expression compared with cells from normal patients. As opposed to IFNγ-exposed cells, IL-4 and IL-13-exposed BEAS-2B concomitantly exhibited increased GATA-3 expression and reduced TLR-4 expression. Downregulation of TLR-4 was also obtained by overexpressing GATA-3 in BEAS-2B, and was partially blocked using GATA-3-specific siRNA vectors.

Deliverables: Atopy and asthma are associated with reduced TLR-4 expression in airway epithelial cells. AEC from asthmatic patients or grown in Th2 conditions were shown to express increased levels of the transcription factor GATA-3. GATA-3 would affect TLR-4 expression, and possibly interfere with microbial patterns-driven immunity.

Relevance: GATA-3 expression by AEC is novel and its role remains to be described. GATA-3 could be a relevant therapeutic target to limit the role of epithelial cell in mounting Th2 immune responses. On the other hand, TLR agonist-based therapies are presently being designed to prevent the development of allergic phenotype. Although the exact role of TLR-4 remains unclear, LPS exposure was reported as being beneficial against the development of atopy.

Overweight May Not Antedate Asthma in a Sub-Group of the 1995 Manitoba Birth Cohort

JLP Protudjer, AL Kozyrskyj, GP Sevenhuysen, AB Becker
Supervisors: AB Becker & GP Sevenhuysen

Objective/Purpose: Asthma and obesity have increased dramatically and concomitantly in recent years. While generally viewed as associated conditions, recent studies suggest the possibility of obesity as a causal factor in the development of asthma in females starting in puberty. During childhood, nearly twice as many boys as girls have asthma. From puberty onward there is gender shift with an excess of asthma in females compared to males. Notably, it is during puberty that females accumulate higher levels of body fat. The biologic activity of adipose tissue is thought to be involved in the development of asthma especially in females. Based on data from the 1995 Manitoba Birth Cohort, we considered the possibility that overweight antedates asthma in 11-12 year old girls, but not 11-12 year old boys.

Methods: At age 8-10, children were assessed for asthma by a pediatric allergist and had anthropometric measurements taken. Heights and weights were converted to age- and gender-appropriate body mass indices, and classified as normal weight (<85th percentile) or overweight (≥85th percentile). At age 11-12, children were assessed by a pediatric allergist for asthma. Incident asthma at age 11-12 years was ascertained for overweight status at age 8-10 year by the odds ratio (OR) and 95% confidence interval (CI). Consideration was given to confounding variables based on parental report collected when children were 8-10.

Findings: 248 (136 [54.8%] boys) were assessed at both age 8-10, and 11-12. There were 15 (7 [4 overweight at age 8-10] boys and 8 [1 overweight at age 8-10] girls) cases of incident asthma. No association was found between overweight at age 8-10 and incident asthma at age 11-12 for boys (OR 2.76 CI 0.59-12.9) or girls (OR 0.45 CI 0.05-3.84). Confounding variables (maternal history of asthma, maternal smoking during pregnancy or infancy, and/ or presence of mould in home) did not impact on this association.

Deliverables: In this birth cohort, overweight did not antedate asthma. This may be due to the small number of children who developed asthma between 8-10 and 11-12, given the nature of the birth cohort, which was weighted to enrolment of children with asthma at age 8-10. Thus, data from this sub-group may not be representative of all Manitoba children.

Relevance: The prevalence of both childhood asthma and overweight has risen over the past two to three decades. Both conditions are associated with substantial direct- and indirect costs. Future work should consider the temporal association between overweight and asthma across socio-economic groups. In addition to contributions to the scientific literature, these results will be communicated to decision-makers and parents through advisory notes and popular press, respectively.

Changes in Upper and Lower Airway Inflammation Following Repeated Nasal Allergen Challenges in Rhinitic Subjects With or Without Asthma

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Objective/Purpose: Asthma and allergic rhinitis may result from a similar allergen-induced inflammatory process, but studies are needed to determine possible mechanisms by which one influences the other. The objective was to determine the influence of repeated nasal allergen challenges on upper and lower airway inflammation in rhinitic subjects with or without asthma.

Methods: Thirty-six subjects with allergic rhinitis were recruited: 21 mild asthmatics (A) and 15 non-asthmatics (R). Subjects underwent a nasal control challenge with normal saline followed by 4 consecutive daily allergen challenges. At each challenge day, increasing allergen dilutions were sprayed into each nostril until a positive response occurred. Induced sputum (IS) and nasal lavage (NL) with differential cell counts were obtained 7 hours following the control, the first and the last challenge.

Findings: No difference between groups was observed in the percentage of IS and NL eosinophils at any points ($p>0.05$). No change in the percentage of IS eosinophils was observed after one and 4 days of challenge compared with control day ($p>0.05$), but some subjects (A=4 (19%) and R=3 (20%)) had an increase ($\geq$2%) after the first and/or the last challenge. An increase in the percentage of NL eosinophils was observed after 4 days of challenge compared with control challenge (mean$\pm$SEM: 16.9±23.0% vs 3.8±9.6%; $p<0.05$), but not after 1 day (8.8±17.1%; $p>0.05$).

Conclusions: Repeated nasal allergen challenge is effective to induce upper airway inflammation, but only induces lower airway inflammation in about one in 5 subjects. The presence or absence of asthma did not appear to make a difference.

Diagnosing Pulmonary Inflammation by Nuclear Magnetic Resonance

Programme B: The Immunology of Allergy & Asthma - CanGoFar & Biomarkers

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Darryl J. Adamko, MD, FRCPC

Objective/Purpose: A number of pulmonary disorders share the common pathology of inflammation. However, there are subtle differences that make each disease unique. Clinically the knowledge of ongoing inflammation is extremely important for diagnosis and the modification of therapeutics to optimize patient care. The analytical method of 1D ^{1}H-nuclear magnetic resonance spectroscopy (NMR) is able to identify metabolites, chemicals, and biomarkers in solution based upon their unique nuclear spin properties. Our hypothesis stated that compounds excreted in the urine from pulmonary patients with differing diseases and disease states would allow for identification and separation of the patient populations.

Methods: Clinical information and urine samples were collected from stable asthma patients at the Stollery Children's Hospital pediatric outpatient asthma clinic, acute asthma patients from the Emergency Department, and bronchiolitis patients at the Alberta University Hospital (n=46, 20, and 18 children respectively, ages 4-16). Age and sex matched controls were also sampled to correlate with each patient cohort. By chart review age, sex, history, physical exam, medication dosage, atopic status, and lung function from each visit were collected in order to correlate clinical presentation with urine NMR data. NMR spectra were collected on a 600MHz spectrometer and metabolite concentrations were determined using Chenomx NMR Suite software.

Findings: Fifty metabolites were referenced to creatinine and analyzed by partial least squares discriminate analysis (PLS-DA). A combination of metabolites excreted in the patients' urine allowed for the separation of acute and stable asthma, bronchiolitis and asthma, as well as the combination of all three-disease states.

Deliverables: Our study demonstrates the ability to non-invasively sample paediatric patients through urine collection and to separate differing pulmonary disease states based upon NMR analysis of metabolite excretion.

Relevance: Currently pulmonary diagnostic and treatment decisions are based upon invasive methods (bronchoscopy), or secondary indications of previous insults (pulmonary function). This method allows for the sampling of current inflammatory states by non-invasive means. Our study may lead to the ability to tailor therapeutics to specific patient needs in order to better control continuing pathology and to prevent future exacerbations.

Relationship between insulin resistance, asthma and airway hyperresponsiveness in overweight and obese children

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Objective/Purpose: Previous studies have shown a positive relationship between obesity and asthma. The nature of this relationship is not fully understood. We hypothesize that insulin resistance is the mechanism that explains this relationship.
The objective of this study was to examine the relationship between insulin resistance (assessed by HOMA-IR and [^{13}C] breath test), asthma and airway hyperresponsiveness (AHR) in 10 year old overweight and obese children.

Methods: Data were obtained from a nested case-control study from the 1995 Manitoba Birth Cohort. All children had fasting serum samples for glucose and insulin levels. Insulin resistance was defined by HOMA_IR and [^{13}C] breath test (Diatest® Isodiagnostica Inc. Edmonton). Asthma was diagnosed by a pediatric allergist and AHR was measured using the methacholine challenge test (PC20 ≤ 8mg/ml).

Findings: 123 overweight and obese children (62.6% boys) participated in this study. The mean age was 10.15 years (SE 0.04); 42.28% asthma, 52.54% AHR and 27.12% had asthma + AHR. Insulin resistance did not differ between those with or without asthma, AHR or asthma + AHR. Even though the mean levels of HOMA-IR and the [^{13}C] glucose breath test were higher in the overweight and obese girls who had asthma, AHR and asthma plus AHR the results were not statistically significant. BMI z-score did not differ for children with or without asthma.

Deliverables: Measures of insulin resistance were not associated with asthma, AHR or asthma + AHR at the age of 10 in overweight and obese children. Long-term follow up will determine if these relationships change as the boys and girls in our cohort enter and complete puberty.

Relevance: The prevalence of obesity and asthma in Canada and all over the world is increasing every year. The positive relationship between these two is well known but the nature and the etiology of it is not fully understood. In this study, we did not find any significant relationship to support our hypothesis. This is a new field of research and additional studies will be required, particularly when the children have completed their pubertal changes.

Alterations in Bronchial Immune Barrier in Response to Environmental Challenges

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Background: Fas and FasL presentation on airway epithelial cells (AEC) is critical for regulating the immune barrier. Respiratory viral infections and exposure to environmental air pollutants are two major triggers that adversely impact the epithelial immune barrier. We hypothesize that exposure to particulate matter (PM10) and Respiratory Syncytial Virus (RSV) would alter Fas/Fas Ligand expression in AEC and that this mechanism may be dysregulated in the asthmatic airway epithelium.

Methods: Differentiated air-liquid interface (ALI) cell culture model (normal and asthmatic) was exposed to RSV or PM10 alone or in combination for 24- 96 hrs, with and without mechanical wounding. Formalin fixed/paraffin embedded sections were stained with H&E and Periodic Acid-Schiff (PAS) reagent. Immunohistochemistry was used to determine Fas and FasL expression and levels were quantified via ImagePro Plus using color segmentation.

Results: Asthmatic ALIs demonstrated a higher level of PAS+ mucin staining compared to the normals. In normal ALIs wounding increased Fas expression significantly ($p<0.05$) by 48hr. Asthmatic ALIs demonstrated a significantly higher Fas expression at baseline compared to normal ALIs, however wounding did not result in any further significant change. FasL expression was similar at baseline for normal and asthmatics. Wounding in combination with allergen challenges reduced FasL expression, with a greater reduction in asthmatics vs. normal.

Conclusion: Increased baseline expression of Fas and reduced FasL expression after injury in asthmatic AECs may expose the epithelium to immune-cell mediated damage, whilst also encouraging immune cell persistence. The response to mechanical wounding of the asthmatic ALI suggests that pre-existing damage exaggerates this dysregulation and the extent to which allergen challenge contributes to airway remodeling in asthma remains to be determined.

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Genetic variation in immune signaling genes differentially expressed in asthmatic lung tissues

Programme A - Theme I: Project # 1.1

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Background: Eight genes of the immune signaling pathway (*ALOX15*, *CD14*, *CD27*, *CXCL12*, *IL2RB*, *IL7R*, *NOS2A* and *SFRP1*), shown to be differentially expressed in asthmatic lung biopsies in our previous microarray experiment, were selected as candidate genes for asthma susceptibility.

Objective: To perform an association study with these genes and asthma-related phenotypes in three independent Canadian familial asthma collections and one Australian asthma case-control study.

Methods: Tagging single nucleotide polymorphisms were selected using the HapMap public database ($r^2 > 0.8$; minor allele frequency > 0.10) and genotyped using the Illumina platform. Family-based association and trend tests for asthma, atopy, airway hyperresponsiveness (AHR) and allergic asthma phenotypes were done in each sample correcting for multiple testing.

Findings: Uncorrected associations with polymorphisms within six genes were detected with one or more of the phenotypes in one or more of the four populations ($0.001 < P < 0.05$). After correction, the 15-lipoxygenase gene (*ALOX15*) associations with AHR and allergic asthma remained significant in two Canadian samples (corrected $P = 0.022$ and 0.049, respectively), and the association of the CD14 antigen (*CD14*) with asthma remained significant in one Canadian sample (corrected $P = 0.042$). In both cases, a protective effect of the minor alleles was observed.

Conclusion: The expression profiling studies are useful to identify candidate genes for asthma as this approach has led to the first report of an association with *ALOX15* in two independent populations. Since the 15-lipoxygenase enzyme is involved in anti-inflammatory processes and produces metabolites that antagonize the leukotriene receptor, further functional and clinical investigations of the role of this biological pathway in asthma is warranted. The *CD14* association confirms previous reports and highlights the importance of gene-environment interactions in the search of genetic determinants for asthma.

Relevance: This original study demonstrates the efficiency of the microarrays in the identification of asthma candidate genes with the *ALOX15* and the *CD14* positive associations to asthma related phenotypes. Moreover, we believe that this hypothesis-driven methodological approach as well as the findings we reported should have a relevant impact in the complex trait genetic research field and should, ultimately, motivate functional and clinical studies with novel target genes such as *ALOX15*. Such studies will improve our understanding of asthma pathophysiology and perhaps, lead to the finding of novel therapeutic targets for the quality of life improvement of subjects suffering from asthma.

Genapha: Knowledge Discovery, Management and Transfer

Programme: A / Theme: I
(Knowledge Discovery, Management and Transfer - AllerGen Theme I Web Resource)

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Supervisors: Drs. Denise Daley and Scott J. Tebbutt

Objective/Purpose: Genetic studies generate enormous volumes of data, including genotypic data on thousands of polymorphisms in hundreds of candidate genes. This presents many challenges, including how to efficiently process genotype information produced by the high throughput SNP genotyping technologies and incorporate this information with phenotype and exposure data, as well as efficiently communicating the analysis results to investigators, collaborators, students, and post-docs located across Canada, the US and Australia. Our objective in meeting such challenges has been to develop a web portal to provide access to the information, thus allowing for novel hypothesis generation using the results generated by analyzing the genotype, phenotype and exposure information collected on the four genetic studies involved in the Theme 1 collaboration (Asthma Prevention Study; Study of Asthma Genes and the Environment; Saguenay Lac-St-Jean founder population; The Busselton Health Study).

Methods: In order to accomplish the objective we are developing a database that combines the analysis results, phenotype, genotype and exposure information collected for all four studies. This database will be interfaced with a web portal that will allow users to access the information via an internet connection. Users will be provided with an inventory of the results from experiments and analyses that have already been completed. For more effective dissemination of the research discoveries to end-users and the general public, education tools are also being developed ("AllerGen Animations") and online questionnaires will be deployed to study the response to the education tool.

Findings: There are real and urgent needs to share and access data among Theme I investigators and with other AllerGen investigators. In addition, some of the genotypic and phenotypic data and analysis results we are producing have value not only to the international Allergy and Asthma research community but to the entire genetic research community.

Deliverables: We have developed a website (www.genapha.ca) to facilitate the Knowledge Discovery, Management and Transfer of information generated by our studies. Since our ultimate aim is to apply the discriminate power of genotyping to clinical medicine, we also need to communicate our plans to the general public and to potential participants in our research. This includes the development of interactive tools that describe in a visually engaging manner the science and technology of genetic testing and pharmacogenetics.

Relevance: Information is of no value unless it is communicated to others, and knowledge transfer is key to the utilization of information. The genapha website will bring the power and resources of the Theme 1 AllerGen collaboration together. The website will facilitate the access and utilization of information, and will be the central resource for distribution of our findings to the AllerGen and scientific community as well as to the general public.

Controlled Human Exposures to Fine Particles ± Ozone Increases Diastolic Blood Pressure and Impairs Vascular Reactivity

Programme A/Theme II (Environments, Populations and Society)

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Supervisors: Paul Corey and Frances Silverman

Rationale: Exposure to fine particulate matter ($PM_{2.5}$) and ozone (O_3), the main contributors to urban smog, have shown strong and consistent associations with increases in cardiorespiratory morbidity and mortality. Using our controlled $PM_{2.5}$ exposure facility, we have demonstrated that short-term exposure to concentrated ambient fine particles (CAPs) + O_3 is associated with an increase in diastolic blood pressure (DBP) during and a decrease in brachial artery diameter immediately after exposure. Expanding on our recent findings, we aimed to examine the pollutant(s) responsible and the time course of response.

Objectives: 1) To elucidate the physiological mechanisms whereby inhalation of $PM_{2.5}$ ± O_3 impairs vascular function/elevates blood pressure (BP). 2) Using single and combined pollutant exposures, to quantify the contributions of $PM_{2.5}$ &/or O_3 to the previously observed findings. 3) To examine respiratory, vascular and systemic inflammatory responses, immediately and 21-hrs following pollutant exposures.

Methods: We studied 33 healthy non-smokers, aged 19-48 years, using a randomized block design. Each subject had four 2-hr exposures, 2 weeks apart, including: 1) filtered air (FA); 2) CAPs alone; 3) O_3 alone; and 4) CAPs+O_3. We obtained BP at 30-min intervals during exposures. Using linear regression, we determined the slope of the line fitted over the five BP measures for each subject's exposure. Brachial artery diameter and vascular reactivity (flow-mediated dilatation, FMD) were assessed using ultrasound imaging immediately before, following, and 21-hrs after exposure. Blood was also taken at these three time points for blood cell counts and differentials. Statistical analyses of exposure-induced changes in outcomes were made using 2-way ANOVAs that included O_3 and CAPs as binary categorical variables, the O_3*CAPs interaction and each subject's ID, as well as t tests for intra-exposure changes.

Findings: DBP increased significantly ($p<0.005$, t test) during CAPs+O_3 & CAPs, with mean slopes of 3.6 and 2.9 mmHg/2-hr, respectively. Relatively small, non-significant increases were seen for O_3 (slope=0.5) and FA (slope=1.3). The ANOVA for DBP slope showed a CAPs-effect ($p=0.01$), but no significant O_3 or interaction effects. FMD decreased immediately after, and more so 21-hrs after, CAPs+O_3 and CAPs ($p=0.02$ for CAPs-effect, 21-hr post - pre-exposure FMD). Following exposure (~1-hr after), the absolute blood neutrophil count was elevated above the pre-exposure value for all pollutant exposures. The post - pre-exposure percent change in neutrophils showed a trend towards a CAPs-effect ($p=0.07$).

Deliverables: DBP increased during while reactive hyperaemia (FMD) decreased 21-hrs after CAPs+O_3 and CAPs exposures. Both responses were mainly PM-induced. The observed PM-induced increased in peripheral neutrophils suggests an inflammatory response to CAPs. The rapid (DBP) and delayed (FMD) response suggest different mechanisms, which we are exploring with measures of autonomic and endothelial function and other measures of systemic inflammation.

Relevance: The results of this study will provide important insights into the biological mechanism(s) linking air pollution with cardiorespiratory disease. Shedding light on this link is an important step forward in bringing this important public health issue to the attention of health care providers and policy makers, and would provide potential intervention strategies**.** Resulting knowledge/understanding of pollutant interactions and health effects translates into more concise recommendations in terms of smog alerts, especially for sensitive populations such as asthmatics, the elderly & young and for health care providers.

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Association of MTHFR with Childhood Asthma and Airway Hyper-responsiveness

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Objective/Purpose: Asthma and related phenotypes are the result of genetic and environmental factors. Genes such as Methylenetaetrahydrofolate Reductase (*MTHFR*) which plays a critical role in the metabolism of dietary folate may be important in the development of asthma, atopy and other allergic disorders. *MTHFR* regulates folate metabolism, directing folates towards either DNA synthesis or methylation cycles. Folates are required for DNA synthesis and repair and *MTHFR* is associated with several complex diseases such as cancer, depression, anxiety, schizophrenia, and major mood disorders, and recently *MTHFR* has been associated with asthma and atopy.

Our objectives were to examine the associations between *MTHFR* and asthma, atopy, atopic asthma, and AHR phenotypes in four well characterized study populations from Canada and Australia.

Methods: We combined the power and resources of four study populations including 1) the Canadian Asthma Primary Prevention Study (CAPPS) cohort, which is comprised of 549 children from 545 families at high risk for developing asthma; 2) the Study of Asthma Genes and Environment (SAGE) cohort composed of 723 families from Manitoba, Canada; 3) the Saguenay-Lac-Saint-Jean and Quebec City Familial Asthma Collection (SLSJ) consisting of a French-Canadian founder population panel of 306 multigenerational families with at least one asthmatic proband; and 4) The Busselton Health Study (Busselton) cohort, a population-based, nested, case-control panel of 1,599 individuals from Australia.

MTHFR was genotyped using both Illumina (Busselton) and TaqMan (CAPPS, SAGE, and SLSJ panels) platforms. We genotyped 14 SNP's in the *MTHFR* gene, two variants associated with folate metabolism (rs1801133 and rs1801131), 3 coding variants (rs2066462, rs2274976, rs6697244), and nine tagSNPs (rs12121543, rs12134663, rs1476413, rs1572151. rs17367504, rs17375901, rs3753582, rs7533315, rs9651118). The analysis was conducted using a maximum likelihood ratio test. Allele frequencies were compared with HapMap (CEU) for each sample.

Findings: We found evidence for association of asthma with *MTHFR* (rs12121543, *P* value=0.03) in both the CAPPS and combined populations. This association does not survive correction for multiple testing (*P* value = 0.67).

MTHFR (rs2066462) is associated with asthma OR=0.20 (corrected p-value= 0.0004) and AHR OR=0.36 (corrected p-value=0.0493) in both the combined (includes all trios), and in the SAGE Caucasians.

Additionally we found that the T allele of *MTHFR* SNP rs1801133 (also known as 677C>T) is enriched in our populations, compared with HapMap. This allele is associated with impaired folate metabolism indicating that *MTHFR* and dietary folate intake may be important in asthma related phenotypes.

Ethnic and population specific risk effects for *MTHFR* are biologically plausible as it is involved in the metabolism of environmental exposures, which have extensive worldwide variation.

Deliverables: Our analysis suggests that *MTHFR* are associated with asthma and AHR. Dietary folate is a modifiable risk factor. Future research will determine if folate supplementation is a viable prevention strategy for asthma and related phenotypes

Relevance: Deficiency of folate has been associated with several disorders characterized by enhanced activation of the cellular immune system. We examined the association between asthma and atopy related phenotypes with several SNPs in the *MTHFR* gene including markers of impaired folate metabolism (rs1801133, rs1801131). Our results demonstrate that *MTHFR* is associated with childhood asthma and airway-hyper-responsiveness in the SAGE cohort.

Microarray analysis of changes in gene expression profiles in response to concentrated ambient particulate pollutants and/or ozone in a murine model of allergic asthma

Theme II: Environments, Populations & Society

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Objectives/Purpose: Exposure to urban air pollutants (particulate matter and ozone) can exacerbate the symptoms of asthma. A comprehensive understanding of the genes that respond to pollutants can aid in understanding the mechanisms underlying the adverse health effects of such exposures.

Methods: Female Balb/c mice were sensitized to ovalbumin on days 0 and 7, and challenged with aerosolized ovalbumin or saline on day 14 for 3 consecutive days (25 min/day), followed by exposure to concentrated ambient particles (CAP), ozone (O_3), or the combination (CAP+O_3) for 4 hours. After characterization of the functional phenotype of the model (i.e., exacerbation of airways responsiveness to methacholine), lungs were harvested for isolation of RNA for application to the Affymetrix Mouse exon 1.0 ST array. Image data was subjected to analysis using Partek Genomics Suite and Ingenuity Pathway Analysis (IPA). Genes that exhibited significant changes in expression ($P<0.05$ by ANOVA) with RMA values ≥1.1 were considered and stratified up to RMA >1.5 to determine the profiles in response to the respective exposures.

Findings: Initial analysis of the expression indicated 264, 253 and 63 genes that were significantly up- or down-regulated by exposure to CAP, O_3 or CAP+O_3, respectively. Of these, significant alterations in expression of 14 genes were common to all three exposure groups. Preliminary IPA analysis identified 8 canonical pathways. Some of the affected genes were related to pathways that could be anticipated, such as those involved in DNA repair or leukocyte migration. However, the remaining pathways indicate novel mechanisms that could be related to susceptibility in this disease model.

Deliverables: Microarray analysis provides a powerful method to screen for gene-expression changes throughout the entire genome. In combination with our murine asthma model, it may significantly advance our understanding of the potential mechanisms involved in pollution-induced exacerbation of symptoms in asthmatics. Additionally, it may help define new pathways that contribute to the development of adverse health effects in susceptible populations.

Relevance: Gene expression profiling of tissues following exposure to pollutants may help define new pathways involved in the deleterious effects of air pollution. In addition to furthering our understanding of the basic mechanisms underlying these adverse effects, the identification of novel pathways may help identify new targets for therapeutic intervention.

Association of SDAD1 and CXC chemokine genes with atopy in a Canadian cohort

Theme1: Genes & Early Life Determinants

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Objective/Purpose: Atopic diseases like asthma and atopy are complex disorders caused by interactions between multiple genes of small to modest effect and equally important environmental factors. Previous research has demonstrated that genetic variants in the D4S3042 region (CXCL9, CXCL10, CXCL11 and SDAD1 genes) of chromosome 4q21 are associated with seasonal allergic rhinitis in a Japanese population. CXCL9, CXCL10 and CXCL11 are IFN-γ–inducible chemokines that preferentially attract T_H1 lymphocytes through the CXC chemokine receptor, which is a G protein–coupled receptor expressed at high levels on T_H1 lymphocytes.

Methods: In an attempt to replicate these associations for additional allergy/asthma phenotypes we examined the relationship between genetic variation in this region and doctor-diagnosed asthma and atopy (defined by skin prick tests to common allergens) in a Canadian population-based case control sample of asthmatic and control children who were recruited into the Study of Allergy Genes and the Environment (SAGE). 725 families were recruited into the study, 247 of whom contained a child affected with asthma, 328 contained a child with atopy and the remainders were controls. Ten tag SNPs were selected to explore the majority of the genetic variation in the region were genotyped utilizing the Illumina platform. A family based analysis was performed using UNPHASED.

Findings: SNP rs4859577 in SDAD1 showed a weak association with asthma (p=0.067). SNP rs12649185 in CXCL11, rs2869460 in CXCL9 and SNPs rs3733239, rs3796482 and rs3796483 in SDAD1 were associated with atopy (p=0.027-0.032). However, these associations were not significant after adjustment for multiple comparisons (p=0.08172 – 0.119).

Deliverables: Polymorphisms in a number of CXC chemokines on chromosome 4 may be associated with atopy in a Canadian cohort but this observation needs to be replicated in another population.

Relevance: Replication of the association of CXC chemokine polymorphisms with allergy phenotypes will provide new therapeutic targets in the future.