TRAINEE POSTER ABSTRACTS

Fourth Annual Research Conference:
innovation from cell to society[4]

Fairmont Chateau Laurier, Ottawa ON
February 15-17, 2009

AllerGen

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

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ALLERGEN 2009 ANNUAL RESEARCH CONFERENCE

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- Programme B – Diagnostics and Therapeutics
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- Programme C – Public Health, Ethics, Policy and Society
  - *Strategic Focus: Allergic disease management and surveillance*

Cross-programmatic research teams in priority areas

Established cross-programmatic teams

- *The Canadian Healthy Infant Longitudinal Development (CHILD) Study*
- *Food Allergy and Anaphylaxis*

Emerging cross-programmatic teams

- *Mind-Body Interactions and Allergic Disease*
- *Work-related Allergy and Asthma*

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PROGRAMME A
GENE-ENVIRONMENT INTERACTIONS

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PROGRAMME A – GENE-ENVIRONMENT INTERACTIONS					
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1A	**Allakhverdi**, Zoulfia	Centre de recherche, **Centre hospitalier de l'Université de Montréal** (Hôpital Notre-Dame)	Dr. Guy JT Delespesse	**Strategic Initiative overlapping Programmes A and B** - 08BS12 : Cross-talk between airway epithelial and CD34 Hemopoietic Progenitor Cells Mediated by TSLP and IL-33 in Asthmatic and Normal Individuals	Effector Function in $CD34^+$ Hemopoietic Progenitor Cells in Allergic Inflammation
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4A	**Keast**, Colleen	**Hospital for Sick Children, Toronto**	Dr. Padmaja Subbarao	**Programme A** - Gene-Environment Interactions 07a7: Mini-CHILD	Non-invasive Assessment of Airways Inflammation in Pedriatric Asthma

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Applicant #	**AllerGen Trainee**	**Institution**	**AllerGen Researcher/ Supervisor**	**AllerGen Research Programme (and funded research project where applicable)**	**Abstract Title**
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Effector Function of CD34$^+$ Hemopoietic Progenitor Cells in Allergic Inflammation

This study is a part of Strategic Initiative overlapping Programmes A (Gene-Environment Interactions) and B (Diagnostics and Therapeutics)

Zoulfia Allakhverdi[1], Michael R. Comeau[2], Dirk E. Smith[2], Dean Toy[2], Leandra M. Endam[3], Martin Desrosier[3], Karen J. Howie[4], Judah A. Denburg[4], Gail M. Gauvreau[4], and Guy Delespesse[1]

[1]Notre-Dame Hospital, CHUM Research Centre, Montréal, QC, CANADA; [2]Inflammation Research, Amgen Inc, Seattle, WA; [3]Department of Otorhinolaryngology, CHUM, Hôtel-Dieu Hospital, Montréal, QC, Canada; [4]Asthma Research Group, McMaster University, Hamilton, ON, Canada

Supervisor: Guy Delespesse

Purpose: In allergic diseases, the bone marrow releases increased numbers of CD34$^+$ progenitor cells that migrate to the site of allergic inflammation where they differentiate into tissue dwelling and classical effector cells of allergy, such as mast cells, eosinophils and basophils. We here provide evidence that in addition to being progenitors, the blood CD34$^+$ cells may display a robust proinflammatory activity and may thereby contribute to the pathogenesis of allergic diseases.

Methods: Highly purified neonatal or adult blood CD34$^+$ cells were examined for the expression of TSLP and IL-33 receptors and for their response to these cytokines as well as to supernatants of primary small airway epithelial cells and nasal explants from rhinosinusitis and control subjects. Sputum of asthmatic patients was examined before and after allergen inhalation for the presence of IL-5 and IL-13 containing CD34$^+$ cells.

Findings: Circulating CD34$^+$ cells expressed receptors for TSLP and IL-33 and responded to these cytokines by rapidly releasing high levels of proinflammatory Th2-like cytokines and chemokines. These cells were activated in a TSLP-dependent manner by the supernatant fluids from activated primary human small airway epithelial cells and from nasal explants of chronic rhinosinusitis patients. Moreover, activated CD34$^+$ cells containing IL-5 and IL-13 could be detected in the sputum of allergic asthmatic individuals, with numbers increasing in response to specific allergen inhalation challenge.

Deliverables: 1) Blood CD34$^+$ cells in addition to being progenitors act as proinflammatory effector cells by themselves and may thereby contribute to the pathogenesis of allergic diseases; 2) The responsiveness of circulating CD34$^+$ cells to TSLP is currently explored as a predictive biomarker of atopic diseases in a collaborative study within the AllerGen network.

Relevance: 1) This study provides the basis for a novel therapeutic approach of allergic diseases aiming to neutralize proinflammatory activity of CD34$^+$ cells. 2) Current anti-allergic treatments including anti-histaminics (anti-H1 receptor), beta-adrenergic agents and glucocorticosteroids do not inhibit the proallergic effector function of CD34$^+$ cells. Our data further explain the relative inefficacy of treatment with anti-IL5 antibody.

Characterization of polymicrobial communities in indoor and outdoor air and dust using molecular genetic techniques

Programme A - Gene-Environment Interactions

Drudge C, Al-Naama M, Konya T, Silverman F, Takaro TK, Scott JA. University of Toronto

Supervisor: James Scott

Objective/Purpose
Development of a methodological platform based on molecular genetic techniques to identify, characterize and compare the diversity of air- and dust-borne microorganisms to assist in exposure assessment and apportionment of immunomodulatory microbial chemicals such as endotoxin, glucans and allergens.

Methods
Field samples of air and dust, from sources such as concentrated outdoor urban air particles (microbial contribution to air pollution), hospital isolation room air filter dust (airborne pathogens), and residential floor dust (microbe-related exacerbation of allergic and immunogenic diseases) were collected through leveraged opportunities. Methodologies were developed for the extraction of microbial DNA from samples and subsequent analysis by Denaturant Gradient Gel Electrophoresis (DGGE) fingerprinting to assess diversity, Polymerase Chain Reaction (PCR) to detect specific species and strains of interest, and cloning and sequencing identifying regions of ribosomal RNA genes of predominant microbes. As part of method development, the impact of size fractionation of samples into coarse and fine fractions on measured diversity was evaluated for both air and settled dust.

Findings
We optimized methods for collection of airborne particles and settled dusts, extraction and purification of bacterial and fungal DNA from these matrices, DGGE conditions, PCR, and cloning and sequencing techniques. Preliminary results suggest that size fractionation of dust samples has no appreciable impact on microbial diversity as assessed using DGGE. However, microbial community composition of airborne particles in concentrated ambient particulate matter appears to be strongly influnced by particle size.

Deliverables
Methodologies have been developed and demonstrated for sample collection, DGGE, PCR, and cloning and sequencing with field air and dust samples.

Relevance
Exposure to particles of microbial origin in air and dust may have an important role in the development of allergic and immune-based airway diseases. Current knowledge of the microbial composition these particles has traditionally been based on culture and microscopy methods, which are limited with respect to the proportion of particles that can reliably be detected and identified. The use of molecular genetic techniques circumvents this limitation, providing a novel means to better understanding the hazards posed by exposure to microbial particles.

Probing the interaction of *Aspergillus fumigatus* conidiospores and human airway epithelial cells by transcriptional profiling of both species

Programme A – Gene-Environment Interactions

Pol Gomez[1], Tillie Hackett[1], Darryl A. Knight[1], Margo M. Moore[2] and Scott J. Tebbutt[1]

[1]University of British Columbia (iCAPTURE Centre), Vancouver, BC, Canada and [2]Simon Fraser University, Burnaby, BC, Canada

Supervisor: Scott J. Tebbutt

Objective/Purpose:
Aspergillus fumigatus is a ubiquitous mould that propagates as airborne conidia, which are readily inhaled and small enough to reach the alveoli. It is a major allergen and is responsible for allergic bronchopulmonary aspergillosis (ABPA), a serious complication present in 2-5% of asthmatics and up to 10% of individuals with cystic fibrosis. Numerous *in vitro* studies have shown the internalization of *A. fumigatus* conidia by airway epithelial cells, but the implications of this remain unclear. Our research aims to shed light on the interaction between *A. fumigatus* conidia and airway epithelial cells using transcriptional (gene expression) profiling in both species.

Methods:
We have developed a cell-culture model for the interaction between the bronchial epithelium and *A. fumigatus* conidia using the cell line 16HBE (human bronchial epithelium) and a genetically engineered, green fluorescent protein (GFP) expressing strain of *A. fumigatus*. Immunofluorescent staining and a nystatin protection assay were used to quantify the internalization of conidia by 16HBE cells. RNA was extracted from 16HBE cells and from *A. fumigatus* conidia, incubated together or alone, and analyzed on whole-genome microarrays specific for both species. In addition, fluorescence-activated cell sorting (FACS) was used to segregate 16HBE cells directly interacting with conidia from 16HBE cells not directly interacting with conidia, from the same co-culture, and RNA was extracted from these two populations and analyzed on whole-genome microarrays.

Findings:
1. Fluorescence microscopy and the nystatin protection assay indicate that 16HBE cells internalize 40% of bound conidia within six hours of co-incubation.
2. FACS analysis indicates that up to 50% of cells bind or internalize conidia within six hours of co-incubation. Sorting of cells by the presence of a GFP signal is up to 90% accurate, based on re-sorting and microscopic visualization.
3. 667 human genes show differential expression between 16HBE cells incubated alone versus with conidia, while 889 genes show differential expression between 16HBE cells directly interacting with conidia versus other cells co-incubated with conidia.
4. Major biological functional themes identified within the human gene lists include chromatin remodeling, cell cycle progression, and chemokine activity.
5. Preliminary analysis reveals differential expression of 386 fungal genes in conidia incubated alone versus with 16HBE cells.

Deliverables:
We have developed a cell culture model of the interaction between *A. fumigatus* conidia and human airway cells allowing for transcriptional profiling in both species. Biologically plausible themes associated with this interaction were identified, thus validating this methodology and warranting further investigation.

Relevance:
The methodology developed will allow further characterization of the interactions between inhaled *A. fumigatus* conidia and airway epithelial cells. In particular, extending these studies to primary cells of normal and asthmatic origins will reveal how these cells respond to conidia, as well as possible adaptations of the conidia to the host environment.

Non-invasive Assessment of Airways Inflammation in Pediatric Asthma

Programme A – Gene-Environment Interactions (Mini-CHILD)

C Keast, RRT[1], R Amin, MD[1], S Balkovec, RRT[1], D Shehnaz, PhD[1], G T Rijkers, PhD[3], S Al-Saleh, MD[1], H Grasemann, MD, PhD[1], E Dompeling, MD, PhD[2], F Ratjen, MD, PhD[1] and P Subbarao, MD, MSc[1]. [1]Sick Kids, Toronto, Ontario, Canada; [2]Maastricht University Medical Center, Maastricht, Netherlands and [3]University Hospital Utrecht, Utrecht, Netherlands.

Supervisor: Padmaja Subbarao

Objective/Purpose
Atopic children with persistent asthma have eosinophilic inflammation responsive to inhaled corticosteroids (ICS) unlike children with viral wheezing or non atopic asthmatics. Our aim was to characterize inflammation among wheezy children by using non-invasive techniques including exhaled breath condensate(EBC), exhaled nitric oxide (FeNO) and induced sputum (IS).

Methods
Children between 7 and 18 years with uncontrolled asthma were recruited from asthma clinic. Patients completed the ISAAC questionnaire, EBC, FeNO, and IS.

Findings
Of the 24 patients enrolled, 18 provided sputum samples. The mean (range) age was 11.4 (7-16) years. Thirteen of the sputum producers were male. The mean (SD) FEV_1 % predicted was 80.4 (14.9). Nine samples were eosinophilic (≥ 2.5%). Eosinophilic and noneosinophilic sputum producers did not differ in age, sex, FEV_1, bronchodilator response or ICS dose. Eight eosinophilic sputum producers were atopic (positive skin prick) as compared to 100% of noneosinophilic patients. The median (IQR) for mean eNO (ppb) was 77.7 (79) and 46.4 (21) for eosinophilic and noneosinophilic sputum producers, respectively. Eosinophilia correlated with mean FeNO among eosinophilic sputum producers r=0.7 (p=0.04); the correlation between noneosinophilic sputum producers and eNO trended towards significance for noneosinophilic sputum producers r =-0.65 (p=0.06). EBC cytokines did not correlate with sputum eosinophilia and neutrophilia or with FeNO. Sputum Il-1a and IL-8 significantly correlated with FeNO. Sputum induction, FeNO, and EBC are feasible in asthmatic children. Atopic status and EBC were not helpful in differentiating between the 2 sputum subgroups. However FeNo was able to differentiate between eosinophilic and noneosinophilic sputum producers.

Deliverables
The conclusions from this study will aid in the development of protocols and standardized methodology for the AllerGen CHILD study.

Relevance
Based on the findings from this study, establishing non-invasive methods to characterize inflammation among children presenting with wheezing episodes will aid in determining appropriate treatment and maintenance therapy.
These findings will be presented at the American Thoracic Society Conference in May 2009.

GM-CSF Airway Overexpression Increases The Susceptibility To Develop House Dust Mite Induced Allergic Asthma

Programme A – Gene-Environment Interactions

Alba Llop-Guevara[1], Ramzi Fattouh[1], Cheryl Lynn Moore[1], Amal A Al-Garawi[1], Tina D Walker[1], Susanna Goncharova[1], Martin R Stämpfli[1,2], Paul O'Byrne[2] & Manel Jordana[1]

Departments of [1]Pathology and Molecular Medicine, or [2]Medicine, McMaster University, Hamilton, ON, Canada

Supervisor: Manel Jordana

Objective: There has been remarkable progress in our understanding of the immunobiological basis of allergic asthma. Yet, its origin and evolution remain unresolved. We propose that the development of asthma depends on *aeroallergen exposure* and the *immune status of the lung* at the time of exposure. Regarding the first, we recently provided a comprehensive computational view of the impact of dose and length of exposure to house dust mite (HDM) on allergic responses (PLoS ONE 2008). For the latter, we hypothesize that perturbations able to alter the lung antigen-presenting cell (APC) compartment in a certain way, such as the expression of granulocyte-macrophage colony-stimulating factor (GM-CSF), may increase the susceptibility to allergic asthma.

Methods: BALB/C mice intranasally received $3x10^7$pfu of an adenoviral vector carrying the transgene for GM-CSF (Ad/GM-CSF) 24h before daily exposure to 0.2, 1, 5 or 25µg of HDM. These doses of allergen *per se* induce absent, incipient, moderate or severe responses and, hence, allow for the investigation of thresholds and degrees of responsiveness. Control groups received empty adenovirus and/or vehicles (PBS or saline). Lung tissue, bronchoalveolar lavage fluid and serum were obtained after 7 consecutive days or 2 weeks of HDM exposure. Lung function was assessed by the flexiVent™ system.

Findings: Exposure to Ad/GM-CSF and 0.2µg HDM for 7 days led to increased numbers of activated myeloid dendritic cells, macrophages and $CD4^+$ T cells. After 2 weeks of HDM exposure, HDM-specific IgG_1, eosinophilia, goblet cell hyperplasia and lung dysfunction were apparent in these mice compared to those that received 0.2µg HDM alone. In addition, exposure to Ad/GM-CSF along with 1, 5 or 25µg HDM remarkably amplified immune-inflammatory responses. Interestingly, a new maximal response was reached already with 5µg of HDM in the context of GM-CSF.

Deliverables: Our findings show that the initial and transient perturbation introduced by the overexpression of GM-CSF in the airways substantially lowers the threshold and enhances responses to HDM. These data suggest that perturbations associated with GM-CSF production, such as viral infections or exposure to environmental pollution, might affect responsiveness to allergens by increasing the susceptibility and the severity of allergen responses.

Relevance: This research provides a qualitative and quantitative illustration of the importance of the lung microenvironment on the development of responses to allergen. In a broader perspective, it furnishes an experimental paradigm on how environmental signals may affect the emergence of allergic asthma.

Inhibition of arginase 1 attenuates methacholine hyperresponsiveness in murine models of allergic airways inflammation.

Programme A: Gene-Environment Interactions

Michelle L. North [1,2,3], Nivedita Khanna[2,3], Philip A. Marsden [1,3,4], Hartmut Grasemann[1,5] & Jeremy A. Scott[1,2,4]

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[2]Divisions of Occupational and Respiratory Medicine and [3]Nephrology, Department and Faculty of Medicine, University of Toronto, Toronto, ON, Canada
[4]Keenan Research Centre in the Li Ka Shing Knowledge Institute and the Gage Occupational and Environmental Health Unit, St. Michael's Hospital Research Centre, Toronto, ON, Canada
[5]Physiology and Experimental Medicine, and Division of Respiratory Medicine, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Supervisors: Jeremy A. Scott and Frances S. Silverman

Objective/Purpose
L-Arginine metabolism by the arginase (ARG) and nitric oxide synthase (NOS) enzymes is important in NO production, and imbalances between these pathways contribute to airways hyperresponsiveness (AHR) in asthma. The objectives of this study were to: 1) investigate the dysregulation of L-arginine metabolism in lung tissues from human asthmatics, 2) investigate L-arginine metabolism in acute (3-week) and chronic (12-week) murine models of ovalbumin (OVA)-induced airways inflammation and evaluate the ability of these models to mimic the dysregulation in human asthmatics, and 3) determine whether arginase inhibition corrects the AHR in these murine models.

Methods
In the acute model, female Balb/c mice were sensitized to ovalbumin (OVA; i.p.) on days 0 and 7, and challenged with aerosolized OVA (OVA/OVA) or PBS (OVA/PBS) starting at day 14, for 7 consecutive days. Mice in the chronic model were sensitized in an identical manner, and challenged with aerosolized OVA or PBS starting at day 14, for 2 consecutive days followed by 2 challenges every 2 weeks, up to 12 weeks. Measurement of methacholine (MCh) responsiveness was carried out 24 hours following the final challenge using the flexiVent system. In a subset of mice from each model, the arginase inhibitor, boronoethyl cysteine (BEC), was nebulized into the airways prior to pulmonary function testing. The protein expression of ARG and NOS isoforms, and other proteins involved in L-arginine metabolism, were determined by Western blotting in lungs from both murine models and human asthma.

Findings
ARG1 expression was increased in human asthma, while ARG2, NOS isoforms, and the other L-arginine-related proteins (i.e., CAT1, CAT2, agmatinase, and ODC) were unchanged. In the acute murine model of allergic airways inflammation, augmentation of ARG1 expression was similarly induced; however, ARG2, NOS1, NOS2 and agmatinase were also increased, while NOS3 was decreased. The chronic murine model revealed an expression profile that more closely paralleled the human asthmatic samples; only ARG1 expression was significantly increased. Arginase inhibition attenuated the methacholine AHR in both the acute and chronic murine models.

Deliverables
This study provides the first comprehensive assessment of L-arginine related protein expression in asthmatic human lungs, and demonstrates that ARG1 is the primary isoform that is upregulated in asthma. The chronic murine model of allergic airways inflammation mimicked the human expression profile for arginine-related proteins and inhibition of ARG1 corrected for the development of AHR .

Relevance
The similarity in arginase expression between human asthma and the chronic model, and attenuation of AHR following *in vivo* treatment with BEC, supports the therapeutic potential of arginase inhibition in asthma, and further refines the relevance of animal models to human asthma.

CD14 and asthma: environmental factors that may alter the genetic effect on childhood asthma

Programme A - Gene-Environment Interactions

Julie Park[1], Ben Tripp[1], David Zamar[1], Jian-Qing He[1], Andrew Sanford[1], Allan Becker[2], Anita Kozyrskyj[2,3], Denise Daley[1]

[1]James Hogg iCAPTURE Center, University of British Columbia, Vancouver, BC, Canada; [2] Department of Pediatrics and Child Health, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada; [3] Department of Pediatrics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

Supervisor: Denise Daley

Objective/Purpose: The hygiene hypothesis proposes that elevated microbial exposure during early life may aid in the development of a mature immune system and reduce the incidence of allergic diseases. CD14 is an innate immunity receptor specific for bacterial endotoxin (LPS). Over 200 studies have examined the associations between genetic variation in CD14 and asthma related phenotypes, with inconsistent results. These inconsistent results may be explained by a complex dose dependent environmental interaction with endotoxin exposure in early life, whereby the T allele of -159C/T (rs2569190) can either confer protection or increase risk, with the direction of effect determined by the level of endotoxin exposure. Association analyses of CD14 variants and four asthma related phenotypes demonstrated that variants in this region were associated with asthma, but not with atopy (p=0.026). We hypothesized that a similar interaction of -159C/T and endotoxin would be observed with pediatric allergist diagnosed asthma in the Study of Asthma Genes and Environment (SAGE) cohort.

Methods: To test our hypothesis we genotyped 6 polymorphisms within the region of CD14 in 723 families (children and parents) from the SAGE study and examined evidence for association with pediatric allergist diagnosed asthma. The SAGE study is a population-based birth cohort of 13,980 children, born in Manitoba, Canada, in 1995. A total of 723 families participated in a nested case-control study. All children with either asthma (n=392), or allergy symptoms (n=192) were invited to participate, as were a representative (urban and rural) sample (n=200) of children with neither condition to serve as a control group. Our analyses were restricted to a group of 309 Caucasian children where we had both CD14 genotypes and endotoxin exposure information available. Genotype based tests were performed using the software package UNPHASED. Endotoxin exposure was stratified into as high and low exposure, using the median (97.00 EU/mg dust) as the cut-point. Endotoxin was evaluated as an effect modifier, and region of residence (urban/rural) was evaluated as a confounder and an effect modifier.

Findings: The interaction effects of endotoxin and CD14 SNPs on asthma were not significant. Carriers of the -159TT genotype had a lower prevalence of asthma than the other 2 genotypes, regardless of endotoxin exposure. A higher exposure to endotoxin was protective against asthma among children with CC and CT genotypes, but was a risk factor with TT genotype (p(interaction)>0.05). A significant regional difference in prevalence of asthma was present for rs778584 (p<0.001) and -159C/T (p<0.001). Urban residence was associated with asthma; 73.3% of asthmatic children in the study lived in urban areas. Adjusting for region of residence did not strengthen the effect of endotoxin (p(interaction)>0.05).

Deliverables: The interaction effect of endotoxin exposure and the -159C/T variant on atopic disease was not replicated with the asthma phenotype. The -159TT genotype was protective against asthma. Endotoxin exposure decreased the risk of asthma among carriers of CC and CT genotypes and slightly increased the prevalence of asthma (< 1%) among carriers of the TT genotype. This result is consistent with the previously reported findings that the TT genotype is protective against wheezing, even though it did not reach the statistical significance possibly due to small sample sizes.

Relevance: Endotoxin exposure activates pathways which involve CD14 signaling and ultimately regulation of serum IgE level. Effective CD14 signaling can protect children against allergic sensitization. Our study reaffirms the need for a better understanding of these complex pathways, which may improve the methods of diagnosis and treatment of childhood asthma and related phenotypes.

Highly robust SNP genotyping using arrayed primer extension

Programme A – Gene-Environment Interactions

Jian Ruan*, Mohua Podder*#, Ben W. Tripp* and Scott J. Tebbutt*
*James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, St. Paul's Hospital, Department of Medicine, and #Department of Statistics; University of British Columbia, Vancouver

Supervisor: Scott J. Tebbutt

Objective/Purpose:

Arrayed primer extension (APEX) is a microarray-based rapid minisequencing methodology that may have utility in 'personalized medicine' applications that involve genetic diagnostics of single nucleotide polymorphisms (SNPs). Robust assay design, chemistry and analysis methodologies have been developed in our laboratory and validated using resources from the International HapMap Project. The use of multiple and redundant genetic probes for each SNP site is combined with robust statistical analysis algorithms which are designed to unambiguously capture the data from this genetic probe redundancy and turn these data into a high-quality genotype call. In contrast to other genotyping platforms, this allows one to obtain extremely high call rates whilst maintaining excellent genotype accuracy. The method delivers robust and accurate genotypes (100% call rate and >99.9% genotyping accuracy) from as little as 5 ng of genomic DNA (for a 50-plex assay).

Our objectives for the research described here were two-fold: **1.** to determine why we did not achieve 100% accuracy; and **2.** to develop a method that could be used to avoid such rare instances of genotyping error in future diagnostic applications where even a single error could have serious medical implications.

Methods:

1. From the results of our previous experiment, involving the genotyping of 50 SNPs across each of 49 randomly chosen Coriell DNA samples, two discordant genotype cases were identified, plus a third genotype case which had a poor quality score. For each of these discordant genotype cases (in three different Coriell samples) we independently re-amplified and sequenced the genetic locus where the SNP site was located, in order to identify additional DNA variation that might have caused the original error through allelic drop-out.

2. Subsequently, we designed a systematic, single-tube, redundant multiplex PCR strategy to reduce the risk of otherwise unidentifiable allelic drop-out. An additional pair of PCR primers were designed for each of the 50 SNP loci. This gave four primers for each SNP locus, or 200 PCR primers in total for the 50 SNPs, all of which would be within the same reaction vessel.

Findings:

1. Following DNA sequencing, we identified additional single nucleotide variant sites that coincided with the positions delimited by the PCR primer sequences used for the original 50-plex reaction, therefore consistent with full or partial allelic drop-out during the PCR. Two of these sites represented hitherto unreported single nucleotide variants adding weight to the concept that previously unknown variants cannot be avoided, and hence genotyping will always be somewhat at risk of error due to allelic drop-out.

2. When we performed our novel, redundant multiplex PCR (with subsequent APEX genotyping) we obtained accurate genotyping results for all three Coriell samples, exactly matching our previous data except for the three discordant genotype cases previously found. These three cases were now correctly genotyped, giving evidence that the allelic drop-out had been eliminated.

Deliverables:

We have further robustified our microarray genotyping methodogy by designing and testing a redundant single-tube multiplex PCR assay that is capable of systematically reducing the influence of hidden (yet to be discovered) genetic variation, whilst retaining highly accurate genotyping.

Relevance:

Our assay design may have useful and important application in clinical genetic diagnostics, either in single-plex or multiplex PCR format, where accurate genotyping of an individual patient across one or many genetic markers may assist in health-care decision-making.

Epigenetic profiling of bronchial epithelial cells

Programme A: Gene-Environment Interactions

Chris Taplin[1], Tillie-Louise Hackett[1], Sarah Neumann[2], Alexandra Fok[2], Luana Avila[2], Darryl Knight[1], Peter Paré[1], Michael Kobor[2]

1) The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, Department of Medicine, St. Paul's Hospital / Providence Health Care-University of British Columbia
2) Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, BC.

Supervisor: Peter D. Paré

Background: Current research suggests that epigenetic control of gene expression contributes to the regulation of cell differentiation, cell cycle progression and apoptosis. One epigenetic regulatory mechanism is DNA methylation, which involves methylation of cytosine residues in CpG dinucleotides (CpGs).

Objective: To determine the changes in DNA methylation of individual CpGs for human bronchial epithelial cells expanded under normal cell culture conditions.

Methods: 1HBE o^-, an immortalized bronchial epithelial cell line and Normal Human Bronchial Epithelial cells (NHBEs, Cambrex) were expanded for a total of six passages. DNA and RNA from each passage was collected. DNA methylation was analyzed using the Illumina GoldenGate Methylation array to interrogate 1505 CpG sites in 800 cancer-related genes and gene expression was studied using Illumina Human Whole Genome-6 BeadChips.

Findings: At baseline, 1HBE o^- cultures had 251 differentially methylated CpGs compared to NHBEs. The methylation status of the 450 CpGs sites that satisfied inclusion criteria (p-detection value < 0.05, Average Passage Methylation >0.05) changed more-so for the primary cell line with 2-fold or greater changes in 13% of the CpG sites for the NHBEs in contrast to 7.5% for the 1HBEs. The CpG sites found to be methylated over passage are key regulators of cell differentiation and apoptosis including: TGF-b1(3-fold change) and Caspase-3 (2-fold change). The CpG sites found to be un-methylated over passage are in gene transcription regulators of apoptosis, cell cycle progression and DNA synthesis, including: RAN (2-fold change) and Caspase-10 (2.5-fold change)

Conclusions: Normal cell culture conditions clearly affect DNA methylation of key regulatory genes of bronchial epithelial cell cycle progression, cell differentiation and apoptosis. These dynamic changes in DNA methylation indicate that studies interested in determining cellular level epigenetic or gene-environment interactions for respiratory diseases should limit the use of cell culture. Further data analysis and a larger sample size are required to validate these findings and to expand our knowledge of DNA methylation regulation.

Relevance: Bronchial airway epithelial cells are ideal candidates for studying gene environment interactions since they are the first point of contact with environmental toxins in the lung and therefore may play critical roles in the pathogenesis of respiratory diseases. The initial findings that bronchial epithelial cell DNA methylation is highly sensitive to normal cell culture conditions is important for all AllerGen researchers that study epigenetics and gene-environment interactions to consider when designing cell-based experiments.

Communicating Results: These results will be communicated to the research community via journal publication and conference presentations.

Concentrated Ambient Fine Particles Induce an Interleukin-6 Inflammatory Response in Asthmatics and Non-Asthmatics

Programme A – Gene-Environment Interactions

Bruce Urch[1,2], Mary Speck[1], David Wasserstein[2], Michael Manno[1], Paul Corey[1,3], Jeff Brook[5], Ling Liu[6], Brent Coull[7], Joel Schwartz[7], Diane Gold[7], and Frances Silverman[1,3,4]

[1]Gage Occupational & Environmental Health Unit, St. Michael's Hospital, Toronto, ON, Canada; [2]Institute of Medical Science, [3]Dalla Lana School of Public Health, [4]Department of Medicine, University of Toronto, ON, Canada; [5]Air Quality Research Branch, Meteorological Service of Canada, Environment Canada, Toronto, ON, Canada; [6]Health Canada, Ottawa, ON, Canada; and [7]Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA.

Supervisors: Paul Corey and Frances S. Silverman

Background. Epidemiological studies have established significant associations between ambient levels of air pollution and cardiorespiratory morbidity and mortality. Fine particulate matter ($PM_{2.5}$) and ozone (O_3), which are present as a mixture, have been identified as key pollutants, but mechanisms for their joint as well as individual effects are not fully understood.

Objectives. To further explore the associations of $PM_{2.5}$ and O_3 with acute inflammatory responses, we carried out a controlled exposure study using our concentrated ambient particle (CAP) facility.

Methods. Subjects included 10 mild asthmatic and 13 non-asthmatic, non-smokers, aged 21-40 years. The study design was a randomized block. Exposures were 2 hours in duration and included filtered air and CAP (range 50-200 $\mu g/m^3$), with and without O_3 (120 ppb). Response measures included pulmonary function and inflammatory indices in induced sputum and blood.

Findings. Sputum IL-6 increased 3 hours after CAP exposures, compared to exposures without CAP, but there was no effect modification by whether O_3 was added to CAP. There was also a systemic IL-6 response at the same 3 hour time point, demonstrating an overall association of increase in $PM_{2.5}$ mass concentration with increase in blood IL-6, but only for exposures without added O_3. For CAP+O_3 exposures there was a significant positive association of change in tidal volume during exposure and the corresponding IL-6 change after exposure. Asthmatic and non-asthmatic responses were similar. No significant adverse changes in other response measures were observed.

Conclusions. We have demonstrated both a respiratory and systemic inflammatory response to $PM_{2.5}$ providing evidence to support the epidemiological findings of associations between ambient levels of $PM_{2.5}$ and cardiorespiratory morbidity/mortality. We hypothesize that modification of the CAP response by concomitant O_3 exposure may be modulated through the respiratory autonomic response to CAP+O_3 in some of our subjects, resulting in reduced tidal volume and consequent decreased PM deposition. Most likely this was an O_3-induced reflex inhibition of respiratory effort, originating from airway C-fibers.

Relevance. Air pollution is ubiquitous and affects everyone, but more so in sensitive populations including the young, elderly, and those with allergies, cardiovascular and respiratory diseases. Research using controlled human studies with single and multi-pollutant exposures provides information and guidance to other researchers (e.g., Canadian Birth Cohort study), health care providers (e.g., physicians) and policy decision makers (e.g., Health Canada). Having a better understanding of the mechanisms of pollutant-induced health effects can provide potential intervention strategies, such as guiding asthma medication usage, and more concise recommendations in terms of smog alerts and advice to minimize the risk of adverse air pollution effects. The results of this study will be presented at scientific meetings, made available to policy makers and published in peer-reviewed journals.

Support. Natural Resources Canada; Health Canada (Toxic Substances Research Initiative); Air Quality Health Effects Research Section, Government of Canada; Allergen NCE Inc.; Ontario Thoracic Society; NIH/NIEHS (P01 ES09825) and U.S. EPA (R832416-010).

Introducing the Genomic Applications for Humanity (Genapha) Website and the Path Software for Exploring Pathway-based Genetic Associations

Programme A – Gene-Environment Interactions
David Zamar[1], Julie Park[1], Ben Tripp[1], and Denise Daley[1]
[1]James Hogg iCAPTURE Centre, University of British Columbia, Vancouver, BC, Canada

Supervisor: Denise Daley[1]

Objective: In order for research to have impact, it must be successfully communicated. In an era of high throughput genome-wide association and candidate gene studies, how do we translate the results from international genomic consortiums effectively? Only a small number of results are published, generally only statistically significant observations, leaving the vast majority of results undisclosed. To facilitate meta-analyses and the identification of genes with smaller effect sizes (OR <1.4) we are releasing association results for 98 candidate genes from our study that includes 5,565 individuals recruited into four studies from Canada and Australia. One way to communicate findings is to utilize the World Wide Web and we present *Genapha* (www.genapha.ca) as a model for knowledge translation from high throughput genomic platforms. *Genapha* is an online database of results, providing rapid, full, and open disclosure of our research findings to the scientific community.

We have also developed a stand-alone application, called *Path*. The *Path* application is primarily designed to help researchers interface their data with biological information from several bioinformatics resources (dbSNPs, OMIM, PubMed, The Genetic Association Database, Innate Immunity, HapMap, Seattle SNPs, PharmGKB, UCSC Genome Browser and KEGG). This information may be used to help generate biologically plausible hypotheses for testing gene-gene interactions. The *Path* software is a first-step bioinformatics approach to investigate gene-gene interactions in genetic association studies.

Methods: The Genapha database has been interfaced with other databases such as dbSNPs, OMIM, PubMed, The Genetic Association Database, Innate Immunity, HapMap, Seattle SNPs, PharmGKB, UCSC Genome Browser, and KEGG. The Genapha website interface and search tools allow users to rapidly filter information and identify genes, single nucleotide polymorphisms (SNPs), and association results most pertinent to their own research, not just from our study, but the body of knowledge that is available in the electronic databases interrogated by *Genapha*. We have implemented novel graphical user interfaces to allow visitors to build custom graphs and plots of interest to them using our results database. Detailed descriptions of the study designs, populations, and allele frequencies along with tutorials are hosted on the website.

Those who wish to store, and explore their own data, as is done on the Genapha website, may download the *Path* application. A summary page is provided for each SNP. Entries for each SNP include basic background information, such as function, gene, chromosome, *etc.*, and a summary of the results of single-SNP associations. Each SNP entry also provides several links to other data such as, pathway context, and previous association study results. A graphical user interface (GUI) is used to explore the data alongside information retrieved from bioinformatics resources and to conduct studies on the SNP-SNP interactions. The software application, UNPHASED is used for all analyses. The imported data and results of the analysis are stored in a local database. *Path* may be downloaded from http://genapha.icapture.ubc.ca/Path/.

Findings: The need to disclose and share data and analysis results among Allergen investigators is vital, but this information is also of interest to the international Asthma, Allergy and Genetic research communities.

Deliverables: We have developed a website (www.genapha.ca) to facilitate the knowledge discovery, management and transfer of information generated by our studies. A freely available software package (*Path*) has been developed to enable other researchers to organize, visualize, and explore their data using the bioinformatic tools in *Path*.

Relevance: There is a need to disclose results from large genome-wide genetic association studies effectively. Integration of bioinformatics information with data from genetic association studies is needed in order to conduct pathway-based gene-gene interaction analyses. For these reasons, we developed the Genapha website as a model for knowledge translation and the Path software application to help users explore their own data and integrate it with the growing amount of bioinformatics information from online resources.

Pneumonia following heterologous pulmonary infection correlates with exacerbated inflammatory cytokines and reduced type-I interferon production

Programme A -- Gene-Environment Interactions

Caleb CJ Zavitz, Carla MT Bauer, Gordon J Gaschler, and Martin R Stampfli
Department of Pathology and Molecular Medicine, Centre for Gene Therapeutics, McMaster University, Hamilton, Ontario, Canada.

Supervisor: Martin Stämpfli

Objective: Bacterial superinfections of influenza virus, and resulting secondary pneumonia are both frequent and in many cases lethal. Mixed bacterial-viral exacerbations of chronic obstructive pulmonary disease and asthma are similarly important in the courses of those diseases, and lead to significant morbidity and mortality. Our understanding of host defense may be incomplete to address these problems, in large part because most experimental studies use specific pathogen-free animals, which are then infected with a single infectious agent. The goal of the present study is to elucidate the mechanisms by which viral insult predisposes to secondary bacterial infection, and the viral-bacterial interactions which lead to increased illness.

Methods: We established a model of heterologous pulmonary infection in which C57BL/6 mice were infected with influenza virus, and then superinfected 5 days later with *Bordetella parapertussis* bacteria. At the time of sacrifice, bronchio-alveolar lavage inflammation was determined and cytospins prepared. Lung tissue inflammation was determined by flow cytometry using enzymatically digested lungs. Bacterial burden and viral titre were assayed by plating homogenized lung tissue onto Bordet-Gengou blood agar plates, or onto MDCK cell monolayers, respectively. Cytokine and chemokine expression were determined by ELISA, and MPO levels were determined by enzymatic activity, using homogenized lung tissue. Type-I IFNs were assayed by VSV-GFP plaque reduction assay, using serially diluted samples of bronchio-alveolar lavage.

Findings: Mice infected with both influenza virus and what would otherwise be a sub-clinical dose of *B. parapertussis* had a greater duration of symptoms, and suffered exacerbated and prolonged weight loss compared to mice infected with either pathogen alone. These impairments were associated with significantly reduced bacterial clearance, despite severe pulmonary neutrophillia, both in the lung and in the broncho-alveolar lavage. We also found dramatically increased neutrophil myeloperoxidase activity in the lungs of heterologously-infected mice, and high levels of the neutrophil recruitment factor MIP-2. In contrast with obviously exacerbated inflammatory cytokine and cellular profiles in the lungs, we found that Type I interferons, crucial mediators of early innate antiviral responses, were significantly reduced in the BAL of heterologously infected mice.

Deliverables: We have established a murine model of heterologous pulmonary infection which recapitulates many of the clinical hallmarks of this disease. We are now pursuing an understanding of the molecular mechanisms underlying the immunopathology associated with heterologous infection, and are positioned to test interventions that may mitigate the toll exacted by these infections.

Relevance: Our findings suggest that influenza infection may predispose to bacterial superinfection, and that exacerbated cytokine and chemokine production, specifically of the neutrophil chemokine MIP-2 may drive the inflammation which contributes to the clinical worsening seen in patients with severe pneumonia. Blocking MIP-2 may thus prove an effective therapy against secondary bacterial pneumonia, providing a potential therapeutic drug target. Intervention studies are in progress and will be reported in a future publication.

PROGRAMME B
DIAGNOSTICS AND THERAPEUTICS

ALLERGEN 2009 ANNUAL RESEARCH CONFERENCE

COMPLETED POSTER APPLICATIONS AND ABSTRACTS (N=25)

[Programme A: 11; Programme B: 8; Programme C: 6][1]

PROGRAMME B – DIAGNOSTICS AND THERAPEUTICS					
Applicant #	**AllerGen Trainee**	**Institution**	**AllerGen Researcher/ Supervisor**	**AllerGen Research Programme (and funded research project where applicable)**	**Abstract Title**
1B	**Bruenahl**, Christian	**McMaster University**	Dr. Petra Arck	**Programme B** – Diagnostics and Therapeutics – 07B3.1: Perinatal stress and programming of allergic responses	Mind-Body interactions during pregnancy: Prenatal stress enhances susceptibility of murine adult offspring toward airway inflammation dependent on gender
2B	**Dawick**, Wojciech	**Dalhousie University**	Dr. Jean S. Marshall	**Programme B** – Diagnostics and Therapeutics – 07B1: Canadian Group on Food and Allergy Research (CanGoFAR)	Role of Mast Cell Activation in the Influx of Dendritic Cell Subsets into the Lymph Node
3B	**Hackett**, Tillie	**University of British Columbia**	Dr. Tony Bai	**Programme B** – Diagnostics and Therapeutics – 07B4.2 and 3.13 – Environmental impact on the epithelial immune barrier in asthma	Epithelial-Mesenchymal Transition occurs in undifferentiated basal Bronchial Epithelial Cells in Normal and Asthmatic Subjects

[1] **AllerGen Strategic Research Programme Foci:** Programme A - Gene-Environment Interactions
- *Strategic Focus: Genetics and gene-environment interactions in allergy and asthma*
- Programme B – Diagnostics and Therapeutics
  - *Strategic Focus: Biomarkers, immune monitoring and drug discovery and development*
- Programme C – Public Health, Ethics, Policy and Society
  - *Strategic Focus: Allergic disease management and surveillance*

Cross-programmatic research teams in priority areas

Established cross-programmatic teams
- *The Canadian Healthy Infant Longitudinal Development (CHILD) Study*
- *Food Allergy and Anaphylaxis*

Emerging cross-programmatic teams
- *Mind-Body Interactions and Allergic Disease*
- *Work-related Allergy and Asthma*

Mind-Body interactions during pregnancy: Prenatal stress enhances susceptibility of murine adult offspring toward airway inflammation dependent on gender

Programme B – Diagnostics and Therapeutics

Christian Andreas Bruenahl [1], Maike Pincus [2], Emilia Solano [1], Evelin Hagen [1,2], Astrid Friebe [1], Russ Ellis [1], Mark Inman [1], Petra Clara Arck [1]

1 McMaster University, St. Joseph´s Healthcare, Hamilton, Canada

2 Charité, University Medicine Berlin, Campus Virchow Klinikum, Berlin, Germany

Supervisor: Petra Arck

Objective/Purpose

Allergies are continuously increasing in developed countries over the past 5 decades. Furthermore, an emerging area of research subsumed as fetal programming evaluates the impact of environmental insults *in utero* on the incidence of diseases in later life. The aim of our research is to identify if and how prenatal exposure to stress, which constitutes a severe environmental insult, affects the susceptibility toward allergic airway inflammation in later life of the children.

Methods

Our experiments were performed on mice.

Findings

They revealed that prenatally stressed offspring show an increased vulnerability toward increased airway response, lung inflammation and neutrophil influx in the bronchioalveolar lavage, which are particularly profound in female offspring. Further, we focused on the identification of biomarkers involved and revealed a maladaptation of the local and peripheral immune response, such as decreased frequencies of regulatory T cells $CD3^+CD4^+CD25^+$forkhead box P3(foxP3)$^+$, upon antigen challenge in prenatally stressed adult offspring.

Deliverables

In conclusion, we provide strong evidence for a link between prenatal stress and an increased susceptibility toward allergic airway inflammation in later life and a gender dependant way.

Relevance

These findings will facilitate future research aiming to identify the individual impact, hierarchy, and redundancy of markers involved in asthma. This also includes epigenetic pathways, strongly encouraged by the observation that the foxP3 locus is under epigenetic regulation. Overall, our outcome driven research questions will foster the substantiation of primary disease-prevention strategies as early as pregnancy.

Role of Mast Cell Activation in the Influx of Dendritic Cell Subsets into the Lymph Node

Programme B Therapeutics and Diagnostics (CanGoFAR Project)
Wojciech Dawicki and Jean S. Marshall, Dalhousie University

Supervisor: Jean S. Marshall

Objective/Purpose

Dendritic cell (DC) subpopulations within the lymph node activate naïve T cells and help in determining the quantity and quality of the ensuing T cell responses. The process of DC activation and migration may be modified in allergic diseases resulting in inappropriate T cell responses that promote sensitization. Mast cells play an important role driving the symptoms of an allergic response by releasing mediators such as histamine and leukotrienes and may be involved in sensitization. The purpose of this study was to investigate the role of the mast cell in the development of T-cell immunity. Initially examining the role of mast cells and mast cell derived mediators in the recruitment of DC subpopulations into the lymph node in response to peptidoglycan from *S. aureus* (PGN).

Methods

DC accumulation in response to PGN was determined by injecting either PGN into one ear pinna and a control diluent into the contralater ear and 18h later harvesting the draining auricular lymph nodes. The number of $CD11b^+$ DC, $CD8^+$ DC and plasmacytoid DC (pDC) in the draining lymph node was determined using flow cytometry. The role of mast cells was determined by comparing the mast cell sufficient and mast cell deficient mice. Local reconstitution, with mast cells, of mast cell deficient mice was employed to further confirm the importance of mast cells in the influx of DC into the lymph node. To determine the importance of various mast cell derived mediators in this process we utilized pharmaceutical inhibitors or mice deficient for cytokines or their receptors.

Findings

An intradermal injection of PGN, into ear pinna, led to significant ($p<0.05$) increases in the numbers of $CD8^+$ DC, $CD11b^+$ DC and plasmacytoid DC(pDC) as well as T-cells and B-cells in the lymph node draining the site of activation. By analyzing mast cell deficient mice as well as those having local mast cell reconstitution we observed that mast cells are required for the recruitment of $CD8^+$ and pDC, but not $CD11b^+$ DC, into the lymph node in response to intradermal administration of PGN. Histamine receptor H2 antagonist treatment revealed that histamine is necessary for the influx of pDC and $CD11b^+$ DC, but not the influx of $CD8^+$ DC, T-cell and B-cells. We also found that IL-6, which is produced by mast cells in response to PGN, is required for the optimal recruitment of $CD11b^+$ DC, but not $CD8^+$ DC, pDC or total LN cells following PGN injection. The absence or inhibition of IL-1 receptor, tumor necrosis factor, cyclooxygenase 1 and 2, or cysLT1 had no effect on the PGN-mediated accumulation of DC subsets, T-cells and B-cells. These data show that mast cells, histamine and IL-6 selectively modulate the influx of individual DC subpopulations from the blood as well as the skin into the lymph node in response to PGN and highlight the potential role of mast cells in the modulation of acquired immune responses.

Deliverables

Better understanding of how mast cell activation impacts the development of an immune response will provide insight on how allergic sensitization is regulated by mast cells and pathogen products with a view to improving preventative strategies. This will be incorporated into epidemiological and cohort studies to aid in the identification of risk factors for the development of allergy.

Relevance

These finding may lead to interventions that help prevent the development and progression of allergic disease through better understanding of the regulation of sensitization.

The AllerGen network will provide an important conduit for the translation of these findings into clinically useful preventative and therapeutic strategies.

Epithelial-Mesenchymal Transition occurs in undifferentiated basal Bronchial Epithelial Cells in Normal and Asthmatic subjects.

Programme B – Diagnostics and Therapeutics

T-L. Hackett, PhD[1], F. Shaheen, BSc[1], D. Stefanowicz, BSc[1], G. Singhera, PhD[1], L.A. Murray PhD[2], R.A. Argenteri, BSc[2], D. Dorscheid, MD, PhD[1], T.R. Bai, MD[1], D.A. Knight, PhD[1].
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Supervisor: Tony Bai

Purpose: Remodeling of the asthmatic airway is believed to be an important early morphologic change and is associated with accumulation of myofibroblasts immediately beneath a thickened epithelial basement membrane. Whether bronchial epithelial cells could contribute to airway remodeling via epithelial-mesenchymal transition (EMT) is still unknown.

Objectives: To evaluate whether EMT occurs in primary airway epithelial cells (AECs), the mechanisms involved, and if this process is altered in asthmatic AECs.

Methods: Primary AECs were obtained from asthmatic (n=8) and non-asthmatic normal subjects (n=10). Monolayers and air-liquid interface epithelial (ALI-E) cultures were treated with TGFβ1 (10 ng/ml) for 48 and 72hrs with/without the pre-incubation of SMAD3 siRNA or neutralizing TGFβ antibody or appropriate controls. Cells were then either immunostained and imaged by confocal microscopy or lysed for RNA and protein for analysis by RT-PCR or immunoblotting for mesenchymal and epithelial markers. Taqman genotyping was performed using TagSingle Neucleotide Polymorphisms (SNP) assays for genes cytokeratin-5 (KRT5) and Ki67 (MiK67) from ABI, on 35 asthmatic, 35 atopic and 35 normal individuals.

Findings: When incubated with TGFβ1 ALI-E cultures from asthmatic patients demonstrated substantially increased numbers of epithelial cells undergoing EMT determined by positive staining for EDA-fibronectin and loss of E-cadherin, concurrent with changes in morphology, compared to normal ALI-E cultures. Immunoblot and gene expression in whole ALI-E lysates. Evaluation of mesenchymal markers EDA-fibronectin, vimentin, SMA-α, collagen-1, αVβ3 and co-ordinate loss of epithelial markers E-cadherin and ZO-1 expression by qPCR and immunoblot confirmed the confocal images. TGFβ1 induced EMT coincided with phosphorylation of SMAD3. Transfection with SMAD3 siRNA or treatment with a TGFβ neutralizing antibody prevented cells from undergoing EMT. In normal and asthmatic ALI-E cultures the cells exhibiting the EMT phenotype were positive for basal cell markers cytokeratin-5, CD151, p63 and Ki67 by confocal microscopy. Analysis of patient matched airway sections demonstrated elevated numbers of basal cells expressing CK-5, CD151, p63 and Ki-67 in the asthmatic airways. In addition TagSNP analysis for Ki67 a gene important in differentiation and proliferation, indicates an association between SNPs MKI67_1101 and MKI67_4750 and asthma (p=0.039 and 0.041 respectively).

Deliverables: Our data show that basal bronchial epithelial cells are increased in number in asthmatic patients and that these undifferentiated cells are able to undergo EMT in a multi-layered epithelium. We demonstrate that Ki-67 expression is dysregulated in asthmatic subjects and is associated with SNPs. These findings suggest differentiation programs are altered in the asthmatic epithelium which could affect airway remodeling in asthma.

Relevance: Our study provides further knowledge on the mechanisms involved in airway remodelling of asthmatic airways, which is an important component in the progression of the disease. Thus this research provides new potential therapeutic targets for asthma therapies to improve the quality of life of asthmatic patients

Maternal distress, atopic dermatitis and skin infections in early life

Programme B – Diagnostics and Therapeutics

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(5) Dept Pediatrics and Child Health, U of Manitoba
(6) Dept Pediatrics, U of Alberta

Supervisor: Anita Kozyrskyj

Objective: Evidence is emerging that maternal distress in early life leads to the development of atopic disease in children, but the mechanisms remain unknown. We examined the association between maternal distress and markers of stress, such as skin infections and atopic dermatitis (AD), in children up to five years of age.

Methods: This study utilized the full health care administrative records of the SAGE birth cohort of children born in Manitoba, Canada, in 1995. These records were accessed using the provincial health care databases at the Manitoba Centre for Health Policy. Variables culled from the database included maternal postpartum distress, skin infections occurring in the first 3 years of life, and a working definition for atopic dermatitis (AD). The working definition for AD was generated using diagnoses ICD9 691, 692 and 693 from hospital and physician claims records and validated against a pediatric allergist diagnosis of AD in the case-control study of SAGE. Measures for early (first 2 years) and late (3yr) onset AD and skin infections were created. Multivariate logistic regression was employed to determine the odds ratio and 95% confidence intervals for the association between maternal postpartum distress, skin infections and AD independent of major confounding factors.

Findings: Of the 13,980 children in the birth cohort, 23.9% of the mothers experienced maternal postpartum distress. The proportion of children who had early onset skin infection and early onset AD was 19.16% and 7.32%, respectively. The proportion of children who had both skin infection and AD within the first year of life was 20.88%. With respect to maternal postpartum distress, 22.42% of the children whose mother's experienced maternal distress had a skin infection diagnosis and 9.78% had AD. Children with a skin infection in the first 2 years were 1.210 (95% CI: 1.089 – 1.345) times more likely to have had a mother with postpartum distress. Children with AD in the first 2 years were 1.424 (95% CI: 1.194 – 1.697) times more likely to be exposed to maternal postpartum distress. These odds ratios were independent of a maternal history of asthma.

Deliverables: These findings are the outcome of a set of analysis conducted to create a valid measure for AD in early life using database measures, and to determine whether AD and skin infections are associated with maternal distress during this period. Our next steps are to include skin infections and AD as markers of stress on their own and in combination with measures of the stress response in later life, such as cortisol levels, in analyses which predict the development of asthma in adolescence.

Relevance: Not only are these results essential for our future analyses, but information on markers of stress in infants and toddlers can help physicians, public health nurses and child health policy makers provide better health care for children at this age.

Cord Blood (CB) Progenitor Cell Toll- Like Receptor (TLR) Expression: An Alternate Innate Immune Pathway In The Development Of Atopy?

Programme B - Diagnostics and Therapeutics

P Reece, L Crawford, R Sehmi, M Cyr, A Baatjes, JA Denburg
Division of Allergy & Clinical Immunology (McMaster University)

Supervisor: Judah A. Denburg

Rationale: Neonatal immune responses to microbial stimuli may modulate atopic development in later life. Although the underlying mechanisms are unknown, TLRs have been implicated in the pathogenesis of allergic development. Infants at risk for atopy demonstrate phenotypic alterations with respect to hemopoietic cytokine receptors (HCR) on CB progenitors. Since hemopoietic mechanisms are involved in atopic development and maintenance, we investigated the co-expression of TLR and HCR on CB progenitors.

Methods: Fresh CB, enriched for CD34+ cells through magnetic cell separation techniques, were stimulated with 10μL lipopolysaccharide (LPS) overnight and stained for surface and intracellular expression of TLR-2, TLR-4, TLR-9, IL-5R, IL-3R and GM-CSFR. Mean percent expression and specific median fluorescence intensity (sMFI) were calculated.

Results: Prior to stimulation, mean expression and sMFI were: TLR-2 (9.7 ± 0.5%, 5.6 ± 1.4), TLR-4 (1.8 ± 0.1%, 4.6 ± 2.8), TLR-9 (86 ± 2%, 22.0 ± 6.4), IL-5R (18.2 ± 10%, 17.2 ± 6.5), IL-3R (4.1± 0.08%, 2.5 ± 1.7), GM-CSFR (29.3 ± 0.7%, 12.2 ± 1.8). After stimulation, mean expression of TLR-2 (1.6± 0.7%) decreased ($p=0.001$, $n=4$); TLR-9 (94.9 ± 0.8%) increased ($p=0.005$, $n= 4$), while mean expression of IL-5R (8.8 ± 3.3%), IL-3R (1.5 ± 0.8%) and GM-CSFR (9.4 ± 2.7%) decreased ($p=0.01$, $n= 4$).

Conclusions: CB progenitor cells have significant TLR expression and TLR stimulation directly affects both TLR and HCR expression. These alterations may have functional consequences for CB progenitor cell differentiation into cells involved in allergic inflammation and disease. These findings may highlight an alternate innate immune pathway of microbial influence on the development of atopy in early life.

Deliverables: I have presented this work at the Canadian Society of Allergy and Clinical Immunology (CSACI) in Hamilton Ontario (October 2008) and will be presenting it at the American Academy of Allergy, Asthma and Immunology (AAAAI) in Washington D.C. (March 2009)

Relevance: With the growing pediatric allergic epidemic, it is imperative that research focuses on preventing the onset of allergy. Hemopoietic processes at birth may have an important role in subsequent development of atopy, therefore investigating changes in progenitor function and phenotype may reveal novel biomarkers and predictors of atopic outcomes, beginning in early childhood. These biomarkers may eventually prove helpful in designing preventative strategies and therapeutics which would not only improve the societal economic burden for patient care, but also the patient's quality of life.

IL-13 signaling through the IL-13 receptor α2 mediates airway epithelial wound repair

Programme B – Diagnostics and Therapeutics

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iCAPTURE Centre, University of British Columbia, St Paul's Hospital, Vancouver, BC, Canada

Supervisor: Tony Bai

Objective: The effects of IL-13 are mediated by a complex receptor system that includes IL-4 receptor α (IL-4Rα), IL-13 receptor α1 (IL-13Rα1) and IL-13 receptor α2 (IL-13R α2). IL-13Rα2 is thought to act only as a decoy receptor but recent investigations have demonstrated that IL-13Rα2 acts as a signaling receptor as well. Our laboratory has demonstrated that IL-13 promotes airway epithelial repair via the autocrine release of HB-EGF and activation of EGFR. Our recent finding shows that unlike IL-13Rα1, neutralization of IL-13Rα2 with specific antibodies inhibited HB-EGF synthesis and release and prevented airway epithelial repair. In the current investigation we further studied the role of IL-13Rα1 and α2 and their downstream effectors in airway epithelial repair.

Methods and Findings: Human airway epithelial ($1HAE^{o-}$) cells were transfected with IL-13Rα1, IL-13Rα2, STAT6 or scramble siRNA. After 48 hr of transfection the medium was replaced by serum free media and monolayers were subjected to multiple linear injuries or treated with IL-13 (10ng/ml). Protein cell lysates were collected at different time points. Non-transfected $1HAEo^-$ cells showed an increase in HB-EGF synthesis 6 hr after injury. IL-13Rα1 knocked-down-cells express the same amount of HB-EGF after injury; however, the expression of HB-EGF was significantly reduced in IL-13Rα2 knocked-down-cells. IL-13Rα1 knocked-down inhibited IL-13 induced MUC5AC expression in $1HAE^{o-}$ cells but has no effect on IL-13 induced HB-EGF expression. IL-13 has been shown to act through IL-13Rα2 to activate an AP-1 variant containing c-jun and Fra-2. Our model showed an over expression of Fra-2 and phosphorylation of STAT6 in response to IL-13. Mechanical injury, however, only induced the expression of Fra-2 and had no effect on expression of p-STAT6 by $1HAE^{o-}$ cells.

Deliverables: Our data suggest that IL-13 signaling through IL-13Rα2 and FRa-2 is involved in HB-EGF synthesis and airway epithelial repair.

Does IgE-mediated mast cell activation reduce oral tolerance to food antigens?

Programme B – Diagnostics and Therapeutics (CanGoFar Project)
Matthew Tunis and Jean S. Marshall, Dalhousie University

Supervisor: Jean S. Marshall

Objective/Purpose

While it is clear that mast cell activation in response to an allergen results in symptoms such as anaphylaxis, it is unclear how this activation impacts the immune response to other foods. The objective of this study is to investigate the role of immunoglobulin E (IgE) mediated mast cell activation in the maintenance or reduction of immunologic oral tolerance to a food antigen.

Methods

Three groups of C57Bl/6 mice were sensitized intraperitoneally with 31μg anti-trinitrophenol (TNP) IgE andibody. Oral tolerance to the egg protein ovalbumin (OVA) was established in two groups of mice by feeding OVA *ad libitum* for one week at 4mg/ml. All three groups were then immunized intraperitoneally with 50μg OVA-alum in order to elicit an anti-OVA antibody response. The sensitized mast cells of one group were activated by the delivery of TNP concurrent with the OVA immunization. Two weeks later all mice were challenged intraperitoneally with 10μg OVA alone. One week after challenge, blood was harvested from all mice by cardiac puncture and analysed for anti-OVA IgG1, IgG2a, IgE, and IgA antibodies by enzyme-lined immunosorbent assay (ELISA). Antibody titers were compared between in order to assess oral tolerance/sensitization.

Findings

Preliminary evidence indicates that the activation of mast cells concurrent with OVA antigen exposure leads to a reduction in oral tolerance. Tolerized animals in which mast cells were activated generated higher levels of anti-OVA antibodies than tolerant control mice. However, some degree of tolerance remained despite mast cell activation particularly in the IgE subclass.

These findings suggests that mast cell activation in response to a food allergen may reduce oral tolerance to other antigens, resulting in potential for a new allergy to a bystander food that is present during the initial allergic reaction to develop. This offers a possible explanation for the sequential development of multiple food allergies often observed clinically.

Deliverables

Increased understanding of the role of mast cell activation in regulating allergic sensitization and tolerance will inform clinical and epidemiological studies directed at investigating this relationship in patients. This research highlights a possible mechanism for allergy development that could aid in the development of preventative measures, such as stabilizing mast cells prophylactically in order prevent the onset of new food allergies. This work may also inform policy decisions regarding the potential consequences of exposure of high-risk individuals to known food allergens.

Relevance

This project is highly relevant to food allergies, their development, and their possible management or prevention. The findings of this study will inform our understanding of how new food allergies may develop and allow us to better understand how mast cell activation in response to an allergen can impact tolerance to new foods present during the initial allergic activation. This research will inform clinical and epidemiological research and the development of preventative therapies and policies geared at minimizing the development of new food allergies among Canadians. Initial dissemination of the study information is aimed at other allergy researchers and clinical groups who can evaluate the results of these experiments in the context of allergy patients.

PROGRAMME C
PUBLIC HEALTH, ETHICS, POLICY AND SOCIETY

ALLERGEN 2009 ANNUAL RESEARCH CONFERENCE

COMPLETED POSTER APPLICATIONS AND ABSTRACTS (N=25)

[Programme A: 11; Programme B: 8; Programme C: 6][1]

PROGRAMME C – PUBLIC HEALTH, ETHICS, POLICY AND SOCIETY					
Applicant #	**AllerGen Trainee**	**Institution**	**AllerGen Researcher/ Supervisor**	**AllerGen Research Programme (and funded research project where applicable)**	**Abstract Title**
1C	**Arrandale**, Victoria	**University of Toronto**	Dr. D. Linn Holness	**Programme C** – Public Health, Ethics, Policy and Society	The Toronto Skin Lung Research Program
2C	**Ben-Shoshan**, Moshe	**McGill University Health Centre**	Dr. Ann Clarke	**Programme C** – Public Health, Ethics, Policy and Society - 07C2: Surveying Canadians to assess the prevalence of common food allergies and attitudes towards food labeling and risk (SCAAALAR)	The Prevalence of Sesame Allergy: A Cross-Canada Study
3C	**Fenton**, Nancy	**McMaster University**	Dr. Susan J. Elliott	**Programme C** -- Public Health, Ethics, Policy and Society – 07C1: Evaluation of the implementation and effectiveness of statutory and regulatory school-based policies for anaphylaxis risk reduction	Constructing Risk: Understanding Anaphylaxis using Children's Illustrations
4C	**Harrington**, Daniel	**McMaster University**	Dr. Susan J. Elliott	**Programme C** – Public Health, Ethics, Policy and Society - 07C2: Surveying Canadians to assess the prevalence of common food allergies and attitudes towards food labeling and risk (SCAAALAR)	Perceptions of risks associated with food allergy and anaphylaxis: Preliminary Results of a National Survey

[1] **AllerGen Strategic Research Programme Foci:** Programme A - Gene-Environment Interactions
- *Strategic Focus: Genetics and gene-environment interactions in allergy and asthma*

o Programme B – Diagnostics and Therapeutics
- *Strategic Focus: Biomarkers, immune monitoring and drug discovery and development*

o Programme C – Public Health, Ethics, Policy and Society
- *Strategic Focus: Allergic disease management and surveillance*

Cross-programmatic research teams in priority areas

Established cross-programmatic teams
- o *The Canadian Healthy Infant Longitudinal Development (CHILD) Study*
- o *Food Allergy and Anaphylaxis*

Emerging cross-programmatic teams
- o *Mind-Body Interactions and Allergic Disease*
- o *Work-related Allergy and Asthma*

The Toronto Skin Lung Research Program

Programme C – Public Health, Ethics, Policy and Society

V.H. Arrandale and D.L. Holness, University of Toronto

Supervisor: Dr. D.L. Holness

Objective:
In 2005, AllerGen and The Centre for Research Expertise in Occupational Disease (CREOD) jointly funded the Skin Lung Workshop in Toronto, Ontario. The purpose of this workshop was to determine future directions for investigations of skin-lung interactions in occupational disease. This abstract summarizes the research projects that have resulted from the AllerGen funded Skin Lung Workshop to demonstrate how the initial funding has been leveraged into external grant funding, student projects and both national and international collaborations.

Methods:
In November 2005, 18 experts from Canada, the USA and Europe gathered in Toronto to discuss the skin, the respiratory system and the evidence for a connection between these systems in allergic disease. The speakers were Dr. Ian Kimber (Manchester), Dr. Mark Boeniger (formerly of NIOSH), Prof. John Cherrie (Scotland), Prof. Benoit Nemery (Belgium) and Dr. Carrie Redlich (Yale) and Denis Sasseville (McGill).

Findings:
The outcome of the workshop was a list of priority projects that would further our collective knowledge of the connection between the skin and respiratory systems. Proposed projects were grouped into four categories: clinical, experimental (mechanistic), exposure assessment and epidemiological studies. Since the workshop three major projects have been undertaken, two with additional external support (Ontario WSIB and Manitoba WCB). The first project was an analysis of existing occupational data on skin and respiratory symptoms in four industrial settings. The results of this work were presented at last year's AllerGen Annual Research Meeting (V. Arrandale). The second project is analysis of clinical patch test data to determine allergens that are implicated in both occupational contact dermatitis and occupational asthma (funded by Workplace Safety and Insurance Board of Ontario). This study is in the final stages and involves researchers from the Universities of Toronto and Ottawa as well as McGill. The final study is an epidemiological study of patients with occupational skin and/or respiratory complaints (funded Manitoba WCB). This study will begin collecting data in early 2009 at occupational health clinics in Toronto and Winnipeg. The small investment by AllerGen and CREOD to support the initial workshop allowed for leading experts to gather in one place for two days and exchange ideas. These ideas were translated into research projects that have been awarded external research funding and that continue to build on the collaborations initiated at the Skin Lung Workshop in 2005.

Deliverables:
These projects involve collaborations between the University of Toronto, (Dr. Holness, Dr. Scott, Dr. Silverman, Dr. Tarlo, Dr. Skotnicki-Grant, Dr DeKoven, Dr. Betschel, Dr. Corey) other Canadian institutions (Dr. Kraut - Manitoba, Dr. Pratt - Ottawa, Dr. Sasseville - McGill) as well as US and European investigators (Dr. Redlich – Yale, Dr. Kimber - Manchester). The results from these projects have been presented and submitted to numerous international conferences (ATS 2008 & 2009, AAAAI 2008, ACDS 2009, OEESC 2009, AllerGen 2008 & 2009). Manuscripts from the first two projects and a workshop proceedings document are currently in preparation.

Relevance:
This research directly contributes to the development of better public policy surrounding occupational exposures and occupational disease. Improved understanding of exposure routes in occupational allergic disease will lead to more refined exposure limits and better control of occupational exposures in the workplace. In addition, the connection between the skin and respiratory systems is a relatively new research area; work in this area may have implications beyond the occupational environment.

The prevalence of sesame allergy: A cross-Canada study

Programme C -- Public Health, Ethics, Policy and Society

Ben-Shoshan M[a], Harrington DW[b], Sicherer SH[c], Fragapane J[d], Soller L[d], Joseph L[d,e] St. Pierre Y[d], Godefroy S[f], Elliot SJ[b] and Clarke AE [d,g]
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[b]School of Geography and Earth Sciences, McMaster University, Hamilton, Ontario, Canada
[c]The Elliot and Rosyln Jaffe Food Allergy Institute, Division of Allergy and Immunology, Department of Pediatrics, Mount Sinai School of Medicine, New York
[d]Division of Clinical Epidemiology, Department of Medicine, McGill University Health Centre
[e]Departments of Epidemiology and Biostatistics, McGill University
[f] Bureau of Chemical Safety, Health Canada, Ottawa, Ontario, Canada
[g]Division of Allergy and Clinical Immunology, Department of Medicine, McGill University Health Centre

Supervisor: Dr Ann Clarke

Background: Sesame allergy is a severe food allergy. Recent studies report a prevalence of 0.79% in the United Kingdom compared to 0.13% in Israel *(JACI 2008;122:984)*, but the prevalence has not yet been assessed in North America.

Objective: To determine the prevalence of sesame allergy in Canada.

Methods: We performed a cross-sectional, nationwide,telephone survey by adapting a questionnaire used by Sicherer et al in the United States to assess the prevalence of other food allergies (*JACI 2003;112:1203 & JACI 2004;114:159*). Telephone numbers were randomly selected from the electronic white pages and a letter describing the study was mailed to all selected households. Respondents were eligible to participate if they were 18 years or older, were living in the household, appeared to have no language-mental-hearing barrier to understanding the questions, and reported that they were capable of responding to queries regarding the health status of all household members. To optimize response rates and minimize selection bias, at least ten attempts were made to contact households and calling was done on different days and at different times during the day. The following a priori criteria were established to define sesame allergy:1) self-report of a convincing history, defined as at least 1 moderate symptom (angioedema, throat tightness, change in voice, coughing, difficulty breathing(other than wheeze), nausea and/or vomiting, and/or abdominal pain) or 1 severe symptom (wheezing, stridor, cyanosis, and/or circulatory collapse) within 2 hours of eating sesame or 2 mild symptoms (pruritus, urticaria, flushing, or rhinoconjunctivitis) occurring within the same time interval or 2) self-report of a physician diagnosis of sesame allergy.

Findings: Of 8478 Canadian households surveyed in 2008, 2961 responded, representing 7940 individuals (34.9% household participation rate). Based on a convincing history and/or physician diagnosis, the prevalence of sesame allergy in Canada was 0.11% (95% CI, 0.05%, 0.22%); 37.5% of reactions were severe and the mean age of diagnosis was 6.5 years. In 57.10% of cases, the first reaction was also the most severe reaction. Out of 7 moderate or severe reactions, 3 (42.85%) were treated with epinephrine and 3 (42.85%) with antihistamines alone.

Constructing Risk: Understanding Anaphylaxis using Children's Illustrations

Programme C – Public Health, Ethics, Policy and Society

Nancy E. Fenton[1], PhD., Susan J. Elliott[1], PhD., Lisa Cicutto[2], ACNP, PhD.
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[2] National Jewish Health and the University of Toronto

Supervisor: Susan J. Elliott

Objectives/Purpose: The objective of this research was to explore the perceptions and experiences of 'school' as a safe place for children and adolescents with anaphylaxis allergies. Sabrina's Law, promulgated in Ontario in January 2008, requires school boards to establish and maintain an anaphylaxis response plan for every affected child. While Ontario chose to take a legislative path, other provinces have chosen alternative modes of risk response. For example, Alberta has recently introduced province wide, school-board based anaphylactic policies while Quebec is characterized by what would be termed a non-response within the risk literature. It is anticipated that the wide range of existing risk response mechanisms in place in Canada around anaphylactic allergies will result in a range of experiences related to notions of safety. While this poster explores the perceptions of Ontario students only, future comparative analysis will be undertaken with students from Alberta, Quebec and Newfoundland.

Methods: Qualitative data were collected from children (n=10, 8-12 year olds) and adolescents (n=10, 13-18 year olds) in Ontario who have anaphylactic food allergies. Participants were recruited using electronic tools from Anaphylaxis Canada (e.g., web site; e-newsletter). Participants were asked to draw a picture of 'what it is like to have a food allergy'. In addition, along with their parents, they were asked to participate in an in-depth interview focused on their experiences of living with a food allergy. The initial stage of analysis involved moving reflexively between the visual illustration and the verbal narrative explaining the image. Thematic analysis using a grounded theory approach was applied in order to uncover common themes and to identify context, process and patterns of connectivity between themes.

Findings: The illustrations provide a meaningful space within which to hear participants' perceptions and to explore feelings and experiences related to living with anaphylaxis. Three key themes emerged representing children's perception of risk: *social/environment risks*, *coping strategies*, and *emotional burden of responsibility*. For the most part, children's perceptions were mentally constructed and their illustrations represented imaginary scenarios about risk. Six themes emerged representing adolescent's perception of risk, the first three themes were the same as the children's group, expanded to include: *adapting*, *balance of responsibility*, and *re-defining normal*. In contrast to the children's group, adolescents' perceptions of risk were socially constructed and their illustrations represented real life experiences dealing with actual risk. These findings not only bring to light differences in risk perception, they reveal the conditions (e.g., informed school personnel; safe eating spaces; informed friends) that can facilitate safe transition from elementary to high school.

Deliverables: This study shows that participant's words and drawings offer a rich and meaningful research method to explore how they make sense of living with anaphylaxis. A process of member checking whereby participants' reviewed their transcript for any corrections and additions was conducted to ensure credibility. An analytic framework was developed and is presently being used to compare the meanings in the drawings with the accompanying narrative explanations. In addition to contributions to the scientific literature, these results will be communicated to policy makers and families through advisory bulletins.

Relevance: One of the most compelling implications for children (and adolescents) living with anaphylaxis is finding a method that empowers them in a process that is meaningful and relevant to their life. For policy makers, an understanding of what risk means to children may well be the most cost-effective way of informing educational and interventional efforts in responding to risk in schools. Finally, it is anticipated that this research will advance our understanding of the societal impacts of anaphylaxis in schools and provide insights into how children and adolescents construct risk within their allergic environments.

Perceptions of risks associated with food allergy and anaphylaxis: preliminary results of a national survey

Programme C – Public Health, Ethics, Policy and Society

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[2] McGill University Health Centre, Montreal, QC

Supervisor: Susan J. Elliott

Objective: The purpose of this research is to understand the perceived risks associated with food allergy and anaphylaxis from the perspective of three different groups: directly affected individuals (i.e. those with food allergies), indirectly affected individual (e.g. parents and other care givers of those with food allergies), and the general public.

Methods: As part of the SCAAALAR national food allergy prevalence survey, respondents were randomly selected and surveyed via telephone (n=1346). Respondents were queried on attitudes and opinions toward environmental health risks in general, and food allergy/anaphylaxis risks in particular.

Findings: Respondents were asked to rank 27 environmental health risks facing the Canadian population. The top ranked risk (i.e. those who rated a risk as either high or moderate) was obesity (93.9%). The risk ranked lowest was laser eye surgery (28.7%). In comparison, almost three-quarter ranked food allergies as a high or moderate risk (73.5%) while over two-thirds (68.5%) rated anaphylactic food allergies as high to moderate risk to the Canadian public. Preliminary analysis reveals significant differences between particular subgroups of the sample. For example, females were found to be 2.0 times as likely to rank food allergies as high or moderate risks [95% CI: 1.6 – 2.6]. Respondents who had not completed their secondary school education were twice as likely to rank food allergy risk as high or moderate in comparison with respondents with at least a secondary school education [OR: 2.0, 95% CI: 1.2 – 3.3]. Respondents living in Quebec [OR: 2.2, 95% CI: 1.6, 2.9] were also more likely to rank food allergy risks as high or moderate in comparison with other regions of Canada. No differences were found between (self-reported) allergic and non-allergic households. Analysis produced similar results for the risks of anaphylactic food allergies.

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

Relevance: Though collection of the SCAAALAR data is ongoing, initial results already have important implications for policy. Understanding the determinants of perceived food allergy and anaphylaxis risk will provide essential information for understanding what the risk of food allergies means to the directly affected, indirectly affected, and general population of Canada. The results of this research will advance our understanding of the societal response to food allergy risk. This information will be important for ensuring policies, media campaigns, and education initiatives appropriately reflect the needs and preferences of all Canadians.

Contextual Pathways: An Examination of the Relationship between Neighbourhood Characteristics and Maternal Stress

Programme C – Public Health, Ethics, Policy and Society

Heather Henley, University of Manitoba
Javier Mignone, University of Manitoba
Anita Kozyrskyj, University of Alberta

Supervisor: Anita Kozyrskyj

Objective/Purpose: To review and assess the literature discussing community-level characteristics that may influence maternal stress, and to develop measures of neighbourhood features.

Methods: Literature searches were conducted using the following databases PubMed and Google Scholar. Further searches were done with the following specifically relevant academic journals: Health & Place, The International Journal of Health & Addiction, Social Science & Medicine, The American Journal of Community Psychology, Journal of Family Psychology, and Environment & Health Perspectives. The search terms included were: neighbourhood effects, neighbourhood impacts, community effects, community contexts, community stressors, contextual effects, psychosocial impacts, psychosocial effects, maternal stress, maternal depression, and maternal health.

Findings: Numerous neighbourhood characteristics are thought to influence psychological stress and individual well-being including neighbourhood violence, fear of crime or personal victimization, and social disorder. Neighbourhood stressors are thought to be mediated by neighbourhood resources that may include social support, sense of safety, perceived social control, neighbourhood social capital, residential stability, social cohesion, and collective efficacy. The synthesis of the literature informed the development of measures of neighborhood features that capture the following constructs: social disorganization; social cohesion, and social capital.

Next steps: Manitoba datasets containing potential neighbourhood-level data will be studied to assess their potential use for the final construction of neighbourhood measures. Once completed, the neighbourhood-level variables will be used in multilevel models of longitudinal analyses that aim to determine whether stress hormones and markers at critical development stages in childhood predict the development as asthma, atopy, and other immune phenotypes at age 8-10 years and at age 12 years (SAGE cohort, corresponding to children born in Manitoba in 1995 and 2001).

Relevance: Better understanding of the contextual pathways between neighbourhood characteristics and maternal distress that may influence the development of asthma in children can help guide preventive community-level interventions and policies. Additionally, understanding the real costs of socially toxic environments may enable decision makers to invest more wisely in disadvantaged communities. Research findings will also help Healthy Child Manitoba (project partner) refine a risk assessment tool for maternal depression in the Families First postnatal screening program. Findings will also be communicated to groups such as the Canadian Research Institute for Social Policy, which publishes an online newsletter on child development.

School personnel knowledge regarding EpiPen® administration in Quebec

Programme C – Public Health, Ethics, Policy and Society

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Supervisor : Ann Clarke

Objective / Purpose With the rising prevalence of food allergy among school-aged children, many strategies have been developed to prevent anaphylactic reactions. However, avoidance measures are not always effective, and the efficacy of anaphylaxis management often relies on proper and rapid administration of epinephrine. In school, allergic children have to rely on school personnel to identify and treat anaphylactic events. In Quebec, school nurses are responsible for ensuring all school personnel are properly educated. Our objective is to describe the ability of school personnel in Quebec to recognize anaphylaxis and administer the EpiPen®.

Methods A school board located within a 1 hour radius of the investigators' office was randomly chosen. Following school board approval, randomly selected schools within the board were visited and school personnel were asked to demonstrate their administration technique using an EpiPen® trainer. Four steps were evaluated: 1. Removal of the grey cap, 2. Placement of the black tip against the mid-outer thigh, 3. Application of moderate pressure until the device clicks, 4. Duration of the contact between the device and the participant's thigh. School personnel were not notified in advance of the EpiPen® demonstration in order to prevent selection bias. Descriptive statistics were used to report data.

Findings Six elementary schools and 2 high schools were visited. All schools had at least one child with a life-threatening allergy. One hundred seventy-one participants (114 from elementary schools, 57 from secondary schools) were assessed. Regarding the demonstration, one person refused and the results for 2 people are unavailable. All participants answered the assessor's questions on indications for usage of the EpiPen®. Twenty-eight (16.7%) participants correctly demonstrated all 4 steps. However, four of these participants read the instructions on the device prior to demonstrating the technique. The accuracy rate of elementary school personnel was higher than secondary school personnel (20.5% vs 8.9%). The most frequent error was the omission of holding the device in place for 10 seconds. If this last step was excluded, 52 (31.0%) people would have demonstrated 100% accuracy of the technique. Other common errors: 31 people forgot to remove the grey cap, 91 did not place the device against the mid-outer thigh correctly (41 put their finger over the hole, 16 injected the wrong end of the device, 14 injected the wrong area of the body, and 20 made more than 1 error in this step), 11 did not press the device firmly enough and 103 did not keep the device in place for 10 seconds. Most (85%) participants reported receiving prior EpiPen® training. This percentage was identical among participants who demonstrated the technique with 100% accuracy. On average, participants had received prior training 6.4 times (7 times for those who did well), typically (94.5%) done by school nurses. Training for 115 participants included hands on use of an EpiPen® trainer. When asked to provide 3 indications for using an EpiPen®, 54.4% (n=93) were able to correctly provide 3 indications. For participants demonstrating 100% accuracy for the EpiPen® technique, this percentage reached 67.9%.

Deliverables Although the vast majority of school personnel in Quebec have attended multiple EpiPen® training sessions, only 16.7% were able to correctly demonstrate EpiPen® administration technique.