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

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

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

Conclusions and relevance: The overall objective of this research is to enhance our understanding of the pathogenesis of asthma by narrowing down genetic association signals to specific causal variants. Not only will this strengthen the evidence for IL1RL1 being an asthma gene but it will also help untangle the association signal from this region. Reaching a greater understanding of the molecular pathogenesis of asthma will eventually pave the way for novel therapies targeting the source of inflammation rather than life-long therapies aimed at dampening inflammation and easing symptoms.
between the major (ACC) and minor (GTT) promoter variants of SNPs rs3138053/rs22233406/rs22233409. Peripheral blood mononuclear cells of homozygous (ACC/ACC) and heterozygous (ACC/GTT) individuals were stimulated with LPS and live cultures of *Streptococcus pneumoniae* (serotype 14) for 3 and 4 hours. PBMCs of NFKBIA homozygotes and heterozygotes were stimulated with various Toll-like-receptor (TLR) ligands of the innate immune cascade to assay for differences in the innate immune response.

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

**Deliverables:** We have shown that the observed association of NFKBIA variants with infectious and inflammatory conditions has functional consequences. Individuals heterozygous for SNPs rs3138053/rs22233406/rs1050851 display allelic imbalance, reduced levels of NFKBIA expression, as well as a hyper inflammatory innate immune response.

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

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

**P3**

Functional genomics of the peripheral blood response to allergen inhalation challenge

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**Objective/purpose:** In asthmatic individuals, airway narrowing represents the early phase of the asthmatic response to allergen inhalation challenge, occurring within thirty minutes [1]. In 50-60% of allergic asthmatic adults, the early response is followed by the late phase asthmatic response, usually starting between 3-4 hours after allergen inhalation challenge [2]. and characterized mainly by cellular inflammation of the airway [3]. The pathways leading to the late response are not completely understood. Understanding these pathways is important for evaluating allergic diseases such as asthma. In contrast to the more transient isolated early response, development of the late response is associated with the hallmark inflammatory features of chronic allergic disease.

**Methods:** Nine adult subjects participating in ethically approved allergen challenge studies were recruited following informed consent. Inclusion criteria included non-smokers with stable, mild to moderate atopic asthma, free of other lung diseases. Subjects developing either an isolated early asthmatic response (≥20% drop in FEV1 within 2 hours) or the dual asthmatic response (early response + ≥15% drop in FEV1 between 3-7 hours) were studied. Peripheral blood was drawn just prior to inhalation challenge and 2-3 hours post-challenge. Gene expression analysis was performed using Affymetrix GeneChip® microarrays.

**Findings:** 1783 genes were differentially expressed between pre- and post-inhalation challenge (p ≤ 0.01). 364 genes remained significant at an FDR of 10%. Within this set, the DNAJC1 gene (p = 7.2e-5) has been previously identified in a GWAS (genome-wide association study) as associated with asthma. Gene ontology showed perturbed activity in immune system process, mast cell secretory granules and immunoglobulin biosynthesis.

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

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

**Acknowledgements:** We would like to thank the research participants for their involvement in this project. This research is made possible by financial support from AllerGen NCE.

**References**


**P4**

Association between filaggrin family member genes, asthma, atopy and atopic asthma with atopic dermatitis history in the subjects from the Saguenay-Lac-Saint-Jean founder population

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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P4

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

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

**Findings:** Positive associations were found between a haplotype block formed by FLG rs2184951 and rs12730241 (H1) and asthma and related phenotypes (see results in Table 1).

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

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

**Reference**

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

Michelle L North1,2,3,4, Hajera Amatullah1,2,3,4, Nivedita Khanna1,3,4, Bruce Urich1,4, Mary Spec5, Hartmut Grasemann1,6, Frances Silverman1,2,3,4,5, Jeremy A Scott1,2,4,5

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Asthma, Genes and the Environment (SAGE) Cohort at 11-13 years

Objective/purpose: The SAGE cohort includes 109 children with allergist-diagnosed atopic asthma (AA), 38 non-atopic asthmatics (NAA) and 185 control patients at the age of 11-13 years. Information on asthma symptoms and home environment was obtained from parents at the age of 11-13 years. Measurements and blood pressure were measured. Statistical analysis was performed with SPSS-17.

Findings: NAA (7.8%) was less common than AA (22.4%). NAA vs. AA was significantly higher systolic blood pressure (115.5 mmHg vs. 113 mmHg) but not statistically significant. Mean waist circumference (73.7 cm vs. 71.2 cm) and weight (54.6 kg vs. 50.7 kg) were higher in the NAA group but also not statistically significant. Early life tobacco exposure had an

Table 1 (abstract P4) Association of FLG haplotype and tagSNPs with asthma and atopy (A) and with asthma and atopy that co-occur with the presence of a personal history of atopic dermatitis (B)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>Asthma</th>
<th>Atopy</th>
<th>Asthmatic asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S.E(S) Z p</td>
<td>S.E(S) Z p</td>
<td>S.E(S) Z p</td>
</tr>
<tr>
<td>rs3126085</td>
<td>A</td>
<td>32.050 2 -3.48 0.0005</td>
<td>35.049 2 -2.94 0.0033</td>
<td>250.072 -2.74 0.0061</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>148.01298 3.48</td>
<td>141.01262 2.94</td>
<td>109.0968 2.74</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP/Haplotype</th>
<th>Allele</th>
<th>Asthma and AD</th>
<th>Atopy and AD</th>
<th>Asthmatic asthma and AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S.E(S) Z p</td>
<td>S.E(S) Z p</td>
<td>S.E(S) Z p</td>
</tr>
<tr>
<td>rs3126085</td>
<td>A</td>
<td>14.024 0.0033</td>
<td>16.0245 0.259</td>
<td>0.0097</td>
</tr>
<tr>
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<td>G</td>
<td>66.056 0.0009</td>
<td>62.0535 2.259</td>
<td>52.0440 2.74</td>
</tr>
<tr>
<td>H1</td>
<td>T G</td>
<td>75.066 2.61</td>
<td>75.064 3.33</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Positively associations were also found between FLG rs2065954 and rs3818831 and asthma (p = 0.0033 and p = 0.0016), atopy (p = 0.0009 and p = 0.0006) and asthmatic asthma (p = 0.0004 and p = 0.0002) in all cases in the presence of AD as well as AD alone (p = 0.0016 and p = 0.0007 respectively).

P5

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

Michelle L North1,2,3,4, Hajera Amatullah1,2,3,4, Nivedita Khanna1,2,3,4, Bruce Urich1,4, Mary Spec5, Hartmut Grasemann1,6, Frances Silverman1,2,3,4,5, Jeremy A Scott1,2,4,5

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Asthma, Genes and the Environment (SAGE) Cohort at 11-13 years

Objective/purpose: The SAGE cohort includes 109 children with allergist-diagnosed atopic asthma (AA), 38 non-atopic asthmatics (NAA) and 185 control patients at the age of 11-13 years. Information on asthma symptoms and home environment was obtained from parents at the age of 11-13 years. Measurements and blood pressure were measured. Statistical analysis was performed with SPSS-17.

Findings: NAA (7.8%) was less common than AA (22.4%). NAA vs. AA was significantly higher systolic blood pressure (115.5 mmHg vs. 113 mmHg) but not statistically significant. Mean waist circumference (73.7 cm vs. 71.2 cm) and weight (54.6 kg vs. 50.7 kg) were higher in the NAA group but also not statistically significant. Early life tobacco exposure had an

P6

Characteristics of atopic asthma and non-atopic asthma in the Study of Asthma, Genes and the Environment (SAGE) Cohort at 11-13 years

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Asthma, Genes and the Environment (SAGE) Cohort at 11-13 years

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

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

Findings: NAA (7.8%) was less common than AA (22.4%). NAA vs. AA was 9.9%/20.8% in females, 6.2%/23.6% in males. Mean FEV1% predicted (87.8% vs. 87.3%) did not differ between the NAA’s and AA’s. Wheezing episodes in the last year (2.3 vs. 2.7 p = 0.426) and episodes of sleep disturbances due to wheeze (0.7 vs. 0.5 p = 0.280) were not significantly different. NAA’s had slightly, but not significantly higher total cholesterol (4.0 mmol/L vs. 3.9 mmol/L), LDL (2.2 mmol/L vs. 2.0 mmol/L) and lower HDL (1.4 mmol/L vs. 1.49 mmol/L). For children with BMI >85%ile (n = 144), NAA’s had higher mean cholesterol (p = 0.08). NAA’s also had marginally higher systolic blood pressure (115.5 mmHg vs. 113 mmHg) but not statistically significant. Mean waist circumference (73.7 cm vs. 71.2 cm) and weight (54.6 kg vs. 50.7 kg) were higher in the NAA group but also not statistically significant. Early life tobacco exposure had an

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http://www.aacijournal.com/supplements/6/53
important influence on our subtypes. Interestingly, mothers of non-atopic asthmatics smoked fewer cigarettes/day in the first year of life (1.11 vs. 1.71 𝑝 = 0.025).

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

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

### P7

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

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**Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P7**

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

**Methods:** Mild asthmatics and normal controls were recruited as study subjects. This crossover study was double-blinded, randomized and counter-balanced to the order of three conditions: diesel exhaust with anti-oxidant, diesel exhaust with placebo, or filtered air with placebo. The subjects were exposed to either filtered air or diesel exhaust (300 μg PM2.5/m³) in a state-of-the-art diesel exhaust exposure facility. An anti-oxidant, N-acetylcysteine (600 mg), or a placebo was taken orally for five days preceding, and on the day of the exposure. Each subject was exposed to each of the three conditions. Peripheral blood samples were taken pre-exposure, and also at 2, 6, and 30 hours after the beginning of exposure. Sputum induction was performed by inhalation of hypertonic saline according to ATS guidelines pre-exposure, and also at 6, and 30 hours after the beginning of exposure. FACS Canto II (BD Biosciences) was used for flow cytometry. A 5-colour, 12-marker (CD3/CD8/CD4/Cy5/CD16/CD19/CD20/CD45/CD56/CD83/CD206/CD326/HLA-DR) combination was used to identify dendritic cells, macrophages, monocytes, neutrophils, eosinophils, and bronchial epithelial cells. Direct immunolabelling was performed on whole peripheral blood. After incubation, red blood cells were lysed. Remaining cells were washed and resuspended in PBS with 0.5% paraformaldehyde. Sputum plugs were homogenized with 0.1% DTT, filtered, and then centrifuged to remove supernatant. Sputum cells were resuspended in PBS at 1 million per mL. Direct immunolabelling was performed. After incubation, cells were washed and resuspended in PBS with 0.5% paraformaldehyde. Spectral compensation for flow cytometry was performed using an automatic calibration technique (BD CompBeads). Cellular debris was eliminated on the SSC/FSC scattergram. A gating strategy was designed to identify the leukocyte sub-populations and bronchial epithelial cells. Surface markers were chosen based on differential cell-specific expression according to existing literature.

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

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

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

### P8

**Respiratory syncytial virus replication induces Indoleamine 2,3-dioxygenase (IDO) activation in human dendritic cells**

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1University of Alberta, Canada

**Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P8**

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

**Methods:** Human dendritic cells (DCs) were infected with sucrese gradient purified RSV with a multiplicity of infection (MOI) rate of 1.0. Flow cytometry and confocal microscopy were used to confirm infection. We measured KYN in culture media by a spectrophotometric method using Ehrlich reagent. We blocked RSV infection with the RSV-mAb, Palivizumab, and UV-inactivation to determine a role for infection. The potent competitive inhibitor of viral RNA polymerase, Ribavirin, was used to block RSV replication and protein synthesis. To evaluate dependency of RSV-induced IDO induction on different cell signaling pathways, we used a variety of specific inhibitors including MEK inhibitor I (120 nM), MEK inhibitor II (4 μM), SB220190 (p38-MAPK, 3 μM), JNK inhibitor II (1 μM), IKK inhibitor II (Wedoralactone, 30 μM), IKK inhibitor III (BMS-345541, 3 μM) and relevant negative controls.

**Findings:** DCs incubated with RSV showed a 35% shift in flow cytometry compared to uninfected control DCs (n = 12) thus confirming infection of DCs. KYN, as a marker of IDO induction, was increased 13.2 fold in supernatants of infected DCs compared with control DCs (43.6 vs. 3.3 μM, n = 6). Inactivation of virus by Palivizumab or UV resulted in 99% decrease in levels of KYN compared to controls (n = 3). Infecting DCs with higher MOI of UV-inactivated RSV (up to 20, n = 3) did not induce IDO. Addition of Ribavirin to culture media reduced KYN release in a dose-dependent manner with 50% reduction at 220 μM (n = 3), without having any blocking effect on positive controls (IFN-γ induced KYN release) at similar concentrations. Except for SB220190, none of the specific inhibitors of signaling pathway including NF-κB, JNK-MAPK and p38-MAPK showed any significant inhibition of IDO induction by RSV (n = 3). SB220190, the specific inhibitor of P38-MAPK, blocked 51% (IC50 = 300 nM) and 92% (3 μM) of KYN release (n = 3); negative controls showed no inhibitory effect.

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

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

P9
Regulation of Thymic Stromal Lymphopoietin (TSLP) receptor expression
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Notre-Dame Hospital, CHUM Research Center, Canada
Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P9

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

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

Findings: Expression of TSLP-R on CD34+ cells was markedly enhanced by IL-1/TNF; this effect was suppressed by IL-4, IFN-γ and TGF-β and augmented by PG2E. TSLP-R was induced by SAC and PGN but was not affected by LPS or other TLR ligands. Most interestingly, dexamethasone slightly induced TSLP-R and IL-7Ra expression and markedly increased the effect of IL-1/TNF. The enhancing effect of dexamethasone was also observed on CD14+ and CD356+ neonatal cells. Isoproterenol had no effect on the regulation of TSLP-R.

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

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

P10
Adhesion molecules in experimental peanut allergy
Jami Bennett1, Steven Malby2, Erin Frohwerk2, Kay Jian1, Helen Merkens3, Mathew Tunis4, Kelly McNagny5
1Biomedical Research Centre, University of British Columbia, Vancouver, British Columbia, Canada; 2Department of Microbiology & Immunology, Dhalhouse University, Halifax, Nova Scotia, Canada; 3E-mail: Kelly@irc.ubc.ca
Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P10

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

Methods: Peanut allergy was induced in mice on the C57Bl/6 background (1A) with 4 weekly oral gavage feedings of peanut protein and cholera toxin. After a two-week rest period, sensitized animals were challenged by intraperitoneal injection with crude peanut extract (CPE) and monitored for anaphylaxis. Clinical indicators of peanut allergy include decreased body temperature, scratching, swollen eyes, decreased movement and responsiveness, and moribund condition. We evaluated plasma histamine, total IgE, peanut-specific IgE, and peritoneal albumin levels as in vivo indicators of mast cell degranulation and vascular permeability.

Findings: CD34+ Normally a surface marker of hematopoietic stem cells, mast cells, eosinophils and DCs, required for efficient cell migration. Mice exhibit attenuation of most mucosal inflammatory disease models. CD103+ Alpha-chain of integrin expressed by mucosal DCs and T cells which facilitates binding to mucosal epithelial cells; Mice exhibit exacerbated Th2 inflammatory responses. PSGL-1-/- and E-Selectin-/-: Adhesion molecules required for efficient homing of inflammatory cells to the sites of inflammation. Mice exhibit attenuated inflammatory responses.

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

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

Acknowledgements: Research funded by CanGoFAR/Hematopoietic Stem Cell Markers in Diagnosis & Prediction of Allergic Inflammation & Disease; Postdoctoral Fellowship, Multiple Sclerosis Society of Canada (JLB), Postgraduate Scholarship-Doctoral, Natural Sciences and EngineeringResearch Council (EJF); Strategic Training Program in Transfusion Science, CIHR/HSFC through CBR (SM), Research Scholar, Michael Smith Foundation for Health Research (KMM), and operating funds from CIHR (Canadian Institutes of Health Research).

P11
CD103 in the development of experimental asthma
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Objective/purpose: CD103 (Alpha-E Beta-7 integrin) is expressed on various cell types involved in the development of asthma, including dendritic cells (DCs) and CD4 and CD8 T regulatory cells. This integrin binds E-cadherin on epithelial cells and plays a role in regulating the migration and proliferation of cells. Also, CD103+ dendritic cells have been reported to direct the development of naive T cells into...
T regulatory cells. However, little is known about the exact role of CD103 in the development of asthma. The objective of this project is to investigate the role of this integrin in asthma pathogenesis, in the hope of finding new molecular targets for the treatment of asthma.

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

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

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

P13

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

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Background: Prenatal stress challenge is a pivotal environmental factor which has been proposed to increase the vulnerability of offspring to develop chronic immune diseases in later life. We analyze the effect of prenatal exposure to stress during late gestation in mice.

Materials and methods: BALb/c mice were exposed to sound stress during late gestation. Maternal serum and placentas were analyzed. Fetal development was scored. Allergic asthma was induced in the offspring by being given an ovalbumin protocol. We analyzed immune cells in lungs, bronchoalveolar fluid (BAL) and lung-draining lymph nodes as well as cytokine concentrations in the BAL. Further, stress-challenged pregnant females were treated with a progesterone derivative, followed by fetal analyses and evaluation of the vulnerability towards asthma in the offspring.

Results: Stress challenge resulted in decreased serum levels of maternal progesterone and testosterone, and increased serum levels of estradiol associated with placental endocrine dysfunction, such as low expression of proliferin. Fetal development was impaired upon stress challenge, especially in females. Prenatally stressed female adult offspring revealed an increased susceptibility toward asthma, mirrored by an increased airway response, influx of inflammatory cells and increased T helper 2 cytokines in the BAL. Further, we observed decreased frequencies of regulatory T cells (CD3+CD4+CD25+FoxP3+). Progesterone supplementation abrogated the impaired intrauterine development as well as the susceptibility toward asthma.

Conclusions: Our study revealed that prenatal stress severely interferes with the intrauterine development, resulting in offspring with an increased vulnerability toward asthma-like symptoms. Supplementation of progesterone during stress-challenged pregnancies abrogates gender-dependently the increased susceptibility toward asthma.

P14

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

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

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

Findings: A higher frequency of difficulty falling or staying asleep most of the time was observed in people with asthma (19.1%), chronic bronchitis (29.7%), or COPD (30.9%) compared to the general population (GP: 12.8%). Fewer patients with these conditions reported finding their sleep “refreshing” most of the time (A: 50.7%; CB: 42.1%; COPD: 45.1%) compared to those without these ailments (62.3%). A difference was also observed in regard to difficulty in staying awake most of the time during the day (A: 8.3%; CB: 10.5%; COPD: 11.0%; GP: 5.7%) and in the degree to which chronic fatigue was reported (A: 1.7%; BC: 3.2%; COPD: 5.2%; GP: 0.8%). Canadians with asthma and COPD report more sleep disturbance and chronic fatigue than healthy people.
Deliverables: Eventually, this study will be published in a journal and will be presented at both national and international conferences.

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

P15 CD34 function in intracellular signaling and mucosal inflammatory disease development
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Objective/purpose: CD34 is a cell surface sialomucin that has been the subject of extensive interest, largely based on its use as a marker for hematopoietic stem cells (HSCs) and vascular endothelia. Despite the almost ubiquitous use of CD34 as a HSC marker, little is known about its cellular function. Our lab was the first to show that CD34 is also highly expressed on mature murine mast cells, and we and other groups have found it to be expressed on eosinophils and dendritic cells. We found that mast cells derived from Cd34+ mice exhibit a marked increase in cell-cell aggregation. Moreover, when Cd34−/− mice were challenged in a mouse model of asthma, immune cell accumulation in the lung was drastically reduced, while the number of immune cells in the lung at baseline was similar to that of their wild-type counterparts. We have since found that deletion of the Cd34 gene in mice renders these animals resistant to a wide range of other mucosal inflammatory diseases, including hypersensitivity pneumonitis (HP), ulcerative colitis, salmonella infection and intestinal tumor development. Our objectives are to examine the specific role of CD34 in cellular function and to see whether or not CD34 is a viable therapeutic target to treat mucosal inflammatory diseases.

Methods: Bone marrow mast cells were derived from wild-type and Cd34−/− mice after four week culture in media containing IL-3. Changes in migration, polarization, degranulation and cytokine production were measured after c-kit and/or FcεRI stimulation. For in vivo studies, we developed transgenic mice that lack the mouse Cd34 gene and instead express, in all the appropriate tissues, the human Cd34 gene. These mice were put through a standard Ovalbumin (OVA) induced asthma model. Airway inflammation severity was assessed by analysis of the bronchoalveolar lavage (BAL) content, histological scoring of H&E stained lung sections and cytokine production of isolated lung inflammatory cells in response to OVA.

Findings: Preliminary experiments have suggested that CD34 plays an important role in c-kit signaling events and FcεRI induced degranulation. Initial testing of our hCd34 mice has shown that expression of the human Cd34 gene in Cd34−/− mice is sufficient to regain susceptibility to both allergic asthma and HP in mouse Cd34-deficient animals. These findings suggest that human CD34 serves a similar function to mouse CD34 in both animal disease models.

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

P16 Analysis of Tie2 function in mast cells
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Background: Mast cells are most widely acknowledged as a central mediator of allergic reactions. Recent literature has also implicated mast cells in a variety of biological and pathological conditions, spurring an interest in the genetic regulation of mast cell function and development. In a survey of global gene expression, we identified higher Tie2 mRNA expression in bone marrow derived mast cells (BMMC) in relation to a LinSca1+cKit− (LSK) bone marrow population. Tie2 (gene name Tek) is a receptor tyrosine kinase more commonly known for its expression on endothelial cells and a receptor for angiopoietins including Ang1 and 2. Our objective is to explore the function of Tie2 in mast cell development and biology.

Materials and methods: The mRNA level of Tie2 in of BMMC were established via a microarray and confirmed at the protein level through FACS and Western Blot. Mature BMMC were stimulated with Ang1 (200 ng/ml) and probed for phospho-Akt and phospho-Erk. Tie-2 deficient mast cells were derived from Tie2−/− and Tie2+/− embryonic stem (ES) cells, as reported previously [1]. Briefly, embryoid bodies were generated from embryonic bodies were generated from ES cells and incubated for 14 days, after which the embryoid bodies were dissociated into single cell suspensions and grown in media containing SCF and IL-3.

Results: BMMC express Tie2 mRNA and Tie2 protein at the membrane surface; to our knowledge, this is the first report of Tie2 protein expression in mast cells. Functional analysis and intracellular signaling response following stimulation with Tie2 ligands has revealed that Tie2 is a functionally active Ang1 receptor, as evidenced by activation of Akt and Erk. ES cell derived mast cells (ESMC) can be derived from Tie2+ and Tie2−/− ES cell lines, and these ESMC do not possess any observable morphological abnormalities.

Conclusions: Given the importance of mast cells in the pathology of human disease, analysis of novel genetic factors regulating mast cell function and development may provide insight into suitable therapeutic targets. To further explore the role of Tie2 in mast cells, future aims include evaluating the ability of Tie2−/− ESMC to reconstitute mast cell-deficient mouse models (eg, Kit−/−) and in vitro assays of Tie2−/− ESMC function such as degranulation and migration.

Reference

P17 Unique properties of RV in an in vitro model of asthma exacerbation
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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P17

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

Materials and methods: Human monocytes, T-lymphocytes, autologous moDC and eosinophils (EOS) were isolated from blood. moDC were

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cultured with RSV, RV, or sham, then washed, and incubated with autologous T-cells. To determine the role for antigen presentation, some moDC were treated with the inhibitor chloroquine before virus culture. In some, no moDC were added and T lymphocytes (CD4, CD8 or a mix) were exposed to RSV, RV or UV-inactivated RV (RV-UV). T-cell activation and apoptosis were measured by flow cytometry (CD25 expression and Annexin-V/TO-PRO-3 staining, respectively), and proliferation by BrDU incorporation (Cell Proliferation ELISA, Roche). EOS, cytokine-lukcy-toxine (Cyts-LT) release was measured by ELISA (Cayman Chemical). Cytoxine release was measured by Searchlight Pierce).

Results: RV presence induced a 5-fold increase in Cyts-LT release from EOS co-cultures with T-cells and moDC, while RSV did not have any effect. In T-cell and moDC co-cultures, RV induced greater increases in IL-1beta, IL-10, IL-12p70, IL-13 and IFN-gamma compared with RSV, T-cells, and more evidently CD8+ T-cells, showed increased apoptosis when incubated with RV but not with UV-RV or RSV. Virus-loaded moDC induced proliferation of T-cells, but, unlike RV, RSV was insensitive to chloroquine.

Conclusions: Our results suggest that RV and RSV induce EOS and T-cell activation differentially. RSV induces proliferation in a MHC-II restricted antigen-presentation-dependent manner, while for RV this is not the sole pathway. In addition, based on cytokine and Cyts-LT release, RV appears to be more potent compared to RSV. Our data are relevant to help understand why RV is so potent in the induction of asthma exacerbation in allergic patients, and will lead to novel potential targets for improved preventative strategies.

Deliverables: Fenretinide has been demonstrated to have great potential as a therapeutic agent. Our findings provide a novel approach to treat allergen-induced asthma and will help us in pursuing these studies towards the use of this drug for patients suffering from allergic asthma.

References:

P18

The protective effect of fenretinide against allergic asthma
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Objective/purpose: Fenretinide (N-4-hydroxyphenyl, 4HPR) is a synthetic retinoid derived from vitamin A with anti-apoptotic and anti-inflammatory properties. Studies have shown that at high concentrations fenretinide is an effective anti-neoplastic agent, regulating cell growth and differentiation. We have recently demonstrated that fenretinide, at doses 5-10x lower than for cancer treatment, has protective effect against bacterial infection [1] and osteoporosis [2] in Ctrf-knockout mice. The effect of fenretinide as a potential treatment for allergic asthma induced inflammation has not been evaluated in asthmatics. The goal of this study was to determine if, by normalizing inflammatory mediators, fenretinide would be able to alleviate the symptoms associated with allergic asthma.

Methods: Hypersensitive to methacholine, mice of A/J strain were sensitized weekly using ovalbumin (OVA) for three consecutive weeks, then age-matched and separated into two study groups and two control groups. For a period of 4 weeks, the study groups were orally treated with fenretinide while the controls were treated with the drug vehicle. During the last week of treatment, the control and study groups were split into two additional groups and underwent allergen challenges for three consecutive days, with either an OVA solution or PBS. Forty-eight hours after the third challenge, resistance of the respiratory system of the mice in response to methacholine was measured using a Buxco plethysmograph. Total IgE in plasma was measured by ELISA. Lung histopathology was observed using H&E and PAS stains.

Findings: Vehicle treated OVA challenged mice exhibited high values of airway resistance, plasma IgE concentration, and immune cell infiltration into the airways compared to PBS challenged animals. Interestingly, fenretinide treated OVA challenged mice had a statistically lower respiratory resistance. In addition, fenretinide treatment abrogated the recruitment of eosinophils to the region surrounding the blood vessels and airways. Similarly, after drug treatment a decrease in goblet cell hyperplasia was also observed through histopathology. However, no difference was observed in the level of plasma IgE between the control and study groups.

P19

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

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

Results: Oral sensitization followed by oral challenge evoked the most clinically potent allergic reaction, as compared with dermal or nasal sensitization (mean clinical scores of 1.7 ± 0.1, 1.0 ± 0.3 and 0.06 ± 0.04, respectively). To investigate peanut-specific humoral immune response we measured serum IgE and IgG subclass levels. Intragastric sensitization with peanut extract induced a significant IgE response (125.9 ± 21.7 ng/mL), whereas nasal or cutaneous priming favored elevated levels of the IgG subclasses. Higher IgG1/IgG2a ratios (10-25 folds within the cross sensitization/challenge groups) indicated Th2 polarization of the immune response. PN sensitization and challenge by all three routes resulted in similar increases of plasma histamine upon secondary challenge. Oral or nasal priming triggered greater inflammatory cell infiltration in the peritoneal cavity upon oral challenge. Dermal and nasal priming preferentially resulted in more severe skin inflammation as assayed by wheal diameter, compared with orally-sensitized animals. Upon
inhalational challenge, orally-sensitized mice exhibited greater cellular infiltration into the airways with predominantly neutrophilic influx, whereas nasal sensitization favored mild eosinophilia in the BAL. However, PN priming via these routes and subsequent challenge did not affect methacholine responsiveness of the airways.

Conclusions: Our observations indicate that 1) oral sensitization is the most likely to elicit a significant allergic response to peanut upon secondary challenge, dermal sensitization is less likely; nasal sensitization results in an intermediate likelihood. 2) Oral sensitization resulted in higher production of allergen-specific IgE antibodies, whereas nasal or cutaneous sensitization induced greater IgG responses; higher IgG1/G2a ratios point to Th2 biased response. 3) Initial exposure via the oral route triggered neutrophilia, while inhalational priming elicited an eosinophilic influx into the target organs. Comparison of the immune and cellular mediators of tolerance and anaphylaxis via the different routes may help to identify the causative mediator/cell population that could lead to novel therapeutic targets for intervention.

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Reference:

P20
A bacterial immune-prophylactic approach against asthma for infants and children
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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P20

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

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

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

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

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

P21
The role of perfluorooctanoic acid (PFOA) in airway hyperresponsive ness
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Objective/purpose: Evidence is emerging that human exposure to environmentally ubiquitous perfluorooctanoic acid (PFOA), used commonly for its household stain repellency characteristics, is associated with immunologic changes. Data from adults near an industrial PFOA disposal site demonstrated a strong negative correlation between blood PFOA concentrations and some immune responses. We propose to test if early life exposures to PFOA are playing a role in modifying airway responses.

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

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

Deliverables and relevance: The importance of studying the effects in pregnancy and early life was demonstrated by observations of PFOA exposures in pregnant mice where offspring died, while the mother seemed unaffected. In that study, mortality of the pups was attributed to pulmonary abnormalities. We have demonstrated that environmentally relevant exposures to PFOA in pregnant mice yield significant increases in liver weight. We will investigate lung function in the offspring of these dams. Pups from exposed and control dams are being weaned on the same diet as their mother. The exposed and control groups have been divided into 2 further groups one of which has been sensitized intraperitoneally at day 29 and 46 and intranasally at days 46, 47 and 48 with ovalbumin. Cytokines and WBC in BALF, IgE in blood and lung mechanics using flexVent will be measured and reported.
P22 Bronchial fibroblasts modulate CD4+Tcells phenotype towards Th17 in asthma

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

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

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

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

P23 CD34 is required for the infiltration of inflammatory cells into the mouse colon during DSS-induced colitis

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

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

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

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

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

P24 Immunoregulatory role of secretory leukocyte protease inhibitor in allergic asthma

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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P24

Background: Asthma is a complex and multi-factorial inflammatory disease [1]. It is one of the most common chronic diseases among children and adolescents [2]. Secretory leukocyte protease inhibitor (SLPI) has shown higher levels in asthmatic patients and its function as an anti-inflammatory protein has been documented in respiratory diseases [3,4]. However, its role in the immunomodulation of the response during allergic asthma has not yet been fully elucidated. The aim of this study was to evaluate the role of SLPI in the development of phenotypes associated with allergic asthma, and the effect of resiquimod treatment on the SLPI and the possible mechanisms of action involved in the disease.

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

Results: Allergic SLPI transgenic mice showed a significant decrease in airway resistance compared to wild-type mice (6.3 ± 1.1 vs. 8.0 ± 2.1 cm H2O 0 × s/ml, p < 0.001), the same effect was observed with inflammatory cell infiltration, eosinophil percentage (24 ± 1.1% vs. 29 ± 3.3%, p < 0.001), goblet cells (6 ± 4.1 vs. 36 ± 4.0%, p < 0.001) in the lungs and IgE levels (2014.1 ± 309.2 vs. 4173.2 ± 685.6 ng/ml, p < 0.001) in plasma. Allergic SLPI knock-out mice displayed significantly higher values compared to wild-type mice. They include lung resistance (8.6 ± 2.7 vs. 6.6 ± 0.5 cm H2O s/ml, p < 0.001), inflammatory cell influx, eosinophils (36.0 ± 2.7 vs. 29.0 ± 1.5%, p < 0.001), goblet cells (40 ± 4.1 vs. 30 ± 1.4%, p < 0.001), cytokine levels in the lungs (p < 0.05) and plasma IgE levels (3598 ± 204.7 vs. 2763 ± 220.3 ng/ml, p < 0.001). Expression of SLPI decreased inflammation in the lungs, plasma IgE levels, and lung resistance, whereas the ablation of SLPI has the opposite effect. Treatment with resiquimod improved airway resistance and inflammation of the lungs in SLPI knock-out and wild type, demonstrating that its effect is independent of the expression of SLPI.

Conclusions: SLPI plays an immunoregulatory role in the respiratory tract by reducing the inflammatory process and by improving lung physiology in a murine model of acute allergic asthma.
P25
Cord blood hematopoietic progenitor cell toll like receptor expression and function: a mechanism underlying allergic inflammation in early life?
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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P25

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

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

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

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

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

P26
Virus memory induces airway hyperreactivity through eosinophil activation
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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P26

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

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

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

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

Relevance: This study will identify the cellular pathway that is involved in a virus induced asthma exacerbation. Knowing this pathway will allow researchers to develop targeted methods that may prevent virus induced asthma exacerbations. Metabolomic characterization of virus induced asthma exacerbations in a Guinea Pig model will enable us to identify relevant markers to be studied in the human version of the disease.
signals through two receptors, IL-13Rα1/IL-4R and IL-13Rα2. IL-13Rα2 has previously been thought to act exclusively as a decoy receptor, however our findings show that IL-13Rα2 can act as a signaling receptor and is involved in mediating airway epithelial repair. Differential signaling via IL-13Rα1 or IL-13Rα2 may determine a remodeling versus repair response in injury to airway epithelial.

Methods: IL-13Rα1 and IL-13Rα2 functions were disrupted in Human Airway Epithelial (HAEo-) cells using specific IL-13Rα1 and IL-13Rα2 blocking antibodies and small interfering RNAs (siRNAs). HAEo- cells were also transfected with activator protein 1 (AP-1) specific and scramble siRNA. Following specific antibody blocking or siRNA transfection, HAEo- cells were either stimulated with IL-13 (10 ng/ml) or mechanically injured. Supernatants and protein lysates were collected at different time points. Expressions of phospho-STAT6, STAT6, Egr1, and AP-1 were determined by AP-1/1-luciferase assay.

Findings: IL-13 stimulation resulted in upregulation of phospho-STAT6, Egr1 and AP-1 expression. AP-1 expression correlated with activity as determined by AP-1/1-luciferase assay. Following mechanical injury, the expression of phospho-STAT6 and Egr1 was inhibited when IL-13Rα1 function was disrupted, while induction of AP-1 expression was unchanged. In contrast, when IL-13Rα2 function was disrupted, HB-EGF and AP-1 expression was inhibited while STAT6/Egr1 signaling remains intact. Gene silencing of AP-1 had no effect on phospho-STAT6 expression in response to injury, however HB-EGF expression was significantly inhibited compared to scramble siRNA treated cells.

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

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

PROGRAMME C : PUBLIC HEALTH, ETHICS, POLICY AND SOCIETY

P28

Does chronic stress predict the development of asthma in pre-adolescents?
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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P28

Objective/purpose: Pediatric asthma has risen over recent decades in developed countries, affecting almost 10% of children. Canada ranks near the top of the list of countries with high rates of asthma and allergic diseases. Predicting which children will develop asthma remains a challenge, but both higher weight and stress may play a role. An association between obesity and asthma has been reported in previous studies among adolescents and school-age children; this connection is more apparent in girls. Children exposed to maternal stress in early life are more likely to develop asthma. If the stress exposure becomes chronic and the body is unable to respond appropriately, allostatic load/overload (AL) can develop. AL is defined as the physical price paid by the body under stress exposure, however AL score higher than 2. There was no statistically significant difference between the mean AL score according to sex, urban/rural location and family history of asthma (p > 0.05). However, overweight children had significantly higher mean AL scores compared to non-overweight children (p < 0.001). None of the risk factors of sex, urban/rural residence, overweight or family history of asthma at baseline had a relationship with asthma development after the follow up period (all p > 0.05). Subsequent analyses are planned to determine the association between AL and the development of asthma in logistic regression models, which adjust for these factors and additional covariates.

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

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

P29

Treatment of allergic reactions to peanut in recent versus initial reaction
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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P29

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

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

Results: See Table 1.

Among 180 individuals reporting both an initial allergic reaction and a recent allergic reaction to peanut, epinephrine was administered in 8.9% (95% CI, 5.2-14.0%) and 17.2% (95% CI, 12.0-23.5%) respectively. Treatments excluding epinephrine were given in 35.6% (95% CI, 28.6-43.0%) of initial reactions and in 62.2% (95% CI, 54.7-69.3%) of most recent reactions. Among those treated only outside health care facilities (HCFs) no participant received epinephrine in initial reactions versus almost 9% (95% CI, 3.9-16.6%) in most recent reactions. However, in initial reactions, 44.8% (95% CI, 26.4-64.3%) of those treated, only in HCFs received epinephrine compared to 20% (95% CI, 2.5-55.6%) in recent reactions. Almost 1/3 (95% CI, 15.6-48.7%) of participants with a severe reaction did not receive any treatment for the initial reaction compared 6.7% (95% CI, 0.8-22.1%) of those with a recent reaction.
Conclusions: Although there is higher use of epinephrine in recent reactions compared to initial reactions, it is still administered in only 40% of severe allergic reactions. Further, our results suggest decreased epinephrine use over time in those treated initially in HCFs concurrent with increased use of other treatments such as anti-histamines. Given that prompt administration of epinephrine is the principal therapy for food-related anaphylaxis, it is crucial to develop and distribute guidelines and education programs that would contribute to increase epinephrine use inside and outside HCFs.

Table 1 (abstract P29)

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine +/- other medications</th>
<th>Other medications (excluding epinephrine) e.g: antihistamines</th>
<th>None</th>
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<tr>
<td>Initial reactions, % (95% CI)</td>
<td>8.9% (5.2, 14)</td>
<td>35.6% (28.6, 43)</td>
<td>55.6% (48, 62.9)</td>
</tr>
<tr>
<td>Mild</td>
<td>0.0% (0, 6.4)</td>
<td>26.6% (17.3, 42.2)</td>
<td>71.4% (57.8, 82.7)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7.7% (3.1, 15.2)</td>
<td>37.4% (27.4, 48.1)</td>
<td>54.9% (44.2, 65.4)</td>
</tr>
<tr>
<td>Severe</td>
<td>27.3% (13.3, 45.5)</td>
<td>42.4% (25.5, 60.8)</td>
<td>30.3% (15.6, 48.7)</td>
</tr>
<tr>
<td>Treated only outside HCF</td>
<td>0.0% (0, 9.3)</td>
<td>100.0% (90.7, 100)</td>
<td></td>
</tr>
<tr>
<td>Treated only in HCF</td>
<td>44.8% (26.4, 64.3)</td>
<td>55.2% (35.7, 73.6)</td>
<td></td>
</tr>
<tr>
<td>Treated outside and in HCF</td>
<td>40% (5.3, 85.3)</td>
<td>60% (14.7, 94.7)</td>
<td></td>
</tr>
<tr>
<td>Location unknown</td>
<td>12.5% (0.3, 52.7)</td>
<td>87.5% (47.3, 99.7)</td>
<td></td>
</tr>
<tr>
<td>Most recent reactions % (95% CI)</td>
<td>17.2% (12, 23.5)</td>
<td>62.2% (54.7, 69.3)</td>
<td>20.6% (14.9, 27.2)</td>
</tr>
<tr>
<td>Mild</td>
<td>5.7% (1.2, 15.7)</td>
<td>64.2% (49.8, 76.9)</td>
<td>30.2% (18.3, 44.3)</td>
</tr>
<tr>
<td>Moderate</td>
<td>16.5% (9.7, 25.4)</td>
<td>63.9% (53.5, 73.4)</td>
<td>19.6% (12.2, 28.9)</td>
</tr>
<tr>
<td>Severe</td>
<td>40% (22.7, 59.4)</td>
<td>53.3% (34.3, 71.7)</td>
<td>6.7% (0.8, 22.1)</td>
</tr>
<tr>
<td>Treated only outside HCF</td>
<td>8.8% (3.9, 16.6)</td>
<td>91.2% (83.4, 96.1)</td>
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</tr>
<tr>
<td>Treated only in HCF</td>
<td>20% (2.5, 55.6)</td>
<td>80% (44.4, 97.5)</td>
<td></td>
</tr>
<tr>
<td>Treated outside and in HCF</td>
<td>64% (42.5, 82)</td>
<td>36% (18, 57.5)</td>
<td></td>
</tr>
<tr>
<td>Location unknown</td>
<td>29.4% (10.3, 56)</td>
<td>70.6% (44, 89.7)</td>
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</tr>
</tbody>
</table>

CI, Confidence interval; HCF, Health Care facility

P30
Wheeze in the absence of asthma at age 8-10 is not associated with atopy in Manitoba children

Mathieu F Cooney1, Jennifer LP Protudjer1,2, Anita L Kozynskey2,3, Allan B Becker1,3
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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P30

Background: Atopy in children with recurrent wheeze is the best predictor for persistent asthma. A high prevalence of atopy was found in children with recurrent wheeze who were at high risk of developing persistent asthma based on the Asthma Predictive Index (API) [1]. A newly modified API now includes allergic sensitization to aeroallergens and to foods as criteria for the risk assessment of persistent asthma in children with recurrent wheeze. However, associations between wheeze and atopy in the absence of asthma have not been extensively examined. Since atopy is considered a risk marker for asthma in children with recurrent wheeze, we predicted that it would not be associated with wheeze when asthma was absent.

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

Results: 723 (404 [55.3%] boys) children were involved in this study (mean age 9.08 ± 0.53). 246 (34.1% [149 (36.9%) boys]) had pediatric allergist-diagnosed asthma. 236/714 (33.1%) children assessed had current wheeze based on allergist notes and 420 (58.4%) had parent-reported wheeze ever; these were not mutually exclusive. There was a significant association between atopy and parent-reported wheeze ever (OR 2.16; 95% CI 1.59-2.94), physician-noted wheeze with a cold (OR 2.23; 95% CI 1.65-3.00) and without a cold (OR 1.82; 95% CI 1.33-2.50). Physician-noted wheeze without a cold was more strongly associated with atopy in girls (OR 2.41; 95% CI 1.48-3.93) compared to boys (OR 1.46; 95% CI 0.96-2.22). In the absence of asthma, the association between atopy and parent-reported wheeze ever, physician-noted wheeze with a cold and without a cold was lost. Further stratification by PC20 category did not yield significant associations.

Conclusions: Atopy is an important diagnostic marker in the pediatric clinical assessment of wheeze. As predicted, wheeze not used in the diagnosis of asthma was not associated with atopy. These results support the use of a modified API that includes allergic sensitization to aeroallergens and foods for its positive predictive value.

Reference

P31
Canadians’ perception of food allergy risk

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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P31

Objective: The purpose of this research is to explore the determinants of the perceived risks associated with food allergy and anaphylaxis.
Methods: Households (n = 3,666) were selected at random, as part of a national food allergy prevalence survey, and data were collected via telephone. In addition to determining household allergy status, respondents were asked about environmental health risks, including those associated with food allergy/anaphylaxis. Multivariate logistic regressions, weighted to the age-sex structure of the Canadian population, were used to determine the characteristics of respondents who ranked the risks of food allergy and anaphylaxis as ‘High’ or ‘Moderate’.

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

Conclusions: Studies of risk perception 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 a need to develop appropriate policy responses that can protect allergic individuals, while accommodating the general population. This research contributes to this need by characterizing the societal response to the prevalence of food allergies and anaphylactic food allergies. Analyses revealed marked differences between Quebec and other provinces. It is becoming increasingly apparent that the policy environment in this province in particular is a key determinant of the experience and perception of allergy-related risk. Results from this research indicate that both the perceptions of affected and unaffected populations are modified in this context. Policymakers need to consider these impacts as advancements in regulations and policy emerge in this area.

P33

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

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Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3):P33

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

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

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

Conclusion: Peanut allergy is more common in children who have asthma, but there is not an additional significant association with parental asthma. Diagnosing peanut allergy in an early childhood is an early marker for increased asthma risk.
**P34**

**Comparative Study of Manitoba CHILD participants and the general Manitoba population**

Jennifer LP Protocol, Jing C Luo, Allan B Becker

1. Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 3P4
2. Manitoba Institute of Child Health, Winnipeg, Manitoba, Canada, R3E 3P4

**Background:** Low socioeconomic status (SES) is a risk factor for a broad array of health outcomes. Moreover, we sought to describe the socioeconomic status of participants in the vanguard and cohort of the Canadian Healthy Infant Longitudinal Development (CHILD) Study and compare these participants’ characteristics to similar characteristics of the general Manitoba population.

**Materials and methods:** Nation-wide CHILD plans to recruit pregnant women at 4 Canadian sites including Winnipeg where 1000 participants will be recruited over the next two years. Participants (including those in the ‘vanguard’, pilot group) completed questionnaires about their health, environment and SES. These data were described using descriptive and x² analyses. General Manitoba population data were obtained from Statistics Canada and Manitoba Health and Healthy Living.

**Results:** To date, 100 women (52 vanguard) have completed questionnaires. Participants were 30.8 ± 4.3 years old, this is similar to the average maternal age (29.7 years) at delivery for the general Manitoba population. Post-secondary training was higher amongst CHILD participants than the general Manitoba population (81.1% vs. 38.8% college/undergraduate and 14.9% vs. 5.1% graduate degrees, respectively). Using Statistics Canada’s income adequacy quartiles, the majority of CHILD participants were from upper middle class families. Notably, vanguard participants were more likely to own their own homes than cohort participants (93.8% vs. 71.4%; p < 0.015). In Manitoba, 1 in 5 women smoke. In CHILD, 8.8% of women currently smoke. Compared to the general Manitoba population, CHILD participants are of higher SES and are less likely to smoke, however more recent CHILD cohort participants represent a slightly broader demographic than vanguard participants. Continued efforts to recruit pregnant women from a broad demographic will provide the CHILD study with a nationally representative study population, which will serve to better understand genetic and environmental influences on early life development. This will be of importance to the AllerGen-supported CHILD study.

**Conclusion:** Given the increasing risk of maternal and infant health issues associated with maternal stress, this study will provide a better understanding of the socio-environmental factors impacting maternal health and child development.

**References:**
1. Statistics Canada: 

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**Does perceived stress in pregnant immigrant women predispose their infants to allergic disease development? - a work-in-progress**

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**Background:** Canada’s Immigration Program [1] reports that Canada has the highest per capita immigration rate in the world. Given the finding that pregnant immigrant women display higher prevalence of depressive and anxiety disorders than their Canadian counterparts, the investigation of health outcomes in this population is warranted. The present study will examine whether prenatal perceived stress and/or physiologic maternal stress responses are associated with the development of allergies in infants, in a diverse group of immigrant women.

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

**Results:** To date, forty-eight women have been recruited into the study. Preliminary data illustrate a wide range of depressive symptoms, perceived stress, and social support. Ten women reported high levels of depressive symptoms (≥17 on the EPDS) and high levels of perceived stress (≥19 on the PSS-10), and an additional four women reported both during early- to mid-pregnancy. Thus, stress and depressive symptoms appear to be distinct phenomena in this population. Participants recruitment and testing are ongoing.

**Conclusion:** A large portion of our (diverse) sample of immigrant women reported high levels of perceived stress and/or depressive symptoms during early- to mid-pregnancy. Whether these adverse perinatal mental states (and their associated dysregulated stress responses) contribute to the development of allergic disease in infants is under active, prospective investigation. A better understanding of the effects of perinatal factors on susceptibility to allergic disease in the infant can lead to development of interventions when plasticity in physiologic development is still relatively abundant [3].

**References:**

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**Subsequent childhood asthma and wheeze amongst small-for-gestational-age infants in Manitoba and India: an International Partnership Initiative**

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**Background:** Globally, asthma and wheeze are increasing. Concurrently, the incidence of infants born small-for-gestational-age (SGA) is rising. Evidence describing associations between these two conditions are conflicting. We sought to explore this phenomenon in two distinct populations: Manitoba, Canada and Bangalore, India.

**Materials and methods:** 1995 Manitoba Birth Cohort nested case-control study: Gestational period and birth weight were extracted from hospital records and classified as per Canadian SGA guidelines. At 8-10 years, asthma status and presence of wheeze were ascertained via pediatric allergist assessment. Parental-reported data included wheeze (ever, current [past year], or during various activities) as per International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, and demographic data. Bangalore Cohort: Gestational period at birth and birth weight were measured, SGA babies were classified as per World Health Organization’s SGA guidelines. At 2-7 years, presence of wheeze was ascertained via physician assessment/prescription record and ISAAC questionnaire. Asthma status was not assessed. All data were analyzed using descriptive statistics and x² tests.

**Results:** In Manitoba, 275 children (56.0% boys) were assessed. Mean gestational period was 39.5 ± 2.12 weeks (non-significant [NS] differences

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by gender. Mean birth weight was 3.38 ± 0.64 kg; girls were significantly smaller than boys (p < 0.006). 114 (16.1%) children (54.4% boys) were SGA. At ages 9.06 ± 0.64 years, 246 (34.1%) of children (149 boys) had asthma. No associations were identified between SGA and asthma, or between SGA and wheeze, when considering both genders combined or amongst boys only. Girls who were SGA were significantly more likely to have wheeze-related sleep disturbances than girls who were non-SGA (OR 0.33; 95% CI 0.12-0.94; p < 0.03).

In Bangalore, 432 children (48.0% boys) were assessed for wheeze-like symptoms. Mean gestational period was 38.68 ± 1.62 weeks and mean birth weight was 2.87 ± 0.69 kg (both NS differences between genders). Participants’ mean age was 3.78 ± 1.30 years. 130 (30.2%) children (47.7% boys) were SGA. 71 (16.4%) of children (38.4%) boys had a doctor diagnosis of wheeze. SGA children had twice the risk of developing wheeze at follow-up (OR 2.19; 95%CI 1.30-3.68; p < 0.003). After stratification by gender, these associations were only significant amongst boys (OR 3.24; 95% CI 1.64-7.31; p < 0.001). Conclusion: Children born SGA are at higher risk of developing wheeze-like symptoms, especially among the Indian boys. There is a small, yet significant association between SGA and wheeze-related sleep disturbances in Manitoban girls. Understanding the associations between SGA and wheeze may lead to enhanced pediatric clinical assessments. Public policy ought to target prevention of SGA.

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Estimating the prevalence of milk, egg, and wheat allergies in the Canadian population

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

Materials and methods: We performed a cross-sectional, nationwide, telephone survey adapted from a questionnaire used by Sicherer in the US to assess the prevalence of other food allergies [1,2]. Telephone numbers were randomly selected from the electronic white pages and an information letter was mailed to households. Respondents were eligible to participate if they were 18 or older, were living in the household, and appeared to have no language-mental-hearing barriers to understanding the questions. To optimize response rates and minimize selection bias, up to ten attempts were made to contact households, calling was done on different days and at different times during the day. Individuals were asked whether they had an allergy to milk, egg, and/or wheat.

Results: Of 10,596 households surveyed, 3666 responded, representing 9667 individuals (35% response rate). Of these, 202 (2.09% [95% CI, 1.81,2.39%]) self-reported an allergy to milk, 77 (0.8% [0.63,0.99%]) to egg, and 74 (0.7% [0.60,0.96%]) to wheat. Egg allergy was more prevalent in children than adults, and wheat allergy was more prevalent in adults than children. Both egg and wheat allergies were more prevalent in households with a post-secondary graduate. Regional differences between allergies to milk, wheat and egg were also evident, with Quebec showing a lower prevalence compared to elsewhere in Canada. The unusually high prevalence of milk and wheat allergy in adults is not consistent with the literature [3,4], and may be due to participant confusion with lactose intolerance and celiac disease, respectively. Currently, our research team is contacting participants from the survey in order to validate their report of allergy to milk, egg and/or wheat.

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

References
More frequent demonstrations of auto injector use during clinic visits are needed. Furthermore, training that better stimulates real anaphylaxis reactions, including acute management, needs to be developed.

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

Relevance: Our study provides direction for improvement during allergy clinic visits, and strategies to address common challenges allergists face.

This in turn will help improve patient care and quality of life for families living with food allergy. We aim to share our findings with patients and families through Anaphylaxis Canada, and with pediatricians across the country at the 2010 Canadian Pediatric Society Annual Conference.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Xu et al. Challenges and strategies in managing food allergy: a patient and allergist perspective. Allergy, Asthma & Clinical Immunology 2010, 6(Suppl 3)P38.