AllerGen Poster Competition

2015 CSACI Annual Scientific Meeting

Book of Abstracts

October 21-24, 2015

Vancouver Convention Centre, Vancouver, BC

ALLERGEN POSTER COMPETITION

#	Presenter Name	Abstract Title	Page
1	**Azad, Meghan**	Breastfeeding and infant wheeze, atopy and atopic dermatitis: findings from the Canadian Healthy Infant Longitudinal Development Study	3
2	**Boucher-Lafleur, Anne-Marie**	*IL33* DNA methylation in bronchial epithelial cells is associated to asthma	4
3	**Chen, Michael**	NRF2 mediates the antioxidant response to organic dust-induced oxidative stress in bronchial epithelial cells	6
4	**Cosic, Nela**	The effects of perinatal distress, immune biomarkers and mother-infant interaction quality on childhood atopic dermatitis (rash) at 18 months	7
5	**Dawod, Bassel**	Examining the immunological mechanisms associated with cow's milk allergy	9
6	**De Schyver, Sarah**	Typtase levels in children presenting with anaphylaxis to the Montreal Children's Hospital	10
7	**Feldman, Laura Y.**	Secondhand tobacco smoke exposure in infancy and the development of food hypersensitivity from childhood to adolescence	12
8	**Hosseini, Ali**	Combined exposure to diesel exhaust and allergen enhances allergic inflammation in the bronchial submucosa of atopic subjects	13
9	**Huq, Saiful**	Comparison of skin-prick test measurements by an automated system against the manual method	14
10	**Khamis (Hamada), Mona**	The accurate identification and quantification of urinary biomarkers of asthma and COPD through the use of novel DIL-LC-MS/S methods	15
11	**Kim, Young Woong**	Systemic immune pathways associated with the mechanisms of Cat-Synthetic Peptide Immuno-Regulatory Epitopes, a novel immunotherapy, in whole blood of cat-allergic people	17
12	**Ladouceur, Erika**	Reducing the health disparities: online support for low-income children with asthma and allergies	18
13	**Larouche, Miriam**	Epigenetic association of PSORS1C1 and asthma in a Saguenay-Lac-Saint-Jean familial collection	20

ALLERGEN POSTER COMPETITION

#	Presenter Name	Abstract Title	Page
14	Legere, Stephanie A.	IL-33 induces cytokine and chemokine production in human mast cells	22
15	Lu, Zihang	Reference ranges for lung clearance index from infancy to adolescence for Canadian population	23
16	North, Michelle	Kingston Allergy Birth Cohort: cohort profile and mother/child characteristics	24
17	Rahman, Tanvir	Cow's milk protein specific IgE, IgA & IgG4 as a predictor of outcome in oral immunotherapy	25
18	Simons, Elinor	Age of peanut introduction and development of reactions and sensitization to peanut	27
19	Singh, Amrit	Multi-omic blood biomarker signatures of the late phase asthmatic response	28
20	Soliman, Mena	Mapping local inflammatory cytokine secretion following a cumulative allergen dose using the Allergic Rhinitis Clinical Investigator Collaborative Nasal Allergen Challenge model	29
21	Stiemsma, Leah	Early life gut microbial alterations in children diagnosed with asthma by three years of age	30
22	Thiele, Jenny	Nasal allergen challenge induced eosinophilia - the Allergic Rhinitis Clinical Investigator Collaborative	31
23	Tran, Maxwell	The relationship between food sensitization and atopic dermatitis at age 1 year in a Canadian birth cohort	32
24	Tworek, Damian	Allergen inhalation enhances toll-like receptor-induced thymic stromal lymphopoietin receptor expression by hematopoietic progenitor cells in mild asthmatics	34
25	Walsh, Laura	The Allergic Rhinitis Clinical Investigator Collaborative - Replicated eosinophilia on repeated cumulative allergen challenges in nasal lavage samples	35
26	Warner, Linda	The CHILD study: optimizing subject retention in pediatric longitudinal cohort research	36
27	Yang, ChenXi (Yolanda)	Differential expression of C3a and C5a in allergic asthma	37

AllerGen Poster Competition

Abstract #1

BREASTFEEDING AND INFANT WHEEZE, ATOPY AND ATOPIC DERMATITIS: FINDINGS FROM THE CANADIAN HEALTHY INFANT LONGITUDINAL DEVELOPMENT STUDY

Meghan B. Azad[1], Zihang Lu[2], Allan B. Becker[1], Padmaja Subbarao[2], Piushkumar J. Mandhane[3], Stuart E. Turvey[4], Malcolm R. Sears[5], the CHILD Study Investigators[6]

[1]Department of Pediatrics and Child Health, University of Manitoba and Children's Hospital Research Institute of Manitoba, Winnipeg, Manitoba, Canada
[2]Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada
[3]Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada
[4]Department of Pediatrics, Child & Family Research Institute and British Columbia Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada
[5]Department of Medicine, McMaster University, Hamilton, Ontario, Canada
[6]Canadian Healthy Infant Longitudinal Development Study

BACKGROUND

The impact of breastfeeding on asthma and allergy development is controversial. We aimed to characterize the association of breastfeeding with childhood wheeze, atopy and atopic dermatitis in the first year of life.

METHODS

We studied 2587 infants with complete breastfeeding data from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. Infant diet and wheezing episodes were reported by mothers at 3, 6, and 12 months. Atopy was determined by skin testing and atopic dermatitis was diagnosed at 12 months. Breastfeeding was classified as exclusive (human milk only), partial (supplemented with formula, other beverages or solid foods) or none.

RESULTS

Breastfeeding rates were 82% at 3 months (52% exclusive, 30% partial), 74% at 6 months (13% exclusive, 61% partial), and 46% at 12 months (partial). In their first year, 21% of infants experienced ≥1 wheezing episode, 14% were sensitized to ≥1 allergen, and 12% were diagnosed with atopic dermatitis. Breastfeeding rates were lower among younger mothers, First Nations women, and those who smoked or did not have a postsecondary degree. Independent of these maternal characteristics, increasing duration and intensity of breastfeeding were associated with a reduced risk of wheezing: adjusted odds ratio (aOR) 0.97 (95%CI 0.94 – 0.99) for each additional month of breastfeeding; aOR 0.68 (0.50-0.92) for exclusive versus no breastfeeding at 3 months. In contrast, breastfeeding was associated with an increased risk of atopy: aOR 1.04 (1.01-1.07) for each additional month; aOR 1.46 (1.02-2.10) for exclusive versus no breastfeeding. Breastfeeding was not associated with atopic dermatitis.

CONCLUSIONS

In the CHILD cohort, increasing duration and intensity of breastfeeding are associated with a lower risk of wheeze and a higher risk of atopy in the first year of life. Ongoing research will address potential confounding by reverse causation, extend analyses through early childhood, and evaluate physician-diagnosed asthma and food allergy.

AllerGen Poster Competition

Abstract #2
IL33 DNA METHYLATION IN BRONCHIAL EPITHELIAL CELLS IS ASSOCIATED TO ASTHMA

Anne-Marie Boucher-Lafleur[1], Valérie Gagné-Ouellet[1], Éric Jacques[2], Sophie Plante[2], Jamila Chakir[2], Catherine Laprise[1]
[1]Département des sciences fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada
[2]Centre de recherche, Institut Universitaire de Cardiologie et de Pneumologie, Ste-Foy, Québec, Canada

BACKGROUND
Inflammation is a key mechanism of asthma pathogenesis [1]. The bronchial epithelium acts as a barrier involved in innate and adaptive immunity through secretion of inflammatory mediators [2]. Interleukin (IL) 33 is an alarmin cytokine released by airway epithelial cells [3] that has been identified as a gene of susceptibility in asthma in several studies [4-6], as well as being a potential severity biomarker of asthma [7]. *IL33* gene expression was increased in epithelial cells from asthmatic individuals [8]. However, less is known about the epigenetic regulation of *IL33* particularly in severe asthma. In this context, we aimed to characterize DNA methylation (DNA-me) and expression signatures of *IL33* in epithelial cells obtained from mild and severe asthmatic individuals.

METHODS
A total of 21 bronchial epithelial cell (BEC) lines from asthmatics individuals (mild n=7; severe n=4) and control individuals (n=10) were used for DNA and mRNA extraction. DNA-me was measured by bis-pyrosequencing and mRNA level was assessed by qRT-PCR. Student t-tests were performed to analyze the association between DNA-me and asthma severity (significant for $\Delta\beta > 5\%$ and $p > 0.05$). Pearson correlations were used to analyze the correlation between DNA-me and mRNA level.

RESULTS
A hypomethylation for mild and severe BEC groups ($\Delta\beta = 22\%$, p=0.003; $\Delta\beta = 17\%$, p=0.090 respectively) was observed. The DNA-me of *IL33* promoter was also negatively correlated with mRNA levels (r=-0.750, p=0.050), suggesting an upregulation of gene expression by DNA-me. The total mRNA level for *IL33* was increased in BECs from mild and severe asthmatic individuals (foldchange =3.4, p=0.017; foldchange=4.7, p=0.060 respectively).

CONCLUSION
Methylation modifications in *IL33* promoter could modulate gene expression in epithelial cells of asthmatic individuals. This study highlighted the relevance of *IL33* as a potential biomarker of asthma severity.

REFERENCES
1. Calhoun WJ: **The Spectrum of Asthma: An Introduction.** In *Heterogeneity in Asthma*. 1st edition. Edited by Brasier AR. Galveston: Springer; 2014 16-19.
2. Erle DJ, Sheppard D: **The cell biology of asthma.** *J Cell Biol* 2014, **205:** 621-631.
3. Cayrol C, Girard J: **IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy.** *Curr Opin Immunol* 2014, **31:**31-37.
4. Moffat MF, Gut IG, Demenais F, Strachan, DP, Bouzignon, E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WO: **A large-scale, consortium-based genomewide association study of asthma.** *N Eng J Med* 2010, **363:**1211-1221.
5. Torgeson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB et al*.*: **Meta-analysis of genome-wide association studies of asthma in ethSnically diverse North American populations.** *Nat Genet* 2011, **43:**887-892.
6. Bønnelykke K, Sleiman P, Neilsen K, Kreiner-Moller E, Mercader JM, Belgrave D, den Dekker HT, Husby A, Sevelsted A, Faura-Tellez G et al.: **A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbation.** *Nat Genet* 2014, **46:**51-55.

AllerGen Poster Competition

7. Guo Z, Wu J, Zhao J, Liu F, Chen Y, Bi L, Liu S, Dong L: **IL-33 promotes airway remodeling and is a marker of asthma disease severity.** *J Asthma* 2014, **51:**863-869.

8. Préfontaine A, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, Martin JG, Hamid Q: **Increased IL-33 expression by epithelial cells in bronchial asthma.** *J Allergy Clin Immunol* 2010, **125:** 752-754.

AllerGen Poster Competition

Abstract #3

NRF2 MEDIATES THE ANTIOXIDANT RESPONSE TO ORGANIC DUST-INDUCED OXIDATIVE STRESS IN BRONCHIAL EPITHELIAL CELLS

Michael Chen[1], Toby McGovern[1,2], Mikael Adner[2], James G. Martin[1]

[1]Research Institute of the McGill University Health Centre, Department of Medicine, McGill University, Montréal, Québec, Canada

[2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

BACKGROUND

Bronchial epithelial cells play an important role in mediating the response to airway injury and repair through the release of chemotactic factors, such as interleukin 8 (IL-8) and antioxidant production. These antioxidant mechanisms are particularly important in the context of neutrophil-mediated pulmonary disease. Inhalation of organic dust (OD) from swine confinement facilities leads to pulmonary neutrophilia, airway hyperresponsiveness and oxidative stress. We sought to examine the role of NRF2, a critical transcription factor involved in mediating the endogenous antioxidant response, in bronchial epithelial cells following OD exposure. We hypothesized that OD exposure would increase NRF2 activity and antioxidant production in human bronchial epithelial cells.

METHODS

A human bronchial epithelial cell line (BEAS-2B) was stimulated for 24 hours with 100 µg OD. Supernatant was retained for evaluation of IL-8 by ELISA. qPCR was performed for NRF2-dependent and NRF2-independent antioxidant genes. NRF2 nuclear translocation was quantified at various time points using an NRF2 luciferase reporter assay and by immunofluorescence.

RESULTS

OD exposure resulted in an increase of IL-8 release. mRNA expression levels of NRF2-dependent antioxidant genes HO-1, NQO1 and GCLM, but not of NRF2-independent antioxidant genes SOD 1, and catalase were increased following OD exposure compared to control cells. OD exposure induced a time and dose-dependent increase in luminescence indicating NRF2 nuclear translocation, a result confirmed by quantification of NRF2 nuclear localization by immunofluoresence.

CONCLUSIONS

OD exposure induces IL-8 release, NRF2 nuclear translocation, and upregulation of antioxidant gene expression in BEAS-2B cells. These results suggest a dual role for bronchial epithelial cells in mediating neutrophil recruitment as the endogenous antioxidant response. We demonstrated that the endogenous oxidative properties of OD are specific to NRF2 and that NRF2 is a critical mediator of OD-induced oxidative stress in bronchial epithelial cells.

AllerGen Poster Competition

Abstract #4

THE EFFECTS OF PERINATAL DISTRESS, IMMUNE BIOMARKERS AND MOTHER-INFANT INTERACTION QUALITY ON CHILDHOOD ATOPIC DERMATITIS (RASH) AT 18 MONTHS

Nela Cosic[1,2], Henry Ntanda[1,2], Gerald Giesbrecht[3], Anita Kozyrskyj[4], Nicole Letourneau[1,2]

[1]Faculty of Nursing, Department of Pediatrics & Psychiatry, University of Calgary, Calgary, Alberta, Canada

[2]Faculty of Medicine, Department of Pediatrics & Psychiatry, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

[3]Faculty of Medicine, Department of Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

[4]Faculty of Medicine and Dentistry, Department of Pediatrics, University of Alberta and School of Public Health, University of Alberta, Edmonton, Alberta, Canada

BACKGROUND

Perinatal psychosocial distress, including stressful life events, anxiety and depression are risk factors for childhood atopic dermatitis (rash) [1]. Maternal psychosocial distress is associated with excess prenatal stress hormones [2] and reduction in the quality of maternal-infant interaction [3], which may affect biomarkers of infant immunity such as interleukin (IL) levels and predispose the growing child to rash [4,5]. This study will: (1) describe the association between perinatal distress and infant interleukins at 3 months of age, (2) describe the association of interleukins and atopic dermatitis (rash), and (3) build a best fit model from the identified associations in (1) and (2).

METHODS

120 women reported distress levels during pregnancy and at 3 months postpartum. Venus blood was collected from their 3-month-old infants to assess plasma interleukin levels. Maternal-child interaction was measured 6 months postpartum with the Nursing Child Assessment Teaching Scale. Presence and number of skin areas affected by rash were assessed *via* parent report at 18 months. Correlation and multiple regression analyses identified the best fit model for rash using forward stepwise regression.

RESULTS

Prenatal depression (r=-0.23, p=0.01) and stressful life events pre- and post-natally (r=0.27, p=0.00) were associated with IL2p70. Pre- and post-natal anxiety were associated with IL8 (r=-0.28, p=0.02) and IL4 (r=-0.24, p=0.04). IL10 was associated with child skin rash (r=-0.25, p=0.01) at 18 months. 20% of the variance in childhood rash at 18 months was explained by the model (Table 1).

Table 1: Multiple regression analysis for variables predicting child skin rash

Variables	Estimate	Standard Error	P-value
Maternal Age	-0.03	0.01	0.023
Maternal Education	0.11	0.06	0.091
IL10	-1.31	0.41	0.002
Perinatal Stress	-0.12	0.06	0.057
Maternal-Infant Interaction Quality	0.02	0.01	0.018

Note: IL10 was transformed by taking the square. *P <0.05

CONCLUSIONS

Perinatal distress is associated with elevated infant ILs. Demographic variables (maternal age, education), IL10, perinatal stressful life events (*e.g.* separation/divorce, family death) and maternal-infant interaction best predicted skin rash in 18-month-old infants. We will now evaluate how maternal-child interaction moderates associations between both (1) perinatal distress and (2) immune biomarkers, and atopic dermatitis in children at 18 months.

AllerGen Poster Competition

REFERENCES
1. Peters JL, Cohen S, Staudenmayer J, Hosen J, Platts-Mills TA, Wright RJ: **Prenatal negative life events increases cord blood IgE: interactions with dust mite allergen and maternal atopy**. *Allergy* 2012, **67**(4)**:**545-551.
2. Giesbrecht G, Campbell T, Letourneau N, Kooistra L, Kaplan B, APrON Study Team: **Psychological distress and salivary cortisol covary within persons during pregnancy**. *Pscyhoneuroendocrinology* 2011, **27**(12)**:**171-180.
3. Letourneau N, Watson B, Duffett-Leger L, Hegadoren K, Tryphonopoulos P: **Cortisol patterns of depressed mothers and their infants are related to maternal–infant interactive behaviours**. *J Reprod Infant Psychol* 2011, **29**(5)**:**439-459.
4. Wright RJ: **Prenatal maternal stress and early caregiving experiences: implications for childhood asthma risk**. *Paediatr Perinat Epidemiol* 2007, **21**(s3)**:**8-14.
5. Von Hertzen LC: **Maternal stress and T-cell differentiation of the developing immune system: possible implications for the development of asthma and atopy**. *J. Allergy Clin Immuno* 2002, **109**(6)**:**923-928.

AllerGen Poster Competition

Abstract #5
EXAMINING THE IMMUNOLOGICAL MECHANISMS ASSOCIATED WITH COW'S MILK ALLERGY

Bassel Dawod[1], Jean Marshall[1, 2]
[1]Department of Pathology, Dalhousie University, Halifax, Nova Scotia, Canada
[2]Department of Microbiology & Immunology, Dalhousie University, Halifax, Nova Scotia, Canada

BACKGROUND
Oral tolerance is a state of unresponsiveness of the immune system to food antigens. Failure of tolerance can lead to future allergic reactions. Milk allergy, is the most common childhood allergy, affecting between two to seven percent of children [1]. Beta-lactoglobulin (BLG) is a major allergen in cow's milk. Several factors, such as transforming growth factor-beta, vitamin A, and soluble toll-like receptors (TLRs) that are found in breast milk, are thought to enhance oral tolerance toward breastmilk-transferred antigens. In contrast, we have shown previously that PAM_3CSK_4, a TLR2 activator, is able to disrupt oral tolerance toward ovalbumin in mice. The variable levels of these tolerogenic or sensitizing factors in breast milk or baby formulas might be critical to the development of tolerance or allergy. The objective of this work was to better understand the impact of these milk factors in the development of tolerance toward milk-antigens.

METHODS
Models of oral tolerance to cow's milk, BLG, and ovalbumin were established. Oral tolerance was assessed in wild type and TLR2-deficient mice through analysis of antigen-specific antibody levels after a systemic antigen challenge. The development of antigen-specific Tregs was also assessed.

RESULTS
Oral administration of doses of skim milk (above 1mg/ml protein for 7 days) or BLG induced the development of oral tolerance, independently of TLR2. Immunoglobulin-E levels in antigen fed mice were 35% of the control (n=15). In contrast to ovalbumin, tolerance induction to BLG in milk was not altered by the addition of PAM_3CSK_4. Heat-killed *Lactobacilli* also did not modify milk tolerance. These results indicate that immunoregulatory factors in milk modify the response to an oral TLR2 activator.

CONCLUSION
This research could provide important insights into the significance of specific milk contents for the development of milk and other allergies, potentially informing both allergy prevention and treatment strategies.

ACKNOWLEDGEMENTS
Funded by AllerGen NCE and Canadian Institutes of Health Research (CIHR).

REFERENCES
1. Bahna SL: **Cow's milk allergy versus cow milk intolerance.** *Ann Allergy Asthma Immunol* 2002, **89**(6)**:** 56-60.

AllerGen Poster Competition

Abstract #6

TRYPTASE LEVELS IN CHILDREN PRESENTING WITH ANAPHYLAXIS TO THE MONTRÉAL CHILDREN'S HOSPITAL

Sarah De Schryver[1], Michelle Halbrich[2], Ann Clarke[3], Sebastian La Vieille[4], Harley Eisman[5], Reza Alizadehfar[1], Lawrence Joseph[6], Judy Morris[7], Moshe Ben-Shoshan[1]

[1]Division of Allergy and Clinical Immunology, Department of Pediatrics, Montréal Children's Hospital, Montréal, Québec, Canada

[2]Division of Paediatric Allergy and Clinical Immunology, Department of Paediatrics, University of Manitoba, Winnipeg, Manitoba, Canada

[3]Division of Rheumatology, Department of Medicine, University of Calgary, Calgary, Alberta, Canada

[4]Food Directorate, Health Canada, Ottawa, Ontario, Canada

[5]Department of Emergency Medicine, Department of Pediatrics, Montréal Children's Hospital, Montréal, Québec, Canada

[6]Department of Epidemiology and Biostatistics, McGill University, Montréal, Québec, Canada

[7]Department of Emergency Medicine, Hôpital du Sacré-Cœur, Montréal, Québec, Canada

BACKGROUND

The study aimed to evaluate tryptase levels in children presenting with anaphylaxis, to examine predictors of elevated tryptase (defined as levels ≥11.4 ng/ml), and to compare tryptase levels during and post-anaphylaxis.

METHODS

Between April 2011 and September 2014, data were collected on anaphylaxis cases at the Montreal Children's Hospital Emergency Department. Cases were recruited either prospectively or identified retrospectively through chart review. Total tryptase levels were measured within 2 hours following onset of symptoms. Levels during reaction and approximately 10 months after reaction were compared using confidence intervals based on paired means using the t-distribution. Logistic and linear regression models were fit to estimate the associations between tryptase levels and sociodemographic and clinical characteristics of anaphylaxis.

RESULTS

Over a three-year period, 165 children were recruited and had serum tryptase levels measured. Among those, the mean tryptase level was 7.6 μg/l (SD 6.4) and 32 cases [19.4% (95% CI, 13.8%, 26.4%)] had elevated tryptase levels. Elevated levels were found more frequently in severe reactions compared to moderate and mild reactions (Table 1).

Table 1. Elevated serum tryptase levels during reaction according to severity of reaction

Grades of anaphylaxis	Elevated Tryptase (N)	Elevated Tryptase %, 95% CI
Overall	32/165	19.4 (13.8, 26.4)
Mild	6/32	18.8 (7.8, 37)
Moderate	19/121	15.7 (9.9, 32.7)
Severe	7/12	58.3 (28.6, 83.5)

Of the 54 children with post-reaction tryptase levels, the mean tryptase level was 8.2 μg/l during reaction and 3.7 μg/l post-reaction; yielding a difference of 3.5 (95% CI, 2.96, and 3.96). Only severe reactions were associated with elevated tryptase levels (≥ 11.4 μg/l) [OR 7.2 (95% CI 2.11, 24.40)]. Factors associated with an increase in tryptase levels regardless of previously published thresholds were severe reactions and milk trigger [beta=10.3 (95% CI, 6.9, 13.7) and 4.3 (95% CI, 0.5, 8.0)], respectively.

AllerGen Poster Competition

CONCLUSIONS

Our study results do not support the role of tryptase as a reliable diagnostic biomarker for the diagnosis of anaphylaxis in children. Assessing the difference between levels during and post-reaction may improve the diagnostic utility of tryptase, mainly in severe reactions or reactions triggered by milk. Future studies are needed to evaluate more reliable diagnostic biomarkers.

ACKNOWLEDGEMENTS

AllerGen Poster Competition

Abstract #7

SECONDHAND TOBACCO SMOKE EXPOSURE IN INFANCY AND THE DEVELOPMENT OF FOOD HYPERSENSITIVITY FROM CHILDHOOD TO ADOLESCENCE

Laura Y. Feldman[1,2], Jesse D. Thacher[3,4], Inger Kull[3,4], Erik Melén[3,4], Göran Pershagen[3], Magnus Wickman[3,4], Jennifer L. P. Protudjer[3,4†], Anna Bergström[3,4†]

[1]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada
[2]Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada
[3]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[4]Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden
[†]Both authors contributed equally to this work.

BACKGROUND

Previous studies have demonstrated a link between early-life exposure to secondhand tobacco smoke (SHS) and allergen-specific immunoglobulin E (IgE) mediated sensitization to food allergens. However, it is unclear whether this association extends to clinical symptoms following food consumption [1]. We aimed to determine if SHS exposure during infancy is associated with food hypersensitivity from childhood to adolescence.

METHODS

Data were obtained from the BAMSE birth cohort of 4,089 Swedish children born in 1994–96 and followed to adolescence [2]. SHS exposure in infancy was assessed through parental report when the children were 2 months old. Food hypersensitivity was defined as the presence of parent-reported symptoms to specific food items at 1, 2, 4, 8, 12 and 16 years. Food sensitization was defined as an IgE ≥0.35 kU_A/l to fx5® – a mix of milk, egg, soy, peanut, wheat and codfish allergens – at 4, 8 and 16 years. Odds ratios (OR) and 95% confidence intervals (95%CI) from generalized estimating equations were used to calculate the overall association between SHS exposure in infancy and food hypersensitivity and/or food sensitization. Estimates were initially adjusted for young maternal age (≤25 years) at birth, exclusive breast feeding (≥4 months), parental allergy and socioeconomic status; they were subsequently adjusted for concomitant asthma.

RESULTS

SHS exposure in infancy was associated with 1.12 times greater odds of reporting food hypersensitivity (OR 1.12; 95%CI 0.96–1.30) and 1.29 times greater odds of food sensitization (OR 1.29; 95%CI 1.06–1.57). With respect to concurrent outcomes, SHS exposure in infancy was associated with 1.43 times greater odds of having both food hypersensitivity and sensitization (OR 1.43; 95%CI 1.06–1.87); this estimate did not change substantially upon adjustment for concomitant asthma (OR 1.39; 95%CI 1.03–1.88).

CONCLUSIONS

SHS exposure in infancy is associated with food sensitization from childhood to adolescence, particularly with concurrent food hypersensitivity.

ACKNOWLEDGEMENTS

REFERENCES

1. Saulyte J, Regueira C, Montes-Martínez A, Khudyakov P, Takkouche B: **Active or passive exposure to tobacco smoking and allergic rhinitis, allergic dermatitis, and food allergy in adults and children: a systematic review and meta-analysis.** *PLoS Med* 2014, **11**(3)**:**e1001611.
2. Wickman M, Kull I, Pershagen G, Nordvall SL: **The BAMSE project: presentation of a prospective longitudinal birth cohort study.** *Pediatr Allergy Immu* 2002, **13**(s15)**:**11-13.

AllerGen Poster Competition

Abstract #8

COMBINED EXPOSURE TO DIESEL EXHAUST AND ALLERGEN ENHANCES ALLERGIC INFLAMMATION IN THE BRONCHIAL SUBMUCOSA OF ATOPIC SUBJECTS

Ali Hosseini[1,2], Tillie L. Hackett[2], Jeremy Hirota[1,2], Kelly McNagny[3], Susan Wilson[4], Chris Carlsten[1,2]

[1]Department of Medicine, University of British Columbia, Vancouver General Hospital, Vancouver, British Columbia, Canada
[2]University of British Columbia, Institute for Heart + Lung Health, Centre for Heart Lung Innovation, St. Paul's Hospital, Vancouver, British Columbia, Canada
[3]University of British Columbia, Biomedical Research Centre, Vancouver, British Columbia, Canada
[4]Histochemistry Research Unit, University of Southampton, Southampton, Hampshire, United Kingdom

BACKGROUND

Asthma is a chronic inflammatory disease of the airways. Diesel exhaust (DE) is a major contributor to ambient particulate matter (PM). There is evidence that PM acts as an adjuvant to biological allergens and aggravates allergic inflammation [1,2]. We aim to elucidate if DE increases allergen-induced inflammation and cellular immune response in the airways of atopic human subjects.

METHODS

We recruited 12 volunteer subjects with allergy to house dust mite (HDM), birch or timothy grass. In a randomized blinded, crossover design, subjects were exposed to DE ($300\mu g$ $PM_{2.5}/m^3$) or filtered air for 2 hours. One hour following the exposure, segmental allergen challenge was performed by instilling into contralateral lung segments through flexible bronchoscopy, either the allergen extract to which the participant is sensitive to, or placebo (sterile saline). Endobronchial biopsies from the same segments were then obtained 48 hours post exposure. Thus, biopsies under 4 different conditions were acquired: filtered air and saline (FAS), DE and saline (DES), filtered air and allergen (FAA), and DE and allergen (DEA). Tissue biopsies were embedded in glycol methanlacrylate acrylic resin and 2 μm sections were cut and used for immunostaining with monoclonal antibodies to CD4, interleukin (IL)-4, tryptase and eosinophil cationic protein (ECP). Aperio ImageScope software was used to quantify the immunohistochemical staining of positive cells in the bronchial submucosa excluding smooth muscle and glands.

RESULTS

The percent positivity for CD4 expression significantly increased from FAS (0.087 ± 0.018) to DEA (0.311 ± 0.06039; p=0.035) in the bronchial submucosa. The percent positivity for IL-4 expression elevated from FAS (0.127 ± 0.062) to DEA (0.548 ± 0.143; p=0.034). The percent positivity for tryptase and ECP expression remained unaffected. Data are presented as mean ± SEM.

CONCLUSIONS

This data suggest that co-exposure to DE and allergen augments $CD4^+$ T cells recruitment and IL-4 expression, thus promoting Th2 polarization in the bronchial submucosa of atopic airways.

ACKNOWLEDGEMENTS

REFERENCES

1. Riedl M, Diaz-Sanchez D: **Biology of diesel exhaust effects on respiratory function.** *J Allergy Clin Immunol* 2005, **115**(2)**:**221-228.
2. Nel AE, Diaz-Sanchez D, Ng D, Hiura T, Saxon A: **Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system.** *J Allergy Clin Immunol* 1998, **102**(4)**:**539-554.

AllerGen Poster Competition

Abstract #9

COMPARISON OF SKIN-PRICK TEST MEASUREMENTS BY AN AUTOMATED SYSTEM AGAINST THE MANUAL METHOD

Saiful Huq[1], Rishma Chooniedass[1], Brenda Gerwing[1], Henry Huang[1], Diana Lefebvre[2], Allan Becker[1]
[1]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada
[2]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

BACKGROUND

Allergic diseases are the earliest of all chronic diseases to develop in children and their prevalence is on the rise [1,2]. Skin prick tests (SPT) are considered an important diagnostic tool for allergies [3,4]. SPT results to a specific allergen are typically calculated by manual measurement of the wheal of a response. The objective of this study is to evaluate a new, automated, image-processing method for wheal measurements against the standard of manual measurements.

The Canadian Healthy Infant Longitudinal Development (CHILD) Study is a general population, longitudinal study that focuses on the development of allergy in early life. For the purposes of this project, we measured SPTs from one-year clinic visits at the Manitoba CHILD site. SPTs were performed for common allergens as well as positive and negative controls, histamine and glycerine respectively.

METHODS

SPTs were performed for 10 common allergens (cat, dog, cockroach, D. farinae, D. pteronyssinus, Alternaria, cow's milk, egg white, soybean and peanut) and controls in 1033 children (mean age: 13 ± 2 months). Of the 12,396 tests, there were measurable wheals for 1180 SPTs, identified by both automated and manual methods. By manual measurements 1105 wheals were $\geq$2mm while by the automated method 1004 were $\geq$2mm. Each child's results were measured by hand and by an automated scan and measure system developed in our laboratory. The overall diameters averaged 2.94 ± 1.0 (range: $0.5 - 7.8$) by the automated method and 3.52 ± 1.1 (range: $0.5 - 9.3$) by the manual method with a correlation coefficient of 0.91.

RESULTS

The results demonstrate the value of the automated measurements. The potential advantages of the automated method are reproducibility, accuracy, and speed when compared with manual measurements.

REFERENCES

1. Boyce JA, Assa'ad A, Burks AW, et al.: **Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel.** *J Allergy Clin Immunol.* 2010, **126**(60)**:** S1-58.
2. Eigenmann PA: **Breaking frontiers for better early allergy diagnosis**. *Allergy* 2004, **59**(9)**:** 895-896.
3. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, Niggemann B: **The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges**. *Clin Exp Allergy* 2005, **35**(3)**:** 268-273.
4. Kanny G, Moneret-Vautrin DA, Flabbee J, Beaudouin E, Morisse, M, Thevenin F: **Population study of food allergy in France**. *J Allergy Clin Immunol* 2001, **108**(1)**:** 133-140.

AllerGen Poster Competition

Abstract #10

THE ACCURATE IDENTIFICATION AND QUANTIFICATION OF URINARY BIOMARKERS OF ASTHMA AND COPD THROUGH THE USE OF NOVEL DIL- LC-MS/MS METHODS

Mona M. Khamis[1], Hanan Awad[1], Kevin Allen[1], Darryl J. Adamko[2], Anas El-Aneed[1]

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[2]College of Medicine, Department of Pediatrics, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

BACKGROUND

With the growing power of analytical capabilities, tens-to-hundreds of potential biomarkers are regularly being identified in discovery experiments. However, only a limited number has actually achieved FDA approval for routine clinical practices. One of the major impediment in biomarker discovery pipeline is the lack of coherent and well-established assays for subsequent validation steps [1].

Asthma and chronic obstructive pulmonary disease (COPD) are of particular importance to practitioners due to their overlapping clinical presentations. Diagnosis in regular outpatient clinics can be difficult. Current diagnostic tests are laborsome, unachievable in some cases, and/or insensitive to changes in airway function [2].

Metabolomics has demonstrated promising potential for discovering new biomarkers. Urine is an ideal matrix for metabolomic studies due to its richness in metabolites and ease of collection. A novel proton nuclear magnetic resonance (^{1}H-NMR) study verified 50 polar urinary metabolites as candidate biomarkers for the differential diagnosis of asthma and COPD patients. Therefore, the next step in the 'new biomarker' pipeline is to accurately quantify and validate these metabolites with respect to their clinical sensitivity and specificity [2].

METHODS

Two liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods were developed to simultaneously quantify 34 metabolites in urine. Differential isotope labeling (DIL) was applied using ^{12}C/^{13}C-labeled dimethylaminophenacyl bromide and dansyl chloride reagents.

RESULTS

DIL has significantly improved the detection of the polar metabolites in urine using a conventional LC-MS/MS platform. Methods were successfully developed to allow for the quantitative separation of the target metabolites. The use of structurally identical internal standards is an ideal option to correct for matrix effects from indigenous compounds within the urine (Figure 1).

CONCLUSION

The developed methods are suitable for quantifying 34 candidate metabolites. Currently, we are validating the LC-MS/MS methods according to the FDA guidelines. This will allow us to quantify the targeted metabolites in the urine of asthma and COPD patients.

AllerGen Poster Competition

Figure 1. LC-MS/MS chromatogram of (A) [12]C- dimethylaminophenacyl-labeled metabolites and corresponding [13]C- internal standards and (B) [12]C- dansyl-labeled metabolites and corresponding [13]C- internal standards, in control urine
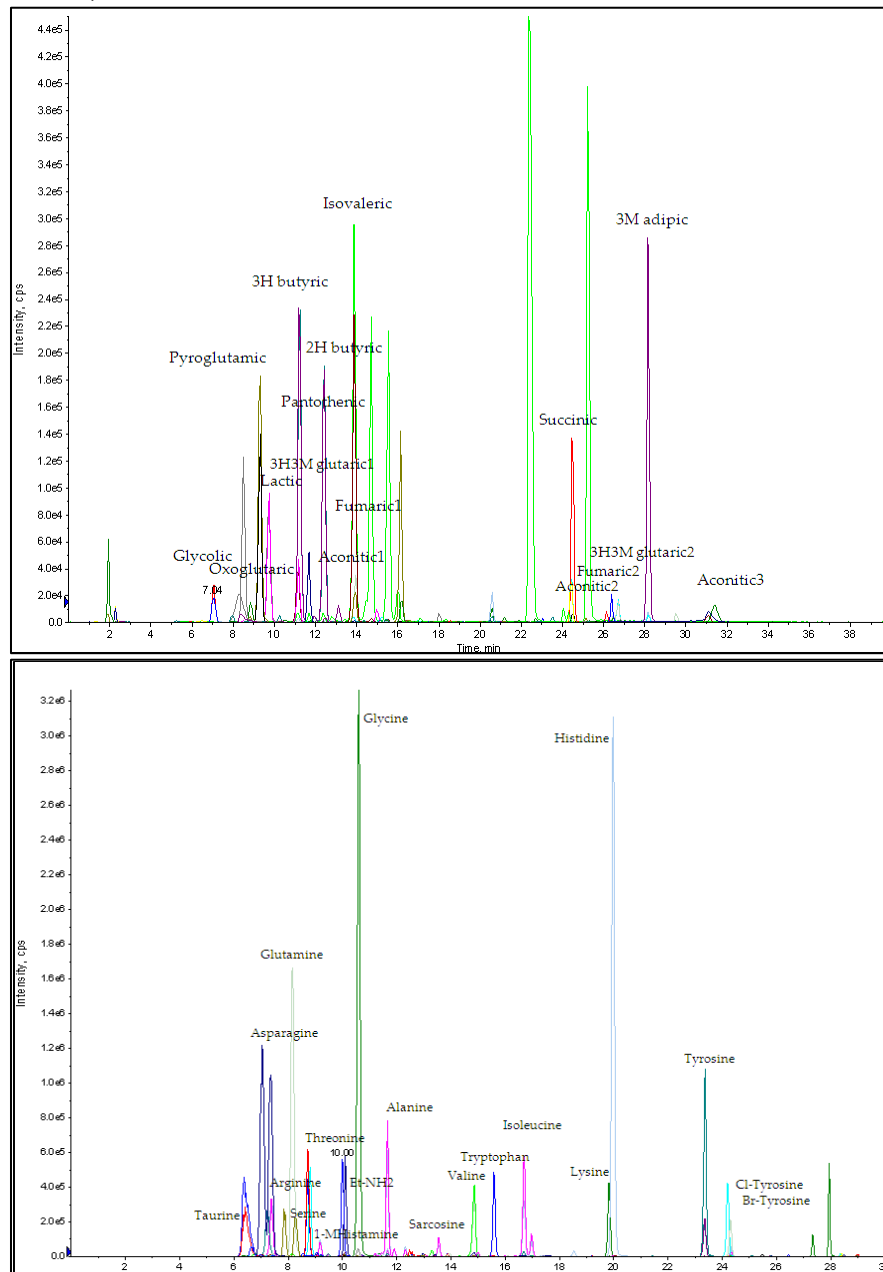
REFERENCES
1. Beger RD, Colatsky T: **Metabolomics data and the biomarker qualification process**. *Metabolomics* 2012, **8**:27.
2. Adamko DJ, Nair P, Mayers I, Tsuyuki RT, Regush S, Rowe BH: **Metabolomic profiling of asthma and COPD: differentiating diseases**. *JACI, accepted manuscript* 2014.

AllerGen Poster Competition

Abstract #11

SYSTEMIC IMMUNE PATHWAYS ASSOCIATED WITH THE MECHANISM OF CAT-SYNTHETIC PEPTIDE IMMUNO-REGULATORY EPITOPES, A NOVEL IMMUNOTHERAPY, IN WHOLE BLOOD OF CAT-ALLERGIC PEOPLE

Young Woong Kim[1,2,3], Daniel R. Gliddon[4], Casey P. Shannon[3], Amrit Singh[1,2,3], Pascal L. C. Hickey[5], Anne K. Ellis[6,7], Helen Neighbour[8,9], Mark Larche[8,9], Scott J. Tebbutt[1,2,3]

[1]Experimental Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[2]James Hogg Centre for Heart Lung Innovation, St. Paul's Hospital, Vancouver, British Columbia, Canada
[3]Prevention of Organ Failure (PROOF) Centre of Excellence, Vancouver, British Columbia, Canada
[4]Circassia Ltd., Oxford, UK
[5]Adiga Life Sciences, Hamilton, Ontario, Canada
[6]Departments of Medicine and Biomedical & Molecular Science, Queen's University, Kingston, Ontario, Canada
[7]Allergy Research Unit, Kingston General Hospital, Kingston, Ontario, Canada
[8]Department of Medicine, McMaster University, Hamilton, Ontario, Canada
[9]Firestone Institute for Respiratory Health, McMaster University, Hamilton, Ontario, Canada

BACKGROUND

Allergic rhinitis (AR) is an IgE-mediated inflammatory condition of the nasal mucosa induced after allergen exposure [1]. Cat allergy affects 10-15% of patients with allergic rhinitis and/or asthma [2]. A novel immunotherapy, Cat-Synthetic Peptide Immuno-Regulatory Epitopes (Cat-SPIRE), composed of seven synthetic peptide T-cell epitopes, acts on allergen-specific T-cells to induce subsequent clinical tolerance to cat allergen.

METHODS

In this study, 19 participants with a clinical history of cat allergies with significant cat exposure received Cat-SPIRE (4x6nmol intradermal injection, 1 dose every 4 weeks). Clinical symptoms were assessed by Nasal Allergen Challenge (NAC) [3]. Whole blood was collected into PAXgene tubes at baseline and post NAC (1, 2, and 6 hours), before and 1 month post treatment. 770 immune genes in the PAXgene blood lysates at 6 hours post NAC were profiled using the nanoString nCounter PanCancer Immune Profiling Panel. After normalization, a statistical comparison of pre- versus post-treatment changes was performed and an enrichment pathway analysis undertaken (Enrichr).

RESULTS

70 immune genes were significantly down regulated compared to pre-treatment at the 6 hours post NAC time point (FDR <10%). These genes were associated with T-cell effector pathways, particularly canonical Th2 cytokines such as IL-4, IL-5, IL-3, IL-6 and TSLP. These changes were accompanied by significant post- versus pre-treatment reductions in clinical symptoms, Total Nasal Symptom Score and Peak Nasal Inspiratory Flow.

CONCLUSION

Following Cat-SPIRE treatment, systemic immune pathways of T-cell effectors were changed in peripheral blood of cat exposed, allergic individuals. The effect on these pathways provides insight towards the mechanism by which Cat-SPIRE induces immune tolerance. Whole blood immune transcriptome profiling in larger sample sizes and/or various time points after NAC may provide biomarkers or tools to discover the immune response or tolerance mechanism.

REFERENCES

1. Pawankar R et al.: **Allergic Rhinitis and Its Impact on Asthma in Asia Pacific and the ARIA Update 2008**. *World Allergy Organ J* 2012, **5**(Suppl 3)**:** S212-7.
2. Patel D et al.: **Fel d 1–derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: A randomized, placebo-controlled study**. *J Allergy Clin Immunol* 2013, **131**(1)**:** 103-9.
3. Ellis AK et al.: **The Allergic Rhinitis – Clinical Investigator Collaborative (AR-CIC): nasal allergen challenge protocol optimization for studying AR pathophysiology and evaluating novel therapies**. *Allergy Asthma Clin Immunol* 2015, **11**(1)**:** 16.

AllerGen Poster Competition

Abstract #12

REDUCING THE HEALTH DISPARITIES: ONLINE SUPPORT FOR CHILDREN WITH ASTHMA AND ALLERGIES FROM LOW-INCOME FAMILIES

Erika Ladouceur[1], Miriam Stewart[1], Josh Evans[2], Jeff Masuda[3], Nicole Letourneau[4], Teresa To[5,6,7], Malcolm King[8]
[1]Faculty of Nursing, University of Alberta, Edmonton, Alberta, Canada
[2]Faculty of Humanities and Social Sciences, Athabasca University, Athabasca, Alberta, Canada
[3]School of Kinesiology and Health Studies, Queen's University, Kingston, Ontario, Canada
[4]Faculty of Nursing, University of Calgary, Calgary, Alberta, Canada
[5]University of Toronto, Toronto, Ontario, Canada
[6]Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada
[7]Institute for Clinical Evaluative Sciences, North York, Ontario, Canada
Division of Pulmonary Medicine, University of Alberta, Edmonton, Alberta, Canada

BACKGROUND
Children of low-income families access more urgent care and less preventive care for asthma [1]. Children with a respiratory disease from low-income families also experience a lower quality of life [2,3]. Our previous online peer/professional support programs have improved children's asthma and allergy self-management skills, and decreased loneliness [4-8]. However, in our previous programs, participants' have come from high-income families [4-8]. Consequently, the objective of this research was to determine low-income participants' perceptions of the impact of the support intervention, the factors influencing these impacts, their satisfaction with the intervention, and recommended changes.

METHODS
Peer and professional mentors who lived with asthma and allergies and had experience with vulnerable families were recruited and trained to facilitate sessions. Participants in the cohort included 16 children aged 7-11, and adolescents 12-17 from across Canada. Children were provided with Internet services for the program duration, chromebooks, and headsets/microphones that they could keep. Eight weekly meetings were offered on the Internet using GoToMeeting®; a secure online meeting platform. Quantitative data on health-related outcomes were elicited by standardized measures administered pre- and post-intervention. Qualitative data on the intervention processes was also collected.

RESULTS
Preliminary analysis post-intervention, has shown an increase in coping skills and perceived support, and reduced loneliness after participating in the online sessions. Even more significant, in comparison to online programs with higher income families, was the increased understanding and appreciation of asthma/allergy triggers in participants. They also learned new ways to manage their triggers. Many parents reported that their children were less resistant to taking their medication and had a higher adherence to medication post-intervention due to an increased understanding about their medications and use.

CONCLUSION
It is evident that online-peer-support programs for lower income families require a different focus compared to programs developed for higher income families, as they face different challenges and have different needs.

REFERENCES
1. To T, et al.: **Health outcomes in low-income children with current asthma in Canada**. *Chronic Dis Can* 2009, **29:**49-55.

AllerGen Poster Competition

2. Cesaroni G, Farchi S, Davoli M, et al.: **Individual and area-based indicators of socioeconomic status and childhood asthma**. *Eur Respir J* 2003, **22:**619-624.

3. Blais L, Beauchesne MF, Levesque, S.: **Socioeconomic status and medication prescription patterns in pediatric asthma in Canada**. *J Adolesc Health* 2006, **38**(5)**:**e9-16.

4. Masuda JR, Anderson S, Letourneau N, Morgan VS, Stewart M: **Reconciling preferences and constraints in building online peer support communities for youth with asthma and allergies**. *Health Promot Pract* 2013, **14**(5)**:**741-750.doi:10.1177/1524839912465083.

5. Stewart M, Letourneau N, Masuda JR, Anderson S, McGhan SL: **Impacts of online peer support for children with asthma and allergies: "It just helps you every time you can't breathe well**. *J Pediatr Nurs* 2013, **28:**439-452.

6. Stewart M, Letourneau N, Masuda JR, Anderson S, McGhan SL: **Online support for children with asthma and allergies**. *J Fam Nurs* 2013, **19**(2)**:**171-197. doi: 10.1177/1074840713483573.

7. Letourneau N, Stewart M, Masuda JR, Anderson S, Cicutto L, McGhan SL, Watt S: **Impact of online support for youth with asthma and allergies: Pilot study. ** *J Pediatr Nurs* 2012*,* **27**(1)**:**65-73.

8. Stewart M, Letourneau N, Masuda JR, Anderson S, McGhan SL: **Online solutions to support needs and preferences of parents of children with asthma and allergies**. *J Fam Nurs* 2011, **17**(3)**:**357-379. DOI 10.1177/107484071141541.

AllerGen Poster Competition

Abstract #13

EPIGENETIC ASSOCIATION OF *PSORS1C1* AND ASTHMA IN THE SAGUENAY-LAC-SAINT-JEAN ASTHMA STUDY

Miriam Larouche[1], Liming Liang[2], Catherine Laprise[1]

[1]Département des sciences fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada
[2]Departments of Epidemiology and Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, United States

BACKGROUND
Asthma is a chronic respiratory disease involving genetic and environmental interactions [1,2]. More than 300 genes have been associated with asthma or related phenotypes, and the number continues to increase [3]. The associated common SNPs represent a small part of the genetic component and the exact nature of the causal genetic variants remains to be discovered [4]. One reason for this "missing heritability" is that many genetic factors act primarily through complex mechanisms involving interactions with other genes and environmental factors and/or epigenetic mechanisms [5]. We have shown that genetic variants related to asthma can be mechanistically studied using epigenetic and functional genomic assessment of individual variants [6,7]. Therefore, we plan to focus on *IL33* pathway following the previous study demonstrating a correlation between methylation signature, expression and asthma in bronchial epithelial cells.

METHOD
DNA methylation (Δβ) has been measured with an epigenome wide association study (EWAS) using Illumina HumanMethylation450K arrays among 69 asthmatic individuals and 91 controls. Data were extracted for 21,720 genes including 26 in the pathway of *IL33* and the association with asthma was assessed by logistic regression using methylation percentage, asthma phenotype, sex, age, IgE level, smoking status and eosinophils, lymphocytes, monocytes, neutrophils, and basophils counts as covariates. Batch effect was corrected for the analysis.

RESULTS
A total of four genes have demonstrated a significant difference of methylation between asthmatic individuals and control ones: *CLSTN2* (Δβ=7.35%, p-value=0.0008), *CDSN* (Δβ=7.39%, p-value=0.02), *PSORS1C1* (Δβ=7.39%, p-value=0.02) and *CCDC57* (Δβ=5.63%, p-value=0.02).

CONCLUSION
This study shows differences in methylation marks between asthmatic individuals and non-asthmatic non-allergic controls for four genes (*CLSTN2*, *CDSN*, *PSORS1C1*, *CCDC57*). Interestingly, *PSORS1C1*, a susceptibility gene for psoriasis, has already been associated with asthma in a genome wide association study in Latino children [8]. The pyrosequencing of this gene will allow us to confirm this association.

REFERENCES
1. Handoyo S, Rosenwasser LJ: **Asthma phenotypes.** *Curr Allergy Asthma Rep* 2009, **9**(6)**:** 439-445.
2. Harb H, Renz H: **Update on epigenetics in allergic disease.** *J Allergy Clin Immunol* 2015, **135**(1)**:**15-24.
3. Lee SH, Park JS, Park CS: **The search for genetic variants and epigenetics related to asthma**. *Allergy Asthma Immunol Res* 2011, **3**(4)**:** 236-244.
4. Laprise C, Bouzigon E: **To define the biological nature of asthma.** *Curr Opin Allergy Clin Immunol* 2011, **11**(5)**:**391-392.
5. Wjst M, Sargurupremraj M, Arnold M: **Genome-wide association studies in asthma: what they really told us about pathogenesis.** *Curr Opin Allergy Clin Immunol* 2013, **13**(1)**:**112-118.

AllerGen Poster Competition

6. Berlivet S, et al: **Interaction between genetic and epigenetic variation defines gene expression patterns at the asthma-associated locus 17q12-q21 in lymphoblastoid cell lines.** *Human Genetics* 2012, **131**(7)**:**1161-1171.

7. Verlaan DJ, et al.: **Allele-specific chromatin remodeling in the ZPBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease.** *Am J Hum Genet* 2009, **85**(3)**:** 377-393.

8. Galanter JM, et al.: **Genome-wide association study and admixture mapping identify different asthma associated loci in Latinos: the Genes-environments & Admixture in Latino Americans study.** *J Allergy Clin Immuno*l 2014, **134**(2)**:** 295-305.

AllerGen Poster Competition

Abstract #14

IL-33 INDUCES CYTOKINE AND CHEMOKINE PRODUCTION IN HUMAN MAST CELLS

Stephanie A. Legere[1], Ian D. Haidl[1], Jean-Francois Legaré[1, 2], Jean S. Marshall[1]
[1]Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada
[2]Department of Surgery, Dalhousie University, Halifax, Nova Scotia, Canada

BACKGROUND

Sterile inflammation is a major mechanism by which tissue damage occurs throughout the body and is also a major determinant of disease progression. It is initiated after sterile physical damage has occurred in local tissues, but can also be initiated in a number of inflammatory disorders, such as asthma and arthritis. Many different cell types appear to be involved in sterile inflammation, but the role of mast cells has been of particular interest. Mast cells are innate immune sentinel cells that reside in many tissues throughout the body that synthesize and secrete many pro- and anti-inflammatory mediators. The alarmin IL-33 is an important component of the sterile inflammatory process and has been shown to directly activate populations of mast cells [1, 2]. Furthermore, IL-33 has been implicated as a potential early-life predictor of childhood asthma and allergy [3]. Therefore, the purpose of this study was to determine the cytokine and chemokine profile of human mast cells activated with IL-33.

METHODS

Cord blood-derived mast cells (CBMCs) were activated for 24 hours with IL-33 at a concentration of 30 ng/mL. To analyse protein production from CBMCs, supernatants were collected and a Luminex assay was conducted to analyse the concentration of 29 cytokines and chemokines. Additionally, gene expression data was analyzed through qPCR to determine gene expression changes upon activation of mast cells with IL-33.

RESULTS

It was found that after IL-33 activation, CBMCs had a statistically significant increase in production of IL-13, but not other T_H2-related cytokines such as IL-4. There was no increase in production of T_H1-related cytokines, such as IFNγ and IL-12. Production of the cytokines VEGF and GM-CSF were also statistically significantly increased after IL-33 activation of CBMCs.

CONCLUSIONS

In conclusion, IL-33 activation of human mast cells results in the production of cytokines supportive of T_H2-driven responses and tissue regeneration.

REFERENCES

1. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G: **Cutting edge: the ST2 ligand IL-33 potently activates and drives maturation of human mast cells.** *J Immunol* 2007, **179:** 2051-2054.
2. Iiukura M, Suto H, Kajiwara N, Oboki K, Ohno T, Okayama Y, Saito H, Galli SJ, Nakae S:
IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest* 2007, **87:**971-978.
3. Ashley-Martin J, Dodds L, Arbuckle TE, Levy AR, Platt RW, Marshall JS: **Predictors of interleukin-33 and thymic stromal lymphoprotein levels in cord blood.** *Pediatr Allergy Immunol* 2015, **26:**161-167.

AllerGen Poster Competition

Abstract #15

REFERENCE RANGES FOR LUNG CLEARANCE INDEX FROM INFANCY TO ADOLESCENCE FOR CANADIAN POPULATION

Zihang Lu[1], Malcolm Sears[2], Theo J. Moraes[1], Felix Ratjen[1], Per Gustafsson[3], Wendy Lou[4], Padmaja Subbarao[1]
[1]Division of Respiratory Medicine, Department of Pediatrics, and Program in Physiology and Experimental Medicine, SickKids Research Institute, The Hospital for Sick Children, and University of Toronto, Toronto, Canada.
[2]Department of Medicine, McMaster University, Hamilton, Canada.
[3]Department of Pediatrics, Central Hospital, Skövde, Sweden.
[4]Dalla Lana School of Public Health, University of Toronto, Toronto, Canada

BACKGROUND

Lung clearance index (LCI), a parameter derived from the Multiple Breath Washout (MBW) test, is a sensitive marker of ventilation inhomogeneity and has been associated with early lung disease. Appropriate representative normative reference data must be available to correctly interpret individual lung function results. We aim to develop a novel Canadian reference equation for LCI and compare it to previously published reference equations.

METHODS

Two hundred and sixty five subjects underwent MBW measurement using an AMIS mass spectrometer and 4% SF6 as a tracer gas. All subjects were free of prior respiratory distress and had no history of wheezing or exposure to maternal smoking during pregnancy. For subjects under 4 years of age, data were obtained from 196 subjects recruited from the Canadian Healthy Infant Longitudinal Development (CHILD) Study. For subjects aged 4-15 years, data were obtained from 69 identified healthy controls. The lamda-mu-sigma (LMS) method was used to construct reference equation, with LCI modelling in terms of age and height.

RESULTS

LCI was found to be independent of race and gender. Compared with reference equation from Lum et al. [1], predicted median LCI based on our reference equation was 0.41 [95% Confidence Interval (CI): 0.39 to 0.43] lower. During infancy to 4 years of age, LCI decreased non-linearly as age and height increased (Table 1). After 4 years of age, LCI remained constant with a mean (standard deviation) of 6.15 (0.39), regardless of change in age and body size. This change in LCI values was associated with a decreased misclassification rate. In a group of well controlled asthmatics from our clinic, 25% (9/36) subjects had an abnormal LCI when using our reference equation while only 11% (4/36) were identified using the Lum et al. reference equation.

Table 1. Reference equation for lung clearance index (LCI) from infancy to 15 years of age

	$Age \leq 4$	$Age > 4$
Skewness(L)	-2.12	-
Predicted (M)	$6.88 - 6.95(Age/10)^2 - 1.16((\log Length)/10)^3$	6.15
Coefficient of variance (S)	0.07	-
Upper Limited of Normal (ULN)	$predicted \times ((1.96 \times 0.07 \times (-2.12)) + 1)^{1/-2.12}$	6.91

CONCLUSIONS

Population-specific equations are necessary for the proper interpretation of clinical results.

REFERENCES

1. Lum S, Stocks J, Stanojevic S, Wade A, Robinson Paul, Gustafsson P, Brown M, Aurora P, Subbarao P, Hooe A, Sonnappa S: **Age and height dependence of lung clearance index and functional residual capacity.** *Eur Respir J* 2013, **41:**1371-1377.

AllerGen Poster Competition

Abstract #16

KINGSTON ALLERGY BIRTH COHORT: COHORT PROFILE AND MOTHER/CHILD CHARACTERISTICS TO AGE 2

Michelle L. North[1,2,3], Elizabeth Lee[1], Vanessa Omana[1], Jenny Thiele[1,2,3], Jeff Brook[4], Anne K. Ellis[1,2,3]

[1]Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario, Canada
[2]Division of Allergy and Immunology, Department of Medicine, Queen's University, Kingston, Ontario, Canada
[3]Allergy Research Unit, Kingston General Hospital, Kingston, Ontario, Canada
[4]Air Quality Research Division, Environment Canada, Toronto, Ontario, Canada

BACKGROUND

The Kingston Allergy Birth Cohort (KABC) was instigated to study the role of gene-environment interactions and epigenetic modifications in the developmental origins of allergy. Kingston General Hospital was chosen as the collection site, as it serves rural/urban residents with diverse socioeconomic status and has documented challenges such as a high rate of maternal smoking.

METHODS

Pregnant women completed a health/environmental survey (n=594). Umbilical cord blood was collected from 454 deliveries and environment/health surveys to age 2 encompassing 327 years of person-time from 235 children. We examined home-environment characteristics by urban/rural residence and socioeconomic status (SES; National Household Survey). Exposure to smoke was active/passive >3 days/week. For rates of parentally-reported skin, respiratory and food symptoms of potential allergic disease, only wheezing/coughing not associated with illness, and rashes that were not cradle cap, diaper rash or infectious were considered.

RESULTS

Maternal and both-parent allergies were self-reported in 27.5%, and 22.3%. The KABC encompassed a high proportion of rural residents (41.3%) and mothers exposed to smoke (25.9%). Rural participants had significantly higher household income, less post-secondary education, more fireplace use, and more indoor dogs. Urban participants were significantly more likely to live near a major road. The homes of lowest-tertile-SES participants were significantly older, more likely to be apartment-style and to be located near a major road, relative to highest-tertile-SES. However, low-SES participants were significantly less likely to cook with gas, have a fireplace or an attached garage. The rate of development of parentally-reported skin, respiratory and food reactions in the KABC was 0.36, 0.17, and 0.17 cases/person-year, respectively.

CONCLUSIONS

We found that both residing in rural/urban and high/low-SES areas affected characteristics of the home and potential environmental exposures. Further analyses will explore rate differences between children with various exposures. Mother/child pairs are returning for skin testing and epigenetic analysis of umbilical cord and peripheral blood.

AllerGen Poster Competition

Abstract #17

COW'S MILK PROTEIN SPECIFIC IGE, IGA AND IGG4 AS A PREDICTOR OF OUTCOME IN ORAL IMMUNOTHERAPY

Tanvir Rahman[1], Duncan Lejtenyi[2], Sarah De Schryver[2], Ryan Fiter[1], Ciriaco Piccirillo[3], Moshe Ben-Shoshan[2], Bruce Mazer[1,2]

[1] Meakins-Christie Laboratories, McGill University, Montréal, Québec, Canada
[2] Division of Pediatric Allergy and Clinical Immunology, Montréal Children's Hospital, Québec, Canada
[3] Division of Allergy and Clinical Immunology, Montréal General Hospital, Montréal, Québec, Canada

BACKGROUND

Cow's milk allergy (CMA) is defined as immunologic adverse reactions to cow's milk proteins (CMP). It affects 2-3% of infants [1]. Approximately 15% of the CMA population retains their allergic status even after 8 years of age [2].

METHODS

Twenty patients were recruited in a case control study of cow's milk (CM) oral immunotherapy (OIT). Blood samples were collected at baseline and at multiple timepoints during the trial. Specific IgE, IgA and IgG4 to casein, β-lactoglobulin (BLG) and α-lactalbumin (ALA) were measured in OIT recipients (n=9) by using Enzyme-Linked Immunosorbent Assays (ELISA).

RESULTS

CMP-sIgE decreased multiple times baseline in 6 of 9 OIT recipients, remained high in 2 subjects, and unaltered in 1 individual due to OIT (data not shown). At the same time, specific IgA and specific IgG4 increased (Figure 1) in parallel with the decrease in CMP-sIgE. Specific IgA increased significantly baseline in 7 of 9 patients in case of casein, and in 6 of 9 patients in case of BLG and ALA. Again, specific IgG4 to casein increased significantly baseline in 8 of 9 patients, and in all 9 patients in case of BLG and ALA.

CONCLUSIONS

In summary, a significant decrease in CMP-sIgE and a concomitant increase in CMP-sIgA and -IgG4 were observed in successful CM OIT recipients. These findings demand an extensive study of pro-inflammatory (*e.g*. T-helper cells) and anti-inflammatory cells (*e.g.* T- and B-regulatory cells) along with their secreted cytokines to elucidate the mechanism of the development of tolerance in CM OIT.

Figure 1. CMP-specific IgA to casein (a) and ALA (c) increased significantly from pre-therapy to 300ml challenge with CM (unpaired t-test, **$P<0.01$ and *$P<0.05$ respectively for casein and ALA). Casein (d), BLG (e), and ALA (f) specific IgG4 increased significantly baseline at the completion of OIT (Unpaired t test, *$p<0.05$, ***$p<0.001$, ***$p<0.001$ respectively for casein, BLG, and ALA).
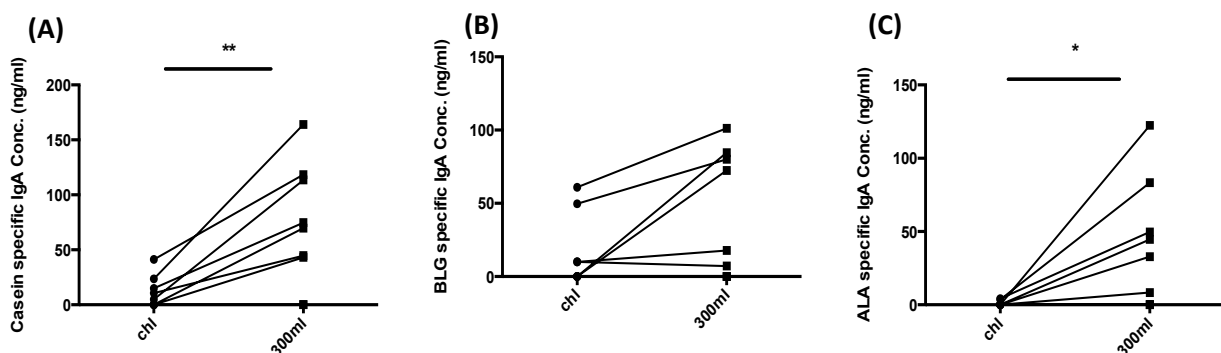
AllerGen Poster Competition
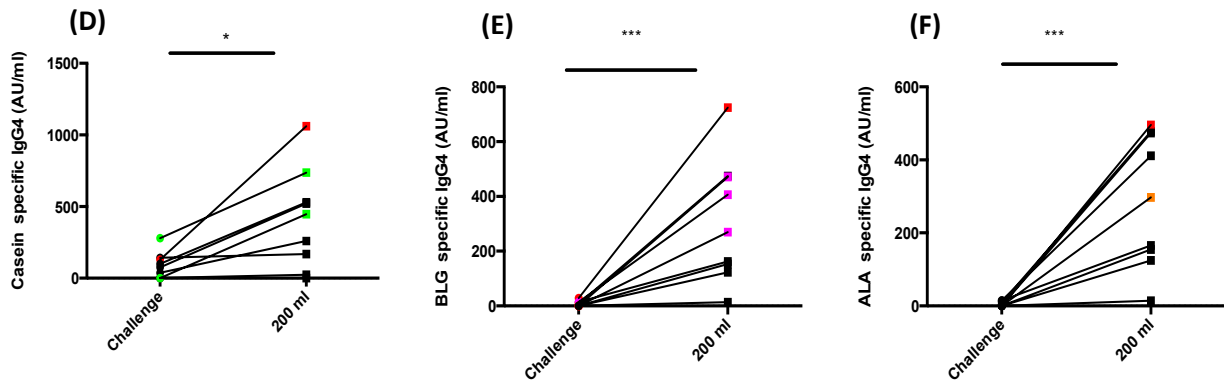
REFERENCES

1. Saarinen KM, Juntunen-Backman K, Järvenpää AN, Kuitunen P, Lope L, Renlund M, Siivola M, Savilahti E: **Supplementary feeding in maternity hospitals and the risk of cow's milk allergy: A prospective study of 6209 infants**. *J Allergy Clin Immunol* 1999, **104**(2)**:**457-46.

2.Saarinen KM, et al.: **Clinical course and prognosis of cow's milk allergy are dependent on milk specific IgE status**. *J Allergy Clin Immuno*l 2005. **116**(4)**:**869-75.

AllerGen Poster Competition

Abstract #18

AGE OF PEANUT INTRODUCTION AND DEVELOPMENT OF REACTIONS AND SENSITIZATION TO PEANUT

Elinor Simons[1], Allan B. Becker[1], Rishma Chooniedass[1], Kyla Hildebrand[2], Edmond S. Chan[2], Stuart Turvey[2], Padmaja Subbarao[3], Malcolm Sears[4]

[1]Section of Allergy and Immunology, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada

[2]Division of Allergy and Immunology, Department of Pediatrics, British Columbia Children's Hospital, Vancouver, British Columbia, Canada

[3]Department of Pediatrics, Hospital for Sick Children, Toronto, Ontario, Canada

[4]Division of Respirology, Department of Medicine, McMaster University, Hamilton, Ontario, Canada

BACKGROUND

Sensitization to peanut places a child at risk for anaphylaxis to peanut, although some peanut-sensitized children tolerate eating peanut. We evaluated the association between timing of peanut introduction in infancy and development of reactions and sensitization to peanut by age 3 years.

METHODS

Caregivers of participants in the population-based Canadian Healthy Infant Longitudinal Development (CHILD) Study prospectively reported their child's first introduction and reactions to peanut starting at age 6 months. At ages 1 and 3 years, the children underwent skin prick testing for sensitization to peanut and other food allergens, and an assessment for eczema/atopic dermatitis. We conducted multivariable logistic regression to determine if later introduction of peanut was associated with increased odds of reactions and sensitization to peanut.

RESULTS

Among participants from the Manitoba and Vancouver sites (n=1610), peanut was introduced into the diet at ages 6 (1.3%), 9 (14.1%), 12 (27.4%) and >12 (42.3%) months; 14.9% had not tried peanut by 18 months. At age 1 year, 2.4% of children had moderate-to-severe eczema. By age 3 years, 5.6% of children had a reaction to peanut and 7.2% had at least one positive skin prick test to peanut, 3.4% to milk, and 9.3% to egg. In the first 3 years, 53.9% of peanut-sensitized children had reacted to peanut and 77.8% of children who had reacted to peanut were peanut-sensitized. Children with later peanut introduction (on a continuous scale from 6-18 months) had higher odds of a reaction (1.04; 95% CI, 1.02-1.06) and sensitization (1.04; 1.03-1.05) to peanut, after adjustment for sensitization to egg and moderate-to-severe eczema.

CONCLUSION

In a population-based sample of Canadian children, later peanut introduction was associated with reported reactions and sensitization to peanut by age 3 years. Further investigation is needed to characterize the association between timing of peanut introduction and persistent peanut allergy.

AllerGen Poster Competition

Abstract #19

MULTI-OMIC BLOOD BIOMARKER SIGNATURES OF THE LATE PHASE ASTHMATIC RESPONSE

Amrit Singh[1], Casey P. Shannon[1], Young Woong Kim[1], Mari DeMarco[1], Kim-Anh Le Cao[2], Gail M. Gauvreau[3], J. Mark FitzGerald[1], Louis-Philippe Boulet[4], Paul M. O'Byrne[3], Scott J. Tebbutt[1]

[1]Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[2]Translational Research Institute, University of Queensland, Brisbane, Australia
[3]Department of Medicine, McMaster University, Hamilton, Ontario, Canada
[4]Centre de Pneumologie de L'Hopital, Université Laval, Québec City, Québec, Canada

BACKGROUND

Individuals with allergic asthma respond differently, but reproducibly, to allergen inhalation challenge. Some individuals develop an isolated early response (early responders, ERs) while others also go on to develop a late response (dual responders, DRs). Since new drugs for allergic asthma are targeted towards attenuating the late phase response, the purpose of this study is to identify a multi-omic biomarker panel that can screen mild asthmatics for those likely to exhibit the late response.

METHODS

32 individuals participated in the allergen inhalation challenge as part of the AllerGen Clinical Investigator Collaborative. 15 (17) participants were classified as ERs (DRs), respectively. Blood samples were collected prior to the allergen inhalation challenge. Measurements of cell counts were obtained using a hematolyzer; gene transcript relative levels using RNA sequencing; metabolite levels using tandem mass spectrometry. Sparse generalized canonical correlation discriminant analysis was used to classify ERs and DRs by integrating all three datasets, adjusting for age, sex and allergen. Geneset enrichment analysis was performed using Enrichr.

RESULTS

After pre-processing and filtering, 5 cell-types, 11,879 gene transcripts and 124 metabolites were retained for downstream analysis. The multi-signature classifier consisted of 2 cell-types, 10 gene transcripts and 10 metabolites and had an error rate of $20\pm8.3\%$ (10x5-fold cross-validation). The cells selected in the multi-signature panel included lymphocytes and monocytes. The selected metabolites were enriched for phosphatidylcholines. The top 10 enriched pathways in the selected gene transcripts included the phosphatidylinositol signalling system.

CONCLUSIONS

Molecular signatures in blood can be used to screen for asthmatic individuals that develop the late asthmatic response. This study implicates the arachidonic acid metabolism pathway in leading to the early or late phase asthmatic response.

AllerGen Poster Competition

Abstract #20

MAPPING LOCAL INFLAMMATORY CYTOKINE SECRETION FOLLOWING A CUMULATIVE ALLERGEN DOSE USING THE ALLERGIC RHINITIS CLINICAL INVESTIGATOR COLLABORATIVE NASAL ALLERGEN CHALLENGE MODEL

Mena Soliman[1], Jenny Thiele[1], Louis-Philippe Boulet[3], Paul K. Keith[4], Harissios Vliagoftis[5], Susan Waserman[4], Helen Neighbour[4], Anne K. Ellis[1,2]

[1]Departments of Medicine and Biomedical & Molecular Science, Queen's University, Kingston, Ontario, Canada
[2]Allergy Research Unit, Kingston General Hospital, Kingston, Ontario, Canada
[3] Institut Universitaire de Cardiologie et de Pneumologie de Québec, Québec City, Québec, Canada
[4]Department of Medicine, McMaster University, Hamilton, Ontario, Canada
[5]Pulmonary Research Group, University of Alberta, Edmonton, Alberta, Canada

BACKGROUND
The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) uses a Nasal Allergen Challenge (NAC) model to study the pathophysiology of allergic rhinitis (AR) and provides proof of concept for novel therapeutics. We aimed to map cytokine responses in allergic versus non-allergic participants using our NAC protocol.

METHODS
10 ragweed allergic participants were screened using 4-fold increases in NAC dose every 15 minutes until a Total Nasal Symptom Score (TNSS) of 8/12 and a Peak Nasal Inspiratory Flow (PNIF) of ≤50% from baseline was achieved. 12 non-allergic participants were included. The Cumulative Allergen Concentration (CAC) of the incremental doses was delivered once at the NAC visit; non-allergics received the highest allergen dose. Nasal secretions were collected using Synthetic Absorbent Matrix placed on the inferior turbinate for 2 minutes at baseline, 1 hour and 6 hours, and processed using Luminex xMAP technology. Mann-Whitney U test compared the two groups at each time point, Friedman's test compared time points within each group, and Spearman's correlation evaluated associations between cytokines.

RESULTS
Up-regulation of pro-inflammatory cytokines was observed in allergic participants; IL-5 was higher at 1 hour ($p<0.01$) and 6 hours ($p<0.00001$) compared to non-allergics. Similar results were observed for IL-13. Allergic participants had significantly greater MCP-1(CCL2) and MIP-1b (CCL4) at 6 hours compared to non-allergic participants ($p<0.01$). G-CSF was also increased at 1 hour ($p<0.01$) and at 6 hours ($p<0.001$) compared to controls. IL-6 was also elevated at 6 hours ($p<0.01$) versus non-allergic participants. Significant correlations exist between cytokines, most notably IL-5 and IL-13 at 6 hours ($p=0.001$, $r=0.891$). IL-13 and MIP-1b correlate at baseline ($p=0.011$, $r=0.782$) and 1 hour ($p=0.003$, $r=0.855$); IL-13 and IL-6 at 1hour ($p=0.015$, $r=0.758$), and IL-13 and IL-10 at 6 hours ($p=0.035$, $r=0.685$).

CONCLUSION
The AR-CIC's CAC protocol is an effective method of studying AR that is capable of generating measurable and robust nasal cytokine responses, enabling better understanding of its pathophysiology. Novel medications can be evaluated with biomarkers, in addition to clinical outcomes.

AllerGen Poster Competition

Abstract #21

EARLY LIFE GUT MICROBIAL ALTERATIONS IN CHILDREN DIAGNOSED WITH ASTHMA BY THREE YEARS OF AGE

Leah T. Stiemsma[1,2], Marie-Claire Arrieta[1,3], Jasmine Cheng[1,2], Pedro A. Dimitriu[1], Lisa Thorson[3], Sophie Yurist[3], Boris Kuzeljevic[2], Diana L. Lefebvre[5, 6], Padmaja Subbarao[7, 8], Piush Mandhane[9, 10], Allan Becker[11], Malcolm R. Sears[6], Kelly M. McNagny[12], Tobias Kollmann[2, 13], the CHILD Study Investigators, William W. Mohn[1], B. Brett Finlay[1, 3, 14], Stuart E. Turvey[2, 13]

[1]Department of Microbiology & Immunology, University of British Columbia, Vancouver, British Columbia, Canada,

[2]The Child and Family Research Institute, Vancouver, British Columbia, Canada

[3]Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada

[4]Department of Statistics, University of British Columbia, Vancouver, British Columbia, Canada

[5]St. Joseph's Healthcare, Hamilton, Ontario, Canada

[6]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

[7]Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada

[8]Hospital for Sick Children, Toronto, Ontario, Canada

[9]Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada

[10]School of Public Health, University of Alberta, Edmonton, Alberta, Canada

[11]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada

[12]The Biomedical Research Centre, University of British Columbia, Vancouver, British Columbia, Canada

[13]Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada

[14]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada

BACKGROUND

Asthma is the most prevalent childhood disease affecting over 300 million people worldwide. Our research group previously associated features of the early life gut microbiota (at 3 months and 1 year of age) in children with risk of active asthma at school age (determined by the asthma predictive index). These features included underrepresentation of four bacterial genera and a decreased production of microbial derived metabolites. We hypothesize that this constitutes a dysbiotic early life microbiota, which is also associated with asthma diagnosed in children by three years of age and sought to identify additional bacterial taxa that may be associated with this asthmatic phenotype.

METHODS

287 children enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study were classified according to the diagnosis of asthma by three years of age. Bacterial 16S rDNA from 3-month and 1-year stool samples from these children was extracted, amplified, and subjected to high throughput Illumina sequencing. Specific bacterial genera and species were quantified by quantitative PCR from all children with asthma and a subset of controls with no history of asthma symptoms. An exact logistic regression model was used to assess the effects of potentially confounding variables (*i.e.* antibiotic exposure, mode of birth).

RESULTS

16S sequence analysis of our sample cohort identified differentially abundant bacterial populations between asthmatics and controls in stool collected at 3 months and 1 year of age. Additionally, qPCR identified significant shifts in the abundance of specific bacterial genera in stool collected at 3 months and 1 year of age in the asthmatic group when compared to controls.

CONCLUSIONS

Shifts in the relative abundance of specific gut bacterial populations in early life are associated with asthma diagnosed in children by three years of age.

AllerGen Poster Competition

Abstract #22

NASAL ALLERGEN CHALLENGE INDUCED EOSINOPHILIA – THE ALLERGIC RHINITIS CLINICAL INVESTIGATOR COLLABORATIVE

Jenny Thiele[1,2], Daniel Adams[2], Mena Soliman[1,2], Lisa Steacy[2], Anne Ellis[1,2]
[1]Departments of Medicine and Biomedical & Molecular Science, Queen's University, Kingston, Ontario, Canada
[2]Allergy Research Unit, Kingston General Hospital, Kingston, Ontario, Canada

BACKGROUND

The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) is a Canadian initiative with the goal of performing standardized nasal allergen challenge (NAC) to study the effects of therapeutic agents for allergic rhinitis (AR), while allowing the identification of mechanisms and biomarkers of AR. Various NAC protocols have been described previously. The multiple cumulative allergen concentration (MCAC) NAC protocol was shown to produce more robust symptom scores than a cumulative allergen concentration (CAC) protocol. Here we examined NAC-induced eosinophilia for these two protocols.

METHODS

17 atopic and 12 non-atopic participants were enrolled for this study. Atopic individuals presented with AR symptoms in ragweed season and a supportive skin test response. During screening incremental concentrations of ragweed allergen were administered until each participant reached the qualifying symptom cut-off. For the subsequent NAC one week later, ten atopics were challenged with one dose of allergen equivalent to the cumulative amount of allergen each received during screening (CAC). Seven atopics received the cumulative of all preceding allergen doses to the qualifying concentration (QC), followed by the QC 15 minutes later (MCAC). Non-atopics were challenged with a 1:2 ragweed concentration. Nasal lavage samples were collected at baseline, 1 hour and 6 hours post-NAC to determine differential counts.

RESULTS

The eosinophil fraction was significantly increased in atopics following NAC when compared to non-allergics at both 1 hour and 6 hours for the CAC-protocol and at 6 hours for the MCAC-protocol.

CONCLUSIONS

Even though the MCAC protocol establishes more robust symptom scores, the CAC protocol appears to produce more pronounced eosinophilia.

AllerGen Poster Competition

Abstract #23

THE RELATIONSHIP BETWEEN FOOD SENSITIZATION AND ATOPIC DERMATITIS AT AGE 1 YEAR IN A CANADIAN BIRTH COHORT

Maxwell M. Tran[1], Diana L. Lefebvre[1], Chinthanie F. Ramasundarahettige[1], Allan B. Becker[2], Wei Hao Dai[3], Padmaja Subbarao[3], Piush J. Mandhane[4], Stuart E. Turvey[5], Malcolm R. Sears[1]

[1]Department of Medicine, McMaster University, Hamilton, Ontario, Canada
[2]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada
[3]Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada
[4]Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada
[5]Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada

BACKGROUND

The relationship between immunoglobulin E (IgE)-mediated food sensitization and the development of atopic dermatitis during infancy is not well-characterized. Past studies have shown that food sensitization is associated with the occurrence of atopic diseases [1-3], including atopic dermatitis, although this relationship has not been examined in a population-based birth cohort at 1 year. Our aim was to address this knowledge gap by analyzing results from nearly 3,000 children enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study.

METHODS

An algorithm based on Child Health questionnaires completed by parents when their children were 1 year old was used to classify children as having definite, possible, or no atopic dermatitis. Atopic dermatitis classification was cross-tabulated with skin prick test (SPT) results to food allergens, specifically peanut, milk, egg, and soybean. A positive SPT was defined by a wheal diameter of ≥2 mm compared to the negative control. In total, 2,626 children who underwent skin testing and had corresponding questionnaire data were included in this project.

RESULTS

Of 2,626 children, 526 (20.0%) had definite atopic dermatitis and 906 (34.5%) had possible atopic dermatitis (Table 1). There was an increasing trend in the proportion of positive SPTs with increasing certainty of atopic dermatitis (Cochran-Armitage trend test, p<0.001). Sensitization to any food allergen was associated with higher odds of having definite or possible atopic dermatitis (OR 2.04, 95% CI 1.64-2.53). Specifically, sensitization to peanut (p<0.0001), egg (p<0.0001), and milk (p=0.0021) were significantly associated with atopic dermatitis, but sensitization to soybean (p=0.3470) was not.

Table 1. Cross-tabulation of food sensitization and atopic dermatitis classification

SPT results	Atopic dermatitis classification			
	Definite	**Possible**	**No**	**Total**
Sensitized to ≥1 food allergen(s)	112 (21.3%)	98 (10.8%)	109 (9.1%)	319 (12.1%)
Non-sensitized	414 (78.7%)	808 (89.2%)	1085 (90.9%)	2307 (87.9%)
Total	526	906	1194	2626

CONCLUSIONS

IgE-mediated sensitization to any food allergen at 1 year was associated with higher odds of having definite or possible atopic dermatitis. Sensitization to peanut, egg white, and milk, but not soybean, were significantly associated with atopic dermatitis.

AllerGen Poster Competition

REFERENCES
1. Chiu CY, Huang YL, Tsai MH, Tu YL, Hua MC, Yao TC, et al.: **Sensitization to food and inhalant allergens in relation to atopic diseases in early childhood: a birth cohort study**. *PLoS ONE* 2014, **9**(7)**:**e102809.
2. Hill DJ, Sporik R, Thorburn J, Hosking CS: **The association of atopic dermatitis in infancy with immunoglobulin E food sensitization**. *J Pediatr* 2000, **137**(4)**:**475-9.
3. Eller E, Kjaer HF, Høst A, Andersen KE, Bindslev-Jensen C: **Development of atopic dermatitis in the DARC birth cohort.** *Pediatr Allergy Immunol* 2010, **21:**307-14.

AllerGen Poster Competition

Abstract #24

ALLERGEN INHALATION ENHANCES TOLL-LIKE RECEPTOR-INDUCED THYMIC STROMAL LYMPHOPOIETIN RECEPTOR EXPRESSION BY HEMATOPOIETIC PROGENITOR CELLS IN MILD ASTHMATICS

Damian Tworek[1], Delia Heroux[1], Seamus N. O'Byrne[1], Paul M. O'Byrne[1], Judah A. Denburg[1]
[1]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

BACKGROUND

Hematopoietic progenitor cells (HPCs) may act as pro-inflammatory effector cells by themselves and directly contribute to allergic inflammation. HPCs express the receptor for thymic stromal lymphopoietin (TSLPR) and respond TSLP by rapidly releasing high levels of pro-inflammatory Th2-like cytokines and chemokines. HPCs also express Toll-like receptors (TLRs)-2, -4 and -9, and TLR ligation induces eosinophil-basophil differentiation from HPCs. In the current study we investigated the effects of allergen inhalation on TLR-induced TSLPR expression by HPCs.

METHODS

Seven mild allergic asthmatics underwent bronchial allergen challenges. All subjects developed a dual response to inhaled allergen. Blood was collected before and 24 hours after the challenge. CD34-enriched peripheral blood (pb) cell populations were stimulated with TLR-2 (lipoteichoic acid, LTA), TLR-4 (lipopolysaccharide, LPS) or TLR-9 (ODN2006) ligands. TLR expression by pb HPCs as well as TSLPR expression by HPCs stimulated with TLR agonists pre- and post-challenge were measured by flow cytometry.

RESULTS

There was no significant change in TLR-2, TLR-4 or TLR-9 expression post allergen inhalation. TSLPR was barely detected on unstimulated HPCs. However, overnight stimulation of HPCs with LPS and ODN2006 induced a significant increase in TSLPR expression compared with unstimulated HPCs ($p<0.01$ and $p<0.05$, respectively). In addition, LPS- and ODN2006-induced TSLPR expression by HPCs was more pronounced post-allergen compared to baseline ($p<0.001$ and $p<0.05$, respectively). No significant effect of LTA on TSLPR expression was noted.

CONCLUSIONS

Allergen exposure changes the response of HPCs to TLR stimulation by enhancing TSLP (and thus pro-inflammatory) responsiveness. These findings suggest that HPCs may be relevant in the pathogenesis of pathogen-related asthma exacerbations.

AllerGen Poster Competition

Abstract #25

THE ALLERGIC RHINITIS CLINICAL INVESTIGATOR COLLABORATIVE – REPLICATED EOSINOPHILIA ON REPEATED CUMULATIVE ALLERGEN CHALLENGES IN NASAL LAVAGE SAMPLES

Laura Walsh[1], Mena Soliman[1,2], Jenny Thiele[1,2], Lisa M. Steacy[2], Daniel E. Adams[2], Anne K. Ellis[1,2]
[1]Departments of Medicine and Biomedical & Molecular Science, Queen's University, Kingston, Ontario, Canada
[2]Allergy Research Unit, Kingston General Hospital, Kingston, Ontario, Canada

BACKGROUND

The Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) is a national initiative conducting standardized nasal allergen challenges (NAC) to investigate the effectiveness of therapeutic agents for allergic rhinitis (AR), as well as analyze biomarkers and potential pathways of the allergic response. The NAC involves direct exposure of the nasal mucosa to the allergen of interest and evaluation of clinical, cellular and molecular outcomes.

METHODS

10 ragweed allergic participants, with a history of AR and a skin prick test to short ragweed ≥3mm than the negative control, were enrolled. Participants were screened for inclusion/exclusion criteria and their qualifying allergen concentration determined. Participants returned 21-28 days later and received the cumulative allergen concentration on two NAC visits separated by 21 days. Nasal lavage samples were collected at baseline, 1 hour, 6 hours, and 24 hours after allergen challenge. The samples were kept on ice until cytospin slides were prepared and stained with Diff-Quik™. Differential cell counts were completed for each sample to determine the eosinophil fraction as a percent of the total white blood cells. GraphPad Prism™ was used for all statistical analysis.

RESULTS

Starting at 1 hour, there was an increase in eosinophils after NAC which was significant at 24 hours at both NAC visits ($p \leq 0.05$). When compared to non-allergic controls, obtained previously under similar study conditions, a significant increase in the eosinophil population of allergic participants was observed for both NAC visits at 1 hour and 6 hours post NAC time points ($p \leq 0.05$). 24-hour samples for non-allergics were not collected in the previous study. No significant differences were identified between the consecutive NAC visits concerning eosinophil fraction in allergics at any time point.

CONCLUSIONS

There were no significant differences in eosinophil fraction between the two NAC visits, indicating repeatability of nasal eosinophilia using the repeated cumulative allergen challenges (RCAC) protocol.

AllerGen Poster Competition

Abstract #26

THE CHILD STUDY: OPTIMIZING SUBJECT RETENTION IN PEDIATRIC LONGITUDINAL COHORT RESEARCH

Linda Warner[1], Mary Ann Mauro[1], Robby Mamonluk[1], Stuart E. Turvey[1]
[1]Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada

BACKGROUND
The Canadian Healthy Infant Longitudinal Development (CHILD) Study is a multicentre birth cohort study following children for at least the first five years of life to determine how environmental and genetic variables impact health, and particularly the development of asthma, eczema, and allergies. Subject retention is essential for optimal scientific integrity.

METHODS
Vancouver-based participants completed surveys after 1 and 5 years. The 1-year surveys focused on what was important to families for their continued participation and reasons for potential withdrawal (reported at the 2013 Canadian Society of Allergy and Clinical Immunology Scientific Meeting). The 5-year surveys reassessed reasons for ongoing participation and evaluated retention strategies.

RESULTS
The first 79 subjects, due for the 5-year clinic visit, were analyzed through April 2015: 69/79 (87%) completed the visit, 1/79 (1%) transferred to the Toronto site, 3/79 (4%) booked future visits, and efforts to schedule the remaining 5/79 (6%) are ongoing. Importantly, no participants have been lost to follow-up at this stage, including 15 families who moved outside of Vancouver. Of the 69 visits completed: 57/68 (84%) of blood samples were collected, including only 2 refusals and 10 failures related to lab closure; 68/69 (99%) of urine samples were collected; 69/69 (100%) skin prick tests were completed; and 68/69 (99%) spirometry assessments were completed.
From survey data, parents identified their highest priority for study participation as: desire to improve child health globally (68%), a positive experience for the child (19%), ease of online questionnaires (6%), monetary reimbursement (4%) and flexibility in scheduling (2%).

CONCLUSION
Successfully engaging participants is crucial to achieving study objectives, particularly in a pediatric longitudinal study where there is a high likelihood for change over time. In our Vancouver experience, ongoing engagement with families through surveys has ensured that challenges are identified and addressed during the course of the study. Ultimately, active participant engagement in the CHILD Study likely enhanced subject retention and protocol completion to optimize scientific integrity.

AllerGen Poster Competition

Abstract #27

DIFFERENTIAL EXPRESSION OF C3A AND C5A IN ALLERGIC ASTHMA

ChenXi Yang[1,2,3], Amrit Singh[1,2], Casey P. Shannon[1,2], Young Woong Kim[1,2], Ed M. Conway[3], Scott J. Tebbutt[1,2]
[1]Centre for Heart and Lung Innovation, University of British Columbia, Vancouver, British Columbia, Canada
[2]Prevention of Organ Failure (PROOF) Centre of Excellence, Vancouver, British Columbia, Canada
[3]Centre for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada

BACKGROUND

Allergic asthma is an airway inflammatory disease characterized by airway obstruction, increasing bronchovascular permeability, and airway hyperresponsiveness. Two phenotypes – early responders (ER), who only experience an acute bronchoconstriction within minutes following the allergen exposure, and dual responders (DR), who also experience a further longer-lasting bronchoconstriction several hours after the initial exposure – are involved in the disease, but the mechanism that leads to the two phenotypes remains elusive. Activation of the complement system, which is part of innate immunity, is strongly associated with the disease. Various models have shown that the complement anaphylatoxins, C3a and C5a, play an important role in regulating the allergic response owing to their pro-inflammatory effects [1]. Therefore, we hypothesized that C3a and C5a are differentially abundant in plasma of ERs, DRs and non-asthmatic controls.

METHODS

14 mild asthmatic and 6 non-asthmatic control individuals participated in our study. Peripheral whole blood samples were collected using EDTA tubes and the samples were further processed to obtain plasma. Quidel MicroVue[TM] C3a Plus and C5a EIA kits were used to measure C3a and C5a expression levels, respectively. Student's t-tests were then used to compare C3a and C5a levels in plasma of ERs, DRs and non-asthmatic controls.

RESULTS

C3a levels were lower in controls compared to ERs ($p<0.01$) and DRs ($p<0.05$) but there was no significant difference between ERs and DRs. C5a levels were up-regulated in ERs compared to DRs ($p<0.01$) and controls ($p<0.05$). The down-regulation of C5a in DRs may be due to the fact that C5a is protective during allergen sensitization.

CONCLUSIONS

Both C3a and C5a expression levels are associated with allergic asthma. C3a is lower in non-asthmatics compared to ERs and DRs; C5a is higher in ERs compared to DRs and non-asthmatics. Further validation is ongoing.

REFERENCES

1. Zhang X, Kohl J: **A complex role of complement in allergic asthma.** *Expert Rev Clin Immunol* 2010, **6**(2)**:**269-277.