



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

***Second Annual Research Conference:
INNOVATION from cell to society²***

Sheraton Hotel, Hamilton, ON
February 11-13, 2007

TRAINEE POSTER ABSTRACTS

ABSTRACTS

Poster #	Name	Topic
1.	Jennifer Protudjer	A Qualitative Exploration of How Children with Asthma Perceive Healthy Eating and Physical Activity
2.	Idongesit Obiefuna	Urinary Metabolites of Inflammation in a Guinea Pig Model of Asthma
3.	Shamima Huq	The Impact of Mold on Asthma and Bronchial Hyperresponsiveness: Differences between First Nations and Non First Nations Children
4.	Ben Tripp	AllerWeb: Knowledge Discovery, Management and Transfer
5.	Anne Ellis	Peripheral Blood Molecular Bio-markers of Eosinophil-Lineage Commitment: Multiplex Q-PCR Analysis of GATA-1, MBP and IL-5 Receptor mRNA Expression Kinetics
6.	Santiago Parent	Endotoxins Detection and Control in Drinking Water Systems
7.	Denise Daley	Candidate Genes for Asthma, Atopy and Allergic Disease: Results from Theme 1
8.	Gurpreet Singhera	The Influence of RSV and Particulate Air Pollution (PM10) on Airway Epithelial Cell Damage and Repair
9.	Troy Mitchell	Mechanisms of Degranulation in Inflammatory Cells
10.	Xiao-Mei Mai	Leptin is Associated with Asthma in Overweight Children
11.	Jamie Haddon	Investigation of Mast Cell and Eosinophil Function in Immune and Allergic Response via Cre-Mediated Conditional Gene Deletion
12.	Jian Zhang	Identification of Functional SNPs in TIM3
13.	Andrea Lo	Proteomic Analysis of Rac2-Mediated Secretion in Neutrophils
14.	Jason Pole	Antenatal Steroid Therapy for Fetal Lung Maturation: Is there an association with childhood asthma?
15.	Mohua Podder	Autocalling Algorithms for Microarray Based Genotyping
16.	Marie-Claire Rousseau	Comparative Responses to Nasal Allergen Challenge in Allergic Rhinitic Subjects With or Without Asthma
17.	Jianqing He	Selection of Housekeeping Genes for Real-Time Quantitative PCR in Normal and Atopic Human Bronchial Epithelial Cells
18.	Katherine Morris	Expression of Anti-Inflammatory Proteins and Peptides in Mind-Body Pathways of Inflammation

ABSTRACTS

Poster #	Name	Topic
19.	Leandro Fritscher	Allergic Asthma: Air Pollution and Allergen Interactions
20.	Shannon Cope	Socioeconomic Factors Related to Asthma Control and Quality of Life in Children
21.	Moshe Ben-Shoshan	Is the Prevalence of Peanut Allergy Increasing? A five-year follow-up study on the Prevalence of Peanut Allergy in Montreal School Children aged 5 to 9 years
22.	Steven Maltby	CD34 Involvement in the Development of Allergic Asthma and Eosinophil Migration in Mice
23.	Tillie Hackett	Characterization of Bronchial Epithelial Side Population (SP) Cells: Progenitors of Multiple Cell Types
24.	Nicole Garzia	Adult Asthma among Canadians in Different Occupations and Labour Force Groups
25.	Nha Uyen Nguyen-Lu	Accidental Exposures to Peanut in Children with Peanut Allergy

A Qualitative Exploration of How Children with Asthma Perceive Healthy Eating and Physical Activity

PROTUDJER JLP, Marchessault GDM, Kozyrskyj AL, Becker AB.

Supervisors: Dr. A. Becker, Dr. G. Marchessault

Objective/ Purpose

The association between asthma and obesity is well documented. We sought to better understand how asthmatic children perceive weight and physical activity in order to enhance knowledge translation concerning weight-management strategies for working with families of children with asthma.

Methods

This is a qualitative study, using semi-structured in-depth interviews. Children were purposefully selected from the Study of Allergy, Genes and the Environment's (SAGE) 1995 Birth Cohort. Participating children will be 10-11 years old at the time of the interview. The purposeful selection was based on Winnipeg residency, completion of a dieting history questionnaire and assessment by a physician for asthma in 2003 and confirmed by a positive reply to the International Study of Allergies and Asthma in Children (ISAAC) question on a history of wheeze or whistling in the 12 months prior to the interview. The first author (JP) will be interviewing 40-60 children (50% male; 50% dieting) with and without asthma. Interviews are on-going; this presentation will focus on 20 interviews of children with asthma, or until saturation (no further emergence of new themes) is reached. To date, interviews with 8 children with asthma have been completed, averaging 25 minutes. All interviews will be audio-recorded and transcribed verbatim. Analysis involves topical coding to identify themes and looking for recurring regularities in the data. Overarching themes will be identified by addressing findings that are consistent and coherent, that increase and broaden our understanding of how children with asthma perceive healthy eating and physical activity and how they cope with asthma, and are useful for an intended purpose

Findings

Preliminary analysis suggests the following themes may be important to explore further: Children are aware of healthful food choices and talk about the importance of high-fat, high-sugar substitutions; children perceive physical activity advice as easier to follow than dietary advice; when asked about barriers to healthful food choices and physical activity, children talk about tempting foods (e.g. "junk food") and shortness of breath, respectively.

Deliverables

These preliminary data indicate that some children with asthma recognize which foods are sound nutrition choices and appreciate physical activity as easier than healthy eating. The presentation will explain how children perceive these topics.

Relevance

This study will provide a further understanding of how children understand healthy eating and physical activity. It will also help elucidate how children with asthma perceive the daily struggles associated with their disease. Understanding these aspects may yield insights useful in developing future programs that assist pre-adolescents living with asthma.

Urinary Metabolites of Inflammation in a Guinea Pig Model of Asthma

Idongesit P. Obiefuna^{1,2}, Erik J. Saude³, Farnam Ajamian¹, Brian D. Sykes³, and Darryl J. Adamko^{1,2}
Pulmonary Research Group, ²Departments of Pediatrics; ³CIHR Group in Protein Structure and Function, Department of Biochemistry; all are members of the Medical Resonance Diagnostics Centre (MRDC) at the University of Alberta, Edmonton, Alberta, Canada.

Objective/Purpose

Asthma is the most prevalent chronic illness of children, causing airway obstruction secondary to inflammation. Corticosteroids are used to control the severity of inflammation. Objective measurements of airway inflammation (e.g. Bronchoscopy, induced sputum) may be helpful in guiding therapy, but these may be difficult or impossible in young children. An easier noninvasive test to measure airway inflammation in children is needed

In an asthma attack, inflammatory cells are recruited to the lungs. We hypothesize that the metabolic activity of airway inflammatory cells during asthma will produce a unique pattern of metabolic molecules in urine, which can be measured using Nuclear Magnetic Resonance (NMR).

Methods

Female Guinea Pigs were used: nonsensitized control (n=8), sensitized alone (10 mg/ml ovalbumin ip; n=8) and sensitized challenged (10 mg/ml ovalbumin ip + 0.5% ovalbumin aerosol, n=6). Airway hyperreactivity (AHR) to histamine and measurements of inflammation (bronchoalveolar lavage fluid (BALF) and lung histology) were determined. Urine was collected and analyzed by NMR for metabolomic analysis.

Findings

Sensitized challenged animals developed AHR and associated increase in airway inflammation in the BALF and lung histology. The increased AHR and airway inflammation correlated with patterns of urine NMR profiles and differences in excreted methylhistamine.

Relevance

Urine NMR can correlate airway inflammation and AHR with the metabolic urine profile in a Guinea Pig model of asthma. NMR may serve as a non-invasive technique to monitor pulmonary inflammation and direct therapeutic treatment in humans.

The Impact of Mold on Asthma and Bronchial Hyperresponsiveness: Differences between First Nations and Non First Nations Children

S Huq, AB Becker, AL Kozyrskyj

Rationale

First Nation's homes have high levels of indoor mold. While indoor mold has been linked to respiratory problems, its association with asthma is controversial. We sought to determine the risk of asthma in children from exposure to indoor mold in First Nations (FN) and non First Nation homes.

Methods

This was the SAGE case-control study of the 1995 Manitoba cohort, consisting of 246 children with pediatric allergist-diagnosed asthma and 477 non-asthmatic controls at age 8-10 years. First Nations status was self-declared in 150 children, 55 % living on reserve. Information on current mold exposure was obtained from home inspection and survey questionnaire. BHR (bronchial hyper-responsiveness) was defined as methacholine < 8 mg/mL. Endotoxin levels were analyzed by LAL assay from dust samples collected during the home inspection. The likelihood (odds ratio, OR) of asthma according to current mold exposure, adjusted for gender, maternal asthma, mold and tobacco smoke exposure at birth, and endotoxin levels, was determined in logistic regression analyses.

Results

68% of FN and 67% of non FN children had current mold in the home. Asthma was 3 times more likely in FN children exposed to indoor mold (95%CI: 1.02-9.70). The adjusted OR for BHR asthma was 4.00 (0.86-18.8) in FN children, and the OR for BHR alone was 1.56 (95% CI: 0.60-4.05). No associations were observed for indoor mold and asthma phenotypes in non FN children.

Conclusions

Indoor mold is associated with asthma in FN, but not FN children. While these results may be subsequent to biased reporting by FN families, the higher risk of BHR asthma suggests that mold may be acting as a respiratory irritant in FN homes. The extent of exposure to mold may be determined by the levels of beta glucan in dust samples which are currently being assayed.

AllerWeb: Knowledge Discovery, Management and Transfer

Ben W. Tripp, Byron Chu, Scott J. Tebbutt, Denise Daley

*The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research,
St. Paul's Hospital & University of British Columbia, Vancouver, Canada*

Objective/Purpose

To develop a secure web portal to provide access to information and facilitate web based analysis experiments, thus allowing for novel discoveries using the genotype, phenotype and exposure information collected on the three studies involved in the Theme 1 collaboration (Asthma Prevention Study, Study of Asthma Genes and the Environment, and the Saguenay-Lac-St-Jean founder population). Genetic Studies generate an enormous volume of data including genotypic data on thousands of polymorphisms in hundreds of candidate genes. This presents many challenges including how to efficiently process genotype information produced by the high throughput SNP genotyping technologies and incorporate this information with phenotype and exposure data. Additionally, we need to provide secure and efficient access to this information to investigators, collaborators, students, and post-docs located across Canada, the US and Australia.

Methods

In order to accomplish these objectives we are developing a database that combines the phenotype, genotype and exposure information collected for all three studies. This database will be interfaced with a secure web portal that will allow users to access the database via a secure internet connection. Users will also be provided with access to statistical applications to conduct web based experiments and facilitate further analysis of the data. Users will be provided with an inventory of the results from experiments and analyses that have already been completed.

Findings

There are real and urgent needs to share and access data among Theme 1 investigators and with other AllerGen investigators. In addition, some of the genotypic and phenotypic data and analyses we are producing have value for the international Allergy and Asthma research community.

Deliverables

We are developing a website "www.genapha.ca" to facilitate the Knowledge Discovery, Management and Transfer of information generated by our studies. Since our ultimate aim is to apply the discriminate power of genotyping to clinical medicine, we also need to communicate our plans to the general public and to potential participants in our research. This will include the development of interactive 3D graphical animations that describe in a visually engaging manner the science and technology of genetic testing and pharmacogenetics.

Relevance

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

Peripheral Blood Molecular Bio-markers of Eosinophil-Lineage Commitment: Multiplex Q-PCR Analysis of GATA-1, MBP and IL-5 Receptor mRNA Expression Kinetics

AK Ellis, L Crawford, P. Lugomirski, JA Denburg; Division of Clinical Immunology & Allergy, Department of Medicine, McMaster University, Hamilton, Ontario, Canada.

Rationale

Using colony assays and flow cytometry, we have shown that eosinophil/basophil (Eo/B) progenitor phenotype and function are associated with both atopic risk at birth and early childhood clinical outcomes. We have further demonstrated that real-time polymerase chain reaction (Q-PCR) can reveal kinetic patterns of expression in cord blood (CB) of several Eo/B lineage-specific genes, specifically GATA-1, MBP and IL-5R α , as surrogate molecular markers of Eo/B differentiation. These same methods have yet to be established in peripheral blood (PB) samples.

Objective

To utilize Q-PCR to determine the kinetic patterns of expression of CB Eo/B-lineage specific genes in PB.

Methods

PB non-adherent mononuclear cells (PB NAMNC) were isolated from random fresh samples, and incubated in the presence of IL-5 (1 ng/mL). At 24, 48, 72h, and 1 week post-stimulation, RNA was isolated, reverse transcribed, and expression of IL-5R α , GATA-1, and MBP was determined utilizing multiplex Q-PCR. Relative expression ratios of stimulated to un-stimulated cells were calculated using the delta-delta Ct method.

Results

Stimulation of PB NAMNC with IL-5 resulted in an up-regulation of GATA-1 expression, peaking at 24h, with a lower peak and a slower return to baseline expression than that observed in CB. MBP expression was minimally altered at all time points, compared to CB, where slow up-regulation, maximal at 72h, had been observed. Preliminary analysis of one week incubation suggests very late up-regulation at this time-point. There was completely stable expression IL-5R α , similar to that seen in CB.

Conclusion

Multiplex Q-PCR analysis of mRNA from PB demonstrates expression of critical Eo/B lineage-specific events. Further investigation of the validity and utility of Q-PCR analyses of PB for surrogate, molecular markers Eo/B differentiation is underway.

Funding Sources: This research was funded by AllerGen NCE and the Hamilton Health Sciences Corporation. AKE is the recipient of an AllerGen/ Bayer/CAAIF Immunodeficiency and Immunomodulation of Allergic Inflammation Clinician-Scientist Research Fellowship.

Endotoxins Detection and Control in Drinking Water Systems

S. PARENT¹, R. GEHR¹, B. MAZER²

¹ *Department of Civil Engineering, McGill University, Montreal, Canada, H3A 2K6*

² *Department of Pediatrics, McGill University, Montreal, Canada, H2X 2P2*

Purpose

Endotoxins are a constituent of the lipopolysaccharide (LPS) complexes present in the outer layer of the cell wall of most Gram-negative bacteria and some cyanobacteria. The ingestion by a typical adult of amounts exceeding 1,000 endotoxin units (EUs) can cause fever, diarrhoea, vomiting, acute respiratory illnesses, and lung inflammation. In contrast, much smaller doses may lead to protective immunity against allergic diseases. Endotoxins can be released in the air as well as in the water. Although many studies on endotoxins in raw and treated drinking waters have been performed, few have assessed seasonal variations and none have been conducted in Eastern Canada. Furthermore, a clear understanding of removal of endotoxins by various water treatment processes is still required.

Methods

Two methods to measure the concentrations of endotoxin were used and compared, the Limulus Amoebocyte Lysate test (LAL) and the recombinant Factor C test (rFC). Raw water samples were taken from various sources around the Island of Montreal. The effects of free chlorine, UV radiation, and ozone were studied in batch experiments on filtered water samples via typical dosages and fluences used in drinking water treatment facilities. Residual concentrations for free chlorine were 0.8 and 1.6 mg/L; ozone doses were 0.5 and 1 mg/L; UV fluences were 40 and 100 mWs/cm². Detention times of 5 and 20 minutes were tested for both chlorine and ozone. Samples from three drinking water treatment plants in the Montreal area were analyzed during the months of June and late August/September 2006 and will be analyzed in January 2007. Processes at these plants include coagulation and flocculation, sand filtration, ozonation and disinfection by chlorine. To test the variation in endotoxin concentrations during a sand filter cycle, samples were withdrawn directly from a filter in one of the treatment plants studied. The complete cycle lasts 72 h. Samples were collected immediately before the backwash, at the beginning and at the end of the ripening period, at the beginning of the filtration cycle and 48 h later, which corresponds to a half cycle period.

Findings

Of the two endotoxins methods used, LAL consistently gave slightly higher values compared to rFC; rFC also required more expensive hardware, but the method was less tedious and reagent costs were lower. Endotoxin levels decreased in raw water samples between June and September. Concentrations ranged from 20 to 50 EU/mL in the summer, and decreased to 10 to 14 EU/mL in September and beyond. For the disinfection processes, the UV and free chlorine doses tested had little or no effect on the endotoxin concentrations, but ozone reduced the concentrations by up to 75%. Sand filtration and flocculation showed significant endotoxin removal efficiencies (50 – 60%). Levels remained around 5 EU/mL throughout the remaining treatment processes, however initial measurements have shown a spike up to 18 EU/mL in endotoxin concentration at the end of the ripening period (i.e. shortly after the beginning of the filtration cycle); tests are continuing.

Relevance

Endotoxin inactivation by free chlorine and UV does not occur with typical doses used in drinking water treatment plants; in contrast, flocculation and sand filtration, as well as ozonation, are much more effective.

Candidate Genes for Asthma, Atopy and Allergic Disease: Results from Theme 1

Denise Daley¹, Peter D. Pare¹, Andrew J. Sandford¹, Anita L. Kozyrskyj², Catherine Laprise³, Yohan Bosse⁴, Alexandre Montpetit⁴, Jianqing He¹, Alan Becker², Karine Tremblay^{5,6}, Thomas J. Hudson⁴, and Mathieu Lemire⁴: 1) University of British Columbia, 2) University of Manitoba, 3) University of Quebec at Chicoutimi 4) McGill University and Genome Quebec Innovation Centre 5) University of Montreal Community Genomic Medicine Centre, Chicoutimi University Hospital, Saguenay (QC), Canada 6) Laval University, Quebec (QC), Canada

Objective/Purpose

Conduct a genetic association study to investigate 165 candidate genes associated with asthma and allergic phenotypes (asthma, atopy and a measure of airway responsiveness).

Methods

Cohorts: 3 Canadian cohorts including; 1) a high risk birth cohort (Asthma Prevention Study ~1200 individuals), 2) a population birth cohort of children from the Study of Asthma Genes and Environment (SAGE) (~1800 individuals), and 3) The Saguenay-Lac St. Jean Quebec Founder Cohort (SLSJ) (~1200 individuals). Selection of Candidate Genes: Genes were selected following a literature search and include novel candidate genes identified by linkage analysis and expression microarrays. We selected prime candidate genes based upon previous associations with early life asthma and allergic disease. Genes include chemokines, innate immunity genes, and Toll-like receptors. For full list of genes see http://www.mrl.ubc.ca/pare/Gene_list2.doc. For each gene a maximally informative set of common single-nucleotide polymorphisms (SNPs) were selected by studying the HapMap dataset and were genotyped using the Illumina GoldenGate assay. Statistical Methods: Genetic analysis is being carried out using Family Based Tests of Association (as implemented in FBAT). This Transmission Disequilibrium Test (TDT) uses the transmission of alleles from heterozygous parents to affected children. There is association if alleles are transmitted to affected children more often than expected by Mendel's law (>.50). A disease allele, or an allele that is in linkage disequilibrium with a disease allele, will be transmitted more often than expected to affected offspring.

Corrections for Multiple Testing: When performing thousands of tests it is vital to account for multiple testing. We utilized a method that utilizes a LD Matrix based upon the pair-wise correlation between SNPs. It then determines the effective number of independent tests that are being performed based upon spectral decomposition of the correlation matrix see <http://genepi.glmredu.au/general/dateN/SNPSpD/>.

Findings

Analysis is complete for two samples, the SLSJ and the Asthma Prevention Study. Genotyping of the affected trios in the SAGE cohort will be completed by Jan 07. We will present preliminary results for all 3 cohorts. Preliminary findings indicate that there are significant associations with genes previously identified by the SLSJ cohort and a few novel associations that have not been previously reported in the literature. Most associations are significant only in a single cohort, but there are a few genes that are significant in two or three cohorts. Combined analyses of the cohorts will be presented.

Deliverables

Identification of susceptibility genes for asthma and allergic diseases will lead to better treatments and interventions and can be used to help guide the CHILD birth cohort.

Relevance

Asthma and allergic diseases are the result of complex genetic and environmental interactions. We must better understand the contribution of genetics and gene-environment interactions in order to understand the mechanism for the development of asthma and allergic disease. Once we understand the mechanisms we can better target treatment, intervention and prevention of these diseases.

The Influence of RSV and Particulate Air Pollution (PM10) on Airway Epithelial Cell Damage and Repair

G.K.Singhera, S. Allahverdian, T-L. Hackett, D.A. Knight, R. Hegele, S. Van Eeden, T.R. Bai, and D.R. Dorscheid. iCapture Centre, UBC, St Paul Hospital, Vancouver, BC, Canada.

Rationale

The bronchial epithelial cell is the first cell contact and a physical barrier to the external environment. Respiratory viral infections and environmental air pollution particles are two major insults that affect the airway epithelium. Detailed cellular examination of bronchial biopsies and BAL fluid has provided convincing evidence of epithelial damage and aberrant repair in asthma. It is therefore important to understand how cellular regulators such as apoptotic proteins and wound repair are affected after exposure to these environmental challenges.

Methods

Primary airway epithelial cells (AEC) in submerged monolayer culture were infected with Respiratory Syncytial Virus (RSV) and/or exposed to particulate matter (PM10). Apoptosis was measured by the detection of histones released by ELISA. Expression of p85, Bcl-xl, Bax, and Annexin II (All) proteins were measured by Western blot. Wound closure rates were measured by time-lapse video microscopy.

Results

Analysis of histone release and p85 (PARP) cleavage showed exposure to RSV and PM10 together produced a greater apoptotic response (2.5 fold increase, $P < 0.05$) compared to control or RSV/PM10 individually at 4 and 24 hours. Infection of cells with RSV alone resulted in increased Bcl-xl:Bax ratio compared to control and RSV and PM10 together ($P < 0.05$). Recent studies have alluded to the role of Annexin II in AEC repair; we found Annexin II was up-regulated by RSV infection however this effect was not demonstrated following RSV+PM10 co-exposure. Finally, the kinetics of repair after wounding was significantly inhibited in the presence of RSV and PM10 together compared to control and RSV alone ($P < 0.05$).

Conclusion

Exposure of normal AEC to PM10 and RSV results in elevated apoptosis and inhibited repair compared to RSV infection alone. RSV infection of normal AEC inhibited apoptosis as demonstrated by an up-regulation of BCL_{xL}/Bax and this did not affect wound repair. The extent to which this natural combination of exposures may contribute to airway remodeling in asthma remains to be determined.

Mechanisms of Degranulation in Inflammatory Cells

Troy Mitchell¹, Andrea Lo², Gary Eitzen¹ and Paige Lacy²

Department of Cell Biology, University of Alberta, Edmonton, AB1; Department of Medicine, and Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton AB2

Objectives/Purpose

Hematopoietic inflammatory cells, including basophils, eosinophils, neutrophils (granulocytes) and tissue resident mast cells, are crucial mediators of pathogenesis in allergic disease. Disease symptoms can be caused by secretion of cytotoxic mediator proteins (degranulation) from these cells, which leads to further granulocyte recruitment and activation, and can result in uncontrolled inflammation, edema and collateral tissue damage. Degranulation occurs by regulated exocytosis (membrane fusion) of mediator-containing secretory vesicles in response to inflammatory cell activation. Our objective is to attenuate allergic responses, and our therapeutic strategy is directed at downregulation of inflammatory cell exocytosis. We have identified the small monomeric Rho GTPase, Rac2, as a key intracellular regulator of the receptor signaling pathway leading to degranulation in neutrophils and mast cells. Rac2 is implicated in the control of an early step of primary granule exocytosis. Rho-GTPases in general have also been implicated in cytoskeletal rearrangement during exocytosis. The purpose of this study is to identify signaling components downstream of Rac2 which are vital to the mechanism that regulates exocytosis. These include cytoskeletal components such as α -actin and microtubules, which undergo continuous remodeling to move secretory organelles to various parts of the cell. Our hypothesis is that Rac2 governs cytoskeletal remodeling which are necessary for exocytosis. Since little is known regarding the regulation of cytoskeletal remodeling in exocytosis, we focused this study on how the cytoskeleton is involved in secretion from neutrophils. Since expression of Rac2 is limited to hematopoietic cells it is an ideal target for precise therapy of inflammatory diseases.

Methods

To determine whether cytoskeletal remodeling is important for degranulation we focused our studies on the use of specific drugs that modulate actin and microtubule dynamics. Neutrophils Human peripheral blood neutrophils were treated with either latrunculin B (destabilizes F-actin), jasplakinolide (stabilizes F-actin), nocodazole (destabilizes microtubules) or paclitaxel (stabilizes microtubules) and stimulated either by adhering them to plastic surface adherence or via CB/fMLF exposure to cells in suspension, both of which have been implicated in activating the Rac pathway. Degranulation of the neutrophils was then examined by probing for the release of specific granule-derived mediator's resident specific to each granule population. Primary granule release was assayed by myeloperoxidase activity, while secondary and tertiary granule secretion was determined by ELISA for lactoferrin and matrix metalloprotease-9, respectively.

Findings

Our current results indicate that there is a variable inhibitory effect, on the differing granules. In general, when cells are in suspension, stabilization of F-actin by jasplakinolide inhibits primary granule release in a dose dependent manner, whereas F-actin destabilization by latrunculin B promotes primary granule release at low concentrations but inhibits at high concentrations. Both microtubule-affecting drugs inhibit primary granule release in a dose dependent manner suggesting that granule dependence on microtubules is a dynamic process. When examining secondary granule release of neutrophils in suspension all four drugs showed some level of inhibition however there was no dose dependent trend. This data suggests that the granule populations may have differing dependence on cytoskeletal reorganization. Results from surface adherence are still pending.

Relevance

Little is known about the molecular mechanisms that regulate inflammatory cell degranulation. This is in spite of the fact that granules of neutrophils and mast cells release tissue-damaging enzymes that significantly contribute to allergy disease burden. Rac2 regulates multiple events in inflammatory cell activation, one of which is primary granule exocytosis. In our search for downstream factors that specifically govern exocytosis we found that Rac2-activated may be target the cytoskeleton to facilitate granule secretion. Since expression of Rac2 is limited to hematopoietic cells it is an ideal target for precise therapy of inflammatory diseases.

Leptin is Associated with Asthma in Overweight Children

Xiao-Mei Mai^{1,2}, *Carla Taylor*³, *Allan B Becker*², *Anita L Kozyrskyj*^{1,2}
*Faculty of Pharmacy*¹, *Pediatrics & Child Health and Manitoba Institute of Child Health*², *Human Nutritional Sciences*³, *University of Manitoba, Canada*

Objective

Leptin is an inflammatory adipokine associated with obesity and cardiovascular disease. Our aim was to evaluate the levels of serum leptin in relation to asthma in overweight vs. normal weight children.

Methods

Serum levels of leptin were analyzed in 87 asthmatic children diagnosed by a pediatric allergist and 126 non-asthmatic controls (8-10 years). Among the 213 children, 69 children were classified as being overweight according to body mass index (BMI) \geq 85th percentile of gender and age specific growth charts. The serum levels of leptin were analyzed by ELISA.

Findings

Overweight was associated with 3 fold increases in leptin compared with normal weight (Geometric Mean 13.87 vs. 4.14 ng/ml, $p < 0.0001$). The levels of leptin were higher in asthmatic than non-asthmatic children (GM 7.32 vs. 5.37 ng/ml, $p = 0.03$). Differences by asthma status were significant in overweight (GM 20.29 vs. 10.49 ng/ml, $p < 0.001$) but not in normal weight children (GM 4.39 vs. 3.94 ng/ml, $p = 0.45$). In overweight children, multiple linear regression analysis showed that leptin was increased 1.7 times in asthmatic vs. non-asthmatic children ($p = 0.003$), independent of gender, age, BMI percentile, maternal and paternal asthma, current passive smoke and breastfeeding. Besides asthma status, female gender and increase of BMI percentile predicted elevated levels of leptin in overweight children ($p < 0.001$ for both).

Relevance

Leptin is increased in children with asthma, particularly those who are overweight and may play a role in airway inflammation in those children. This adds important information on our understanding of the mechanism of asthma in obese individuals.

Investigation of Mast Cell and Eosinophil Function in Immune and Allergic Response via Cre-Mediated Conditional Gene Deletion

Haddon, D.J.¹, Maltby, S.¹, Kerfoot, S.², Kubes, P.², Lahti, J.³ & McNagny, K.¹. ¹Biomedical Research Centre, University of British Columbia, Vancouver, BC, ²Immunology Research Group, University of Calgary, Calgary, AB and ³Department of Genetics and Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN.

Objectives/Purpose

Mast cells and eosinophils play important roles in tissue damage and inflammation associated with allergic and immune disease. These cell types are present in affected tissues and express molecules known to have damaging effects, such as proteases, peroxidases and nucleases. While their involvement in allergy and asthma has been confirmed, little is known about their regulation in the disease state. To better study the physiology of both cell types, we will create transgenic mouse lines capable of mast cell and eosinophil specific gene deletions. Further, the transgenic lines will enable visualization of these cells *in vivo*. Using these mice, we aim to delete mast cell and eosinophil associated genes to determine their role in the regulation of the immune response. Ultimately, we hope these projects will lead to the discovery of novel target molecules to control allergic and immune disease.

Methods

The mast cell and eosinophil transgenic mice will be created by microinjection of the recombinant DNA constructs into fertilized mouse oocytes, resulting in adult mice containing the transgene. The constructs have been designed to limit expression specifically to mast cells by using the promoter from Mast Cell Carboxypeptidase A (MCCPA), and to eosinophils by using the promoter from eosinophil peroxidase or the IL-5 receptor. Each construct contains the coding sequence for Enhanced Green Fluorescent Protein (EGFP) and Cre recombinase. EGFP is a protein that fluorescently marks the cell it is expressed in when viewed under specific wavelengths of light. We will use EGFP to identify and track transgenic cells both *in vivo* and *in vitro* using fluorescent microscopy and Fluorescence Activated Cell Sorting (FACS). When Cre is expressed it binds to loxP DNA sites, causing excision of an intervening gene. The Cre/loxP system requires two transgenic mouse lines, the first being our tissue specific Cre expressing line, and the second having the gene of interest flanked by loxP sites. When the two lines are crossed, the gene of interest remains intact in all other tissues in which Cre is not expressed.

Findings

Analysis of transgenic animals will be performed to ensure limited expression of GFP and Cre is specific to mast cells and eosinophils, in the respective mouse lines. To demonstrate the efficiency and specificity of Cre in the transgenic mast cell and eosinophil mouse lines, they will be bred with ROSA-YFP reporter mice. ROSA-YFP mice contain a reporter construct, which only expresses Yellow Fluorescent Protein (YFP) in cells in which Cre-mediated gene deletion has occurred. Crosses of our transgenic animals with various LoxP-containing transgenics will be performed to determine candidate molecules to target for allergic treatments. By performing cell-specific gene deletions for adhesion molecules, signaling receptors and transcription factors, we will gain a better understanding of mast cell and eosinophil differentiation, homing and regulation. After performing gene deletions, we will induce asthma, allergy and immune disease models in the transgenic mice to determine the effect on pathology. Gene deletions resulting in altered cell infiltration and pathology may result in identification of target molecules for future research.

Deliverables

We plan on generating transgenic mouse lines capable of mast cell and eosinophil specific gene deletions for the investigation of allergic and immune disease.

Relevance

Currently, no genetic tools exist to analyze the regulation and physiology of mast cells and eosinophils and their involvement in allergic and immune disease. This project addresses the need for tools to allow characterization of specific genes' activities in these cell types. By identifying target genes involved in disease pathology, we hope to identify novel therapeutic targets for treatments of asthma, allergy and immune disease.

Identification of Functional SNPs in TIM3

Jian Zhang, University of British Columbia

Rationale

Numerous genetic studies have mapped asthma susceptibility genes to a region on chromosome 5q31-33 in several populations. This region contains a cluster of cytokines and other immune-related genes important in the immune response including TIM1 (T cell immunoglobulin mucin molecular 1) and TIM3. One positional cloning study using congenic BALB/c-HBA mice mapped the *Tapr* (T cell and airway phenotype regulator) locus to the region of the TIM family and found major variants in the TIM family that completely cosegregated with *Tapr*. In two association studies, 3 SNPs in TIM3 showed association with atopy and eczema using Caucasian, Hispanic and Korean samples.

In adaptive immunity CD4⁺ T cells differentiate into T helper 1 (T_H1) and T_H2 cells in response to different antigenic stimulation. Tim-3, a T_H1-specific type 1 membrane protein, is not expressed on the surface of naive T cells but emerges on the cell surface of fully differentiated T_H1 cells. TIM3 and its ligand, Galectin-9, regulate both peripheral tolerance and the expansion of effector T_H1 cell populations.

Aims

To identify the functional SNP(s) in the TIM3 promoter region which affect the rate of transcription.

Methods

5' RACE was performed to isolate full-length cDNA. Genotyping was done with RFLP (Restriction Fragment Length Polymorphism) and direct sequencing using 28 normal Caucasian samples. An expression analysis was performed with RT-PCR. Plasmids were constructed containing different sizes of fragments of the TIM3 promoter from -2199 bp to +51 bp and different haplotypes were made with standard methods. Reporter gene assays was used to measure promoter activity.

Results

TIM3 was expressed extensively at the RNA level. It was expressed strongly in placenta, lung, kidney, spleen, and leukocytes. An additional 20 bp was extended to the 5' side of the known cDNA sequence but no novel exon was found. There were 6 polymorphisms including one novel deletion and 4 haplotypes in the promoter region. Two promoter regions were identified. One is from -214 bp to -58 bp and another is from -914 bp to -1.6 kb relative to the transcription start site.

Future plan

To transfect the vectors containing different haplotypes into a cell line to compare their relative promoter activities. If a significant difference is found then EMSA and specific-allele RT-PCR will be performed.

Proteomic Analysis of Rac2-Mediated Secretion in Neutrophils

Andrea N. Lo¹, Troy Mitchell², Greg Downey³, Gary Eitzen², and Paige Lacy¹

¹Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB; ²Department of Cell Biology, University of Alberta, Edmonton, AB; and ³Department of Medicine, University of Toronto, Toronto, ON.

Objectives

Neutrophils are leukocytes that are important mediators in the pathogenesis of many inflammatory disorders. Diseases such as asthma, rheumatoid arthritis, acute lung injury and sepsis are exacerbated by the secretion of granule-derived compounds from these cells. Furthermore, these cytotoxic proteins cause collateral damage to adjacent healthy tissue. Our lab has identified Rac2, a Rho GTPase, as a crucial signalling molecule for the exocytosis of primary granules. Rho GTPases are well known for their roles in signaling to the cytoskeleton via kinase cascades. Thus, we hypothesize that Rac2 gene deletion will lead to aberrant regulation of cytoskeletal remodeling and kinase activity required for the release of primary granules in neutrophils.

Methods

To investigate the Rac2 signalling pathway, bone marrow neutrophils from wild-type (WT) and Rac2 knockout (KO) mice were examined. Cells were either unstimulated or exocytosis was induced with cytochalasin B/f-Met-Leu-Phe (CB/fMLF). Cell pellets were lysed and proteomic analysis was performed using the Amersham Ettan DIGE 2D electrophoresis method to globally identify differences in protein abundance between the two samples. Spots showing statistically significant differences were sequenced by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) and compared to a database of known proteins. For determining additional signalling components of the secretory pathway such as kinases, we also analyzed phosphorylation events using a custom Kinexus phospho-site screen.

Findings

Over 3500 spots were revealed by 2D gel electrophoresis. Of those, 37 were chosen to undergo sequencing by MS because they showed significant differences in abundance between wild-type stimulated and unstimulated samples, and this trend was not apparent in Rac2-KO samples. Only species-specific matches were considered and 10 known proteins were identified: coronin, HSPs, GAPDH, chitinase, beta-actin, myeloid granule proteins, and actin capping proteins. Two unknown proteins were also identified. Furthermore, the Kinexus screen showed significant changes in the activity of several different protein kinases in Rac2 KO neutrophils stimulated to undergo degranulation. Optical density measurements of phosphorylated residues were screened by Western blot analysis and PAK1/2/3 was identified as a kinase that remained downregulated in both resting and stimulated KO neutrophils when compared to WT.

Deliverables

To attenuate an inflammatory allergic response, an effective therapeutic strategy would be one that is directed at blocking key molecules to downregulate degranulation of leukocytes. Our objective is to develop inhibitors of key regulatory molecules in the signalling pathway that regulate exocytosis using specific antagonists. The potential outcome of our research is the development of specific drugs that can block the damaging effects of inflammation in allergy. The benefit of this research to Canadians is also likely to be high since allergy is a major disease in this country.

Relevance

Proteomic analysis is a powerful technique that can be used to study secretion pathways. We have identified proteins that may be important in neutrophil exocytosis. Confirmation of their role in degranulation responses will be done by modifying protein functions using transfection techniques established in our lab. Moreover, there is the potential diagnostic use for proteomics to identify biomarkers by comparing healthy versus diseased states.

Antenatal Steroid Therapy for Fetal Lung Maturation: Is there an association with childhood asthma?

Jason D. Pole^{1,2,3}, Cameron A. Mustard^{1,2}, Teresa To^{1,3}, Joseph Beyene^{1,3} and Alexander C. Allen⁴

1. Department of Public Health Sciences, University of Toronto, Toronto, Ontario Canada. 2. Institute for Work & Health, Toronto, Ontario Canada. 3. The Hospital for Sick Children, Toronto, Ontario Canada. 4. Perinatal Epidemiology Research Unit, Departments of Obstetrics and Gynecology and Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada.

Objective

This study was designed to test the hypothesis that fetal exposure to corticosteroids in the antenatal period is an independent risk factor for the development of asthma in childhood.

Methods

A population-based cohort study of all pregnant women who resided in Nova Scotia, Canada, and gave birth to a singleton fetus between January 1989, and December 1998, and lived to discharge was undertaken. After exclusions, 79,395 infants were available for analysis. Using linked health care utilization records, incident asthma cases between 36 to 72 months of age were identified. Generalized Estimating Equations were used to estimate the odds ratio of the association between exposure to corticosteroids and asthma while controlling for confounders.

Findings

Over the 10 years of the study corticosteroid therapy increased by 3-fold. Exposure to corticosteroids during pregnancy was associated with a risk of asthma in childhood: adjusted odds ratio of 1.23 (95 % confidence interval: 1.06, 1.44).

Relevance

Antenatal steroid therapy appears to be an independent risk factor for the development of asthma between 36 and 72 months of age. Further research into the smallest possible steroid dose required to achieve the desired post-natal effect could be undertaken to reduce the risk of developing childhood asthma.

Autocalling Algorithms for Microarray Based Genotyping

Mohua Podder¹, Will Welch², Ruben Zamar², Scott J. Tebbutt¹

¹iCAPTURE Centre for Cardiovascular and Pulmonary Research and ²Department of Statistics, UBC; Vancouver

Introduction

Single nucleotide polymorphisms (SNPs) represent the most common form of inherited genetic variation, and are defined by a change of a single nucleotide (A, C, G, or T) at a specific position of a genome sequence. Accurate and fast genotyping has been an integral part of any SNP related research. In the last decade or so, many researchers have started work on several medium to high-throughput microarray based genotyping technologies. Among them, GeneChip®, Tagged/ZipCode arrays and mini-sequencing arrays are three commonly used microarray genotyping protocols which have been designed optimally to give accurate genotypes for a medium to large number of analyzed SNPs.

Objective

We will first briefly discuss these three microarray genotyping platforms, and then describe the genotype calling algorithm based on a mini-sequencing array using both classical arrayed primer extension (APEX) probes and allele-specific APEX (ASO) probes, developed by our research group.

Existing technologies

For all these genotyping protocols, the microarray signal intensity data are obtained using different levels of hybridizations of the sample templates with the corresponding oligonucleotide probes arrayed on a solid support. Fluorescent intensity signals associated with each probe spot are measured by a scanning device followed by digital imaging software. Our laboratory has developed a robust and redundant microarray platform generating multiple signals from multiple probes [APEX and ASO probes for both DNA strands] corresponding to a single SNP.

Method

From this APEX microarray platform, four groups of classifiers are generated; each consists of two explanatory variables specific to two possible alleles in a particular SNP site. Using a set of 32 Coriell DNA samples plus three negative PCR controls as a training data set, we have developed a fully-automated genotyping algorithm based on simple linear discriminant analysis (LDA) using dynamic variable selection. We have tested our algorithm on a completely independent data set of 270 DNA samples, with validated genotypes, and our method automatically achieves a concordance rate of 98.9% with a 99.6% call rate for a set of 96 SNPs.

Relevance

To our knowledge, APEX is the only chemistry by which the on-chip assay can be performed in 20 minutes, making APEX potentially suitable for rapid genetic diagnostics in clinical settings. Building an optimum genotype-calling tool using signals from all the four channels (including background signals) for this APEX based microarray platform will allow quick genetic diagnostics for a large number of SNPs specific to any disease, including asthma and allergy.

Comparative Responses to Nasal Allergen Challenge in Allergic Rhinitic Subjects With or Without Asthma

Rousseau MC¹, Boulay ME¹, Goronfolah L², Lin M², Ferrie P², Denburg JA², Keith P², Boulet LP¹.
¹Centre de recherche de l'Hôpital Laval, Institut universitaire de cardiologie et de pneumologie de l'Université Laval, Québec (Qc), Canada. ²McMaster University, Health Sciences, Hamilton (On), Canada.

Rationale

Nasal allergen challenge is a non-invasive method used to study pathophysiological mechanisms in rhinitis. Since single dose allergen provocation may not adequately approximate natural exposure, multiple nasal challenges may be more helpful.

Objective

To determine the effect of daily nasal allergen challenge over 4 consecutive days on clinical parameters in rhinitic subjects with or without asthma.

Methods

Seventeen subjects with allergic rhinitis were recruited: 9 with mild asthma and 8 without asthma. Subjects underwent a nasal control challenge with normal saline followed by 4 consecutive daily allergen challenges. During each challenge day, determined allergen dilutions (1:10,000 to non-diluted) were sprayed into each nostril until a positive nasal response occurred. Patients recorded their symptoms (stuffy nose, itchy eyes, sneezing, runny nose, cough) on a Likert scale, as well as oral peak expiratory and nasal peak inspiratory flows, allowing an assessment of a nasal blockage index (NBI), for a period of 7 hours.

Findings

Compared with the control day, there was a significant increase in NBI and symptom scores 10 minutes after each last daily allergen exposure for the two groups ($p < 0.05$). Similar allergen concentrations caused nasal responses which were not significantly different in magnitude on the 4 test days. NBI and symptom scores were similar for the 2 groups except for cough: the rhinitics without asthma had greater symptom scores for cough in the first 45 min post-challenge than asthmatics ($p < 0.05$). No late nasal responses were observed on any day in either group.

Relevance

Multiple nasal allergen challenges may be useful to investigate the mechanisms and consequences of allergic rhinitis, in particular its potential influence on the lower airways.

Selection of Housekeeping Genes for Real-Time Quantitative PCR in Normal and Atopic Human Bronchial Epithelial Cells

Jianqing He¹, Darryl Knight^{1,2}, Anthony Kicic³, Stephen M. Stick³ and Peter D Paré¹

1. James Hogg iCAPTURE Center for Cardiovascular and Pulmonary Research, St. Paul's Hospital, and 2. Department of Anesthesiology, Pharmacology, and Therapeutics, University of British Columbia, Vancouver, Canada. 3. Department of Respiratory Medicine, Princess Margaret Hospital for Children, Perth; School of Pediatrics and Child Health, The University of Western Australia, Nedlands; Telethon Institute for Child Health Research, Subiaco, Western Australia, Australia

Background

Real-time quantitative PCR is a very powerful technique to examine gene expression profiles under different biological conditions. The stability of housekeeping genes is critical when comparing gene expression profiles in normal and disease tissues. To date, there have been no studies that have no studies which have systematically compared the stability of common housekeeping genes in normal and atopic human bronchial epithelial cells.

Methods

We measured the expression levels of twelve housekeeping genes and analyzed the stability of their expression in bronchial epithelial cells of healthy children (n = 10), atopic children without asthma (n = 10) and atopic asthmatic children (n = 10). The twelve housekeeping genes studied were 18S rRNA, Acidic ribosomal protein (PO), Beta-actin (β A), Cyclophilin (CYC), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Phosphoglycerokinase (PGK), β 2-Microglobulin (β 2m), β -Glucuronidase (GUS), Hypoxanthine ribosyl transferase (HPRT), Transcription factor IID, TATA binding protein (TBP), Transferrin receptor (TfR) and Guanine nucleotide binding protein (GNB2L1).

Results

All twelve housekeeping genes were expressed in human airway epithelial cells. However, two of these genes (HPRT and TBP) were excluded from stability analysis due to relative low expression levels. The stability of the remaining ten genes was determined using geNorm applet. The three most stable genes were CYC, GNB2L1 and 18S rRNA in healthy children; GNB2L1, β A and CYC in atopic children without asthma; β A, GAPDH and CYC in atopic asthmatic children. There were no differences in the expression levels of the ten housekeeping genes among the three groups of subjects.

Conclusion

The results of this study suggest that CYC may be the most suitable housekeeping gene in gene expression studies of human bronchial epithelial cells derived from normal and atopic children.

Expression of Anti-Inflammatory Proteins and Peptides in Mind-Body Pathways of Inflammation

Katherine Morris, Paul Forsythe, Sam Hariforoosh, Ron Mathison, and A. Dean Befus, Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, Alberta; University of Calgary, Calgary, Alberta; McMaster University, Hamilton, Ontario

Objective

The sympathetic nervous system modulates the function the submandibular gland (SMG), altering its release of bioactive peptides that act systemically to regulate pulmonary inflammation. In rats, a cleavage product of submandibular rat 1 (SMR1), TDIFEGG, has potent anti-inflammatory activities. The tripeptide FEG and the metabolically stable D-isomeric feG exert anti-inflammatory activities in rat, mice, dogs, sheep, and isolated human neutrophils. feG is effective in animal models of pancreatitis, spinal cord injury, and pulmonary inflammation. Little is known about the endogenous regulation of FEG-containing proteins in animals or humans. Our aim is to develop methods to evaluate the expression and processing of SMR1 and other proteins that contain similar biologically relevant motifs. These methods will be used to evaluate the endogenous regulation of FEG-containing proteins in humans and to evaluate changes to processing of FEG-containing proteins that may occur in pathologies including asthma.

Methods

- Rabbits were immunized with one or a combination of synthetic peptides to regions of the SMR1 protein. Purified IgG fractions of the antibodies were produced.
- Submandibular glands from male Sprague-Dawley rats or human SMG biopsy specimens were homogenized and resolved in one or two dimensions on SDS-PAGE.
- Western blots were performed with each of the novel SMR1 antibodies or anti-phospho threonine antibodies.
- Lysates were subjected to denaturation and treatment with N-glycanase prior to electrophoresis.
- RNA was extracted from rat SMG and quantitative RT-PCR was performed.

Findings

- Three antibodies showed similar reactivity to at least 4 protein bands ranging from 10-25 kDa on 1-D Western blots.
- The SMR1 protein in rat SMG is detected on 2-D gel electrophoresis in 52 spots that have ranges of 10-25 kDa in molecular weight and 4-7 in pI.
- The SMR1 protein in rat SMG is N-glycosylated, but the major species of SMR1 are not significantly phosphorylated.
- SMR1 mRNA was expressed in rat SMG at levels approximately 5-fold greater than in rat lungs.
- A human submandibular gland lysate contained several bands that were immunoreactive with an antibody designed to the biologically relevant C-terminus of SMR1.

Deliverables

- Methods have been developed to quantitatively evaluate the expression of SMR1 mRNA and protein
- 2-D SDS-PAGE and Western blotting techniques allow us to monitor the post-translational processing of SMR1 and its fragments
- The antibodies produced will allow us to evaluate the expression and processing of other proteins containing similar potentially biologically relevant motifs, including human proteins.

Relevance

feG is anti-inflammatory in animals and on human cells. In rats, a neuroendocrine pathway regulates the expression and processing of FEG-containing proteins, thus modulating inflammation. Using the methods we have developed, we aim to identify the molecular components involved in this pathway in humans. Investigating the endogenous regulation of biologically relevant FEG-containing human proteins will allow us to better understand the therapeutic potential of feG and identify additional therapeutic targets for the treatment of asthma. Additionally, this research will provide valuable information about the regulation and mechanisms of mind-body pathways.

Allergic Asthma: Air Pollution and Allergen Interactions

Fritscher L¹, Urch B^{1,3}, Taday M¹, Speck M^{1,2}, Manno M^{1,2}, Guha P^{1,2}, Lukic KZ^{1,2,7}, Fila M^{1,3}, Denburg J⁵, Koutrakis P¹⁰, Evans G^{1,3}, van Eeden S⁶, Palaniyar N^{3,7}, Brook JR^{3,8}, Liu L⁹, Corey P^{1,3}, Scott James^{1,3}, Scott Jeremy^{1,3}, Tarlo S^{1,3,4}, Silverman FS^{1,2,3}, Gage Occupational and Environmental Health Unit¹, St. Michael's Hospital², University of Toronto³, The Toronto Hospital⁴, McMaster University⁵, The James Hogg iCAPTURE Centre⁶, The Hospital for Sick Children⁷, Environment Canada⁸, Health Canada⁹, and Harvard School of Public Health¹⁰

Objectives

To determine: 1) airway reactivity to inhaled allergen and 2) airway and systemic inflammatory cellular and molecular responses after exposure to urban concentrated ambient fine particles (CAP) + ozone (O₃); as well as 3) the association of CAP constituents with biological responses.

Methods

The study is conducted at the CAP exposure facility of the Southern Ontario Centre for Atmospheric Aerosol Research located at the Gage Occupational & Environmental Health Unit, St. Michael's Hospital/University of Toronto. The facility includes an ambient fine particle concentrator and subject enclosure for delivery of controlled levels of CAP+O₃. Twenty allergic asthmatic non-smokers 18-49 yrs old will be exposed to "real life" ambient pollutants (150 µg/m³ CAP + 200 ppb O₃) and filtered air (FA), in a randomized single-blind crossover design. Exposures are 1-hr in duration, followed by an allergen inhalation challenge to determine the allergen provocation concentration causing a 20% decrease in FEV₁ (PC₂₀). Inclusion criteria are: physician-diagnosed asthma, methacholine PC₂₀ < 8 mg/ml, FEV₁ > 70% of predicted normal value¹ (FEV₁%), positive skin-prick testing to grass, ragweed pollen, cat dander or house dust mite (HDM) and a subsequent (next day) positive allergen inhalation challenge (AIC)^{2,3,4}. The exposure protocol includes 3 consecutive days of testing, with a 3-week washout period between exposures. Day-1 tests include a methacholine challenge (MC) and sputum induction^{5,6}. Day-2 is the exposure day and tests include: pre-exposure blood sampling (CBC, cytokines/mediators, markers of bone marrow stimulation^{8,9,10}, IgE, surfactant proteins¹¹ A & D), spirometry & lung volumes; during-exposure spirometry, capnography and symptom assessment¹²; post-exposure spirometry^{13,14}, lung volumes & symptoms, followed by an AIC (to 2-fold increasing allergen concentrations with extrapolated determination of PC₂₀). Day-3 is used for 24-hr post-exposure testing, including a MC, sputum induction, blood sampling, and symptom assessment.

Findings

To date, 9 individuals have been screened, 5 did not meet all the entry criteria. Two subjects have completed the study and 2 are currently enrolled. Among the two completed subjects, for subject 1 grass pollen was used in subsequent inhalation challenges and the baseline PC₂₀ was 1/2116. Subject 2 had an HDM AIC and the PC₂₀ was 1/217. Both subjects had mild asthma: baseline FEV₁% and methacholine PC₂₀ of 111% & 1.8 mg/ml (subject 1), and 90% & 2.3 mg/ml (subject 2). Responses to the 1-hr CAP+O₃ exposure, including spirometry, lung volumes, CBCs and symptoms were similar to those for FA. For subject 1, the grass PC₂₀ was 1/1317 after FA (1st exposure) and 1/1316 after CAP+O₃ exposure. For subject 2, the HDM PC₂₀ was 1/117 after CAP+O₃ (1st exposure) and 1/66 after FA. Results for the induced sputum demonstrated an increase in %eosinophils from the day before to the day after for both exposures. Subject 2 exhibited the greatest increase after CAP+O₃ exposure (0.25 to 16.25% vs. 0.25 to 2.00% for FA), while subject 1 showed the greatest increase after FA exposure (1.25 to 18.25% vs. 0.00 to 9.00% for CAP+O₃). Subject 1 was virtually deficient in functional SP-D & A obtained from induced sputum before & after both exposures, but 24-hrs post CAP+O₃ exhibited high levels of cleaved or non-functional SP- and was not detectable using ELISA; some small fragments of SP-A may also have been present. Subject 2 exhibited intact SP-D & A pre & post for both exposures; and ELISA for SP-D increased after both exposures.

Conclusions-Relevance

The results provided to date are only descriptive, as 16 subjects remain to be tested to complete the study, at which time statistical analyses will be performed. There is a strong and consistent association between air pollutants, such as particulate matter and O₃, and asthma morbidity (e.g. increased hospital admissions & asthma symptoms). Air pollutants may exacerbate asthma by enhancing the response to allergen(s) in allergic asthma. We are examining whether exposure to urban CAP+O₃ increases airway reactivity to inhaled allergen in allergic asthmatics, resulting in enhanced lung and systemic inflammatory responses. From a public health perspective it is important to understand the interaction between air pollutants and allergy: critical for risk management and the development and implementation of Air Quality Standards.

Socioeconomic Factors Related to Asthma Control and Quality of Life in Children

*Shannon Cope, Health Policy, Management and Evaluation, University of Toronto,
Dr. Wendy Ungar, Hospital for Sick Children*

Objective/Purpose

The main objective was to determine the socioeconomic predictors of asthma control in children, as defined by the 2003 Canadian Pediatric Asthma Consensus Guidelines (CPACG). The secondary objective was to determine the relationship between asthma control and health-related quality of life (HRQL) in children.

Methods

A cross-sectional design was used to analyze data from a completed CIHR-funded study that recruited participants from seven sites in the Greater Toronto Area from 2000-2003. The following information was collected on 879 children aged 1 to 18 years with a documented diagnosis of asthma and a prescription for an asthma medication in the previous year: demographics, medical history, medication use, health services use, asthma education, allergen exposures and HRQL using the Pediatric Asthma Quality of Life Questionnaire (PAQLQ). Multiple linear regressions were used to analyze asthma control (based on six CPACG control parameters including daytime symptoms, night-time symptoms, need for B2-agonists, physical activity level, exacerbations and school absences). The impact of the following factors was investigated in the regression models: income adequacy, drug plan, parent education, parent employment, ethnicity, parent immigration, language, parent marital status, and physical environment characteristics. These analyses were adjusted for demographic, community, need, and healthcare utilization factors. The number of control parameters satisfied according to the CPACG and Global Initiative for Asthma guidelines was compared using a chi-square and weighted kappa statistic. The number of asthma control parameters and the PAQLQ scores were examined using the Pearson correlation coefficient.

Findings

Results indicate that only 11% of patients met the requirements for acceptable control by satisfying all six parameters. Among remaining patients, 19% satisfied five parameters, 24% satisfied four parameters and 46% satisfied three or fewer parameters. The multiple regressions indicated that income adequacy had a substantial impact on asthma control. Children from families in lower income adequacy levels, especially those in the middle income, tended to have worse control. Higher numbers of asthma triggers, increased physician or specialist visits, and daily use of anti-inflammatories, were associated with lower levels of control. The CPACG and GINA guidelines had a high level of agreement (Weighted kappa=0.74, $p < .0001$), although it was more difficult to achieve acceptable asthma control in the CPACG compared to the GINA guidelines. The number of asthma control parameters that were satisfied demonstrated a strong correlation with the PAQLQ domains (Emotional Function: $r = 0.47428$, Activity Limitations: $r = 0.46735$, Symptoms Domain: $r = 0.50653$, $p < 0.0001$).

Deliverables

A review of the international asthma guidelines demonstrated limitations for assessing asthma control in children and provided recommendations to improve the Canadian Pediatric Asthma Consensus Guidelines. A framework for evaluating asthma control based on an adaptation of the Andersen Model for Health Care Utilization was created, which incorporates environmental factors, population characteristics, health behaviours and health outcomes. This model may be relevant for other chronic pediatric conditions. A unique strategy to analyze multiple explanatory variables using several regression approaches was developed, which may demonstrate promise as an improved methodology.

Relevance

Despite the established effectiveness of inhaled corticosteroids in the prevention of asthma exacerbations, poor control remains a problem which continues to lead to unnecessary morbidity and health care costs in Canada, as well as internationally. Results suggest that targeting programs for children in the middle income adequacy quintiles may be important to provide effective access to improve control.

Is the Prevalence of Peanut Allergy Increasing? A five-year follow-up study on the Prevalence of Peanut Allergy in Montreal School Children aged 5 to 9 years

Moshe Ben-Shoshan, Rhoda S Kagan, Reza Alizadehfar, Lawrence Joseph, Elizabeth Turnbull, Yvan St. Pierre, Ann E. Clarke (supervisor), McGill University Health Center

Background

Peanut allergy represents a major health problem and is receiving increasing attention in the medical literature. Our research team published the first study to estimate the prevalence of peanut allergy in Canada (Kagan et al. JACI 2003; 112:1223-8). We estimated that the prevalence was 1.50% (95% confidence interval [CI] 1.16% - 1.92%). Although some recent studies have suggested that the prevalence of peanut allergy is increasing, these results have not yet been substantiated in North America.

Objective

Our aim was to determine if the prevalence of peanut allergy in Montreal is increasing by conducting a follow up study between 2005 and 2007, using the identical methodology and sampling frame of our 2000/2002 study.

Methods

Our original study was cross-sectional, involving a random sample of kindergarten - grade 3 classrooms in the public and private schools of Montreal. In our follow up study, we are re-visiting the schools that participated in the original sample and randomly selecting classrooms within these schools. The diagnosis of peanut allergy was made (as in our original study) only if one of the following was fulfilled: 1) a child who had never or rarely ingested peanuts or had an uncertain history of an IgE-mediated reaction to peanut had EITHER a positive skin prick test (SPT) to peanut AND a serum peanut-specific IgE of ≥ 15 kU/L OR a positive SPT to peanut AND a positive double-blind placebo-controlled food challenge (DBPCFC) with peanut or 2) a child who had a convincing history of an IgE-mediated reaction to peanut had EITHER a positive SPT OR a peanut-specific IgE ≥ 0.35 kU/L OR a positive DBPCFC. To determine whether children fulfilled the criteria for peanut allergy, questionnaires were administered regarding peanut ingestion to their parents which enabled children to be stratified as: 1) peanut tolerant, 2) never/rarely ingested peanut, 3) convincing history of peanut allergy, or 4) uncertain history of peanut allergy. Children in groups 2, 3 and 4 required SPT to determine if they were allergic to peanut. For groups 2 or 4, if the SPT was negative, the child was not allergic. If the SPT was positive, these children underwent measurement of peanut-specific IgE and potentially a DBPCFC, depending on the results. Children in group 3 with a positive SPT were considered allergic to peanut without further testing. Children in group 3 with a negative SPT required measurement of peanut-specific IgE and, potentially, a DBPCFC. A preliminary point estimate of the prevalence was based on the observed fraction of participants with peanut allergy of the total number of participants who completed the questionnaire and necessary testing (i.e., full responders). Selection bias-adjusted estimates were also derived by using the information provided by those who withdrew prior to completion of the necessary testing (i.e., partial responders) and also by those who did not complete the questionnaire (i.e., nonresponders) through a Bayesian bias correction technique called multiple imputation.

Findings

Year 1 of the 2-year study has been completed. Within the 25 public schools participating, of 3157 children surveyed, 2001 responded, 93% in group 1. Among full responders, the prevalence was 1.99% (95% CI, 1.42%-2.72%); among full and partial responders, the prevalence was 2.32% (95% CI, 1.70%-3.09%); and among full, partial, and nonresponders, the prevalence was 2.09% (95% CI, 1.53%-2.77%). Thus, the differences between the prevalence in 2005/2007 and 2000/2002, stratified by subset of responders were 0.49% (95% CI, -0.23%-1.20%) in full responders; 0.57% (95% CI, -0.21%-1.34%) in full and partial responders; and 0.75% (95% CI, 0.09%-1.41%) in full, partial, and nonresponders.

Deliverables

This study is the first to document temporal trends in the prevalence of peanut allergy in North America by corroborating history with confirmatory tests. The results from Year 1 of our follow up study, suggest that there might be an increase in the prevalence of peanut allergy over a five year period. However, with the inclusion of approximately 2500 additional patients in the second year of our study, we should be able to determine with certainty if the prevalence of peanut allergy has indeed increased.

Relevance

Determining whether the prevalence of peanut allergy is increasing is crucial to understanding its etiology, developing preventive measures, ensuring the allocation of appropriate health care resources and informing educational institutions and industry about the needs for policies and products which will minimize inadvertent peanut exposure.

CD34 Involvement in the Development of Allergic Asthma and Eosinophil Migration in Mice

Steven Maltby, Marie-Renée Blanchet, Jamie Haddon, Kelly M. McNagny.
The Biomedical Research Center (BRC), University of British Columbia, Vancouver.

Objective/Purpose

In the mouse model, asthma is characterized by the infiltration of hematopoietic cells into the lung tissue and bronchial-alveolar space, associated with remodeling and airway hyperresponsiveness. Prominent among infiltrating hematopoietic cells are mast cells and eosinophils, which are hypothesized to directly cause tissue inflammation and damage by local release of mediators. Previously we showed that expression of CD34 is essential for efficient mast cell migration leading us to examine its role in the development of asthma.

Methods

CD34^{-/-} and wild-type C57Bl/6 mice were sensitized and challenged with chicken ovalbumin (OVA) as has been previously reported. To assess asthma severity, airway hyperresponsiveness was tested on a Flexivent analyzer following methacholine challenge and differential hematopoietic cell counts were performed on broncho-alveolar lavage (BAL) cells. Histological tissue preparations were stained with hematoxylin/eosin and toluidine blue for evaluation of tissue inflammation and mast cells counts. BAL eosinophils were also stained for CD34 expression, sorted and evaluated via in vitro migration assays.

Findings

We found that CD34^{-/-} mice had far fewer infiltrating cells than Bl/6 controls (1.162 ± 0.32 compared to $2.946 \pm 0.417 \times 10^6$ cells/ml in WT; $p = 0.0015$). All hematopoietic subsets were significantly reduced and histological analysis revealed attenuation of both inflammation and mast cell counts in CD34^{-/-} mice (inflammation score: 6.57 ± 1.15 vs. 10.33 ± 0.92 in Bl/6; $p = 0.03$ and mast cell counts: 6.28 ± 1.5 /section vs. 18.17 ± 1.5 in WT; $p = 0.0002$). Similarly, airway hyperresponsiveness in CD34^{-/-} OVA-challenged mice was lower and comparable to that of unsensitized Bl/6 mice. Interestingly, BAL and tissue-derived eosinophils in Bl/6 mice were found to express significant levels CD34 and we found a 57.2% reduction in the ability of CD34^{-/-} eosinophils to migrate towards eotaxin in an in vitro migration assay compared to Bl/6 eosinophils. Follow-up studies indicate that this difference in migration efficiency is not due to differences in binding to P, E or S selectins.

Deliverables

Current research on the role of CD34 in asthma remains preliminary and we aim to further characterize the differences CD34^{-/-} and Bl/6 animals. However, future studies into CD34 function could yield interventions to suppress the asthmatic response.

Relevance

Taken together our results suggest CD34 plays an important role in mast cell and eosinophil migration, presumably by reducing adhesion and enhancing invasiveness. This decreased homing efficiency results in a significant decrease in asthma pathology, in a mouse model, hinting at potential therapeutic interventions to act directly on mast cell and eosinophil infiltration into the asthmatic lung.

Characterization of Bronchial Epithelial Side Population (SP) Cells: Progenitors of Multiple Cell Types

T-L. Hackett, PhD¹, F. Shaheen, BSc¹, G. K. Singhera, PhD¹, D. R. Dorscheid, MD¹, T. Bai, MD¹, S. Van Eeden, MD¹, R. Hegele, MD¹ and D.A. Knight, PhD¹. ¹iCAPTURE centre, Vancouver, B.C., Canada.

Introduction

The bronchial epithelial cell is the first cell of contact and a physical barrier to the external environment. We and others have provided evidence of epithelial damage and aberrant repair in asthma. One way to investigate epithelial repair is to identify and characterize tissue resident progenitor cells that may contribute to the repair process. The rapid efflux of the fluorescent DNA-binding dye Hoechst 33342 identifies a side population (SP) of cells, which are enriched for stem/progenitor cell activity. SP cells are very rare, representing less than 0.5% of epithelial cells. For this reason, we have used the bronchial airways of sheep to identify SP cells and characterize their phenotype.

Methods

Epithelial cells obtained from sheep airways via pronase digestion were stained with Hoechst 33342 (5ng/ml) and PI, and sorted using FACS. SP and non-SP cells were then collected and plated 1×10^3 cells/ml for clonal expansion. In situ fluorescence staining was used to characterize SP cells in culture.

Results

The bronchial epithelium contained a viable SP population of cells comprising <0.1% of total epithelial cell population, which contained both CD45⁻(85%) and CD45⁺(15%) subsets. When placed in culture, a single SP cell gave rise to a heterogeneous colony of >40 cells for 16 passages, whereas non-SP cells failed to attach and grow. SP cells were ^ΔNp63, CK-19, isolectin B4, E-cadherin, ZO-1 positive and 10% of cells remained BRCP-1 positive following repeated passage. Furthermore, SP cells produced a differentiated epithelium when placed in air-liquid interface culture.

Conclusions

Our findings illustrate that bronchial epithelial SP cells have the potential to produce a diverse number of epithelial cells. We speculate that these cells may play an important role in both homeostasis and repair of the airways and further work is required to characterize these cells.

Adult Asthma among Canadians in Different Occupations and Labour Force Groups

NA Garzia, M Koehoorn, SM Kennedy, University of British Columbia, Vancouver BC
Supervisor: SM Kennedy

Objectives

The identification of Canadian subpopulations that are likely to have or experience the onset of asthma is required to alleviate the impact of the disease on sufferers and the public health care system. Population-based studies have the ability to identify these vulnerable subpopulations. Our project investigates where the burden of asthma lies among adults in different occupations and labour force groups.

Methods

Cross-sectional data from the Canadian Community Health Survey (CCHS) 2.1 was used to identify the adult population, 15-65 years of age (n=101,123 household residents from all provinces in 2002/03). Current labour force status (employed, unemployed, not in labour force) was determined for all adults, using pre-determined labour force definitions derived for users of National Population Health Survey. Labour force status groups were further broken down to separate full-time employed from part-time employed, and non-working full-time students not in the labour force from other persons not in the labour force. Adults who reported "yes" to having asthma in 2002/03 were classified as current asthmatics. Adult asthmatics who reported "yes" to having asthma symptoms or attacks in the past 12 months OR who reported "yes" to taking asthma medication in the past 12 months or the past 1 month were classified as having active asthma. All jobs reported by employed adults in 2002/03 were grouped into occupational categories based on job similarities. The jobs were also classified as high-risk (HR) and low-risk (LR) based on an asthma specific Job Exposure Matrix, blinded to asthma status.

Findings

Among Canadian adults, estimates indicate that asthma prevalence for men and women combined is highest among adults not in the labour force (9.93%, $p < 0.0001$); about 23% of all adults are not in the labour force. Women showed the highest asthma prevalence across all labour force status groups when compared to men, with the highest prevalence (10.78%) among the unemployed group. Prevalence of active asthma was also higher among women than men across all labour force groups, and for the unemployed, active asthma among women was more than double that of men (9.78% v 4.39%, $p < 0.0001$). Among employed adults, comparisons of job risk group (HR v LR) produced opposite conclusions for asthma and active asthma prevalence compared to those seen when adult- and childhood-onset asthma could be defined among asthmatics as part of a prior analysis of the National Population Health Survey, Longitudinal Household Component. In the current analysis, job at time of asthma onset could not be determined since we did not have the survey information to differentiate between adult- and childhood-onset, causing results to be potentially biased by the healthy worker effect. Occupational categories with the highest prevalence of asthma included: Domestic & Personal Service Occupations (9.92%); Food Services, Preparation, Manufacturing Occupations (8.97%); Scientists & Professionals in Chemical, Biological & Life Sciences (8.96%); Forestry, Wood Processing, Pulp & Paper (8.84%); Nurses & Nursing Aides (8.74%); Art, Culture, Recreation & Sport (8.61%); and Retail, Wholesales and Other Sales or Services Jobs (8.60%). Women attributed more than 50% of the asthma in all of these occupational categories, except for the Forestry, Wood Processing, and Pulp & Paper category.

Deliverables

Overall, women had higher asthma and active asthma prevalence across all labour force status groups, when compared to men. Unemployed women, specifically, had the highest prevalence of asthma and active asthma when compared to women in other labour force status groups. When assessing differences in asthma and active asthma prevalence, comparisons by job risk group are biased by the healthy worker effect when asthmatics cannot be differentiated into adult- and childhood-asthma, and job at time of asthma-onset cannot be determined for adult-onset asthmatics.

Funding Source: AllerGen NCE

Accidental Exposures to Peanut in Children with Peanut Allergy

Nha Uyen NGUYEN-LUU¹, Rhoda KAGAN¹, Reza ALIZADEHFAR¹, Nathalie NICOLAS², Claire DUFRESNE³, Laurie HARADA⁴, Mary ALLEN⁵, Elizabeth TURNBULL², Yvan ST-PIERRE², Ann CLARKE^{1,2} (supervisor)

¹Division of Clinical Immunology and Allergy, McGill University Health Center, ²Division of Clinical Epidemiology, McGill University Health Center, ³Association Québécoise des Allergies Alimentaires (AQAA), ⁴from Anaphylaxis Canada (AC), ⁵Allergy and Asthma Information Association (AAIA)

Objective/Purpose

Peanut allergy is a serious condition affecting about 1 to 2% of children. Often lasting lifelong, it can be potentially life-threatening and is a source of considerable anxiety for affected children and their parents. The mainstay of peanut allergy management is complete avoidance of peanut-containing products, achieved through education of patients and caregivers. However, strict peanut avoidance is difficult and accidental exposure to peanut remains an important concern. The objective of our study is to determine the incidence of accidental exposures in Canadian children with peanut allergy.

Methods

Children with peanut allergy were identified from two sources: 1) the Allergy Clinics at the Montreal Children's Hospital and 2) provincial and national advocacy organizations for food allergic patients, i.e. *Association Québécoise des Allergies Alimentaires (AQAA)*, *Anaphylaxis Canada (AC)*, and the *Allergy and Asthma Information Association (AAIA)*. Peanut allergy diagnosis was defined using the following criteria: 1) a convincing clinical history of an allergic reaction to peanut and a positive skin prick test (SPT) to peanut or peanut-specific IgE ≥ 0.35 kU/L or 2) no history of peanut ingestion or an uncertain clinical history of peanut allergy and either a positive SPT to peanut and a peanut specific IgE level ≥ 15 kU/L or a positive SPT and positive food challenge with peanut. Parents of participating children completed annual questionnaires on demographics and the child's comorbid medical illnesses, personal history of atopy, and accidental exposures to peanut over the preceding year. Descriptive statistics were compiled for all continuous variables and the annual incidence rate of accidental exposure was expressed as the number of events divided by the patient-years at risk.

Findings

521 patients completed a questionnaire at study entry and 266 also completed a questionnaire at one year follow up. The participants were predominantly boys (64%). The mean age (SD) at diagnosis was 2.9 (2.7) years. Eighty-three accidental exposures occurred in 67 children over a period of 745 patient-years, yielding an annual incidence rate of accidental exposure of 11.1% (95%CI, 8.9% to 13.8%). Thirty reactions occurred at home, 9 at school, including 7 in schools prohibiting peanut, 3 in daycare, 10 in restaurants, 20 at the home of a relative or friend, and 11 at other or unknown places. Fifty-seven reactions resulted from ingestion, 18 from skin contact, 4 from mucosal contact, and 4 from other or unknown reason. Thirty-four reactions were mild (pruritus, urticaria, flushing and/or rhinconjunctivitis), 42 were moderate (angioedema, voice change, coughing, nausea and/or vomiting and/or abdominal pain), and 7 were severe (wheezing, stridor, cyanosis and/or circulatory collapse). No treatment was given for 7 mild reactions and 6 moderate reactions. Forty-six reactions, including 2 severe reactions, were treated at home. Epinephrine was used in only 11 of 49 moderate and severe reactions.

Deliverables

Children with peanut allergy have an annual incidence rate of accidental exposure of 11.1%. This finding is consistent with our previous study which involved only 252 patients recruited from a single site. Although our rate of inadvertent exposures is lower than that reported by other studies, it should be noted that a significant number of accidental exposures occurred in schools not allowing peanut and in the patient's home. Furthermore, many moderate and severe reactions were managed inappropriately. A further reduction in accidental exposure is desirable and likely can be attained through better education of caregivers and patients, enforcement of more stringent food manufacturing standards, and more accurate food labeling. To further define potential diagnostic, preventive, and management strategies for peanut allergy, we are continuing our recruitment of peanut allergic children throughout Canada.

Relevance

Increased societal awareness of peanut allergy and its consequences and the creation of safer food products and environments for peanut allergic children may have contributed to the lower rate of inadvertent exposure we have observed in this study of Canadian children with peanut allergy. More research is needed to better identify determinants of accidental exposure and to develop efficacious prevention strategies. Since a significant number of accidental exposures occurred in schools prohibiting peanut, school-based policies for anaphylaxis-risk reduction should also be reviewed.