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Poster #01

Molecular Markers of Eosinophilopoiesis: Multiplex Q-PCR Analysis of GATA-1, MBP and IL-5 Receptor mRNA Expression in Peripheral Blood

*AK Ellis, L Crawford, JA Denburg; Division of Clinical Immunology and 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 also recently 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, in order to evaluate surrogate markers of Eo/B differentiation.

Methods

PB non-adherent mononuclear cells (PB NAMNC) were isolated from random fresh samples, and incubated in the presence of IL-5. At 24, 48 and 72h post-stimulation, RNA was isolated, reverse transcribed, and expression of IL-5R α , GATA-1, and MBP was determined utilizing comparative Q-PCR in a multiplex reaction. 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 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. 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 and their relationship to atopy and atopic outcomes are underway.

Funding Sources: This research was funded by AllerGen NCE, and also in part by the AllerGen/ Bayer/CAAIF Immunodeficiency and Immunomodulation of Allergic Inflammation Clinician-Scientist Research Fellowship

Poster #02

The Effect of Respiratory Rate, Tidal Volume and Temperature On Exhaled Breath Condensate Volume

*R Amin MD, S Balkovec RRT, F Ratjen MD PhD, and P Subbarao MD MSc
The Hospital for Sick Children and The University of Toronto, Toronto, Ontario, Canada*

Background

Exhaled breath condensate (EBC) has been proposed as a non-invasive marker of lower airways inflammation. To assess its potential applicability in infants, we developed an in vitro system to test the effects of respiratory rate (RR), tidal volume and temperature of the cooling device on EBC collection volume.

Methods

A mechanical ventilator (*Evita XL, Drager, Germany*) was used to modulate tidal volumes and respiratory rates over the range expected for infants. The ventilator delivered 100% relative humidity. The temperature at the EBC collection point was 34 °C. The ventilator was connected via a modified two way valve (*Hans-Rudolph Inc, Kansas*) separating inspiratory and expiratory flow to a modified R-Tube for EBC collection. EBC was collected for tidal volumes from 20-120mL in increasing increments of 20mL. For each tidal volume, EBC was collected over 20 minute periods for respiratory rates ranging from 20-70 breaths per minute in increments of 10 breaths/min. Temperature of the cooling device was modulated by storing the R-Tube sleeve at -80 °C, -20 °C and -8 °C for at least 1 hour prior to sample collection. Data was analyzed using factorial ANOVA and linear regression.

Results

EBC volume ranged from 0.37mL to 3.8mL. Using factorial ANOVA, both tidal volume and respiratory rate were found to be significant factors in determining EBC volume ($p < 0.05$). A trend towards significance was observed for the sleeve temperature ($p = 0.053$). EBC volume had the strongest correlation with tidal volume ($R = 0.74$, $p < 0.05$) followed by respiratory rate ($R = 0.13$, $p = 0.4$). The linear regression model found that tidal volume had the greatest effect on EBC volume, followed by respiratory rate; temperature had a minimal effect.

Conclusions

Exhaled breath condensate collection rates using a modified R-Tube in an in vitro model collects adequate amounts of fluid for analysis. Tidal volume had the strongest effect on EBC.

Poster #03

A Case of Hemolytic Anemia Following Intravenous Immunoglobulin Administration in a Patient with Chronic Urticaria

*Sari M. Herman*¹, *Stephen Betschel*², *Gordon Sussman*²

¹*Department of Medicine, McMaster University,*

²*Division of Clinical Immunology and Allergy, Department of Medicine, University of Toronto.*

Purpose

To report an unusual case of haemolytic anemia following intravenous immunoglobulin (IVIG) administration. We review the published literature examining haemolytic anemia with the use intravenous immunoglobulin and the evidence for its use in chronic urticaria.

Design

Case report

Methods

We report a case of a 54-year-old man with a seven year history of chronic urticaria refractory to conventional therapy who was treated with IVIG.

Results

A three day course of treatment with IVIG (1 g/day) was initiated. After the first infusion our patient developed a headache. A week after completion of therapy he developed arthralgias and jaundice. Laboratory studies revealed a normocytic anemia (haemoglobin 106 g/l) with polychromasia and few spherocytes present, hyperbilirubinemia (total bilirubin 79 umol/l), and a low haptoglobin (less than 0.0583 g/l) despite a negative direct Coombs test. Laboratory values returned to normal approximately 2 weeks after the onset of the hemolytic reaction. Complete clearing of his urticaria occurred within 2 days after the IVIG treatment. Recurrence of his urticaria appeared two months later.

Conclusions

IVIG has been used for the treatment of chronic urticaria in patients unresponsive to conventional therapies with short-term results. Although IVIG therapy is generally safe, hemolytic anemia is a potentially serious complication that is often overlooked. Clinicians should be aware of possible adverse effects related to intravenous immunoglobulin therapy. Close monitoring of haemoglobin levels with IVIG therapy is recommended

Poster #04

Asthmatic and Non-Asthmatic Children have Similar Levels of Body Dissatisfaction

Authors: JLP Protudjer, GDM Marchessault, C Benoit, AL Kozyrskyj, AB Becker

Rationale

Body image is important to pre-adolescents. Youth with asthma may have experiences that decrease or alter their body image, resulting in greater body dissatisfaction; however, the few studies that have explored this association suggest that asthmatic children often have greater body dissatisfaction than non-asthmatics.

Methods

Children enrolled in a cohort study born in Manitoba in 1995 were assessed by pediatric allergists for asthma at age 8-10. We administered the Children's Body Shape Drawing Questionnaire to assess overall body dissatisfaction (absolute difference between perceived current body shape and desired body shape) and queried feature-specific satisfaction using the Healthy Youth Survey (1=very satisfied; 5=very dissatisfied) in both asthmatic and non-asthmatic boys and girls.

Results

In total, 532 children were assessed for both asthma status and body dissatisfaction. Using the Wilcoxon Mann-Whitney U test, we found no difference ($p=0.69$) in body dissatisfaction in asthmatic versus non-asthmatic children. Neither asthmatic boys and girls ($p=0.22$) nor non-asthmatic boys and girls ($p=0.26$) had differences in overall body dissatisfaction. Asthmatic and non-asthmatic boys had the same median level of body dissatisfaction (median=9.00; range=4 (most satisfied) to 27 (most dissatisfied)), as did asthmatic versus non-asthmatic girls (median=8.00). No differences in facial ($p=0.91$), muscle ($p=0.44$), weight ($p=0.37$) or height ($p=0.48$) dissatisfaction were found between asthmatics and non-asthmatics.

Conclusions

Asthma does not affect body dissatisfaction in pre-adolescent children. Based on other literature, we speculate that other socio-cultural factors, such as peer pressure and media, and biologic factors, such as puberty, influence body dissatisfaction in pre-adolescent children independent of a chronic disease such as asthma.

Funding: Canadian Institutes of Health Research, AllerGen, Manitoba Institute of Child Health, National Training Program in Allergy and Asthma

Poster #05

A Role for CD34 in the Development of Allergic Asthma in Mice

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

Migration of mast cells and eosinophils to the lung during allergic asthma leads to persistent inflammation and the development of airway hyperresponsiveness, presumably due to the release of potent inflammatory factors. Previously we showed that expression of CD34 is essential for efficient mast cell migration and therefore we examined the role this sialomucin in allergic inflammation.

CD34KO and wild type (WT) mice were sensitized and challenged with chicken ovalbumin (OVA). Airway hyperresponsiveness was tested using a metacholine challenge. Differential cell counts were performed on broncho-alveolar lavage (BAL) cells. Histological 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.

We found that CD34KO mice had far fewer cells in BAL than WT 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 CD34KO mice (inflammation score: 6.57 ± 1.15 vs 10.33 ± 0.92 in WT; $p = 0.03$ and mast cell counts: 6.28 ± 1.5 /section vs 18.17 ± 1.5 in WT; $p = 0.0002$). Similarly, airway hyperresponsiveness in CD34KO OVA-challenged mice was lower and comparable to that of unsensitized WT mice. Interestingly, BAL and tissue-derived eosinophils in WT mice were found to express significant levels CD34 and we found a 57.2% reduction in the ability of CD34KO eosinophils to chemotax towards eotaxin in an *in vitro* migration assay compared to WT eosinophils.

Taken together our results suggest CD34 plays an important role in mast cell and eosinophil migration presumably by reducing adhesion and enhancing invasiveness and that this mucin may offer a target for therapeutic intervention.

Poster #06

Is there a correlation between skin prick tests and bronchial hyperresponsiveness in children?

JJ Liem MD¹, R Rabbani PhD², AL Kozyrskyj PhD³, AB Becker MD¹

¹Section of Allergy and Clinical Immunology, Department of Pediatrics and Child Health, University of Manitoba

²The Manitoba Institute of Child Health, Department of The Children's Hospital Foundation, University of Manitoba

³Departments of Community Health Sciences and Pediatrics & Child Health, Faculties of Medicine and Pharmacy, University of Manitoba

Background

Allergists frequently use the skin prick test to detect sensitization to aeroallergens in patients with asthma. It is assumed that sensitization on the skin reflects inflammation in the airways, resulting in bronchial hyperresponsiveness.

Rationale

To determine if allergen sensitization is associated with bronchial hyperresponsiveness in children.

Methods

The SAGE cohort is a birth cohort study of all children born in the province of Manitoba, Canada in 1995. A case control group of these children were assessed by asthma specialists and diagnosed with asthma, rhinitis or neither. They were then allergy tested to common aeroallergens and underwent a methacholine challenge to determine the concentration of methacholine required to drop their FEV1 by 20 % (PC20). Spearman's correlations were determined between the total number of positive skin prick tests with PC20.

Results

723 children were assessed from across the province of Manitoba (urban and rural locations). 4 children refused skin prick testing and 58 children did not perform proper methacholine challenges. The correlations between the number of skin prick tests and total mean wheal diameters with PC20 were -0.296 (p<0.0001) and -0.310 (p<0.0001) respectively. When stratified by gender, the correlation for boys was -0.310 (p<0.0001) and -0.331 (p<0.0001) for number of skin prick tests and total mean wheal diameters respectively. For girls, the correlation was lower at -0.260 (p<0.0001) and -0.269 (p<0.0001) respectively.

Conclusion

The total number and mean wheal diameters of positive skin prick tests have a weak correlation with bronchial hyperresponsiveness. The correlation was stronger in boys than girls. Sensitization to allergens is associated with airway hyperresponsiveness.

Poster #07

The Relationship between Sputum Eosinophils and Exercise-Induced Bronchoconstriction in Asthma.

M Duong¹, P Subbarao^{1,2}, E Adelroth³, G Obminski¹, T Strinich¹, MD Inman¹, S Pedersen⁴, PM O'Byrne¹.

¹McMaster University, Hamilton, Canada; ²University of Toronto, Canada; ³Umea University, Umea, Sweden; ⁴University of Southern Denmark, Kolding, Denmark.

Background

Airway eosinophils are important effector cells in the inflammatory processes of asthma. In the present study, the relationship between eosinophilic airway inflammation and exercise-induced bronchoconstriction (EIB), and the responses to inhaled corticosteroid therapy was examined.

Methods

Twenty-six steroid naïve asthmatics with EIB were randomised to two-3 week periods of low (40ug or 80ug) and high dose (160ug or 320ug) inhaled ciclesonide in a double-blind crossover trial, with two parallel arms and a washout period of 3-8 weeks. Baseline and weekly exercise challenges and sputum analyses were performed during each treatment period (total of 4) to assess response.

Results

Baseline sputum eosinophils $\geq 5\%$ were found in 10 subjects. Sputum eosinophils were significantly correlated to EIB severity (Spearman's rho = -0.61; $p < 0.01$). Sputum eosinophil count was also a significant predictor of the temporal response of EIB to high (160ug and 320ug), but not low dose (40ug and 80ug) ciclesonide therapy. The latter was characterised by a steep slope of improvement at week 1, with little additional improvement thereafter, irrespective of sputum eosinophil counts. In contrast, subjects with sputum eosinophilia demonstrated an improvement in EIB, which continued to increase with time without plateau by 3 weeks of high dose therapy. Whereas, non-eosinophilic subjects receiving high dose therapy demonstrated a limited and time-independent improvement similar to that of low dose therapy.

Conclusions

These results suggest that eosinophilic inflammation significantly contributes to the mechanisms of EIB and is a useful marker in the prediction of EIB response to inhaled corticosteroid therapy.

Poster #08

Efflux of Peripheral Blood Basophils After Allergen Inhalation by Mild Asthmatics Using Cell Surface Staining with an Antibody to CD203c

*M.Y. Evans, BSc, E.J. Farrell, BSc, T.X. Strinich, BSc, A.J. Baatjes MSc, M.Y. Duong, MD and G.M Gauvreau, PhD
McMaster University, Hamilton Ontario, Canada*

Introduction

Eosinophils and basophils are circulating granulocytes that play a central role in the pathogenesis of allergic inflammation. In response to inhaled allergen there is an accumulation of eosinophils in the airways which is coincident with an efflux of eosinophils from the circulation. Basophils have also been shown to accumulate in allergen challenged (AC) airways, however these cells have not routinely been measured in the circulation due to their low numbers and lack of commercially-available basophil markers. We evaluated different staining methods to determine which can most accurately track basophils in peripheral blood (PB).

Methods

PB was obtained at baseline, 0.5h, 2h, 4h, 6h and 24h post allergen and diluent inhalation, from eight mild asthmatic subjects. Basophils were stained by three methods: a) direct surface immuno-staining with anti-CD203c (Immunotech) and enumeration by flow cytometry; b) indirect surface immuno-staining with an antibody cocktail (Becton Dickinson) and enumeration by flow cytometry, and c) chemical staining with alcian blue and enumeration using a hemocytometer. Eosinophils were enumerated by flow cytometry using CD45/SSC plots and gating on the eosinophil population.

Results

There was a decrease in PB eosinophils after AC when compared to diluent ($p < 0.05$), with a maximal decrease occurring at 2h, and complete resolution by 24 h post AC. There was also a decrease in PB basophils after AC when compared to diluent when staining with CD203c ($p < 0.05$), with a maximal decrease at 6h, and complete resolution by 24 h. We did not observe a decrease in PB basophils after AC compared to diluent using the BD antibody cocktail or alcian blue ($p > 0.05$).

Conclusion

We observed efflux of basophils from PB of asthmatic subjects after inhaled allergen challenge using direct immunostaining of PB basophils with anti-CD203c.

Poster #09

Socioeconomic factors related to Asthma Control in Children

Shannon Cope, Dr. Wendy Ungar, and Dr. Richard Glazier.

Despite the established effectiveness of inhaled corticosteroids in the prevention of asthma exacerbations, poor control remains common in children with asthma in Canada and has led to unnecessary morbidity and health care costs, especially among low-income, inner-city and minority families. The objective is to determine the socioeconomic predictors of asthma control, as defined by the 2003 Canadian Pediatric Asthma Consensus Guidelines (CPACG), in children. 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 health related quality of life. Multiple linear regression is being used to analyze asthma control (based on six CPACG control parameters including daytime symptoms, nighttime symptoms, need for β_2 -agonists, physical activity level, exacerbations and school absences). Logistic regression on unacceptable asthma control is also being conducted. The impact of the following factors is being investigated using stepwise backward analysis: income adequacy, drug plan, parent education, parent employment, ethnicity, parent immigration, language, parent marital status, and physical environment characteristics. These analyses are being adjusted for demographic, community, need, and healthcare utilization factors. The relative importance of the control parameters is also being explored using linear regression models. Three levels of asthma control (acceptable, poor, and unacceptable) are being compared to the level of HRQL using a weighted kappa statistic. Results indicate that only 3.1% of patients met the requirements for acceptable control by satisfying all six parameters. Among remaining patients, 19.5% satisfied five parameters, 22.2% satisfied four parameters and 55.2% satisfied three or fewer parameters.

Poster #10

The Risk of Peanut Allergy in Siblings of Peanut Allergic Children

S Huq, JJ Liem, JLP Protudjer, AL Kozyrskyj, AB Becker

Rationale

Parents are frequently concerned about development of peanut allergy in younger siblings of a peanut allergic child. We sought to determine the risk of peanut allergy in these children.

Methods

In 2005/06, a survey was sent to 441 households of children born in 1995 in Manitoba, Canada (as part of the SAGE project). Parents were asked whether their 10-year old child (index child) had any recognized food allergies, and were asked to list siblings and any possible food allergies that they may have. Skin prick tests +/- RASTs were performed the index children. The likelihood (odds ratio, OR) of peanut allergy in siblings of peanut allergic children vs non-peanut allergic children was determined.

Results

370/441 (83.9%) of the surveys were completed (urban, rural and First Nations communities). 29 (7.8%) children were peanut allergic (physician diagnosis +/- Skin prick test/RAST/oral challenge). 5 children were sensitized but not allergic to peanut. 9 children did not have siblings. There were 43, 9 and 568 siblings of peanut allergic, peanut sensitized not allergic and non-peanut allergic children respectively. The number of siblings with peanut allergy was 13 (2.1% of total siblings). The risk of a sibling of a peanut allergic sibling was increased (n=4, 9.3%) compared to siblings (n=9, 1.6%) of a non-peanut allergic child. (OR=6.37, 95%CI=1.88-21.62).

Conclusions

Children are more likely to be allergic to peanut if they have a peanut allergic sibling. Clinicians must be aware of this risk and consider testing of younger siblings before peanut is introduced to the diet.

Poster #11

Development of an Algorithm to Better Predict Clinical Responsiveness to Peanut

*Andrea Marrin, Kevin Sun, Susan Wasserman, Manel Jordana, Kent HayGlass
Department of Allergy & Immunology, McMaster University, Hamilton, Ontario,
Department of Immunology, University of Manitoba, Winnipeg, MB.*

Objective

The only treatment for peanut allergy is avoidance, however the majority of peanut allergic people will accidentally ingest peanut. Skin tests and peanut specific serum IgE (ImmunoCap) cannot definitively predict who is allergic. This study examines the relationship between skin prick tests, peanut specific serum IgE levels, and cytokine production by mononuclear cells (PBMC) to generate an algorithm to better predict clinical responsiveness to peanut.

Methods

4 groups were identified. 1) True positives (TP): subjects with a clinical history of peanut allergy and a positive peanut skin test 2) False positives (FP): subjects who tolerate peanut but have a positive peanut skin test. 3) Unknown reactivity (U): subjects with a positive skin test but no history of having ingested peanut 4) Non atopic controls (C). PBMCs were isolated and cultured in the presence and absence of peanut. Cytokines were measured at baseline and in the presence of crude peanut extract. Analysis of the supernatants was performed using Luminex™ multiELISA system.

Findings

85 subjects have so far been recruited. TP: 30, FP: 17, U: 16, C: 22. Peanut specific IgE levels were 0 to >100 kU/L in TP and U and 0 - 27 kU/L in FP. C had undetectable peanut specific IgE. There was increased expression of Th2 cytokines (IL5, IL9, and IL13) in TP compared to C. The Th1 cytokine IFN-gamma was also increased in TP compared to C.

Relevance

Peanut allergic patients demonstrated an increase in Th2 cytokine expression compared to non-atopic controls. The variability in peanut specific IgE levels among peanut allergic individuals demonstrates the difficulty with this measurement for predicting peanut allergy. The next phase will consist of the development of a diagnostic algorithm to be tested in patients with unknown reactivity (Group 3).

Poster#12

The Effect of Early Life Stress on Airway Inflammation Later on in Life

Vig RS, Befus AD, Vliagoftis H.

Rationale

Asthma exacerbations develop during periods of stress in 20 to 35% of asthmatics. Stress also strongly correlates with difficult to manage asthma, frequent and lengthier hospitalizations, and functional disability in children. Although psychological stress in adults can induce asthma exacerbations, little is known about the effects of asthma in the neonate. Neonatal stress may induce permanent psychological, neurological, and physiological changes that may also affect the immune system. We hypothesize that mice stressed early in life will develop augmented airway inflammation and airway hyperresponsiveness (AHR) to an allergen challenge compared to unstressed mice.

Methods

Mouse litters underwent a daily 3 hr. maternal separation on days 1-10 after birth during which other litters were left unhandled. On day 31 and 36, mice were sensitized to chicken egg ovalbumin (OVA) via an IP injection with aluminum hydroxide and were later challenged intra-nasally (day 42 and 44) with OVA or saline. Measured outcomes included bronchoalveolar lavage fluid (BAL) cell count, BAL differential cell counts, BAL cytokine levels, and AHR (measured by whole body plethysmography).

Results

All OVA challenged mice had increased AHR and total BAL inflammation compared to saline challenged animals. Interestingly, OVA challenged maternally separated males showed a significant reduction (~50%) in BAL inflammation compared to unstressed controls. Reduced inflammation was also present in females. However, AHR of stressed mice following OVA challenge were comparable to unstressed groups. Cytokine analysis of OVA challenged BAL fluid show significant reductions in maternally separated Interferon-gamma and Interleukin (IL)-4 levels, with no change in IL-5, 6, 9, 13.

Conclusions

These findings suggest that neonatal stress downregulates airway inflammatory responses and alters the cytokine milieu in BAL fluid. We are assessing factors associated with this reduction in inflammation such as plasma corticosteroids and developmental changes in the lung like alveolar size that may be a result of early-life stress in mice.

Poster #13

Airway Hyper-Responsiveness Predicts Increased Th1-like Anti-Viral Immunity in Children With and Without Asthma

Douville R¹, Liem J^{1,3}, Li Y^{2,4}, Bastien N⁴, Becker A^{1,2,3}, Kozyrskyj A^{1,3,5} and HayGlass KT^{1,2,3}

CIHR National Training Program in Allergy and Asthma Research¹, Depts of Immunology¹, Medical Microbiology² and Pediatrics/Child Health³, Univ. Manitoba, Canadian Science Center for Human and Animal Health⁴, Dept. of Community Health Sciences, Faculties of Medicine and Pharmacy⁵, Winnipeg, Canada.

Rationale

Severe Respiratory syncytial virus (RSV), and more recently human Metapneumovirus (MPV), infections causing bronchiolitis have a substantial epidemiologic association with asthma pathogenesis. Emerging evidence suggests they may also be triggers of asthma exacerbation. Here, we evaluated the relationship between human recall cytokine responses to these viruses, current clinical status and airway hyper-responsiveness as measured by methacholine challenge (PC₂₀).

Methods

Peripheral blood mononuclear cells (PBMC) from ≈300 children (8-9 year old) were cultured with live RSV and MPV. The frequency and intensity of Th1-like (IFN γ □□CXCL10), Th2-like (IL-13), CCL5 and IL-10 virus-specific recall responses in the supernatants were quantified. Clinical parameters, such as allergist-diagnosed asthma and airway hyper-responsiveness (PC₂₀) were related to virus-specific cytokine responses. Subsequently, virus-specific responses were stratified based on corticosteroid use.

Results

Children with allergist-diagnosed asthma and evidence of airway hyper-responsiveness demonstrated increased production of Th1 cytokines and IL-10 in response to MPV compared to healthy controls (IFN γ ; p<0.05 and IL-10; p<0.05). Non-asthmatic children with airway hyper-responsiveness demonstrated similarly increased Th1 anti-viral responses. Weak negative correlations between anti-viral IFN γ and IL-10 responses and PC₂₀ values was observed in this child cohort (p<0.01). Concomitantly reduced CCL5 production was observed among hyper-responsive asthmatic children compared to healthy controls. Both asthmatic status and airway hyper-responsiveness contributed to this phenotype.

Conclusions

Airway hyper-responsiveness predicts increased Th1-like anti-viral immunity in children both with and without asthma. Asthmatic status can further amplify these immunoregulatory changes.

Support: CIHR, Canada Research Chair in Immune Regulation, Tom and Mindel Olenick Award in Immunology, Manitoba Health Research Council.

Poster #14

Epigenetic Regulation of the Balance of Th1/Th2- Recall Responses in Atopic Asthmatic Individuals

Ruey-Chyi Su^{*}, Allan Becker^{*,†}, Anita L Kozyrskyj^{†,‡}, Kent T. HayGlass^{*,†,2}

^{*}CIHR National Training Program in Allergy and Asthma Research, Departments of Immunology, [†]Community Health Sciences, and [‡]Pediatrics/Child Health, University of Manitoba, Winnipeg, Canada.

Chromatin remodeling, including histone-tail modifications and DNA methylation, regulates cytokine expression during *in vitro* differentiation of Th1/Th2 cells. Murine studies demonstrate that increased histone acetylation leads to increased cytokine gene expression. However, little is known about its role in human cytokine gene regulation. We hypothesized that epigenetic modifications play a regulatory role in maintaining Th2-like recall responses in atopic asthmatic humans.

Specifically, we examined how Th1/Th2 recall responses are affected by inhibiting histone deacetylase (HDAC) activity with trichostatin A (TSA). Subjects were asthmatic (allergist-diagnosis), positive HDM (house dust mite)-specific skin prick test, and methacholine-challenge. *Ex vivo* PBMC from 31 atopic asthmatic and 29 healthy controls 8-10 year old children were treated with TSA to increase cellular histone acetylation before stimulation with HDM or streptokinase (SK, a bacterial recall antigen). Ag-specific stimulation resulted in IL-5, IL-13, IFN γ and CXCL-10 responses in both populations. Inhibiting HDAC activity resulted in enhanced IL-5 and IL-13 production (median increases 50 to 70%, $p \leq 0.0003$) and concomitantly reduced IFN γ and CXCL-10 responses (median reductions 20 to 80%, p values <0.0001).

Secondly, we found that atopic asthmatics are significantly less sensitive to the effects of increased histone acetylation during HDM recall responses than healthy controls. Both Th1 decreases and Th2 increases were consistently less ($p < 0.035$ to 0.0001) than in control individuals. Finally, increasing acetylation resulted in a broad shift towards increased Th2 bias (ie decreased IFN γ :IL-13 and IFN γ :IL-5 ratios) in both HDM and SK recall responses.

Collectively, these findings demonstrate that (i) interfering with endogenous acetylation results in an immunological shift towards increased Th2 expression in human Ag-specific recall responses and (ii) atopic asthmatic individuals exhibit a more rigid pattern of regulation of cytokine/chemokine genes in HDM-responsive cells than do non-atopic individuals. This study provides the foundation for better understanding of epigenetic mechanisms that are involved in the maintenance of allergen specific cytokine and chemokine responses.

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Poster #15

Vitamin D Stimulation of Bronchial Smooth Muscle Cells Induces Autocrine, Contractility and Remodelling Processes

Yohan Bossé¹, Karim Maghni², Thomas J Hudson¹

¹McGill University and Génome Québec Innovation Centre, Montréal, Québec, Canada;

²University of Montréal, Sacré-Coeur Hospital, Research Centre, Montréal, Québec, Canada

Genetic variants in the vitamin D receptor (VDR) gene were recently associated with asthma. The biological mechanisms explaining this association is unknown, but are likely to involve many cell types given the pleiotropic effect of its ligand, vitamin D. Considering the prominent role of bronchial smooth muscle cells (BSMC) in the pathogenesis of asthma, experiments were conducted to explore the gene regulatory effects of vitamin D in these cells. Using RT-PCR and Western blot, it was shown that VDR is present both at the mRNA transcript and protein levels in human BSMC. The functionality of the receptor was then demonstrated by showing a more than 200-fold change in the expression of the 24-hydroxylase (CYP24A1) gene following vitamin D stimulation. Microarray experiments were then performed to identify differentially regulated genes and pathways in BSMC treated or not with vitamin D. A total of 729 probe sets on the U133 plus 2.0 Affymetrix GeneChip showed fold-change differences above the 1.5 threshold using the Robust Multichip Average (RMA) intensities. This corresponds to 231 unique genes that were up-regulated and 215 unique genes that were down-regulated following vitamin D stimulation. A high similarity between microarray and real-time PCR results was observed for 13 random genes, with a concordance correlation coefficient of 0.91. Real-time PCR was also performed to confirm the regulation of asthma candidate genes. To identify the biological relevance of this regulation, biological pathways analyses were performed. The most significant network of up-regulated genes included genes involved in morphogenesis, cell growth and survival as well as genes encoding structural proteins such as VEGF, IL6, FN1 and COL1A1, which are potentially involved in airway remodelling.

Poster #16

Fast Food Consumption, Overweight and Asthma in Preadolescents

Xiao-Mei Mai^{1,2}, Elizabeth AC Sellers², Allan B Becker², Anita L Kozyrskyj^{1,2} Faculty of Pharmacy¹, Pediatrics & Child Health and Manitoba Institute of Child Health², University of Manitoba, Canada

Background

Fast food consumption and being overweight are suggested to be associated with asthma in children.

Aims

To evaluate the interactive effect of fast food consumption and overweight on asthma in preadolescents.

Material and methods

A case-control study comprised 246 children with pediatric allergist-diagnosed asthma and 477 non-asthmatic controls at age 8-10 years. Information on fast food consumption was obtained from survey questionnaire, and defined as eating burgers/fast food once or twice per week on average in the last 12 months. Overweight was defined as body mass index \geq 85th percentile of age and gender-specific growth charts. The likelihood of asthma according to fast food consumption and overweight status, adjusted for inclusive covariates, was determined in logistic regression analyses.

Results

Fast food consumption was significantly associated with asthma (crude OR 1.70, 95% CI 1.23-2.34). The association was significant in boys and not in girls. Fast food consumption was not linked to overweight. Children who had fast food consumption and were overweight had a two times increased risk for asthma (adjusted OR 1.99, 95% CI 1.18-3.34). Children who had fast food consumption and were not overweight had a similar risk (adjusted OR 1.74, 95% CI 1.14-2.64). Children who were overweight and rarely had fast food consumption (never or occasionally) were not at a significant risk for asthma (adjusted OR 1.43, 95% CI 0.80-2.54). The combined effect and the effect of fast food consumption alone were significant in boys. The combined effect and the effect of overweight alone were at a marginal significance in girls.

Conclusions

The interactive effect of fast food consumption and overweight was weak. Fast food consumption alone may increase the risk of asthma in boys.

Poster #17

The Validity of a Measure of Postpartum Depression in Health Care Database Studies of Childhood Asthma

Authors: Yallop L, McGrath P, MacNeil B, Becker AB, Kozyrskyj AL.

Rationale

To determine the validity of a health care database definition of postpartum depression for future use in population-based studies on the origin of asthma.

Methods

Using health care database records, postpartum depression in mothers was defined in the 1995 Manitoba birth cohort, on the basis of physician visits, hospitalizations or prescription medications for depression or anxiety. A sample of mothers solicited in 2005/06 from the birth cohort was queried on the presence of postpartum (1-item, 4-point, self-report Likert scale on feelings of depression or hopelessness) and of depression 10 years after giving birth (PHQ-9, a validated 9-item depression scale). Sensitivity and specificity of the database definition for postpartum depression were determined, using maternal survey response as the gold standard.

Results

Maternal reports of postpartum depression were available for 418 mothers of children recruited for study. 20% of the mothers in the sample had postpartum depression as defined by the database measure. 47% of mothers had some amount of postpartum depression according to self report. Of the mothers that reported postpartum depression, 17% continued to experience depression ten years after giving birth, as measured by the PHQ-9. Using maternal report as the gold standard, the sensitivity of the database definition of postpartum depression was 78% and the specificity was 82%.

Conclusions

A database definition of postpartum depression, based on prescription use and health care visits for depression within one year following birth, is a valid method to identify postpartum depression in population-based health care database studies.

Poster #18

Peripheral Blood Leucocytes from Asthmatic Horses (heaves) Present an Aberrant Response to Bacterial Extracts

A. Lavoie-Lamoureux¹, J. Martin², J.-P. Lavoie¹

Rationale

It has been suggested that susceptibility of allergic individual to LPS and other bacterial wall products found in house dust predispose asthmatic individuals to the onset of clinical exacerbations. Here, we compared the response to bacterial wall components of neutrophils and other peripheral blood (PB) leukocytes from horses with heaves to that of healthy controls.

Methods

Neutrophils from control (n=5) and heaves-susceptible horses (n=6), isolated from peripheral blood (PB) using an immunomagnetic technique, were stimulated for 5 hr. in the presence of LPS (100 ng/mL) and fMLP (10⁻⁸ M). Differential mRNA expression was studied using Real-Time PCR. In separated experiments, neutrophils were stimulated for 1 hr. with LPS (100 ng/mL or 1 µg/mL) to assess integrin expression (CD18) using flow cytometry. Concurrently, the neutrophil-depleted peripheral blood (PB) leucocytes fraction was cultured in the presence of LPS and fMLP for 5 hr. to evaluate gene expression.

Results

Compared to control animals, LPS and fMLP stimulated neutrophils from heaves susceptible-horses had a significant decreased expression of IL-8, and an increased expression of TNF-α. Moreover, neutrophil-depleted PB leucocytes from heaves-affected horses had a greater expression of pro-inflammatory genes both before (TNF-α) and after stimulation with bacterial extracts (IL-8, IL-1β). No difference between groups was observed concerning neutrophils expression of surface integrins.

Conclusion

Neutrophils and other PB leucocytes from heaves-affected horses have an abnormal inflammatory response to bacterial extracts *ex vivo*. These results suggest that LPS and other bacterial wall components present in house dust may lead to inappropriate inflammatory response in susceptible individuals. Further experiments are needed to determine the association of this altered response to the development of allergic airway diseases.

¹ Dept. of Clinical Sciences, Vet. Medicine Faculty, U. of Montreal, Quebec, Canada

² Dept. of Physiology, Meakins-Christie Laboratories, McGill U., Quebec, Canada

Poster #19

The Molecular Basis for Glucocorticoid Mediated Survival of Primary Human Neutrophils

*Arash S Saffar, Stéphane Dragon, Peyman Ezzati, Lianyu Shan, Abdelilah Soussi Gounni
Department of Immunology, Faculty of Medicine, University of Manitoba, Winnipeg,
Manitoba, Canada*

Glucocorticoids are potent anti-inflammatory drugs that inhibit apoptosis of human neutrophils through unknown mechanisms. In this study we show that dexamethasone, a classical glucocorticoid, significantly inhibited apoptosis of primary human neutrophils by inducing protein neosynthesis specifically through the glucocorticoid receptor independently of transrepression. In dexamethasone treated neutrophils, enhanced levels of the pro-survival protein Mcl-1 were associated with decreased translocation and cleavage of the pro-apoptotic molecules Bid and Bax into mitochondria. Furthermore, dexamethasone inhibited release of Smac from mitochondria, indicating maintenance of mitochondrial membrane integrity. Among the inhibitor of apoptosis proteins, XIAP levels were maintained by dexamethasone, correlating with decreased protease activity of caspases- 8, 9 and 3. In conclusion, our results demonstrate that in contrast to most immune cells, human neutrophils mount a robust anti-apoptotic response to glucocorticoids by enhancing pro-survival proteins and blocking the intrinsic pathway of apoptosis.

Poster #20

The ¹³C Glucose Breath Test in Children: A Simple, Non-Invasive Tool to Assess the Relationship Between Insulin Resistance, Obesity and Asthma

N. Saurek-Aleksandrovskaja, AL Kozyrskij, R. Rabbani, AB Becker, EAC. Sellers.

Rationale

To validate the ¹³C glucose breath test in children as a measure of insulin resistance (IR), which has been proposed as a mechanism to explain the association between obesity and asthma.

Methods

Data were obtained from a case-control study of the 1995 Manitoba birth cohort. Fasting blood glucose and insulin were obtained to calculate the homeostasis model assessment of insulin resistance (HOMA-IR, a validated measure of IR in children) and the ¹³C glucose breath test was administered. Asthma was diagnosed by a pediatric allergist and the BMI z-score was calculated from height and weight measurements. The ¹³C breath test was compared with HOMA-IR and multiple linear regression analysis was conducted to determine best predictors of HOMA-IR.

Results

415 children were recruited (152 asthmatic, 263 non-asthmatics). 15% had a significant degree of IR as measured by HOMA-IR, 6% as measured by the ¹³C breath test. Using HOMA-IR as a standard, the sensitivity of the ¹³C glucose breath test was 16% (0.07-0.27), and the specificity was 96% (0.93-0.98). The Pearson correlation between ¹³C breath test and HOMA-IR was $r = -0.345$, $p < 0.0001$. From the multiple regression results ($r^2 = 0.278$), significant predictors of HOMA-IR were the BMI z-score ($B = 0.611$, $p < 0.0001$) and the interaction between BMI z-score and ¹³C values ($B = -0.019$, $p < 0.0001$). No significant associations were found between ¹³C and asthma status, even following stratification for overweight status.

Conclusions

The ¹³C breath test is a specific, but not sensitive measure of IR and best predicts IR in combination with BMI z-score. It may be a useful, simple, non-invasive tool to measure IR in epidemiologic studies. However, at this preliminary stage of data collection we found no association between IR status and asthma in children.

Poster #21

Exogenous Nitric Oxide Regulates Cyclooxygenase-2 Expression and Prostaglandin D2 Generation In Mouse Bone Marrow-Derived Mast Cells

Tae Chul Moon, A. Dean Befus

Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

Mast cells (MC) are important effector cells in allergic and inflammatory responses. They play these roles through selective secretion of various mediators after IgE-dependent and/or IgE-independent activation. Nitric oxide (NO) is important signaling molecule that regulates MC function. NO can depress MC allergic responses such as leukotriene (LT), cytokine and chemokine production as well as MC degranulation. However, the role of NO on prostaglandin (PG) D2 production, an important lipid mediator produced in MC, is unclear. In PG synthesis, cyclooxygenase (COX) is important enzyme. There are two isozymes of COX, constitutively expressed COX-1 and inducible COX-2. It is well established that mouse bone marrow-derived mast cells (BMMC) exhibit biphasic PGD2 biosynthesis; COX-1-dependent immediate and COX-2-dependent delayed PGD2 production following COX-2 expression, when BMMC are stimulated with stem cell factor (SCF), IL-10 and IL-1 β .

The role of NO on COX-2 expression and PGD2 generation in BMMC was investigated using NO-donors, S-Nitrosoglutathione (SNOG) and S-Nitroso-N-acetylpenicillamine (SNAP). We observed that exogenous NO augmented COX-2 protein expression and increased COX-2-dependent PGD2 generation in response to SCF, IL-10 and IL-1 β . Even though both of SNAP and SNOG augmented COX-2 protein expression and COX-2-dependent PGD2 generation, no effects of NO-donors were observed on COX-2 mRNA expression after 2h of activation. For COX-1, NO-donors did not affect its protein expression. However, in contrast to the augmentation of COX-2 expression and activity, we observed that SNOG (100 to 500 μ M), but not SNAP (up to 500 μ M) inhibited COX-1-dependent PGD2 generation.

These results suggest that exogenous NO regulates PGD2 production by MC in inflammatory states through regulation both of COX-1 and COX-2. Furthermore, these findings help us to understand the role of NO in MC function and the regulatory mechanisms of lipid mediator generation in MC in inflammatory disease.

Supported by CIHR and the Korea Research Foundation (KRF-2005-214-C00111)

Poster #22

Endotoxin Protection Against Atopy: Phenotypic Differences Between First Nations and non First Nations Children

S Huq, AB Becker, AL Kozyrskyj

Rationale

Endotoxin has been shown to be protective against atopic phenotypes in some environments, but not others. We sought to determine the association between indoor endotoxin levels and atopic phenotypes in First Nations (FN) and non First Nation children.

Methods

This was the SAGE case-control study of the 1995 Manitoba cohort, consisting of 246 asthmatic and 477 non-asthmatic controls at age 8-10 years. First Nations or Metis status was self-declared in 150 children, 55 % living on reserve. Atopic dermatitis, allergic rhinitis and asthma were diagnosed by a pediatric allergist. Atopy was defined as one or more positive skin prick tests to common allergens and BHR (bronchial hyper-responsiveness) as methacholine < 8 mg/mL. Endotoxin levels were analyzed by LAL assay from dust samples collected during a home inspection. The likelihood (odds ratio, OR) of asthma phenotype according to endotoxin level quartiles <61 eu/g, 61- 97 eu/g , 98- 141 eu/g and > 141 eu/g , adjusted for gender, maternal asthma, mold and tobacco smoke exposure at birth, and current mold, was determined in logistic regression analyses.

Results

Mean endotoxin levels were significantly higher in FN homes than in non FN homes (189.9 eu/g vs. 108.7eu/g $p=0.03$). Endotoxin was protective against atopic dermatitis in non FN children, with adjusted ORs of 0.47 (95%CI: 0.23-0.97) for mid-quartile levels, and of 0.23 (95%CI: 0.09-0.55) for the highest levels. Third quartile levels also suggested protection against BHR asthma (OR=0.52, 95%CI: 0.27-1.00), BHR (OR=0.60, 95%CI: 0.34-1.05) and atopy (OR=0.63, 95%CI: 0.37-1.08). In FN children, the adjusted OR for atopy was 0.19 (95%CI: 0.05-0.75) for exposure at the highest level. No other associations were observed for endotoxin and atopic phenotypes.

Conclusions

Endotoxin protects against atopic dermatitis in non FN children and against atopy in FN children. These findings may be the outcome of genetic differences superimposed on environmental exposures.

Poster #23

IL-17 Enhances IL-1 β Mediated CXCL-8 Release from Human Airway Smooth Muscle Cells

*Stéphane Dragon**, *Muhammad Shahidur Rahman**, *Jie Yang**, *Helmut Unruh‡*, *Andrew J. Halayko†*, *Abdelilah Soussi Gounni*1*

**Departments of Immunology, † Physiology, and Sections of Respiratory Diseases & ‡ Thoracic Surgery, University of Manitoba, Winnipeg, Manitoba, Canada;*

Recent studies into the pathogenesis of airway disorders such as asthma have revealed a dynamic role for airway smooth muscle cells in the perpetuation of airway inflammation via secretion of cytokines and chemokines. In this study, we evaluated whether IL-17 could enhance IL-1 β mediated CXCL-8 release from human airway smooth muscle cells (HASM) and investigated the upstream and downstream signaling events regulating the induction of CXCL-8. CXCL-8 mRNA and protein induction were assessed by real-time RT-PCR and ELISA from primary HASM cultures. HASM transfected with site mutated AP-1/NF κ B CXCL-8 promoter constructs were treated with selective p38, MEK-1/2 and PI3-K inhibitors to determine the importance of MAPK and PI3-K signaling pathways as well as AP-1 and NF- κ B promoter binding sites. We demonstrate IL-17 induced, and synergized with IL-1 β to up-regulate CXCL-8 mRNA and protein levels. Erk-1/2 and p38 modulated IL-17 and IL-1 β CXCL-8 promoter activity however IL-1 β also activated the PI3-K pathway. The synergistic response mediating CXCL-8 promoter activity was dependent on both MAPK and PI3-K signal transduction pathways and required the cooperation of AP-1 and NF- κ B cis-acting elements upstream of the CXCL-8 gene. Collectively, our observations indicate MAPK and PI3-K pathways regulate the synergy of IL-17 and IL-1 β to enhance CXCL-8 promoter activity, mRNA induction and protein synthesis in HASM via the cooperative activation of AP-1 and NF- κ B trans-acting elements.

Poster #24

Eosinophil-Derived Tryptophan Catabolites Inhibit Lymphocyte Survival via Glutamate Receptors

M. Ghaffari¹, *S. O. Odemuyiwa*¹, *V. Lam*¹, *A. Ghahary*², *M. Duszyk*³, *R. Moqbel*¹

¹*Department of Medicine, Pulmonary Research Group, University of Alberta, Edmonton, AB, CANADA,* ²*Department of plastic surgery, University of British Columbia, Vancouver, BC, CANADA,* ³*Department of Physiology, Pulmonary Research Group, University of Alberta, Edmonton, AB, CANADA.*

Aim of study

Eosinophils express indoleamine 2,3 dioxygenase, which catalyses the catabolism of tryptophan to kynurenines (KYN), leading to the inhibition of T cell proliferation. Although tryptophan depletion was suggested to cause T cell death, the mechanism is still poorly understood. However, exposure of T-cells to NMDA results in T-cell death via activation of NMDA glutamate receptors. Quinolinic acid, a catabolite of tryptophan, is a NMDA receptor agonist. We, therefore, investigated the expression of glutamate receptors by eosinophils and T cells to understand the mechanism of KYN-induced T cell inhibition.

Methodology

RT-PCR, flow cytometry and Western blotting were used to detect the expression of glutamate receptors in eosinophils and T cells. Intracellular Calcium was measured in fura-3-loaded eosinophils and T-cells via flow cytometry. Apoptosis was assessed using Vybrant apoptosis detection kit (Molecular Probes).

Results

Eosinophils but not T-cells express mRNA group III metabotropic receptors (mGluR2 and mGluR7). Resting lymphocytes express only NMDA receptors that are upregulated following activation with phytohaemagglutinin. Activation of eosinophils and lymphocytes with glutamate, NMDA and Quinolinic acid resulted in significant calcium flux, a response enhanced in activated lymphocytes. ACPD, an agonist of metabolic glutamate receptors, increased intracellular calcium only in activated lymphocytes. While 100pg/ml GM-CSF resulted in a viability of 95% in human eosinophils after 36 hours of incubation and incubation with glutamate resulted in 60% viability. Only 25% of eosinophils remained viable during this time period. In contrast treatment of activated lymphocytes with NMDA resulted in T-cell apoptosis.

Conclusions

Human eosinophils express functional glutamate receptors. Activation of glutamate receptors increases the survival of human eosinophils in culture while glutamate, and tryptophan catabolites, through glutamate receptors, may induce cell death in T cells. Thus, eosinophil-dependent tryptophan-induced T-cell death may result from activation of glutamate receptors by Quinolinic acid, in addition to tryptophan depletion.

Funding: AllerGen NCE Inc.

Poster #25

Allergen-Specific T&B Cells in Allergic Patients are not Increased Compared to Non-Allergic Persons

*Ueno A., Lu C., Williams L., Serushago B., Bowen T., Storek J.
University of Calgary, Calgary, Alberta, Canada*

Background

Allergic disease is thought to be caused by increased (compared to non-allergic state) production by plasma cells of IgE against innocuous substances like pollens. These pathogenesis could be one of the following:

1. Allergic individuals may have similar numbers of allergen-specific T and B cells, but the propensity of their B cells to differentiate into plasma cells producing large amounts of IgE may be increased; *or*
2. Allergic individuals may have increased numbers of allergen-specific B cells or T cells helping B cells differentiate into IgE-producing plasma cells.

Methods

We studied blood mononuclear cells from 10 individuals with allergic disease (with symptoms of asthma, rhinitis, or eczema *AND* a positive skin prick test to at least one of nine common aeroallergens) and 10 non-allergic individuals (with no symptoms of asthma, rhinitis, or eczema *AND* with a negative skin prick test to the nine common aeroallergens). Using flow cytometry, allergen-specific T (B) cells were defined as CD3⁺ and CD4⁺ (CD19⁺ or CD20⁺) cells proliferating (diluting CFSE) when stimulated for 7 days with the nine aeroallergens. Allergen-specific T_{H2} cells were defined as CD3⁺ and CD4⁺ cells proliferating upon the stimulation with the aeroallergens and producing interleukin-4.

Results

The counts of allergen-specific T cells, Th2 cells, and B cells were similar in the allergic patients and the non-allergic controls.

Conclusion

This suggests that allergic and non-allergic B cells differ not in the number but in their propensity to differentiate into plasma cells producing large amounts of IgE.

Poster #26

Effects of Nitric Oxide–Mediated Tyrosine Nitration on Enzymatic Activity of Mast Cell Aldolases

Yokananth Sekar and A. Dean Befus.

Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, Canada.

Mast cells (MC) are effector cells of IgE-mediated allergic diseases. MC can produce nitric oxide (NO) and both endogenous and exogenous NO have regulatory effects on MC. We hypothesized that protein tyrosine nitration, a post-translational modification mediated by NO plays a regulatory role in MC. In a hypothesis-generating proteomic approach, nitrated proteins of HMC-1, a human mast cell line were assessed using two-dimensional electrophoresis and western blot with anti-nitrotyrosine antibody. Mass spectrometry was used to characterize proteins selectively nitrated upon treating the cells with SNOG, a NO donor. A 500 μ M of SNOG for 4 hr selectively nitrated aldolase A in HMC-1 cells. Western blot analysis with anti-aldolase antibody revealed that there are multiple isoforms of aldolases with same Mr but different pI in HMC-1. Some of the isoforms are constitutively nitrated, whereas others show SNOG-induced nitration. Mass spectrometric analysis of aldolase spots that are constitutively nitrated confirmed presence of peptides of two isoforms A and C of aldolase in HMC-1, whereas the form of aldolase selected for study from SNOG-induced nitration revealed peptides of aldolase A only. RT-PCR using isoform-specific primers confirmed mRNA level expression of aldolase A and C in human MC, HMC-1 and LAD-2. SNOG-induced nitration of aldolase reduced the enzymatic activity in the supernatant of the HMC-1 cell homogenate. As the effect of nitration of aldolase depends upon the tyrosine residues that are nitrated, identification of those specific tyrosine molecules among the 13 tyrosine residues of aldolase is of functional significance. Using mass spectrometry we are working to characterize the tyrosine residues of MC aldolase that are targets for nitration. Thus the decrease in enzymatic activity of MC aldolase upon its nitration may play a regulatory role on MC phenotype and function.

Acknowledgement: The Canadian Institutes of Health Research and Alberta Lung Association

Poster #27

RSV Infection Influences the Immune System Towards a Th2 Response: the Role of IDO

Farnam Ajamian, Francis Davoine, Solomon O. Odemuyiwa, Redwan Moqbel, Darryl Adamko

Pulmonary Research Group, Department of Medicine, University of Alberta, Canada

Background

Asthma is the most common chronic disease of childhood. Lower respiratory tract infection with Respiratory Syncytial Virus (RSV) during infancy is linked with asthma. The airway inflammation observed in asthma is associated with predominance of Th2 lymphocytes and related cytokines. Indoleamine 2,3-dioxygenase (IDO) induction in dendritic cells (DCs) is a major mechanism of DC-induced Th1 cell apoptosis, which likely contributes to the Th2 polarisation seen in asthma. IDO is the rate-limiting enzyme in extrahepatic catabolism of tryptophan and is induced by IFN- γ . Kynurenine (kyn) is the main product of this catabolism. Viral infection of macrophages, eosinophils and dendritic cells in the respiratory airway can induce IFN- γ release and this could result in increased IDO activity with subsequent perpetuation of Th2 imbalance in asthmatics.

Hypothesis

RSV infection induces IDO that contributes to Th2 bias in asthma.

Methods

We incubated Human monocytic cell line (THP1) and primary human DCs with RSV (MOI: 0.1-10). Flow cytometry and confocal microscopy was used to confirm infection. Kynurenine level was measured in culture media using a spectrophotometric method based on Ehrlich reaction.

Results

RSV infects up to 25% (n = 3) of DCs and 35 % (n = 3) of THP1 cells. DCs infected with RSV (MOI = 5) for 4 days, increased kynurenine release (90 uM) compared to uninfected control DCs (10 uM) (n=2).

Conclusion

RSV infects THP1 and DCs *in vitro*. DCs infection with RSV results in increased IDO activity as measured by kynurenine release. This observation provides a new mechanism through which respiratory infection with RSV in childhood might contribute to skewing the immune system toward Th2 response.

Poster #28

Airway Side Population (SP) Cells: Progenitors of Multiple Cell Types

T-L. Hackett, F. Shaheen, G. Singhera, D. Dorscheid, S. Van Eeden, R. Hegele, DA. Knight, and T. Bai.

Introduction

The bronchial epithelial cell is the first cell of contact and a physical barrier to the external environment. These cells are continuously exposed to, and injured by pollutants, allergens and viruses as part of their normal function. Detailed cellular examination of bronchial biopsies and BAL fluid has provided convincing evidence of epithelial damage and aberrant repair in asthma. This excessive epithelial damage and fragility can arise from an enhanced susceptibility to injury and/or an inadequate repair response or a combination of both. It is therefore important to understand the regulatory mechanisms involved in mucosal repair. Although several cell types have been postulated as having progenitor function in the airways, the identity of the resident stem/progenitor cell(s) in human airways is still unclear. The rapid efflux of the fluorescent DNA-binding dye Hoechst 33342 identifies a rare side population (SP) of cells (<1% of epithelial cells), which are enriched for stem/progenitor cell activity. For this reason, we have used the bronchial airways of sheep to identify and characterise bronchial epithelial stem/progenitor cells.

Methods

Epithelial cells (40×10^6) obtained from sheep airways via pronase digestion were stained with Hoechst 33342 and propidium iodide and then sorted using fluorescence-activated cell sorting. SP and non-SP were then collected and plated for tissue culture.

Results

The bronchial epithelium contained a viable population of cells that showed the SP phenotype and comprised <0.1% of total epithelial cell population (200,000 cells). The SP population were comprised of both CD45+ (85%) and CD45- (15%) subsets. When placed in culture, a single SP cell gave rise to a heterogeneous colony of cells, whereas non-SP cells failed to grow.

Conclusions

Our findings illustrate that bronchial epithelial SP cells have the potential to produce a diverse phenotype of 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 characterise these cells.

Poster #29

Exacerbation of Pulmonary Symptoms in a Mouse Model of Allergic Asthma Following Exposure to Concentrated Ambient Particles and Ozone

***Nivedita Khanna**^{1,2}, Pascal Djiadeu³, Stacey A. Ritz⁴, Nades Palaniyar³, Frances Silverman^{1,2,5} & Jeremy A. Scott^{1,2,5}*

Divisions of Occupational¹ and Respiratory² Medicine, Department of Medicine, University of Toronto. ³Hospital for Sick Children Research Institute, Toronto. ⁴Northern Ontario School of Medicine, Sudbury, ON. ⁵Gage Occupational & Environmental Health Unit, St. Michael's Hospital Research Centre, Toronto, ON

Rationale

Asthma is an inflammatory disease of the airway and air pollution is known to have a significant impact on asthma-related morbidity. In this study we sought to investigate whether co-exposure to concentrated ambient particles (CAP) and ozone affects the airway hyper-responsiveness and associated inflammation in an acute murine model of allergic asthma.

Methods

We have established an ovalbumin (OVA) -sensitization and -challenge model of allergic asthma in mice. Animals were sensitized by injection of OVA (i.p.; 25 µg/mouse in alum) or PBS at day 0 and 7, followed by aerosol challenge (6% OVA in PBS or PBS alone) on days 14-16 (25 min./day). Twenty-four hours after the last exposure to the allergen, conscious, freely-moving animals were exposed to CAP (200-1000 µg/m³) using the Harvard Ambient Particle Concentrator and ozone (200 ppb-2 ppm) for 4 hours. Pulmonary function and airway responsiveness was subsequently assessed using a ventilator-based system (flexiVent). Bronchoalveolar lavage was performed for further assessment of immune end-points with Western blotting and ELISA (i.e., cytokine profiles, surfactant protein (SP) A, and SP-D).

Results

We observed increased maximum responsiveness (p = 0.007) and a trend towards decreased EC₅₀ for methacholine, indicative of increased airway responsiveness, in the pollution exposed OVA-sensitized and -challenged animals (n=4/group). Collectin levels were altered differentially between the CAP and ozone exposed and unexposed mice.

Conclusions

We conclude that ambient particle exposure + ozone exacerbated airway hyperresponsiveness to methacholine and altered surfactant protein secretion in airway-sensitized mice.

Support: AllerGen NCE, National Sanitarium Association

Poster #30

Th2 Inflammation Seen in Allergic Disease Inhibits Toll-like Receptor 4 Expression and Regulatory Functions in T Cells

AllerGen Research Project # 3.11: TLR4+ T cells in Children

Préfontaine D, Fiset PO, Banville-Langelier A-A, Mazer M, Hamid Q, Mazer B Meakins-Christie Laboratories, McGill University, Montreal, Qc, Canada

Background

In support of the Hygiene Hypothesis, bacterial lipopolysaccharide (LPS)-induced signaling through 'Toll-like receptor 4' (TLR4) would promote TLR4+ CD4+ T 'helper' lymphocytes (possibly 'regulatory' T or T-reg cells) which would limit Th2 inflammation seen in allergic diseases. In turn, Th2 inflammation downregulates expression of TLR4, but the mechanism is currently unclear.

Hypothesis

We hypothesized that CD4+ T cells either from allergic children, or stimulated under Th2 conditions, would display reduced TLR4 expression compared with non-atopic controls or unstimulated cells, and that Th2 transcription factors will correlate with TLR4 downregulation. We hypothesized that atopy and Th2 conditions inhibit T-reg cell phenotype and function.

Methods

Peripheral blood mononuclear cells (PBMC) isolated from atopic and non atopic children (2-18 years old) were incubated for 24 h with or without IL-4 or LPS. RNA extracted from CD4± fractions (isolated by MACS) or from PBMC was used for RT-PCR. The following gene expression profiles were determined by real-time qPCR: TLR4, IL-4, IL-10, STAT6, GATA-3, and FOXP3. Fluorescent ICC was used to colocalize FOXP3+TLR4+ cells in PBMC incubated with medium, IL-4 or LPS.

Results

IL-4 stimulation of PBMC specifically reduced TLR4 expression in CD4+ T cells. IL-4 stimulation increased IL-4 and Th2 transcription factors (STAT6 and GATA-3) transcript levels more importantly in CD4+ cells from atopic patients. TLR4 promoter analysis revealed the presence of STAT6 and GATA3 binding sites. IL-4 upregulated FOXP3 gene expression in CD4+ cells regardless of atopic status, and increased its expression among TLR4+ PBMC. LPS also upregulated both FOXP3 and IL-10 regulatory cytokine mRNA levels, whereas IL-4 downregulated IL-10.

Conclusions

These data suggest that Th2 inflammation and atopy reduce the expression of TLR4 especially in CD4+ T cells possibly through Th2 transcription factors. Although IL-4 promoted the development of FOXP3+TLR4+ T-reg cells, it may reduce their immunosuppressive function through downregulation of IL-10.

Poster #31

Role of L-Arginine Metabolism in Murine Allergic Asthma

Michelle L. North^{1,2}, *Nivedita Khanna*^{1,2}, *Hartmut Grasmann*^{2,3}, *Frances Silverman*^{1,2,4}
& *Jeremy A. Scott*^{1,2,4}

Divisions of Occupational¹ and Respiratory² Medicine, Department of Medicine, University of Toronto, ³Hospital for Sick Children, and ⁴Gage Occupational & Environmental Health Unit, St. Michael's Hospital Research Centre, Toronto ON.

Rationale

The semi-essential amino acid, L-arginine, plays an important role in smooth muscle function and tissue homeostasis. The nitric oxide synthase (NOS) and arginase biochemical pathways both utilize L-arginine as substrate to produce nitric oxide/L-citrulline and ornithine/urea, respectively. Disruption of the delicate balance between these competing pathways likely contributes to the pathogenesis of airway disease, including asthma. Therefore, we performed a comprehensive examination of the enzymes and transporters involved in L-arginine uptake and metabolism in a murine model of allergic asthma.

Objective

To examine alterations in the arginine biochemical pathway in a murine model of acute allergic asthma.

Methods

We used an acute ovalbumin (OVA)-sensitization and challenge model of allergic asthma. Mice were sensitized on days 0 and 7, and challenged with nebulized OVA (6%) on days 14-20. Pulmonary function testing and methacholine responsiveness was performed using a ventilator-based system (flexiVent). Western blotting was used to examine the expression profiles of proteins related to L-arginine uptake and metabolism (i.e., arginases 1 and 2, nNOS, iNOS, eNOS and the cationic amino acid transporters) and arginase activity was determined in lung homogenates.

Results

Ovalbumin-sensitized and challenged in our acute model of allergic asthma exhibited altered pulmonary function and increased airway responsiveness to MCh. ArgI, ArgII, iNOS and nNOS protein expression levels were increased in the OVA-sensitized and challenged mice. These changes in expression were paralleled by a concomitant increase in arginase activity, whereas the mechanisms of uptake were unaffected (i.e., cationic amino acid transporters).

Conclusions

These results support that the arginase and NOS isozymes may compete for substrate in asthma. L-arginine metabolism could contribute to the pathogenesis of asthma and may be a possible drug target for therapeutics.

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Poster #32

The Transcription Factor Wilms Tumor 1 Regulates Matrix Metalloproteinase-9 through a Nitric Oxide-Mediated Pathway

Marcelo Marcet-Palacios, Marina Ulanova, Florentina Duta, Lakshmi Puttagunta, Samira Munoz, Derrick Gibbins, Marek Radomski, Lisa Cameron, Irvin Mayers, Dean Befus.

Department of Medicine, University of Alberta. Edmonton. T6G 2S2, Canada.

Matrix metalloproteinase-9 (MMP-9) is released by lung epithelial cells (LEC) in conditions such as asthma and COPD. Expression of MMP-9 correlates with severity of these disorders. However, transcriptional regulation of this enzyme is poorly understood. We have reported that nitric oxide (NO) is required for MMP-9 gene induction. NO activates soluble guanylate cyclase (sGC) to produce cGMP, which can activate protein kinase A (PKA). PKA then translocates to the nucleus and phosphorylates numerous transcription factors thus mediating gene activation.

We observed a highly conserved CA repeat in the 5' flanking region of the MMP-9 promoter. Phylogenetic analysis identified a 100% homology in human, cow, rat and mouse. This highly conserved region contained potential binding sites for a transcription factor called Wilms tumor 1 (WT1) as detected with the transcription factor search system. WT1 is regulated by a PKA-mediated phosphorylation that reduces WT1 DNA binding affinity resulting in its subsequent translocation to the cytosol. We postulate that WT1 is an MMP-9 gene repressor, regulated by a NO-mediated pathway.

Immunohistochemistry analysis in normal human lung identified WT1 in the epithelium. Additionally WT1 was expressed in five human LEC lines. Neither TNF nor a cocktail containing LPS, PMA and IFN-gamma changed WT1 expression. These treatments however induced WT1 translocation from the nucleus to the cytosol. Translocation was blocked with the NO synthase inhibitor 1400W that also reduced MMP-9 gene expression and enzyme activity. WT1 knock down through small interfering RNA (siRNA) upregulated MMP-9 activity in the presence of 1400W. Additionally chromatin immunoprecipitation (ChIP) revealed a decreased level of WT1 binding to the MMP-9 promoter after TNF treatment *in vivo*.

Thus, WT1 is an MMP-9 gene repressor in LEC. NO prevents this repression, potentially through a PKA-mediated pathway. These findings will help us understand the regulatory mechanisms controlling MMP-9 gene expression in health and lung disease.

Poster #33

Lung Function and Response to Acute Allergen Challenge in Caveolin-1 Knockout Mouse

S.Martin, S.Basu, Dr. Ganesh Srinivasan and Dr. Andrew Halayko. University of Manitoba and Manitoba Institute of Child Health, Winnipeg, Manitoba, Canada.

Rationale

Caveolin-1 (Cav-1) is enriched in membrane caveolae; foci for regulating receptor-mediated signalling. We have shown that loss of Cav-1 suppresses airway smooth muscle contraction by decreasing M3 muscarinic receptor mediated intracellular Ca^{2+} release. We used Cav-1 knockout mice (Cav-1 KO) to investigate the role of Cav-1 on lung function before and after acute allergen exposure.

Methods

Female, 8-10 week old Cav-1 KO animals and genetic controls (B6129SF2/J) were sensitized twice *i.p.* with ovalbumin (OVA) (4 μ g/ml)/ AlOH₃ (8mg/ml), then twice intranasally to OVA (50 μ L @ 50 μ g/ml). Lung function was estimated 48 hrs after OVA challenge with Enhanced Pause (Penh) measured by whole body plethysmography. Bronchoalveolar lavage fluid (BAL) was collected for differential cell counts. Histology and electron microscopy were performed to assess lung morphology.

Results

Cav-1 KO mice showed increased alveolar pneumocytes, alveolar septal thickening, plus endothelium and airway epithelium hyperplasia. Trichrome staining showed prominent airway smooth muscle and excessive extracellular matrix around airways and blood vessels. Baseline Penh values were 50% lower in Cav-1 KO mice for methacholine exposure up to 50 mg/ml (6.09 ± 0.64 for Cav-1 KO, 12.09 ± 1.45 for B6129SF2/J). This difference was unaffected by intranasal allergen exposure. In B6129SF2/J, sensitization and challenge decreased macrophages ($85 \pm 2\%$ to $9 \pm 1\%$) and increased eosinophils (4% to $63 \pm 1\%$), while Cav-1 KO showed less decrease in macrophages ($83 \pm 4\%$ to $53 \pm 1\%$) and a greater increase in eosinophils (1% to $43 \pm 1\%$).

Conclusion

These data reveal Cav-1 has significant effects on lung structure and function. Also, absence of Cav-1 is associated with key differences in inflammatory response to inhaled allergen.

Poster #34

Mechanisms of Leukotriene Regulation in Human Mast Cells

Ahmed El-Karmalawy, Tim Olynych, Wenda Greer, and Jean S. Marshall

Departments of Pathology and Microbiology & Immunology, Dalhousie University

Introduction

Leukotrienes (LT) are lipid mediators that have been studied extensively in the context of allergic response due to their rapid effects on bronchoconstriction and vascular permeability. Certain infections can lead to the production of leukotrienes as well as the recruitment of immune effector cells, which may also exacerbate allergic disease. The regulation of leukotriene production is critical to understanding the body's response to allergens. In this study, mechanisms of leukotriene regulation were examined via toll-like receptor (TLR) signaling in three mast cell models, primary cultures of cord blood-derived mast cells (CBMCs), the basophilic cell line KU812, and the human mast cell line HMC-1.

Methods

RNA and protein extracts from CBMCs, KU812, and HMC-1 were analyzed for the expression of the enzymatic machinery (5-lipoxygenase [5-LO], 5-LO activating protein [FLAP], LTA₄ hydrolase, LTC₄ synthase) involved in leukotriene synthesis. The cell models were also examined for their expression of TLR2 and TLR6. CBMCs, KU812, and HMC-1 were stimulated with calcium ionophore A23187 or zymosan (the yeast cell wall component and TLR2 ligand) for 20 min., after which supernatants were collected and analyzed for leukotriene C₄ (LTC₄) content.

Results

RT-PCR revealed the presence of 5-LO, FLAP, LTA₄ hydrolase, and LTC₄ synthase in all the cells examined. Immunoblot probing specifically for 5-LO showed its presence in CBMCs and HMC-1 at the protein level. CBMCs and HMC-1 also showed expression of TLR2 and TLR6 mRNA. Endogenous LTC₄ was produced in all the cells examined. LTC₄ levels were elevated in all the cells at least 20-fold through A23187 stimulation. Zymosan induced similar LTC₄ production in CBMCs, but yielded little to no LTC₄ production above controls in KU812 and HMC-1, consistent with their lack of TLR2 protein expression.

Conclusions

Results indicate that the mast cell models being studied are capable of producing cysteinyl leukotrienes in response to appropriate cell activation. However, primary mast cells were capable of responding to a wider range of stimuli. The molecular basis for these differences, including the role of TLRs and co-receptors, is currently under investigation.

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